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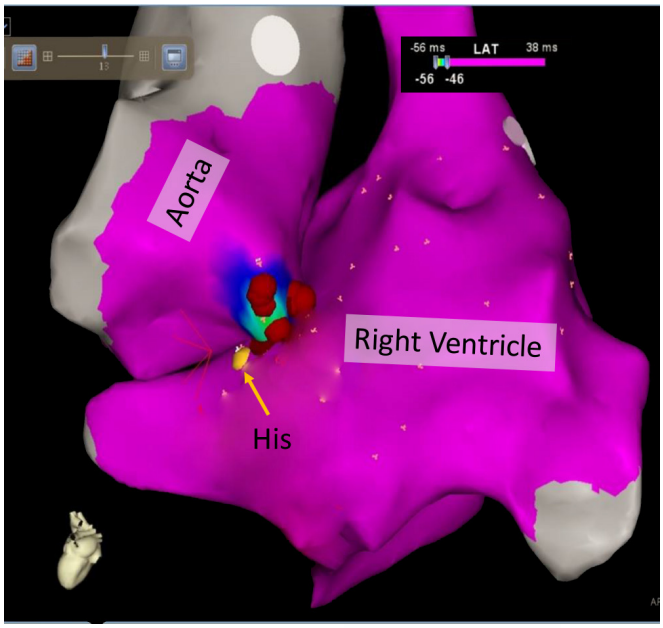

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Arquivos Brasileiros de Cardiologia
JOURNAL OF BRAZILIAN SOCIETY OF CARDIOLOGY - Published since 1943

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The Astronaut and the Jabuticaba

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Every two years, of the thousands of applications from all over the world, only 100 are considered eligible to undergo medical, physical and psychological examinations at NASA for astronaut training. Similar to what happens in medical schools, the selection process to identify which candidates are qualified to fly on space missions is extremely competitive. Only 0.1% of applicants are accepted. Comparable to what happens with medical students, some of the candidates cancel their application once they become aware of the rigorous workload and risks of becoming an astronaut. Aspiring physicians and astronauts have similar traits – they need to be motivated, laser focused on tasks at hand, able to complete exhaustive training, and appreciate the possible catastrophic consequences associated with misconduct.

Physicians, like astronauts, are frequently perceived as exceptional individuals who are capable of making pragmatic and prompt decisions based on the best available information. Medical decision making, like a shuttle launch, requires thorough preparation rather than blind faith that with keeping one's fingers crossed everything will be ok. Patients look to a physician who can make informed decisions coupling evidence-based medicine, guidelines and professional experience. However, variations in clinical practice are common. While it is easy to separate the extremes of excellent care from flagrant malpractice it remains a large gap between these two boundaries, where medical decisions are often made and adequate quality control is difficult.

This lack of oversight in "grey"[1,2] zones has become clear during the COVID-19 pandemic. From rectal ozone therapy – funny, if it were not tragic – to studies showing the inefficacy of several therapies, many physicians and institutions have made therapeutic decisions based on anecdotal experience or personal belief and, not rarely, on political conviction. In this context, to exempt themselves from their regulatory responsibilities, *some* medical councils, with honorable exceptions, have advocated that interventions without proven efficacy could be accepted if consensus between the doctor and the patient exists. However, if unanimity subjugates legislation, and if science cannot prevail over personal impressions, what is the value of such councils? Instead of promoting fruitful debates and adoption of evidence-based practice, a strategy of "if it is not bad, why not?" became acceptable.

Keywords

Pandemics/prevention and control; Dexamethasone; Anti-Inflamatory; Hydroxychloroquine; COVID-19; SARS-CoV-2.

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Jabuticaba

Brazil has a peculiar medical environment. Although interventions like hydroxychloroquine and ivermectin disappeared from the international scientific debate once disproved in clinical trials, Brazilian practitioners continue to debate the validity of these studies. Like *jabuticaba* – a fruit native to and predominant (but not exclusive) in Brazil – this debate is still current only in this country. Previous attempts to discount the results of studies with the argument that they had been conducted abroad and could not be extended to the Brazilian population can no longer be justified as many of these studies have included Brazilian patients. As individuals, we do understand the difficulty in accepting evidence opposing someone's conviction; however, as a doctor, this attitude is indefensible.

In this context of conviction and belief over evidence and data, several practitioners have created websites and even solicited the government to support and disseminate their practice despite proven inefficacy of the proposed interventions. Even though many are excellent physicians in their fields, this situation has only been possible because, under the auspices of trying to help, almost anything has been allowed in Brazil. If there were any regulation proposing a fine or termination of medical license to those who supported unproven medical practices, none of this would have happened. This regulation would be, in fact, similar to previous decisions of ethics committees in cases of charlatanism, when medical practices based on consensual decisions are not accepted as justification to exempt the infringer. However, these types of regulations are defective or absent in Brazil. Curiously, the term "accountability", that in English means an obligation or willingness to accept responsibility for one's actions, does not exist in Portuguese. On the other hand, there is no English word for "*jabuticaba*".

Those promoting unproven medical practices are likely unknowingly participating in the political non-sense debate surrounding the COVID-19 pandemic. Their engagement creates an unsafe atmosphere around both population and the press, as they irresponsibly suggest an alliance between the pharmaceutical industry, important medical scientific journals, and researchers to approve high-cost strategies and exclude less privileged populations. This conspiracy theory, common in situations of crisis, became almost a certainty when two non-randomized trials were published in two of the most important international medical journals today. But the scientific peer-review process was shown to be very effective,

critical and resolute in response to the concerns from other independent physicians and researchers. In only two weeks, the studies were retracted by the authors and the company that had provided the data disappeared. Furthermore, the main authors were sharply rebuked by the medical schools where they work, because in an effort to help, they disregarded the basic principles of scientific methodology. This is to be contrasted with the fact that Brazilian physicians who have stood against science suffer no consequence. These groups have spent enormous energy on the anti- science movement in Brazil, trying to convince the general population about their opinions. Silently rejected by most physicians, the anti-science movement has gained traction outside the academic realm, the latter which they deem as irretrievably corrupted. Instead, they should have positioned themselves to help answering important questions to benefit the whole population. However this is a lot of work! It is always easier to resist, complain and protest than to produce something scientifically relevant.

Brazilian Studies

Which three words have created more victims, "in my opinion" or "randomized clinical trials"? Although experience, or the "art of medicine", is valuable, it should complement the interpretation of scientific data and help apply results of scientific studies to specific patients and clinical situations. This contrasts with the belief that the "art of medicine" is simply a tool to promote one's anecdotal experience and recent memory of medical practice as strategies to establish standards of care. This approach devalues the extraordinary work of Brazilian researchers, who in a few weeks published several papers in renowned medical journals receiving global recognition. Their work set Brazil apart from other nations who have not been able to scientifically answer as many important questions about the COVID-19 pandemic as the Brazilian medical community.

The only way to advance medical practice is through well done clinical investigation. Few countries have been able to coordinate the processes necessary to perform well done, impeccable investigation to answer the challenges of COVID-19. Brazil is one of the countries that was up to it. Today, the *Coalition* group, consisting of leading hospitals and more than 50 national centers, is a global reference. Nearly 11 studies have been conducted on COVID-19 treatment.[3] Thanks to Brazil, physicians have learned that hydroxychloroquine with or without azithromycin did not improve clinical status of patients with mild-to-moderate COVID-19 (COALITION I),[4] and that azithromycin is not effective in severe COVID-19 also (COALITION II).[5] In addition to teaching us what not to do, the group has also confirmed that severe COVID-19 can be treated: hospitalized patients with moderate or severe acute respiratory distress syndrome (ARDS) due to COVID-19 benefited from intravenous dexamethasone, increasing the number of ventilator-free days (COALITION III).[6] Besides the COALITION studies, Brazilian researchers have produced high-quality epidemiological work, developed clinical trials in precarious conditions, answered the global question of how to treat COVID-19 patients receiving angiotensin-converting enzyme inhibitors and angiotensin receptor blockers[7] – yes, these therapies may be continued – and have been testing and producing vaccines that will potentially help millions of people. In the next six months, new studies will evaluate the efficacy of hydroxychloroquine in the out-of-hospital setting (hopefully it has a prophylactic effect), the role of different anticoagulants and the antiviral effect of tociluzumab. This is an astonishing and unprecedented achievement for Brazil.

The COVID-19 pandemic has revealed the best and worst of Brazilian medicine. The unscientific approach to medicine is unacceptable, places the population at risk, creates fake-news, and overshadows excellence in scientific endeavors within our country. After the pandemic, our success will seem natural, obvious, and inevitable; the efforts and methods to obtain all the answers will seem excessive; but dogmas will remain.

The use of garlic to treat patients with influenza is likely a remnant of the Black Death, when it was believed that transmission of the disease occurred through bad odors – phlegm -, and garlic and other essences could prevent the disease. Centuries later, this popular belief persists. Who has never eaten garlic to treat a flu?[8] Numerous studies have suggested that garlic has an antiviral effect. Although there are no randomized studies showing these effects, the myth still exists, since: 1) "it is probably not bad, so why not?"; 2) "it may not cure you, but it could help you"; 3) "a friend of mine used it and got better"; 4) "it seems to work in other diseases"; 5) "I am just trying to help". These are almost the same level of evidence on which some interventions in COVID-19 have been based. In the future, many patients could genuinely prefer to take ivermectin or zinc in case of severe influenza, since "if it could be effective for COVID-19, why would it not be effective for a common flu?" The trickle-down effect of unvalidated practices is not trivial.

The anti-science movement is currently divided among 3 groups: 1) the "converted" ones, 2) those who will be off the scene and show up again using the same strategy when another pandemic issue arises, and 3) those who take financial advantage of the situation by prescribing these drugs of unknown efficacy and encouraging these practices. Consequences to population health can be disastrous. Thus, it does not seem fair that only those who refuse to place their trust in the scientific method have a say today. Clinical research in Brazil has evolved greatly during the pandemic: it has become clear that the scientific community within our country has the capacity to overcome colossal challenges. The population may and should trust Brazilian medical science when properly understood and applied. And who knows, perhaps the key element to treat COVID-19 will be found in the *jabuticaba* extract?! What really matters is the message to the new generation of Brazilian doctors and researchers: just like for astronauts, rigor and training always prevail in the end.

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Strength Training Reduces Cardiac and Renal Oxidative Stress in Rats with Renovascular Hypertension

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Abstract

Background: Strength training has beneficial effects on kidney disease, in addition to helping improve antioxidant defenses in healthy animals.

Objective: To verify if strength training reduces oxidative damage to the heart and contralateral kidney caused by the renovascular hypertension induction surgery, as well as to evaluate alterations in the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) endogenous antioxidant enzymes.

Methods: Eighteen male rats were divided into three groups (n=6/group): sham, hypertensive, and trained hypertensive. The animals were induced to renovascular hypertension through left renal artery ligation. Strength training was initiated four weeks after the induction of renovascular hypertension, continued for a 12-weeks period, and was performed at 70% of 1RM. After the training period, the animals were euthanized and the right kidney and heart were removed for quantitation of hydroperoxides, malondialdehyde and sulfhydryl groups, which are markers of oxidative damage. In addition, the activity of SOD, CAT, and GPx antioxidant enzymes was also measured. The adopted significance level was 5% ($p < 0.05$).

Results: After strength training, a reduction in oxidative damage to lipids and proteins was observed, as could be seen by reducing hydroperoxides and total sulfhydryl levels, respectively. Furthermore, an increased activity of superoxide dismutase, catalase, and glutathione peroxidase antioxidant enzymes was observed.

Conclusion: Strength training is able to potentially reduce oxidative damage by increasing the activity of antioxidant enzymes. (Arq Bras Cardiol. 2021; 116(1):4-11)

Keywords: Hypertension, Renovascular; Resistance Training; Antioxidants; Oxidative Stress; Renal Arterial Obstruction; Oxidation-Reduction.

Introduction

Renovascular hypertension, a type of hypertension caused by total or partial renal artery stenosis due to genetic factors or atherosclerosis, is an important cause of secondary hypertension.[1] In this type of hypertension, the increase in arterial pressure (AP) is triggered by the greater release of renin by the ischemic kidney as a result of the reduction of blood flow to this organ, due to the stenosis of the renal artery.[1,2]

Renin is responsible for the conversion of angiotensinogen to angiotensin I, which is cleaved by the angiotensin-converting enzyme (ACE), producing angiotensin II (Ang II).[3,4] Thus, the elevation of renin triggers an increase in Ang II release. Ang II, in turn, activates the NADPH oxidase[3] and xanthine oxidase[4] enzymes, increasing the production of superoxide anion (O_2^-), a highly reactive pro-oxidant signaling molecule that can cause oxidative damage to lipids, proteins, and DNA, as has been described in renovascular hypertension.[5,6] Increased oxidative damage in the kidney and heart may lead to increased fibrosis of the tissue, leading to a reduction of its function,[2] and, eventually, leading to the failure of the kidney that was not affected by stenosis and cardiac dysfunction.

It is reported in the literature the protective action of strength training in the treatment of several diseases, among them arterial hypertension.[7,8] Among the benefits generated by strength training, it has already been seen that it promotes the improvement of the cardiac function,[9] as well as increased

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activity and/or expression of the enzymes involved with the synthesis of nitric oxide.[10,11] These changes result in an increased release of nitric oxide, an improvement of vascular tone,[10,11] and a reduction in AP in normotensive[12] and hypertensive animals.[13]

In addition, reports in the literature have also described the protective action of strength training in oxidative stress, improving the antioxidant defense in the liver[14] and skeletal muscle.[15] However, the effects of strength training on the heart and contralateral kidney to renal artery stenosis are unknown. Hence, the present study sought to verify if strength training reduces the oxidative damage to the heart and contralateral kidney caused by renovascular hypertension induction surgery, as well as to evaluate the alterations in the activity of the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) endogenous antioxidant enzymes.

Methods

The experimental protocol of the present study was approved by the Animal Research Ethics Committee (CEPA - #54/2015) of the Federal University of Sergipe, in compliance with the Ethical Principles of Animal Experimentation adopted by the National Council for Animal Experimentation Control (CONCEA).

Sample

Male Wistar rats aged 10 to 12 weeks and body mass between 240 and 270 g were obtained from the animal facility of the Federal University of Sergipe. The animals were housed in collective cages (five animals/cage), kept under controlled temperature conditions (23 ± 1ºC) and a light-dark cycle of 12 hours, with feed and water ad libitum.

Experimental groups

Eighteen animals were randomly divided, through an online software, into three experimental groups (n = 6 per group): sham, hypertensive, and trained hypertensive. The sample size was defined by convenience.

Renovascular hypertension induction

Induction to hypertension was performed in the animals from the hypertensive and trained hypertensive groups, applying the renal artery clipping model, developed by Goldblatt et al.,[16] following the adaptations proposed by Cangiano et al.[17] Thus, with animals under deep anesthesia (ketamine 90 mg/kg and xylazine 10 mg/kg, intraperitoneal), an incision was made in the left flank of the animals' back to exteriorize the left kidney, and a ligation of the renal artery was performed with a 4.0 sterile cotton surgical line. The animals of the Sham group underwent surgery only to exteriorize the left kidney so as to mimic the stress generated by the surgery in the animals from the hypertensive and trained hypertensive groups. All animals received painkillers (Flunixin meglumine, sc, 1 mg/Kg, every 24h) for four days following post-surgery.

Strength training protocol

Three weeks after the hypertension induction surgery, the animals from the hypertensive and trained hypertensive groups were adapted to the training apparatus for five days, keeping the animals attached to the equipment for 10 minutes each day. Thereafter, a maximum repetition test (1RM) was performed in the animals of both groups and every two weeks in the trained hypertensive group, in order to determine the load used in the training sessions. The test was performed again in the sedentary hypertensive group at the end of the experimental protocol only.

The maximum repetition tests were performed following the American College of Sports Medicine guidelines[18] for humans, with three attempts per test. The first 1RM test was performed with 3x the animal body weight, adjusting up or down for the next try depending on the animal's performance in the attempt. The animals were allowed to rest for three minutes between each try.

Strength training was performed as described by Tamaki, Uchiyama, and Nakano,[19] and as used in other studies.[20-22] Briefly, this strength training model is performed in a squat-mimetic apparatus, where the torso of rats is fitted with a canvas jacket keeping them in an upright position (Figure 1). The canvas jacket was attached to an aluminum bracket, which is held by the wooden arm holding weights for the animals to lift, and an electro-stimulator was connected to their tail in such a way that the animals received an electrical stimulus (10-15v, 0.3s duration, 3s interval).[12,20-22]

The training period lasted 12 weeks and was started 48 hours after the 1RM test. Each strength training session was done with a 70% overload of 1RM, with four sets of 12 repetitions, and ninety-second intervals. The animals of the hypertensive group received only electrical stimulation without performing strength training. Training and electrostimulation were always performed at the beginning of the active/dark cycle (18-20 h), as it is during the dark cycle that the animals presented better tolerance to exercise.[23]

Arterial pressure (AP) measurement

Twenty-four hours after the training period, the hypertensive animals were again tested for 1RM and, 48 hours after the test of 1RM, the AP of the animals was measured. The AP of the animals was measured by implantation of a catheter in the femoral artery through a pressure transducer (Edwards Lifescience, CA, USA) attached to a preamplifier (BioData, Model BD-01, PB, Brazil).

The pulsatile AP signals were recorded for 30 minutes with the animals awake (Advanced Codas/Windaq, Dataq Instruments Inc., OH, USA), allowing pulse-beat-to-beat analysis to identify heart rate (HR), systolic AP (SAP), and diastolic AP (DAP). The mean AP (MAP) was determined through SAP and DAP in the recording software itself.

Oxidative damage

After the AP evaluation, the animals were euthanized by decapitation without anesthesia,[24] and the heart and right kidney were harvested for the oxidative damage and antioxidant enzyme activity assays.

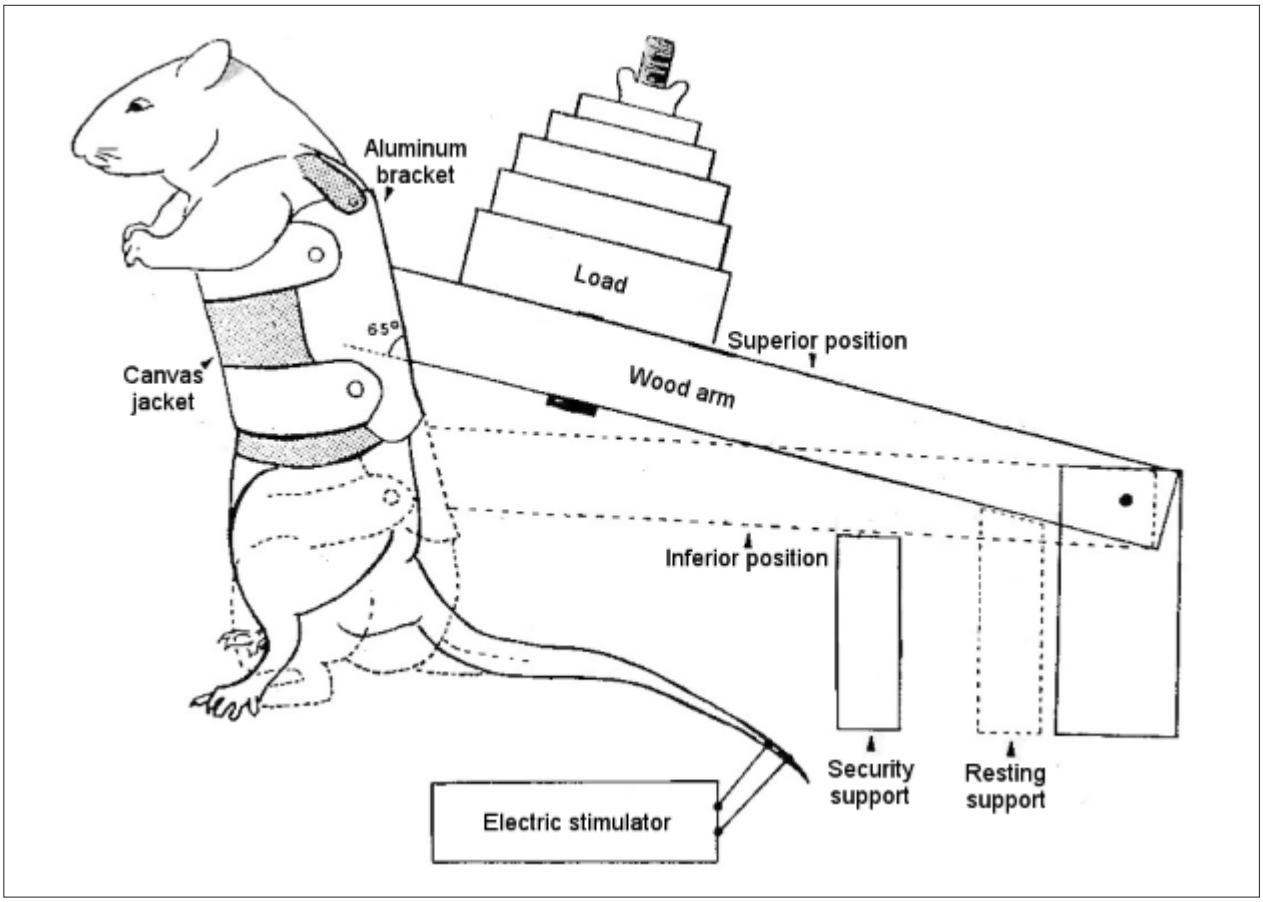

Figure 1 – *Representative illustration of strength training apparatus. (Adapted from Tamaki et al., 1992).*

To determine oxidative damage to lipids, the products of lipoperoxidation were measured by oxidation of xylenol orange, in which the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) occurs under acidic conditions, by the hydroperoxides lipids.[25] In addition, malondialdehyde was measured by the quantification of the thiobarbituric acid reactive substances.[26]

Sulfhydryl groups, which are structures associated with proteins and are highly susceptible to oxidative damage, have also been measured. Through its quantification, it is possible to estimate the protein damage in the tissues. The determination of sulfhydryl groups was performed by reacting 5'5-dithio-bis-2-nitrobenzoic acid (DTNB) with free sulfhydryl of the cysteine side chain.[27]

Antioxidant enzyme activity

SOD activity was determined by the ability of the tissue enzyme to dissociate the superoxide anions derived from pyrogallol self-oxidation and their reaction reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and forming formazan crystals.[26,28]

CAT activity was estimated by the rate of degradation of hydrogen peroxide (H_2O_2) according to the protocol previously described by Nelson and Kiesow.[29] GPx activity was assessed by oxidation of NADPH, as described by Paglia and Valentine.[30]

Determination of protein concentration

The protein concentration was determined in this study's tests by applying the technique set forth by Lowry et al.,[31] quantifying the concentration of proteins present in the homogenate of the samples by comparing this to a standard curve made with serum albumin.

Statistical analysis

The normality of the data was verified by applying the Shapiro-Wilk normality test. Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed through the one-way analysis of variance (ANOVA), followed by the Bonferroni post-hoc test. A value of $p<0.05$ was considered as statistically significant. Statistical analyses were performed using the GraphPad Prism™ 8.0.

Results

To validate our model of renovascular hypertension induction, hemodynamic parameters were assessed. These parameters were measured through the pulsatile AP with the animals awake. The induction of renovascular hypertension was successful and caused the increase of SAP, DAP, MAP, and HR, whereas the strength training was able to counteract the effects of renovascular hypertension (Table 1).

Table 1 – Arterial pressure alteration caused by renal artery stenosis

	Sham	Hypertensive sedentary	Hypertensive trained
SAP (mmHg)	133±2	187±5***	150±10##
DAP (mmHg)	92±1	151±6***	121±5**,##
MAP (mmHg)	114±2	165±5***	138±8*,#
HR (BPM)	337±4	385±9**	338±4##

*All data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared with sham; #p<0.05, ##p<0.01 compared with hypertensive sedentary, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons. SAP: systolic arterial pressure, DAP: diastolic arterial pressure, MAP: mean arterial pressure, HR: heart rate, BPM: beats per minute.*

We also evaluated the effectiveness of strength training through the measurement of 1RM, which measures the maximum strength of the animals. Strength training promoted an increase in the load lifted by the trained hypertensive animals after 12 weeks of training ($p<0.0001$; Figure 2). Nonetheless, as expected, there was no change in the strength of the sedentary hypertensive rats ($p>0.05$).

Increased oxidative stress is another hallmark of hypertension. In this light, we measured the oxidative damage to lipids and proteins by measuring hydroperoxides, malondialdehyde, and sulfhydryl groups. Again, it was possible to validate our model of hypertension since hypertension increased the damage to lipids and proteins in the contralateral kidney and heart ($p<0.01$; Figure 3A and C), through the increase of hydroperoxides and reduction of sulfhydryl group levels. However, trained animals showed protection against oxidative damage with low levels of hydroperoxides and the preservation of sulfhydryl groups in both the right kidney and the heart. In addition, no significant change was observed in the level of malondialdehyde ($p>0.05$; Figure 3B).

To further identify the effects of strength training on oxidative stress in renovascular hypertension, the activity of the endogenous antioxidant enzymes was measured. Strength training increased SOD activity in the heart and rescued SOD activity in the kidney ($p<0,01$; Figure 4A), as well as catalase activity in both tissues ($p<0,01$; Figure 4B), whereas GPx activity was only normalized in the heart ($p<0,01$; Figure 4C).

Discussion

The main results of the present study demonstrated that 12-week strength training with a moderate intensity reduced oxidative damage to the heart and contralateral kidney in renovascular hypertension by increasing the activity of endogenous antioxidant enzymes as well as by reducing blood pressure.

Renovascular hypertension models are well-known for renin-angiotensin system activation, increasing angiotensin II levels and consequent increases in AP.[16,17,32,33] As occurred in the present study, the animals that underwent hypertension induction presented elevated AP values, demonstrating that the experimental hypertension induction model was successfully performed.

Furthermore, the strength training model was performed, as described by Tamaki, Uchiyama and Nakano,[19] which has been reported to show beneficial effects that are similar to those found in humans who practice this type of physical training.[9,12,19-22,34] In the present work, it was found that moderate strength training was efficient in increasing the strength of the trained animals. Demonstrating that triggered beneficial changes, as was also seen by the reduction of AP. In addition, the beneficial effects could also be observed by reducing lipid damage and preserving the sulfhydryl groups in the heart and kidney. It has been reported in the literature that aerobic swimming training performed with moderate intensity reduces oxidative damage in the kidney contralateral to renal artery stenosis.[35]

Other studies have also demonstrated this protective effect of physical exercise on oxidative stress. As has been reported, aerobic treadmill training with progressively increasing intensity reduces renal oxidative damage in other models of experimental hypertension,[36] as well as in another models of chronic kidney diseases.[37] Similar effects have been also shown in other strength training models.[38,39] This protection promoted by physical exercise is important to prevent the occurrence of fibrosis, a process that occurs through the deposition of collagen in the areas that suffered oxidative damage.[40] These damages are increased in renovascular hypertension due to the hyperactivation of the renin angiotensin aldosterone system, generating oxidative stress.[2,41]

However, the organism has mechanisms to prevent the occurrence of these oxidative damages; one of these mechanisms occurs through the activation of the endogenous antioxidant enzymes.[42,43] By means of this mechanism, the antioxidant enzyme SOD catalyzes the dismutation of O_2^- to H_2O_2. Subsequently, the H_2O_2 is reduced to H_2O and O_2 by the peroxidases, GPx, or CAT.[42,43] In healthy individuals, these enzymes are expressed in different ways in different organs, depending on the metabolic and functional processes that occur in them. Nevertheless, these antioxidant enzymes are reduced during arterial hypertension.[44,45]

In the present study, reduced activity of antioxidant enzymes was observed in the animals from the hypertensive group. Other studies corroborate these findings, showing that both the activity[6] and the gene expression of these enzymes are reduced in this model of renovascular hypertension.[5] Aerobic swimming training[35,46] has been shown to increase the activity of SOD and CAT enzymes in the heart and contralateral kidney of animals with induced hypertension, using the same renovascular hypertension model. Although the effects of strength training on contralateral kidney oxidative stress have not yet been studied, it has been shown that climbing

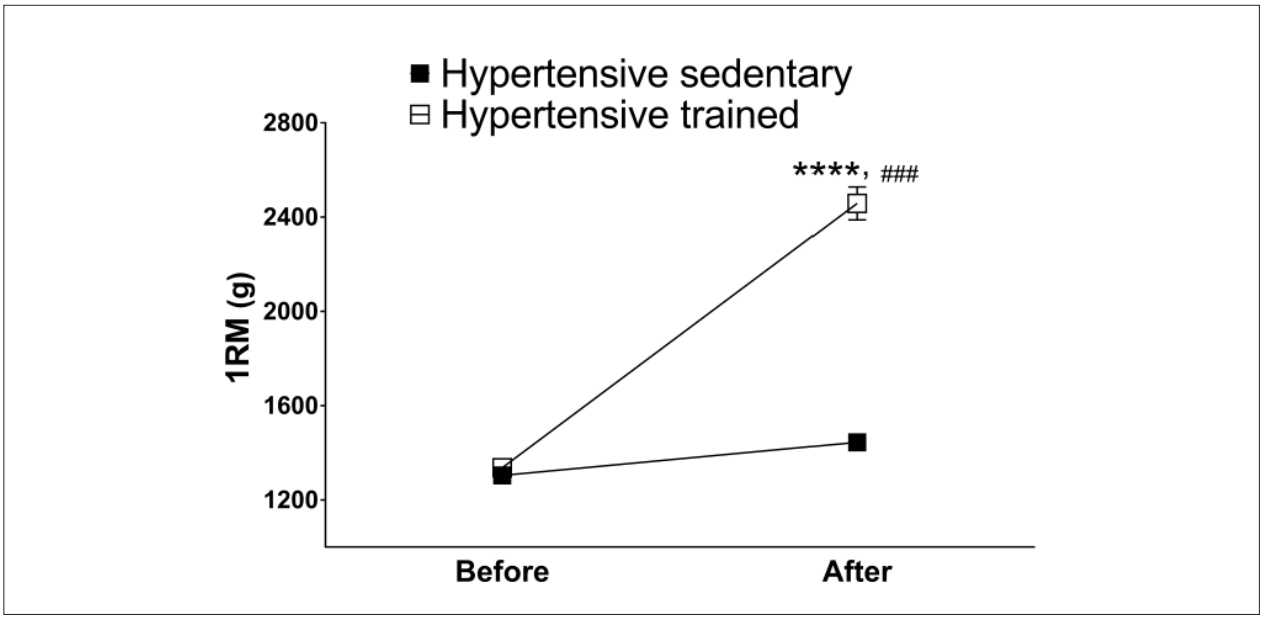

Figure 2 – *Absolute values of the maximum strength test. All data represent mean ± SEM. ****p<0.0001 compared with before training; ###p<0.001 compared with hypertensive sedentary before, calculated by two-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons. 1RM: maximum repetition test.*

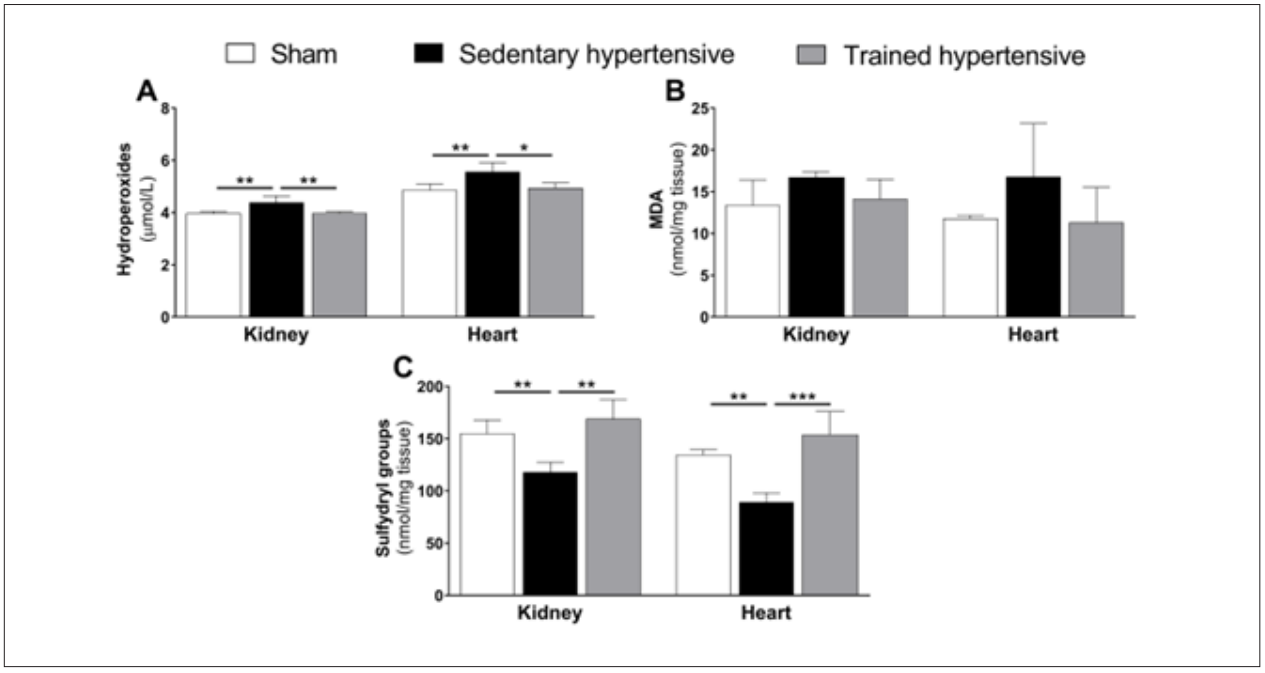

Figure 3 – *Effects of renovascular hypertension and strength training on the markers of oxidative damage in the contralateral kidney and heart. All data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons. MDA: malondialdehyde.*

strength training promotes an increase in antioxidant enzymes in skeletal and cardiac muscles.[15,38,39]

This study presents limitations since, for technical reasons, we were not able to monitor the time-course of change in AP not the baseline measurement of other parameters for a better understanding of the therapeutical action of strength training. Despite the limitations, our results demonstrate, in a rat renovascular model, that strength training has a protective effect, as has already been observed in other modalities of physical exercise. Strength training increased the activity of SOD and CAT enzymes in the contralateral kidney and heart, reestablishing this antioxidant activity to values found in

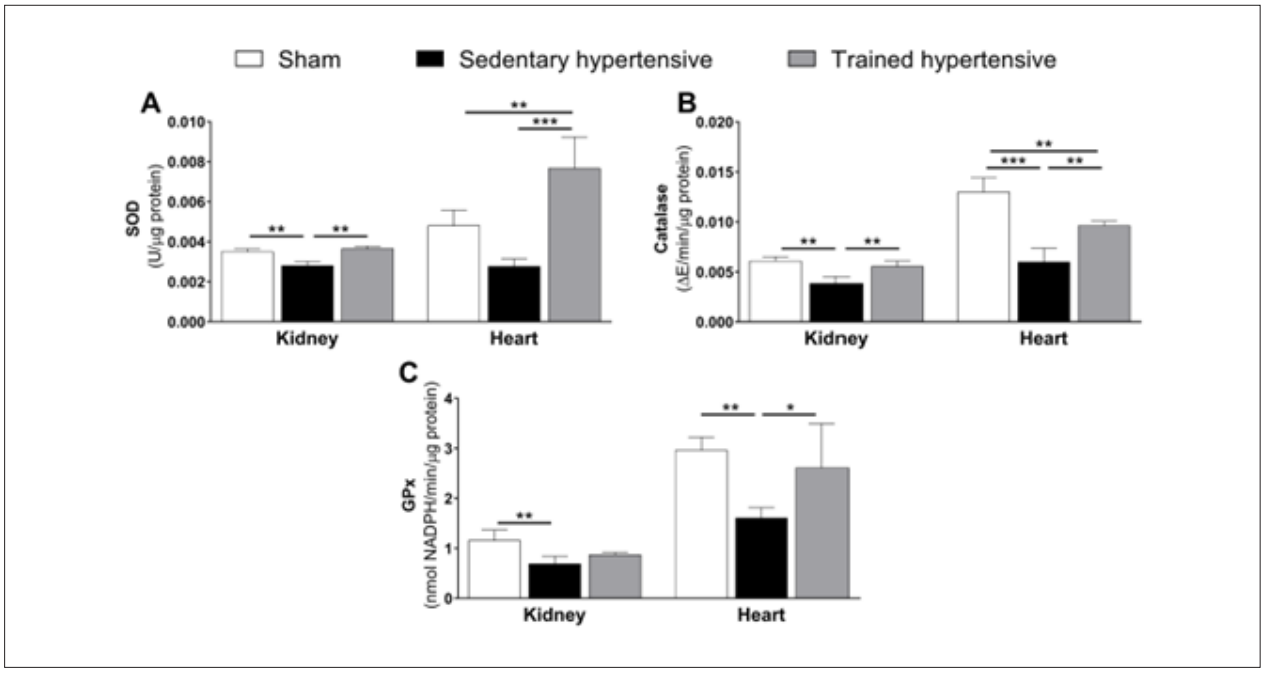

Figure 4 – *Effects of renovascular hypertension and strength training on the antioxidant enzyme activity. All data represent mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, calculated by one-way ANOVA followed by the post hoc Bonferroni test for pairwise comparisons. SOD: superoxide dismutase; GPx: glutathione peroxidase.*

healthy animals (Sham group), indicating that this is a possible mechanism by which strength training is able to reduce oxidative damage in renovascular hypertensive animals.

Conclusion

The results found in the present study allow us to conclude that strength training is able to counteract oxidative damage produced by renovascular hypertension in the contralateral kidney and heart. This reduction is due, in part, to the increased activity of the antioxidant enzymes SOD and CAT promoted by strength training. Therefore, these results suggest that strength training is an important non-pharmacological tool for the treatment of renovascular hypertension, potentially preventing the progression of damage to the heart and kidney without renal artery stenosis.

Author contributions

Conception and design of the research: Miguel-dos-Santos R, Santana-Filho VJ, Wichi RB, Lauton-Santos S; Data acquisition: Miguel-dos-Santos R, Santos JF, Macedo FN; Analysis and interpretation of the data: Miguel-dos-Santos R, Santos JF, Macedo FN, Wichi RB, Lauton-Santos S; Statistical analysis and Critical revision of the manuscript for intellectual content: Miguel-dos-Santos R, Santos JF, Macedo FN, Marçal AC, Santana-Filho VJ, Wichi RB, Lauton-Santos S; Obtaining financing: Lauton-Santos S; Writing of the manuscript: Miguel-dos-Santos R.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Moderate-Intensity Resistance Training Improves Oxidative Stress in Heart

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Short Editorial related to the article: Strength Training Reduces Cardiac and Renal Oxidative Stress in Rats with Renovascular Hypertension

Renovascular hypertension (RVHT) is one of the main causes of secondary hypertension, often leading to resistant hypertension, that is, that does not respond well to aggressive medical treatment. This condition is defined as systemic hypertension that manifests as a result of compromised blood supply to the kidneys.[1,2] In an epidemiological context, RVHT accounts for 1 to 5% of all cases of hypertension and 5.4% of secondary hypertension among young adults.[3]

Studies have shown an association this disease with low levels of physical activity or physical fitness in hypertensive individuals.[4,5] It is known that physical training has a protective action against cardiovascular diseases.[6-8] In 2016, the 7th Brazilian Guideline of Arterial Hypertension, by the Brazilian Society of Cardiology,[9] stated that blood pressure reduction is the most effective measure to decrease cardiovascular risk and slow kidney damage progression, regardless of the antihypertensive drug used. Endurance/aerobic exercise training promotes an important hypotensive effect in hypertensive patients and, therefore, has been recommended as the preferential type of exercise for arterial hypertension prevention and treatment.[9,10]

However, there is now a growing scientific interest in the cardiovascular effects of another type of exercise: the resistance exercise training.[11,12] Resistance/strength training is an activity whose effort is performed against a specific opposing force generated by resistance and which is designed specifically to increase muscular strength, resistance, and/or endurance.[11] The beneficial effects of resistance training encompass improved maximum oxygen consumption, muscle strength and endurance, in addition to being a powerful oxidative stress modulator.[13]

Keywords

Hypertension; Oxidative, Stress; Ventricular Remodeling; Resistance Training; Blood Pressure/prevention and control.

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In the current edition of ABC, we read with great interest the important study "Strength Training Reduces Cardiac and Renal Oxidative Stress in Rats with Renovascular Hypertension",[14] which addresses the potential impact of a resistance training protocol on oxidative damage and endogenous antioxidant enzymatic systems in the heart and contralateral kidney in response to RVHT. Indeed, the animals submitted to RVHT induction showed important characteristics of hypertension, including increase in systolic (SBP) and diastolic (DBP) blood pressure, mean blood pressure (MBP) and heart rate (HR). Sedentary hypertensive animals presented with an elevated concentration of hydroperoxides and reduced levels of sulfhydryl groups.[14]

The authors used a resistance training protocol with 70% overload of 1-repetition maximum (1RM), with four sets of 12 repetitions and ninety seconds intervals over a period of 12 weeks. As a result, the animals in the hypertensive group submitted to the resistance training protocol showed a reduction in the values of SBP, DBP, MBP, and HR.[14] Possibly, the resistance training has increased the availability of nitric oxide and its synthesis by endothelial cells, thus contributing to the modulation of vascular tone.[15] As a consequence, increased bradycardic response could decrease the sympathetic activity in the heart, leading to a reduction in HR at rest, in cardiac output, and in blood pressure levels.[4]

Other findings were reduction in the concentration of hydroperoxides and preservation of sulfhydryl groups in the right kidney and heart in trained hypertensive animals. The trained group presented enhanced superoxide dismutase (SOD), catalase and glutathione peroxidase activities in the heart. Regarding kidney in hypertensive animals, SOD and catalase activities were improved in response to resistance training, although glutathione peroxidase activity was unchanged.[14] Regular exercise elevates reactive oxygen species (ROS) production to a level that may induce tolerable damage, which in turn, can induce beneficial adaptations by upregulating cellular antioxidant systems and stimulating oxidative damage repair systems.[13]

Therefore, the results found by the authors indicate that resistance training of moderate intensity can be an effective intervention in the treatment of cardiometabolic diseases, especially renovascular hypertension. However, further studies are needed so we can understand the molecular mechanisms related to oxidative balance in response to resistance training.

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Survival of Patients with Acute Heart Failure and Mid-range Ejection Fraction in a Developing Country – A Cohort Study in South Brazil

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Abstract

Background: Heart Failure with mid-range Ejection Fraction (HFmEF) was recently described by European and Brazilian guidelines on Heart Failure (HF). The ejection fraction (EF) is an important parameter to guide therapy and prognosis. Studies have shown conflicting results without representative data from developing countries.

Objective: To analyze and compare survival rate in patients with HFmEF, HF patients with reduced EF (HFrEF), and HF patients with preserved EF (HFpEF), and to evaluate the clinical characteristics of these patients.

Methods: A cohort study that included adult patients with acute HF admitted through the emergency department to a tertiary hospital, reference in cardiology, in south Brazil from 2009 to 2011. The sample was divided into three groups according to EF: reduced, mid-range and preserved. A Kaplan-Meier curve was analyzed according to the EF, and a logistic regression analysis was done. Statistical significance was established as $p < 0.05$.

Results: A total of 380 patients were analyzed. Most patients had HFpEF (51%), followed by patients with HFrEF (32%) and HFmEF (17%). Patients with HFmEF showed intermediate characteristics related to age, blood pressure and ventricular diameters, and most patients were of ischemic etiology. Median follow-up time was 4.0 years. There was no statistical difference in overall survival or cardiovascular mortality (p=.0031) between the EF groups (reduced EF: 40.5% mortality; mid-range EF 39.7% and preserved EF 26%). Hospital mortality was 7.6%.

Conclusion: There was no difference in overall survival rate between the EF groups. Patients with HFmEF showed higher mortality from cardiovascular diseases in comparison with HFpEF patients. (Arq Bras Cardiol. 2021; 116(1):14-23)

Keywords: Survivorship; Heart Failure; Stroke Volume; Prognosis; Mortality; Medication Adherence; Epidemiology.

Introduction

Heart Failure (HF) is a complex syndrome considered one of the major causes of hospital admission, morbidity, and mortality worldwide.[1-3] Observational studies have described mortality rates from HF ranging from 4% to 12% during hospitalization and 20% to 30% one year after discharge. Readmission rates are also high ranging from 20% to 30% in 90 days and up to 60% in one year.[3-6] Advances in cardiovascular therapy have been associated with a higher life expectancy and increased prevalence of HF in the elderly population, creating the need for a better knowledge of epidemiology, diagnosis and therapeutics of this important public health disease in developed and developing countries.

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Although ejection fraction (EF) is not an ideal parameter to stratify HF patients, it has been historically used to guide therapy and determine prognosis in clinical practice.[7,8] To stimulate research and better categorize HF patients, the European Society of Cardiology created a new EF category in its recent guideline – HF with mid-range EF (HFmEF) – addressing patients with EF between 40-49%.[1] This new classification was also adopted by the Brazilian Society of Cardiology by the 2018 guideline on HF.[3] Since then, many studies have described the clinical outcomes and characteristics of the HFmEF population, with conflicting results.[9] While some studies with acute and chronic HF patients have shown similar survival among the three EF categories,[10-14] others have shown better survival of HFmEF and HF with preserved EF (HFpEF) as compared with HF patients with reduced EF (HFrEF).[15,16]

Data about HFmEF patients in Brazil and in developing countries are scarce in the literature. The objective of this study is to analyze survival and clinical characteristics of patients with HFmEF in comparison with patients admitted with acute HF (AHF) presenting reduced or preserved EF.

Methods

Study Design and Population

This was a prospective cohort study, derived from a clinical registry of 424 consecutive patients admitted with AHF to the emergency department of São Lucas hospital / Pontifícia Universidade Católica do Rio Grande do Sul, during the period from January 2009 to December 2011 (Figure 1). The inclusion criteria were: 1) age above 18 years old; 2) AHF diagnosis defined by the Framingham criteria and later confirmed with transthoracic echocardiography. Patients who did not realize an echocardiography during the hospital stay were excluded. The clinical registry protocol was approved by the Research Ethics Committee of São Lucas Hospital (city of Porto Alegre) and a databank of AHF was developed. An informed consent form was obtained from participants.

Sample size calculation was based on the Meta-Analysis Global Group in Chronic Heart Failure (MAGGIC), published in 2012. To observe a difference in mortality, it would be needed between 330 and 364 patients, with an 80% power and a 5% alpha error (Roasoft and WinPepi Sample Size Calculator Software).

Clinical Assessment and Data Collection

Clinical assessment and treatment of patients included in the study were conducted by the emergency physician and the cardiology team on call according to the institutional routine protocol, without interference from the researchers. Data collection was done using a structured research form and medical chart reviews.

Patient's initial signs and symptoms were registered at arrival to the emergency department by assessment of clinical status, hemodynamic profile, vital signs and New York Heart Association functional class, prior to admission. In addition to the treatment prescribed during the hospital stay, medications used at home and prescribed on discharge were also evaluated.

Causes of HF decompensation were analyzed: myocardial ischemia (if any type of myocardial revascularization was performed during hospital stay); uncontrolled hypertension (if hypertension stage ≥ II on arrival); arrhythmia (any non-sinus rhythm, except for permanent atrial fibrillation with controlled ventricular rate); poor medication adherence; infection (diagnosis during hospital stay).

Ischemic etiology of HF was considered when previous or recent myocardial revascularization was performed; functional test with ischemia higher than 10%; and anatomical examination revealing stenosis greater than 50% in the left main coronary artery or 70% in the proximal left anterior descending artery or other two coronary vessels. Self-reported comorbidities or those diagnosed during hospital stay were also registered.

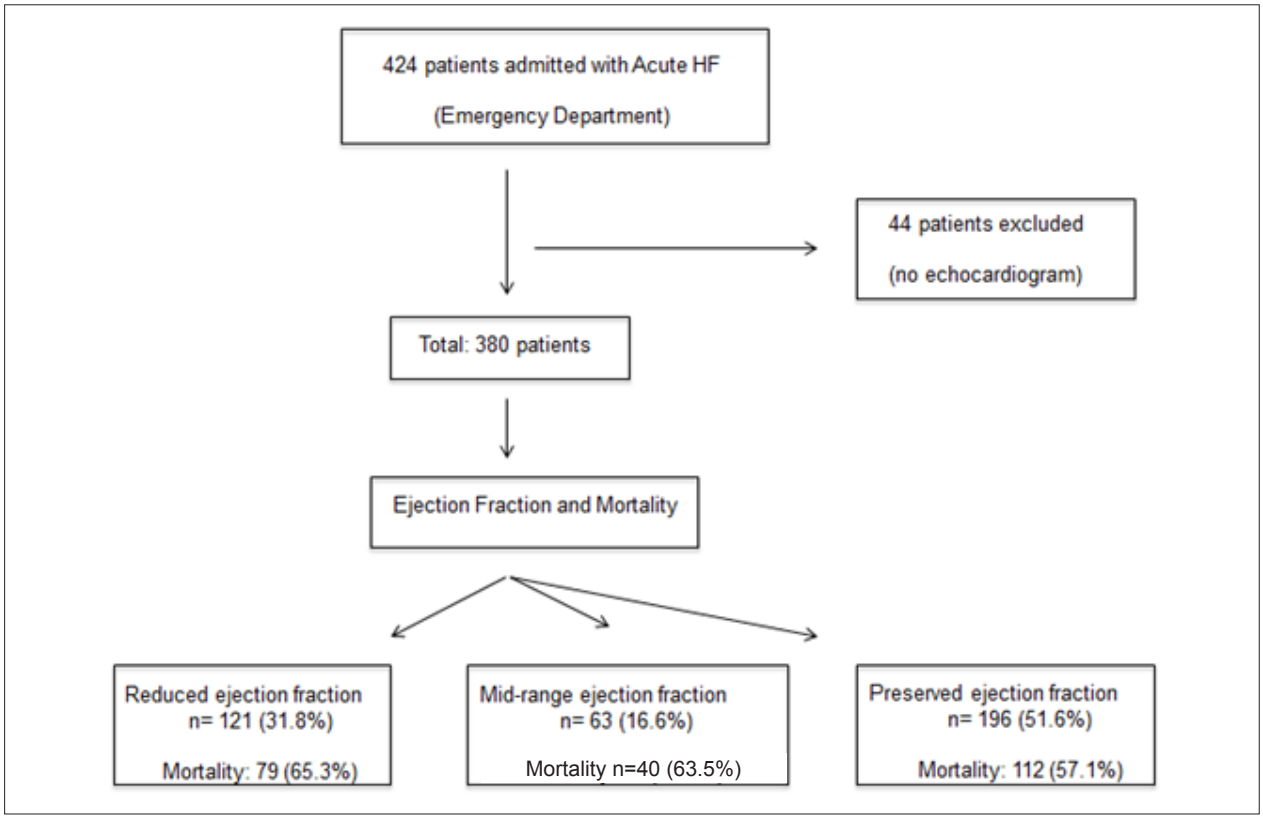

Figure 1 – *Study population with median follow-up of 4.0 years; HF: heart failure.*

As part of the institutional protocol, every patient underwent a 12-lead electrocardiography, chest radiography, laboratory exams (complete blood count, electrolytes, renal function, lipid profile, glucose, and urine analysis) and a transthoracic echocardiogram with measurement of EF by Simpson's method.

The sample was divided in three groups according to left ventricular EF measured on echocardiogram: reduced (<40%), mid-range (40-49%) and preserved (≥ 50%). The diagnosis of HFpEF was made according to existing guidelines, based mainly on atrial diameter, left ventricular mass and diastolic function.

Follow-up and Outcomes

Outcome data were obtained through medical chart review and through the Mortality Information System of the Health Center Information of the Rio Grande do Sul state to identify mortality and cause of death until December 2017.

Direct cause of death was established according to the International Classification of Diseases 10th edition.

The primary outcome assessed was overall mortality and secondary outcome was mortality from cardiovascular causes (acute myocardial infarction, HF, stroke, and arrhythmia).

Statistical Analysis

Continuous variables with normal distribution (analyzed by the Kolmogorov-Smirnov test) were expressed as average and standard deviation or median and interquartile range, as appropriate. Comparison between categorical variables was performed by the chi-square test, and comparison between continuous variables was performed by analysis of variance (ANOVA) and Bonferroni post hoc test. Survival curves were estimated by the Kaplan-Meier method, using the log rank test statistics to compare EF categories. Univariate and multivariate logistic regression were assessed to determine the main variables related to mortality. Statistical significance was established with a p value < 0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) Statistics, version 21.0.0.

Results

Of 424 patients admitted with AHF, 380 patients were studied (Figure 1). Most of patients had HFpEF (51.6%), followed by HFrEF (31.8%) and HFmEF (16.6%). Average age was 68 ±13 years old, mostly females (53%). The median follow up time was 4.0 years (interquartile range: 0.92 – 7.62 years).

Clinical Characteristics

The patient population with HFpEF was mostly older women with higher levels of blood pressure and lower heart rate and left ventricle dimensions. The HFrEF group was mostly composed of young men with lower levels of blood pressure and higher heart rate and left ventricle dimensions. Patients with HFmEF presented intermediate characteristics between HFpEF and HFrEF population regarding to age, gender, blood pressure, heart rate and ventricle dimensions (Tables 1 and 2).

In the population with HFmEF patients, plasma potassium levels were higher at admission and myocardial ischemia was the main HF etiology (Table 1). Patients with HFmEF had a smaller prevalence of chronic obstructive pulmonary disease, tobacco and alcohol use. Patients with HFrEF had a higher use of angiotensin converting enzyme inhibitor, antimineralocorticoid, digoxin and loop diuretics, and more implantable electronic cardiac devices (Tables 2 and 3). Most patients presented with a "wet and warm" hemodynamic profile on admission, with no difference between the EF groups.

Poor adherence to medical therapy was the main cause of HF decompensation, followed by infection in patients with HFrEF and HFpEF respectively (Table 4).

Outcomes

In-hospital mortality was 7.6%. Overall mortality in the eight years of follow- up was 60.7%, with no significant difference between the EF categories (Figure 2).

Mortality in the EF groups through the follow-up time is described in Table 5.

Mean survival rate was 4.7 years (CI 95%: 3.7 – 5.6), with the tendency of a gradual increase with the EF (reduced EF: 4.3 years; mid-range EF: 4.7 years; and preserved EF: 4.9 years). Cardiovascular mortality was responsible for nearly half of the deaths (54.1%). There was a statistically significant difference between the EF groups when cardiovascular deaths were analyzed separately (p=0.031) – reduced EF: 40.5%; mid-range EF: 39.7%; and preserved EF: 26% (Figure 3).

Univariate Analysis

When univariate logistic regression was analyzed with categorical variables, the presence of atrial fibrillation and urea levels higher than 92 mg/dL were identified as risk factors. When analyzed as a continuous variable, higher values of systolic blood pressure were identified as a protective factor. Data collected at arrival to the emergency department are described in Table 6.

Multivariate Analysis

Multivariate logistic regression revealed that there was no difference in clinical characteristics or mortality rate between the groups of EF categories and HF etiologies. When cardiovascular death was analyzed, HFrEF, HFmrEF and atrial fibrillation were identified as risk factors (Table 7).

Discussion

There is a debate about how to better evaluate the prognosis in HF patients beyond EF, also considering ischemic etiology, ventricular remodeling, comorbidities, among others.[7,17,18] It is also known that EF is a dynamic measure with an intra- and inter-observer variability of 7%, making it possible to reclassify 80% of the HF patients.[3,19-21] In its last 2016 guidelines on HF, the European Society of Cardiology recommends identifying those patients with HFmEF. The American Heart Association / American College of Cardiology / Heart failure Society of America, in the 2013 guideline

Table 1 – Demographic data and comorbidities of patients with heart failure according to ejection fraction

Characteristics	Total	Ejection fraction < 40%	Ejection fraction 40-49%	Ejection fraction ≥ 50%	p
	% (N = 380)	31.8% (N=121)	16.6% (N=63)	51.6% (N=196)	
Demographics					
Age (mean in years)	68.1 ±13.8	64.0 ±12,6[b]	66.6 ±15,3[ab]	71.3 ±13,4[a]	<0.001
Female	52.9% (201)	35.5%(43)[b]	52.4%(33)[ab]	63.8%(125)[a]	<0.001
Body Mass Index (mean in Kg/m²)	28.1 ±6,5	26.6 ±6,1	29.2 ±6,3	28.6 ±6,6	0.100
Comorbidities					
Ischemic etiology	40.0% (152)	46.3% (56)	52.4% (33)	32,1% (63)	0.004
Hypertension	93.2% (354)	90.1% (109)	92.1% (58)	95.4% (187)	0.176
Dyslipidemia	74.8% (243)	76.2% (80)	76.9% (40)	73.2% (123)	0.796
Chronic Renal Disease	46.2% (156)	42.1% (45)	57.9% (33)	44.8% (78)	0.135
Diabetes Mellitus	45.9% (169)	43.9% (50)	50.8% (32)	45.5% (87)	0.668
Valvulopathy	35.1% (99)	28,1% (25)	36.2% (17)	39.0% (57)	0.230
Chronic Obstructive Pulmonary Disease	32.2% (111)	42.1%(45)	21.1% (12)	29.8% (54)	0.014
Implantable Cardiac Device	20.7% (78)	27.3% (33)	24.6% (15)	15.5% (30)	0.031
Atrial Fibrillation	20.0% (76)	5.8% (22)	2.1% (8)	12.1% (42)	0.085
Left Bundle Branch Block	16.3% (62)	7.1% (27)	2.9% (11)	6.3% (24)	0.133
Stroke	17.5% (62)	16.2% (18)	12.3% (7)	19.8% (37)	0.390
Hypothyroidism	18.0% (49)	16.7% (14)	28.9% (13)	15.4% (22)	0.112
Alcohol abuse	19.4% (67)	32.4% (34)	12.5% (7)	14.1% (26)	<0.001
Smoking	17.7% (63)	25.9% (29)	11.9% (7)	14.6% (27)	0.021
Cancer	12.0% (43)	12.4% (14)	3.4% (2)	14.5% (27)	0.070

Statistical analysis: Chi-square test with adjusted residual and ANOVA with Bonferroni test when applied (small letters a and b).

Table 2 – Clinical, laboratory and image data on admission

Characteristics	Total	Ejection fraction < 40%	Ejection fraction40-49%	Ejection fraction ≥ 50%	p
	% (N = 380)	31.8% (N=121)	16.6% (N=63)	51.6% (N=196)	
Demographics					
Systolic Blood Pressure (mean in mmHg)	140 (±35)	128 (±26)b	139 (±33)ab	147 (±39)a	<0.001
Heart Rate (mean in bpm)	91 (±23)	96 (±22)a	89 (±20)ab	88 (±22)b	0.006
Hemoglobin (mg/mL)	12.0 (±2.6)	12.6 (±2.5)a	11.9 (±2.3)ab	11.6 (±2.6)b	0.004
Creatinine (mg/dL)	1.8 (±1.2)	1.9 (±1.5)	1.9 (±0.9)	1.8 (±1.2)	0.615
Urea (mg/dL)	71 (±46)	70 (±48)	76 (±40)	71 (±50)	0.766
Sodium (mg/dL)	137 (±17)	139 (±4.4)	139 (±3.1)	137 (±2.5)	0.324
Potassium (mg/dL)	4.3 (±0.7)	4.4 (±0.8)ab	4.5 (±0.6)a	4.2 (±0.7)b	0.017
Left Ventricle Systolic Diameter (cm)	3.5 (±1.8)	5.0 (±1.6)a	4.0(±1.5)b	3.1 (±0.8)c	<0.001
Left Ventricle Diastolic Diameter (cm)	4.7 (±2.0)	5.7 (±1.8)a	5.2 (±1.9)b	4.8 (±0.9)b	<0.001
Left Atrium Diameter (cm)	3.9 (±1.7)	4.3 (±1.3)	4.0 (±1.5)	4.3 (±0.9)	0.182

Statistical analysis: ANOVA test - with Bonferroni test when applied (small letters a, b and c).

Table 3 – Medications at home

Medications	Total	EF < 40%	EF 40-49%	EF ≥ 50%	p
	% (N = 380)	31.8% (N=121)	16.6% (N=63)	51.6% (N=196)	
Loop diuretic	60.1% (218)	67.0% (77)	66.7% (38)	53.9% (103)	0.043
Angiotensin converting enzyme inhibitor	51.5% (187)	63.5% (73)	38.6% (22)	48.2% (92)	0.043
Betablocker	49.0% (179)	50.0% (58)	45.6% (26)	49.5% (95)	0.641
Acetylsalicylic Acid	40.7% (149)	44.0% (51)	45.6% (26)	37.3% (72)	0.367
Statin	43.3 (156)	43.0% (49)	50.0% (28)	41.6% (79)	0.533
Digoxin	25.6% (93)	40.0% (46)	24.6% (14)	17.3% (33)	<0.001
Oral antidiabetic	20.9% (76)	19.1% (22)	17.5% (10)	23.0% (44)	0.568
Insulin	19.3% (70)	20.9% (24)	24.6% (14)	16.8% (32)	0.370
Mineralocorticoid Receptor Antagonist	18.5% (67)	27.0% (31)	22.8% (13)	12.0% (23)	0.003
Calcium Channel Blocker	16.9% (61)	8.8% (10)	15.8% (9)	22.1% (42)	0.011
Thiazide Diuretic	14.6% (53)	14.0% (16)	14.0% (8)	15.2% (29)	0.954
Oral anticoagulation	14.0% (51)	14.7% (18)	10.5% (6)	14.1% (27)	0.660
Angiotensin Receptor Blocker	12.2% (44)	5.2% (6)	17.5% (10)	14.7% (28)	0.019

Statistical analysis: Chi-Square test with adjusted residual.

Table 4 – Causes of decompensation

Characteristics	Total	EF < 40%	EF 40-49%	EF ≥ 50%	p
	% (N = 380)	31.8% (N=121)	16.6% (N=63)	51.6% (N=196)	
Causes of decompensation					
Medications	30.5% (116)	42.1% (51)	27.0% (17)	24.5% (48)	0.003
Infection	27.1% (103)	19.0% (23)	19.0% (12)	34.7% (68)	0.003
Arrhythmia	18.7% (71)	15.7% (19)	19.0% (12)	20.4% (40)	0.721
Hypertension	14.5% (55)	9.1% (11)	15.9% (10)	17.3% (34)	0.120
Myocardial Ischemia	7.6% (29)	8.3% (10)	12.7% (8)	5.6% (11)	0.174
Salt overload	7.4% (28)	7.4% (9)	7.9% (5)	7.1% (14)	0.978
Unknown	18.2% (69)	18.2% (22)	23.8% (15)	16.3% (32)	0.407

Statistical analysis: Chi-square test with adjusted residual.

for the management of HF, use the term "borderline" for patients with clinical characteristics similar to HFpEF, and "improved" for ischemic patients with improved EF after the acute event, but both as HFpEF subclassification. The focused 2017 update does not mention a new EF classification.[1] The Brazilian Society of Cardiology in its latest 2018 HF guideline, also adopted the term HFmEF in a dynamic manner, with a prevalence of approximately 10-20%, in agreement with the 17% prevalence in the present study.[3,7,18]

In regard to clinical characteristics, patients with HFmEF have intermediate prevalence of comorbidities in relation to HFrEF and HFpEF patients.[3,13,14,21] The prevalence of ischemic etiology seems to be similar in HFmEF and HFrEF patients, in agreement with the present study.[3,7,14,21] However, other studies have reported similar prevalence of comorbidities between patients with HFmEF and HFpEF.[13,14]

The I Brazilian Registry of Acute Heart Failure (BREATHE) published in 2015 showed a hospital mortality of 13%, while American and European registries have reported 4% hospital mortality rate. This data indicates important differences regarding in-hospital mortality between developed and developing countries. In the present study, in-hospital mortality was 8%. This may be explained by the place of the study, a tertiary care hospital, reference in cardiology, with a coronary care unit. As in the BREATHE study, poor medication adherence and infection were the main causes of HF decompensation. The first was more representative in the HFrEF population, while the second

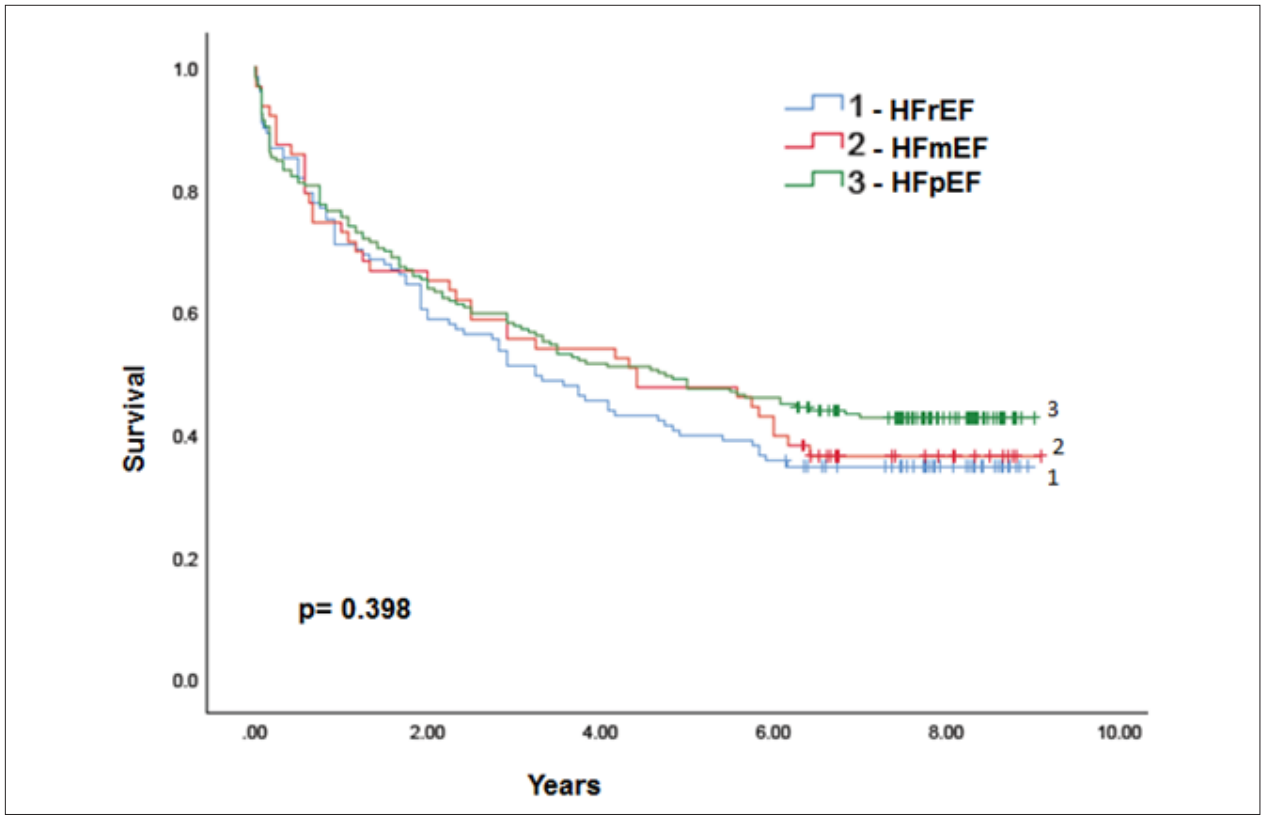

Figure 2 – *Overall survival curve. HFrEF (heart failure with reduced ejection fraction); HFmrEF (heart failure with mid-range ejection fraction); HFpEF (heart failure with preserved ejection fraction).*

Table 5 – Mortality during study follow up

Overall Mortality	Total	EF < 40%	EF 40-49%	EF ≥ 50%	p
	% (N = 380)	31.8% (N=121)	16.6% (N=63)	51.6% (N=196)	
In Hospital	7.6% (29)	6.6% (8)	4.8% (3)	9.2% (18)	0.453
1 month	10.8% (41)	10.7% (13)	7.9% (5)	11.7% (23)	0.699
3 months	14.7% (56)	13.2% (16)	14.3% (9)	15.8% (31)	0.814
12 months	26.6% (101)	28.5% (35)	27.0% (17)	25.0% (49)	0.742
5 years	55.0% (209)	60.3% (73)	52.4% (33)	52.6% (103)	0.439
8 years	60.7% (231)	65.3% (79)	63.5% (40)	57.1% (112)	0.398

Statistical analysis: ANOVA test.

in the HFpEF. Patients with HFmEF had a higher tendency to decompensate due to myocardial ischemia, which may explain why this population had a higher ischemic etiology. Recent studies with acute HFmEF patients did not investigate the cause of decompensation.[13,14,16]

There is a classical understanding that the higher the EF, the higher the survival rate, supporting an important prognostic role of EF.[8] Recent studies that analyzed mortality in HFmEF patients showed conflicting results.[3,24,25] In some of these studies, there was no difference in overall mortality between the groups,[10,13,14] while in others, showed mortality rates between HFrEF and HFpEF [7,8,21] or similar with HFpEF patients.[12,16,20,23] In the present study, there was no difference in overall mortality between the three EF categories. However, when cardiovascular deaths were analyzed, patients with HFmEF had a worse prognosis, similar to HFrEF patients. This may be explained by the fact that most of HFmEF patients had myocardial ischemia, a poor prognostic factor.[17] In our study, we were unable to proof a direct relation between mortality related to ischemic etiology through logistic regression. Another possible interference is the impact of comorbidities on non-cardiovascular deaths in HFpEF patients.

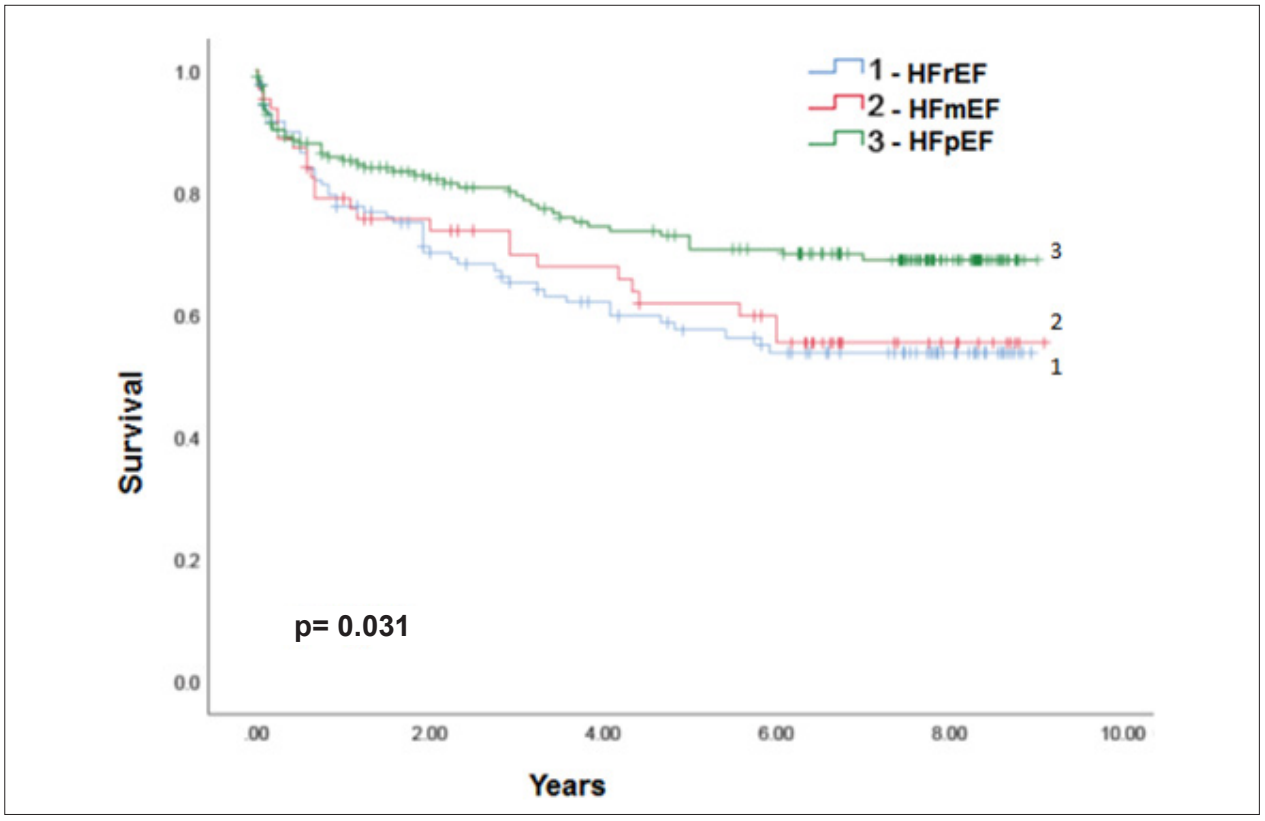

Figure 3 – *Survival curve for cardiovascular cause. HFrEF (heart failure with reduced ejection fraction); HFmrEF (heart failure with mid-range ejection fraction); HFpEF (heart failure with preserved ejection fraction).*

Table 6 – Univariate logistic regression in relation to overall mortality

Univariate Logistic Regression	p	Odds Ratio	Confidence Interval 95%
HFrEF	0.245	1.44	0.78 - 2.65
HFmEF	0.62	1.2	0.58 - 2.49
HFpEF	_	1	_
Ischemic Etiology	0.775	1.07	0.66 - 1.74
Diastolic diameter > 5.6 cm	0.421	1.26	0.72 - 2.12
Systolic Blood Pressure < 115 mmHg	0.494	1.22	0.69 - 2.12
Systolic Blood Pressure	0.006	0.99	0.98 - 0.99
Creatinine > 2.75 mg/dl	0.741	1.15	0.51 - 2.58
Urea > 92 mg/dl	0.034	2.00	1.05 - 3.80
Atrial fibrillation	0.028	1.98	1.08 - 3.64
Left bundle branch block	0.921	1.03	0.54 - 1.97

HFrEF: heart failure with reduced ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFpEF: heart failure with preserved ejection fraction.

Table 7 – Multivariate logistic regression and cardiovascular mortality

Multivariate Logistic Regression	p	Odds Ratio	Confidence Interval 95%
HFrEF	0.003	2.23	1.13 - 3.78
HFmEF	0.034	2.04	1.06 - 4.08
HFpEF	_	1	_
Atrial Fibrillation	0.004	2.31	1.31 - 4.08

HFrEF (heart failure with reduced ejection fraction); HFmrEF (heart failure with mid-range ejection fraction); HFpEF (heart failure with preserved ejection fraction).

Univariate logistic regression was made to identify the prognostic value of some characteristics of HF patients regarding overall mortality. An elevated level of urea was identified as a risk factor and a higher blood pressure was identified as a protective factor. This data agrees with the ADHERE score (Acute Decompensated Heart Failure National Registry) in patients admitted with acute heart failure that demonstrated worse prognosis in patients with systolic blood pressure below 115mmHg, levels of creatinine above 2.75 mg/dL and urea above 92 mg/dL.[5] Atrial fibrillation was also a risk factor in the univariate and multivariate analysis, which also agrees with previous studies.[26,27] In the multivariate analysis with cardiovascular mortality data, HFrEF and HFmEF showed a twofold mortality risk when compared with HFpEF patients in agreement with recent studies,[14,16] but in discordance with studies that did not show a difference in mortality between EF categories.[10-12,15]

The 'Global action plan for the prevention and control of noncommunicable diseases 2013-2020' was created by the World Health Organization with the intention to reduce the impact of these diseases manly by risk factor reduction. When comparing data on cardiovascular disease and mortality, including HF patients, there have been differences when comparing developed and developing countries.[28] In Brazil, HF is mainly caused by ischemic, hypertensive and valve diseases, and still represent an important cardiac manifestation of Chagas disease and rheumatic disorders. The resources and management required by HF patients that are often not met by local public health systems, causing negative impact on hospitalization and mortality, as shown in this study, when compared with developed countries. Observational studies and registries become extremely important to help guide effective public health strategies according to local demands and resources.[29] In a recent 'state of the art' study about HFmEF, the authors reported various findings regarding clinical characteristics and phenotypes, and outcomes and treatment in patients with HFmEF, justifying the complex analysis of this patient population. We hope that our study can add to a better understanding of this issue.[30]

Limitations

The small sample of 380 patients may explain the fact that the logistic regression model was not able to show statistical significance about important characteristics of HF patients. The study was conducted in a single tertiary center, reference in cardiology, which may limit the external validation of the study. As mortality was verified through the Mortality Information System, losses to follow-up may have occurred. Due to logistic difficulties, no contact was made with any of the patients after hospital discharge to verify readmission, an important outcome.

Conclusion

There was no difference in overall survival between HF patients with reduced, intermediate, and preserved EF. HFmEF and HFrEF patients had a higher mortality from cardiovascular cause when compared with HFpEF patients. Hospital mortality was higher when compared with developed countries. HFmEF patients had clinical characteristics intermediate between EF categories, and ischemia as the main cause of HF.

Author Contributions

Conception and design of the research: Petersen LC, Danzmann LC, Bodanese LC, Miglioranza MH; Acquisition of data: Petersen LC, Donay BG, Magedanz EH, Azevedo AV, Porciuncula G; Analysis and interpretation of the data: Petersen LC, Oliveira EB, Bodanese LC, Miglioranza MH; Statistical analysis: Petersen LC, Miglioranza MH; Obtaining financing and Writing of the manuscript: Petersen LC; Critical revision of the manuscript for intellectual content: Petersen LC, Danzmann LC, Miglioranza MH.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Heart Failure Mid-Range Ejection Fraction

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Short Editorial related to the article: Survival of Patients with Acute Heart Failure and Mid-range Ejection Fraction in a Developing Country – A Cohort Study in South Brazil

Heart failure (HF) is a clinical syndrome with typical symptoms caused by structural and/or functional cardiac abnormalities. It has a prevalence of up to 1-2% in adults from developed countries with high mortality due to cardiovascular causes.[1,2] Elevated morbidity and mortality can also be seen in developing countries such as Brazil.[3]

The main terminology used to classify HF is based on left ventricular ejection fraction (LVEF) values. In 2016, the European Society of Cardiology (ESC) Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure introduced a new HF class consisting of patients with an LVEF between 40 and 49%, which was called HF with mid-range ejection fraction (HFmrEF).[1] A grey area between heart failure with reduced (HFrEF) and preserved (HFpEF) ejection fraction had been recognized in previous studies. The introduction of this new HF classification led to a rapid increase in the number of studies on HFmrEF over the next few years,[4,5] with many conflicting results in terms of survival and the clinical characteristics of HFmrEF being reported in literature. Although mortality and morbidity in HFrEF has been reduced by improving treatment in the last thirty years, similar results were not seen in HFpEF and few studies were specifically designed to evaluate mortality in patients with HFmrEF.[6]

In the current edition of *Arquivos Brasileiros de Cardiologia*, we read with great interest the study by Petersen et al.[7] about the clinical characteristics and survival rate of HF patients, comparing HFmrEF with reduced and preserved ejection fraction. The cohort study followed up 380 adult patients with acute HF admitted via the emergency department to cardiology in a reference tertiary hospital in South Brazil. Interestingly, patients with HFmrEF showed intermediate age, blood pressure, and ventricular diameter characteristics between those of HFpEF and HFrEF. Most patients with HFmrEF had arterial hypertension and myocardial ischemia. Although a Kaplan-Meier curve showed no differences in overall survival rate between the different ejection fraction groups, mortality due to a cardiovascular cause was higher in patients with HFmrEF than those with HFpEF, and lower than those with HFrEF. The study had the strength of a considerable sample size and a long median follow up time of approximately four years.

The results of their study are in accordance with previous observational research and clinical records, which have shown that patients with HFmrEF usually present an intermediate clinical profile between preserved and reduced LVEF.[8] However, the prognosis of HFmrEF patients is still a matter of discussion, particularly considering that LVEF changes over time, raising the question about the transitional status of HFmrEF between HFpEF and HFrEF.[8] A longitudinal evaluation of LVEF using the Swedish Heart Failure Registry showed that HFmrEF patients moved to HFpEF, HFrEF, or remained as HFmrEF in approximately the same proportions.[8,9] Furthermore, recent studies have shown both reduced or similar event rates in HFmrEF compared to HFrEF.[8]

The pros and cons of an LVEF-based classification for patients with HFmrEF have recently been discussed.[8] The use of other echocardiographic parameters including a detailed evaluation of systolic and diastolic function could help to better define the phenotype and prognosis of patients with HFmrEF. In a long-term experimental model, by using a combination of cardiac structural and echocardiographic LV systolic and diastolic functional parameters, it was possible to non-invasively diagnose HF in post-infarction rats.[10] The inclusion of additional variables such as other imaging parameters and biomarkers, HF etiology, age, and co-morbidities to characterize HF patients should improve understanding in the gray area of HFmrEF.[11]

Palavras-chave

Heart Failure/mortality; Stroke Volume; Prognostic; Medication, Adherence.

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Sleep Quality Associated with Habitual Physical Activity Level and Autonomic Nervous System of Smokers

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Abstract

Background: **Few studies have examined the relationship of one's habitual physical activity level and autonomic nervous system (ANS) modulation on sleep quality in smokers.**

Objective: **The aim of this study was to identify changes in the sleep quality of smokers and its relation with their habitual physical activity level and ANS modulation.**

Methods: **Forty-two smokers were divided into two groups according to the 50th percentile of the moderate-to-vigorous physical activity (MVPA). Sleep quality was assessed using the Mini-sleep Questionnaire, and ANS modulation was assessed by indices of heart rate variability (HRV). To examine the possible mean differences, the analysis of covariance (ANCOVA) was used, adjusted for age, sex, body composition, pack-years, beta-blockers, anxiety, and depression in log base 10, not including qualitative data, such as sex and beta-blockers. Correlations were made by using the Spearman rank correlation. The statistical significance was set at 5%**

Results: **The smokers who were less active showed poor sleep quality (p=0.048) and insomnia (p=0.045). Furthermore, the less active group presented decreased parasympathetic modulation [HF (un; p=0.049); RMSSD (ms; p=0.047) and SD1 (ms; p=0.047)] and an increased LF (un) index (p=0.033) and LF/HF ratio (p=0.040). A positive correlation between the total Mini-sleep score with LF (un) index (r=0.317, p=0.041) and LF/HF ratio (r=0.318, p=0.040) and negative correlation with HF (un) index (r= -0.322, p=0.038).**

Conclusions: **Smokers with lower levels of habitual physical activity showed poor sleep quality and alterations in autonomic nervous system modulation; (Arq Bras Cardiol. 2021; 116(1):26-35)**

Keywords: **Sleep; Sleep,Quality; Exercise. Heart Rate; Tobacco Use Disorder; Autonomic Nervous System Diseases.**

Introduction

Smoking is considered a major public health problem worldwide, despite being a major cause of preventable death worldwide.[1] The global burden of chronic diseases is increasing and smoking represents an important risk factor for the development of these diseases.[1]

Smoking may also be responsible for neurobehavioral alterations, such as reduced working memory, lapses of attention, depressed mood, and sleep disturbances.[2] In the latter respect, several studies conducted with adults reported a negative association between smoking and sleep quality, such as insomnia,[3] hypersomnia, sleep fragmentation,[4] daytime sleepiness,[5] and poor nocturnal sleep quality.[6]

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Sleep restriction due to smoking can be caused by several mechanisms, the most prevalent of which is the impact of nicotine.[7] During sleep, the nicotine levels decrease, triggering withdrawal symptoms, which are dependent on the number of cigarettes smoked per day, the nicotine dependence level, and the rate of nicotine withdrawal. Moreover, carbon monoxide levels and the elimination of nicotine levels in the blood decrease during sleep.[7-10]

During sleep, the autonomic nervous system (ANS) modulation presents changes along transitions between wakefulness and sleep. The cardiac parasympathetic modulation increases approximately two hours before sleep onset, reaches the peak at sleep onset, and decreases in the sleep period, while sympathetic modulation does not change at sleep onset but does decrease during the deeper stages of sleep. These changes produce decreased heart rate and increased heart rate variability (HRV).[11,12]

Smokers show changes in ANS characterized by reductions in parasympathetic modulation,[13,14] suggesting that in addition to smokers presenting sleep disturbances due to cigarette consumption, the decrease in parasympathetic modulation in these individuals may also affect one's sleep quality.

The literature suggests that a healthy and active lifestyle is able to induce an increase in parasympathetic modulation,[15] promoting ANS regulation and balance.[16] Thus, a habitual active lifestyle, is one of the benefits to sleep quality due to its effects on ANS regulation,[17,18] which can also happen with smokers.[19] Investigating the relationship between sleep quality and ANS modulation according to the habitual physical activity level of smokers can promote valuable information to identify the importance of a more active lifestyle in this population. Moreover, improvement in sleep quality may increase the chances of success rates during smoking cessation. Therefore, the present study aimed to assess the sleep quality in smokers and its relationship with the habitual physical activity level and ANS modulation.

Materials and Methods

Participants and Procedures

Participants were recruited through announcements in the media. Smokers, 18 to 60 years of age and regardless of sex, were selected. The inclusion criteria were: 1) consume at least 10 cigarettes/day, for at least one year; 2) absence of known pre-existing chronic cardiorespiratory diseases that significantly influence the ANS (e.g arrhythmias, uncontrolled hypertension, chronic cough, chronic bronchitis, pulmonary emphysema, or FEV_1/FVC <70%); 3) No excessive use of alcohol or other illicit drugs; 4) No use of nicotine replacement products and/or antidepressants as an aid to stop smoking. The exclusion criteria were: 1) incomplete assessments and 2) Outliers (more than 3 standard deviations away from mean, indicating error in collected HRV data).

A total of 239 smokers expressed interest in participating in the study. Thus, 83 participants were included, but 41 participants were excluded due to incomplete assessments (n = 29) and participants who had a standard deviation greater than 3 in the HRV indices (outliers, n = 12). Therefore, 42 participants were then divided into two groups according to the 50th percentile of the moderate-to-vigorous physical activity (MVPA) (Figure 1).

Participants were previously informed about the research objectives and procedures and, after agreement, signed the consent form. This study was approved by the Research Ethics Committee of Sao Paulo State University (CAAE: 54550116.6.0000.5402).

The assessment was performed on two non-consecutive days; all procedures were performed in the morning under controlled temperature and relative humidity (22.0±2.2ºC, 56.6±6.9%), and all participants were instructed not to ingest alcohol, caffeine, anesthetics, or barbiturates, nor to perform moderate or vigorous exercise 24 hours prior to assessment. Measurement of exhaled carbon monoxide (exCO), with a cut-off point of 10 ppm,[20] was performed to prove nicotine abstinence from 24 hours prior to the assessments.[21] On the first day, the participants took part in an assessment to collect personal data, as well as data regarding smoking status, pulmonary function, and anthropometric and body composition, along with an analysis of anxiety, depression, and sleep quality. On the second day, all participants underwent an assessment of ANS modulation by HRV indices and of the habitual physical activity level performed by the accelerometer. Previously trained professionals accompanied all the assessments.

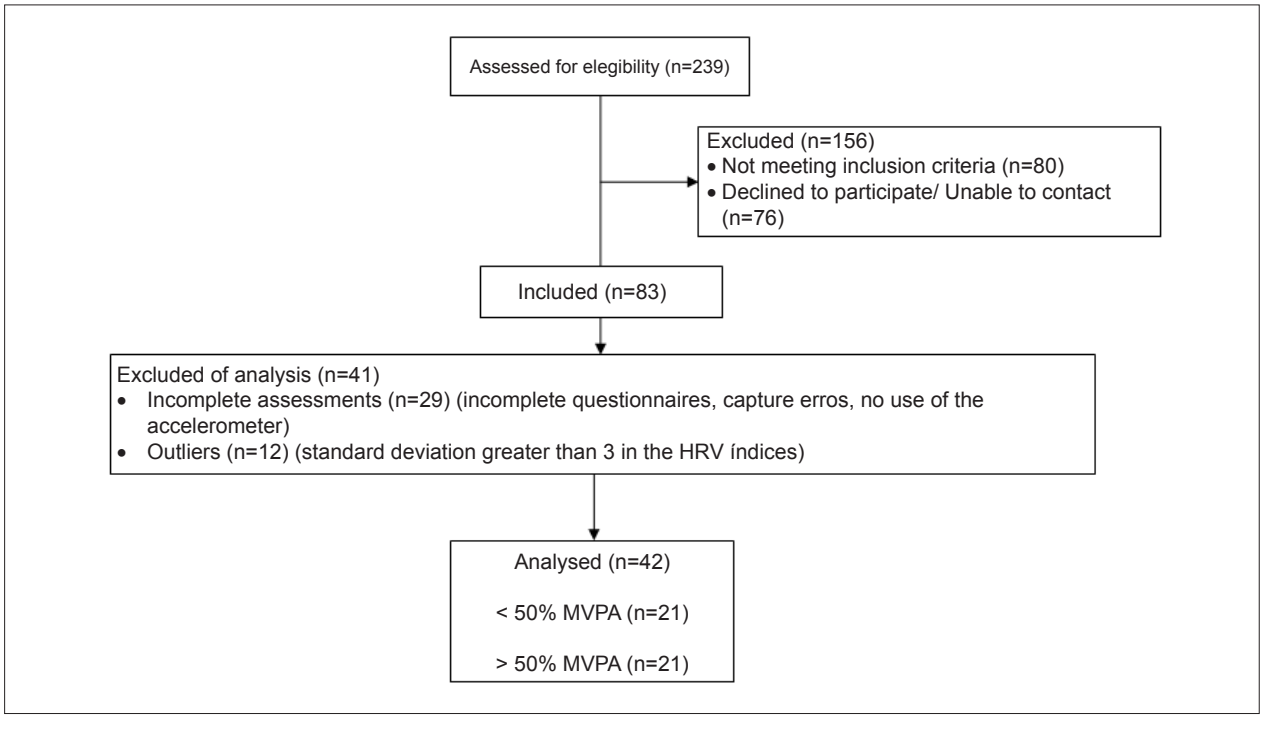

Figure 1 – *Flow chart of the study. HRV: heart rate variability, MVPA: moderate-to-vigorous physical activity.*

Smoking Status

All participants answered questions from the Fagerström questionnaire concerning the number of cigarettes consumed per day, time of smoking, and nicotine dependence.[22] The pack/years was calculated by the formula: number of cigarettes consumed per day divided by 20 and multiplied by the time of smoking.

Pulmonary Function

The pulmonary function was analyzed using a portable spirometer (Spirobank 3.6 Medical International Research, Rome, Italy). The interpretation was made considering the standards of American Thoracic Society and European Respiratory Society,[23] with normal values recorded for the Brazilian population.[24]

Body Composition

The InBody 720 octopolar apparatus (Copyright®, 1996–2006, by Biospace Corporation, USA) was used to calculate the body mass index (BMI), percent fat mass (%FM), skeletal muscle mass (SMM), and fat mass (FM). The InBody 720 uses eight electrodes, two in contact with the palm (E1 and E3) and thumb (E2 and E4) of each hand and two in contact with the front (E5 e E7) and heel (E6 and E8) of each foot.[25,26]

Anxiety and Depression

Anxiety and depression were assessed by applying the Hospital Anxiety and Depression Scale (HADS) questionnaire.[27] This instrument consists of a 14-item scale, seven exclusive for anxiety and seven exclusive for depression.

Sleep Quality

The sleep quality was assessed by a Mini-sleep Questionnaire,[28] validated for the Brazilian population by Falavigna et al.,[29] which consists of 10 self-reported questions with seven possible answers (never = 1, very rarely = 2, rarely = 3, sometimes = 4, often = 5, very often = 6, and always = 7). Insomnia (questions 1, 2, 3, and 7) and hypersomnia (questions 4, 5, 6, 8, 9, and 10) are also assessed in this instrument.

Habitual Physical Activity Level

Participants were instructed to wear an ActiGraph GT3X+ (AG) accelerometer, (ActiGraph LLC, Pensacola, FL USA) for a 7-d period, including while sleeping, only removing the devices when coming in direct contact with water (i.e. bathing or swimming).[30] The AG was attached to an elastic waistband and positioned on the right hip. The AG device is a triaxial accelerometer and measures acceleration in 3 planes, generating activity counts for each axis and a vector magnitude representing the combination of all 3 axes. In the current study, the raw data was collected at a frequency of 80 Hz. Data from the AG device was downloaded using the low extension filter from the ActiLife software (version 6.13, ActiGraph LLC), not including the steps/day, which was downloaded using the normal filter. For data analysis, raw accelerometer data was converted into counts and summed over a 60 sec period with the low frequency extension enabled.

A previously validated algorithm was applied to the AG accelerometer data to separate sleep wear time from awake wear time.[31,32] Data from sleep wear time was not used in the analysis of the activity patterns described below. Periods of non-wear (identified using the AG accelerometer data) were defined as consecutive blocks of at least 60 min of zero activity counts, including up to 2 consecutive minutes of activity counts less than 100, in line with the National Health and Nutrition Examination Survey (NHANES) criteria.[33] A complete day of acclerometer use was defined as at least 10 hours of wake wear time. A minimum of 4 days (including at least 1 weekend day) of wear data was necessary in order for participants to be included in the data analysis.

After initial inspection and processing, accelerometer data from awake wear time was analyzed to determine how much time participants spent in moderate-to-vigorous physical activity (MVPA) using the cut point by Troiano et al. > 2020 counts/min (equivalent to 3 METs), vigorous intensity (5,999 counts or 6 METs).[33] Each period was classified as sedentary time if vertical axis counts were < 100 counts/min.[34]

Autonomic Nervous System (ANS) Modulation

To analyze the indices of ANS modulations, the heart rate was captured beat by beat, using a cardio-frequency (Polar S810i, Finland) equipment, which had been previously validated for the capitation of one's heart rate, beat by beat, and its use for calculating HRV indices.[35]

All participants were instructed not to consume stimulating substances, such as tea, coffee, soda, chocolate, and alcohol for 24 hours prior to this analysis. While recording the heart rate, participants were instructed to remain silent, awake, at rest, without performing movements and conversations during execution, and with spontaneous breathing for 20 minutes while sitting. The circulation of people was not allowed in the room during the execution of data collection in order to avoid capture errors and reduce the anxiety of volunteers.

Data obtained by monitoring were transferred to the computer using the software Polar ProTrainer 5 (version 5.41.002) and each five minutes of the chart were analyzed, with at least 256 RR intervals, selected from the most stable part of the chart, after digital filtration, completed via manual filtering to eliminate artifacts and ectopic beats; only series with over 95% of sinus beats were included in the study.

HRV indices were calculated in the time and frequency domains and the Poincaré plot. In the time domain (TD), the indices of Root Mean Square of Successive Differences (RMSSD) and Standard Deviation of Normal to Normal intervals (SDNN) were calculated, both expressed in milliseconds (ms). In the frequency domain (FD), this study used the low frequency spectrum component (LF, 0.04 – 0.15 Hz) and the high frequency (HF, 0.15 – 0.40 Hz), in absolute values (ms^2) and in normalized units (un), as well as the LF/HF ratio.[36,37] The spectral analysis was calculated using the fast Fourier transform algorithm.[38]

The Poincaré plot was used to calculate the following indices: SD1 (standard deviation of the instantaneous beat to beat variability); SD2 (standard deviation of the long-term continuous R-R intervals); and the SD1/SD2 ratio, which shows

the ratio between short and long-term variations of the RR intervals.[39,40] To analyze the HRV index, the Kubios software (University of Kuopio, Finland) was used.[41]

Data Analysis

Previous research was used to determine the sample size. A correlation of r = 0.43 between sleep quality, physical activity level, and ANS was estimated, with an alpha error of 5% and a sample power of 80%. Hence, a sample of 41 participants was deemed appropriate.[42]

The sample was divided into two groups according to the 50th percentile (26.65 min) of the MVPA (<p50 or >p50). The Shapiro-Wilk test analyzed data normality. The continuous variables were described as mean and standard deviation or as median and interquartile range (IQR), except for categorical variables which were described as frequency (f) and percentage (%). Unpaired t test or Mann-Whitney test were applied in the comparison between the percentiles in the characterization variables of the sample. Comparison of sleep quality, habitual physical activity level, and HRV between percentiles was performed using covariance analysis (ANCOVA) adjusted for age, sex, BMI, %FM, SMM, pack-years, beta-blockers, anxiety, and depression in log base 10 (log10) to decrease the variability of nonparametric variables, not including qualitative data, such as sex and beta-blockers. The assumptions for comparing two independent samples were tested by examining the normality of the data, homogeneity between the groups, according to the Levene's test, and the linear relationship between the covariates and the dependent variables. Correlations between sleep quality, HRV, and habitual physical activity level were calculated using Spearman's rank correlation, which was used because, according to the Shapiro-Wilk test, the data proved to be nonparametric. All analyzes were performed using software SPSS (version 22.0) and statistical significance was set at 5%.

Results

Table 1 presents information about the general characteristics of the studied population. The group of less active smokers (<p50 MVPA) had more women (81%) than men (19%), as compared to the more active group (>p50 MVPA). The % FM was higher in the <p50 MVPA (p=0.017), whereas, the SMM was higher in the >p50 MVPA group (p=0.015).

Table 2 shows the variables of sleep quality, habitual physical activity level, and HRV of smokers in the <p50 and >p50 percentiles of MVPA, which was adjusted for confounding factors, such as age, sex, BMI, %FM, SMM, beta-blockers, pack-years, anxiety, and depression. It was observed that less active smokers (<p50), as compared to those with a higher MVPA level (>p50), showed poor sleep quality according to the total scores regarding Mini-sleep, insomnia, lower MVPA, and steps/days. As regards the HRV indices, the less active group (<p50) showed a decreased parasympathetic modulation, expressed by the RMSSD, HF(un), and SD1 indices, and an increased LF(un) and LF/HF ratio when compared to the more active group (>p50).

Figure 2 shows that there was a moderate negative correlation between MVPA (min) and total Mini-sleep score and insomnia.

Figure 3 shows that there was a weak to moderate positive correlation between the total Mini-sleep score with Mean HR (l/min), LF (un) index, and LF/HF ratio, as well as a weak to moderate negative correlation with Mean RR (ms) and HF (un) index.

Discussion

This study aimed to assess smokers sleep quality and its relationship to one's habitual physical activity level and ANS modulation. Therefore, our results showed that smokers with a lower level of habitual physical activity had poor sleep quality and insomnia, as well as a decrease in the parasympathetic modulation and an increase in the LF (un) index and LF/HF ratio.

Smokers are more likely to develop sleep disturbances than nonsmokers.[4,10,42]

The literature indicates that nicotine is one of the main mechanisms responsible for sleep disturbances in smokers, due to the independent and interactive effects of their neurotransmitters on the central mechanisms that regulate the sleep-wake cycle, increasing sleep latency.[10,43,44] According to McNamara et al.,[44] for each cigarette consumed, there is a decrease of 1.2 min in total sleep time, which suggests a possible influence of nicotine as a potential cause of this dose-response relationship. Furthermore, the decrease in nicotine levels during sleep produces symptoms related to the withdrawal syndrome, which increases insomnia in this population.[7–9]

Sleep disturbances in these individuals may also occur due to the presence of pulmonary diseases that may arise due to smoking (e.g, lung cancer and chronic obstructive pulmonary disease)[45] and behavioral variables, i.e., when the individual uses cigarettes as stress relief, because of a likelihood of a poor quality of life, and due to the appearance of depression and anxiety symptoms.[2,3,46]

Given the strong evidence about smoking on poor sleep quality, some studies have investigated the influence of physical activity on improved sleep quality.[19,47] According to Chen et al.,[19] inactive smokers (0-999 kcal/week) have a higher rate of insomnia when compared to active smokers (≥1000 kcal/week), when considering leisure and non-leisure activities. Masood et al.,[47] observed that heavy smokers were more likely to have less than five hours of sleep per day and more likely to take on unhealthy behaviors, such as a sedentary lifestyle, poor diet, and alcohol consumption. In addition to these studies, our results showed that smokers with moderate to vigorous physical activity levels below 26.65 min/day presented poor sleep quality and insomnia. However, there is still a need to investigate the different levels of physical activity in this condition.

One of the hypotheses to improve sleep quality through the regular practice of physical activity involves physiological adaptations, such as mood improvement, decreasing cortisol secretion, increase in energy consumption, and fatigue that increases the need to sleep for energy restoration, besides changes in body composition.[18,48] Regarding this last point, our results showed that more physically active smokers with good sleep quality present lower %FM and higher SMM.

Table 1 – General characteristics of smokers in 50th percentiles of MVPA (<p50 or >p50)

Demographic characteristics	<p50 (N=21)	>p50 (N=21)	p value
Sex (F/M)	17/4	8/13	0.005†*
Age (years), mean (SD)	42.0 (10.8)	44.3 (8.9)	0.644§
Body Composition			
Height (cm), mean (SD)	1.6 (0.1)	1.7 (0.1)	0.138§
Weigh (kg), mean (SD)	70.1 (12.6)	74.6 (15.1)	0.302§
BMI (kg/m²), mean (SD)	26.6 (4.5)	26.5 (4.2)	0.893§
%FM, mean (SD)	34.4 (6.6)	29.0 (7.6)	0.017§*
SMM (kg), median (IQR)	23.3 (22.2–27.2)	29.5 (24.2–34.7)	0.015‡*
FM (kg), mean (SD)	24.5 (7.6)	22.0 (8.5)	0.323§
Smoking status			
Smoking duration (years), mean (SD)	25.3 (11.5)	26.5 (9.2)	0.724§
Cigarettes days, median (IQR)	20.0 (12.0–20.0)	20.0 (10.0–30.0)	0.827‡
Pack-years, median (IQR)	22.0 (13.5–31.9)	24.8 (13.3–35.0)	0.537‡
Nicotine dependence, mean (SD)	5.2 (2.3)	5.6 (2.3)	0.594§
HADS			
Anxiety, mean (SD)	7.4 (4.5)	9.3 (3.8)	0.144§
Depression, mean (SD)	6.1 (4.0)	6.1 (2.7)	1§
Spirometric indices			
FVC (% pred), mean (SD)	94.1 (12.4)	94.4 (19.4)	0.968§
FEV1 (% pred), mean (SD)	93.5 (12.1)	91.1 (19.1)	0.629§
FEV1/FVC (% pred), mean (SD)	99.0 (6.0)	96.2 (5.5)	0.120§
PFE (% pred), median (IQR)	76.0 (72.0–87.0)	76.5 (58.8-90.3)	0.657‡
FEF25-75% (% pred), mean (SD)	94.7 (31.8)	86.3 (26.5)	0.365§
Current medications, f (%)			
Cardiovascular	6 (29)	4 (19)	0.469†
Beta-blockers	1 (17)	1 (25)	
AT1-blockers	4 (67)	3 (75)	
ACE inhibitors	1 (17)	0 (0)	
Antidepressant	7 (33)	3 (14)	0.147†
Metabolic	1 (5)	1 (5)	1†

*Data expressed as mean and std. deviation or median and interquartile range (IQR) and frequency (f) and percentage (%). F/M: Female/Male; BMI: body mass index; SMM: skeletal muscle mass; FM: fat mass; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; FEV1/FVC: ratio of FEV1 and FVC; PEF: peak expiratory flow; FEF25-75%: expiratory flow between 25% and 75% of FVC. * p-value for significant statistical difference; †Chi-square test; § Unpaired t test; ‡ Mann-Whitney test. Source: author himself*

Furthermore, the practice of physical activity, especially that performed continuously, is capable of causing changes in HR and HRV.[49] In trained individuals, increased parasympathetic modulation occurs, which may be related to one's improvement in mood, sleep quality, latency time, and use of medications to improve sleep quality in both adults and the elderly.[17,49,50]

Individuals with insomnia present increased HR during sleep, decreased total sleep time, and decreased HRV indices, which may hinder transitions of the stages of sleep, in turn requiring parasympathetic activity to achieve deeper stages.[51] In smokers, these changes may be more evident, because smoking may lead to a reduction in HRV.[13,14,52–54] Bodin et al.[52] evaluated smokers in periods in which they consumed and did not consume cigarettes for 12 hours and observed that after smoking the participants presented a reduction in HRV, with a decrease in HF and RR intervals when compared to non-smoking periods. In heavy smokers, Santos et al.,[14] observed increased LF(un) and LF/HF indices and a decreased HF(un) index and SD1/SD2 ratio when compared to moderate smokers.

However, our results demonstrated that the physical activity level in smokers was associated with HRV even though it is a population with changes in HRV due to smoking. More physically active smokers presented increased parasympathetic modulation, expressed by the RMSSD, HF (un), and SD1

Table 2 – Sleep quality, physical activity level, and autonomic cardiac modulation of smokers in 50th percentiles of MVPA (<p50 or >p50)

Mini-sleep	< p50 (N=21)	> p50 (N=21)	p†
Total, median (IQR)	34.0 (28.5–38.5)	29.0 (22.5–32.5)	0.048*
Insomnia, median (IQR)	14.0 (8.0–19.0)	10.0 (7.0–14.0)	0.045*
Hypersomnia, median (IQR)	20.0 (16.5–22.5)	17.0 (13.0–22.0)	0.113
Physical activity level			
MVPA (min), median (IQR)	14.0 (7.4–19.1)	38.0 (30.4–48.6)	<0.0001*
Sedentary (min), mean (SD)	450.5 (147.0)	466.4 (100.3)	0.939
Steps/Day. median (IQR)	7058.0 (5874.5–8431.0)	9753.0 (7977.5–11354.5)	0.020*
HRV			
Mean RR (ms), mean (SD)	751.8 (71.2)	805.3 (96.6)	0.161
SDNN (ms), mean (SD)	32.2 (12.7)	33.2 (14.4)	0.982
Mean HR (bpm), mean (SD)	80.7 (7.9)	75.6 (9.0)	0.147
RMSSD (ms), median (IQR)	14.6 (10.1–26.4)	18.8 (14.6–31.5)	0.047*
RR triangular index, mean (SD)	8.7 (3.1)	9.1 (3.6)	0.970
TINN (ms), mean (SD)	142.9 (57.8)	138.8 (66.6)	0.648
LF (ms²), median (IQR)	220.0 (91,5–607.0)	264.0 (71.5–526.0)	0.530
HF (ms²), median (IQR)	101.0 (23,5–206.0)	114.0 (47.5–269.5)	0.351
LF (nu), median (IQR)	74.5 (57.3–82.3)	70.4 (54.0 –79.0)	0.033*
HF (nu), median (IQR)	25.5 (17.5–42.6)	28.7 (21.0–45.9)	0.049*
LF/HF (ms²), median (IQR)	2.9 (1.4–4.8)	2.5 (1.2–3.8)	0.040*
SD1 (ms), median (IQR)	10.3 (7.2–18.7)	13.3 (10.3–22.3)	0.047*
SD2 (ms), mean (SD)	43.5 (17.0)	43.6 (18.8)	0.670
SD1/SD2 (ms²), median (IQR)	0.3 (0.3–0.4)	0.3 (0.3–0.4)	0.457

*Data expressed as mean and std. deviation or median and interquartile range (IQR). MVPA: moderate to vigorous physical activity; nu: normalized units; RR: between successive heart beats; SDNN: Standard Deviation of Normal to Normal interval; HR: heart rate; RMSSD: Root Mean Square of Successive Differences; TINN: triangular interpolation of RR intervals; LF: low frequency; HF: high frequency; SD1: standard deviation of the instantaneous beat to beat variability; SD2: standard deviation of the long-term continuous R-R intervals. * p-value for significant statistical difference; †ANCOVA adjusted for age, sex, BMI, %FM, SMM, pack-years, Beta-blockers, anxiety, and depression. Source: author himself.*

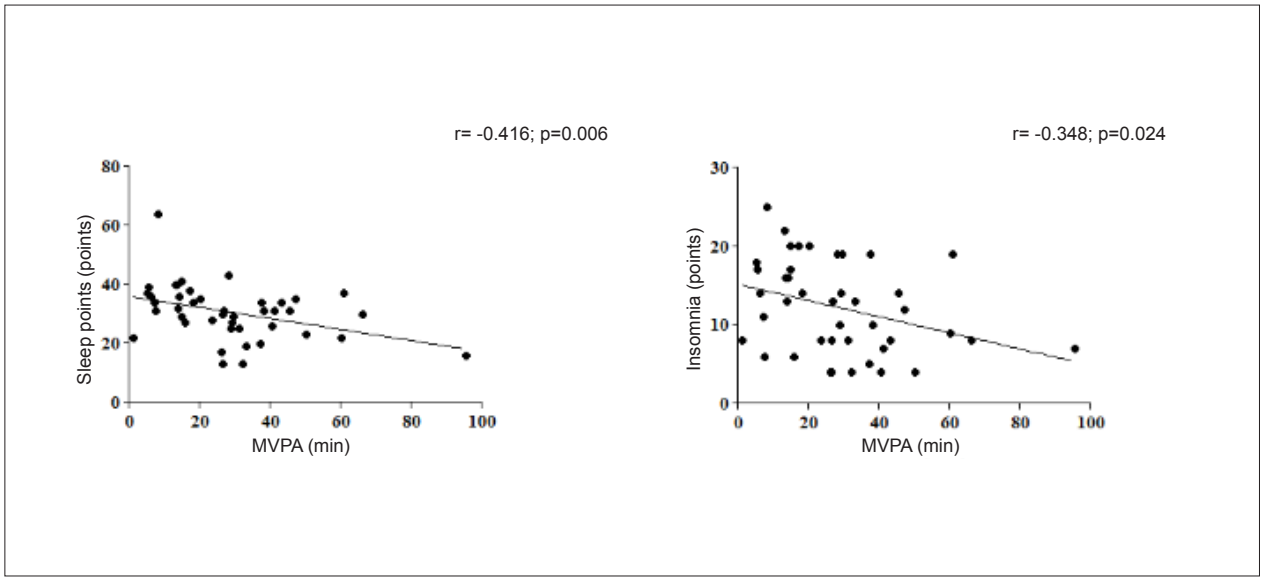

Figure 2 – *Correlation analysis between sleep quality and habitual physical activity level. MVPA: moderate-to-vigorous physical activity; r: Spearman's rank; p: statistical significance (0.05).*

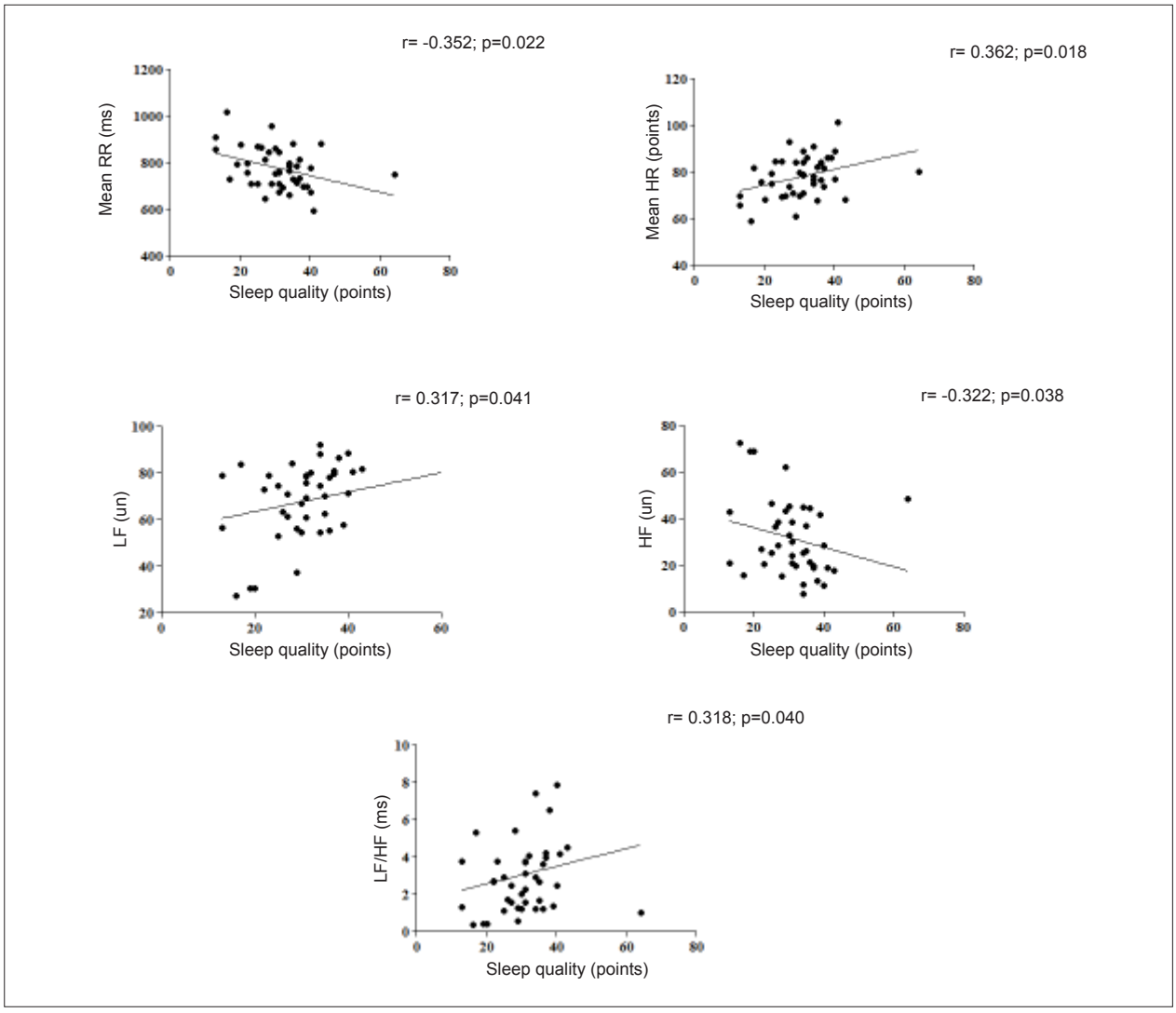

Figure 3 – *Correlation analysis between sleep quality and HRV. RR: between successive heart beats; HR: heart rate; LF: low frequency; HF: high frequency; r: Spearman's rank; p: statistical significance (0.05).*

indices, as well as a decrease in the LF (un) index and LF/HF ratio when compared to less active smokers. This finding suggests that the practice of physical activity in this population improves sleep conditions; such evidence may, at least partly, be related to changes in the ANS.

In the analysis of correlation between sleep quality and HRV indices, it was observed that poorer sleep quality was associated with higher levels of heart rate, LF(un) index, and LF/HF ratio, as well as lower levels of parasympathetic modulation, suggesting that poor sleep quality and insomnia may be correlated with a reduction in HRV, especially in less active smokers.

Limitations of this study include the lack of a control group of non-smokers to better evaluate the influence of smoking on the studied aspects, the non-determination of the phase of menstrual cycles of women in premenopause, and antidepressant medication, which may influence the ANS. Future studies on these issues are warranted. Furthermore, HRV indices are influenced by age, sex, and cardiovascular medication, which may have influenced the results. However, the analyzes were adjusted for potential confounding factors.

Conclusion

In summary this study showed that the sleep quality of smokers was associated with one's physical activity level and ANS modulation. Thus, in addition to nicotine, the poorer sleep quality may be associated with a lower level of physical activity and alterations in autonomic nervous system modulation, suggesting that promoting physical activity in smokers may help improve sleep quality and better autonomic control. However, there is a need for new studies that evaluate different levels of physical activity in ANS modulation during sleep as compared to healthy individuals, which may prevent sleep disorders and encourage a healthy lifestyle by encouraging patients to stop smoking.

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Author Contributions

Conception and design of the research: Trevisan IB, Vanderlei LCM, Proença M, Ramos EMC, Ramos D; Acquisition of data: Trevisan IB, Vanderlei LCM, Barreira TV, Santos CP, Gouveia TS; Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Trevisan IB, Vanderlei LCM, Proença M, Barreira TV, Santos CP, Gouveia TS, Ramos EMC, Ramos D; Statistical analysis: Trevisan IB, Proença M, Barreira TV, Ramos D; Obtaining financing: Trevisan IB, Ramos D.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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A Collaboration to Stop Smoking

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Short Editorial related to the article: Sleep Quality Associated with Habitual Physical Activity Level and Autonomic Nervous System of Smokers

The association between smoking and physical inactivity is an essential expression of morbidity and mortality in wide age groups.[1]

The present study[2] enhances the recognized risks of smoking, correlating it to the quality of sleep, and autonomic dysfunctions with levels of physical activity. Parameters considered as risk factors for cardiovascular pathologies.[3] It is worth mentioning the accurate methodology contributing to the legitimacy of this study. In the most active group (p> 50), higher values of skeletal muscle mass were observed, related to better performance and influencing the reflex metabolite.[4] Statistical treatment demonstrated significant correlations between smoking, sleep quality, and autonomic dysfunction. We believe that a higher number of candidates for the study can obtain stronger associations. We suggest, as a continuation of this study, to evaluate the recovery of heart rate in the first minute of post-exercise recovery, an indicator of parasympathetic autonomic adaptation of prognostic value in cardiovascular diseases.[5] Also, a better quantification of physical activity that characterizes active or non-active behavior is also valid.[6] The current bibliography deserves mention, with about 16% of authors from our country.

We reiterate the originality, design, and conclusions of this study,[2] with broad practical applicability, when it demonstrates that the active smoker exhibits favorable parameters related to the quality of sleep and dysautonomia, being able to collaborate with the interruption of the smoking habit.

Keywords

Atrial Fibrillation/physiopathology; Arrhythmias Cardiac/physiopathology; Risk Factors; Obesity; Sedentarism; Combined Modality Therapy.

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Assessment of Peripheral Blood Mononuclear Cells Senescence and Endothelial Dysfunction among Adults with High Cardiovascular Risk

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Abstract

Background: Cardiovascular diseases (CVD) are one of the leading causes of mortality and morbidity worldwide. Biological aging has been associated with the occurrence of adverse cardiovascular outcomes; however, the underlying mechanism of this process remains unknown.

Objectives: This study sought to evaluate if peripheral blood mononuclear cell (PBMC) senescence and endothelial biomarkers could influence cardiovascular (CV) risk and be suitable markers for the early detection of cardiovascular diseases in adults.

Methods: In this cross-sectional study patients free of CVD were classified as lower (n=32) and higher Interheart Risk (IHR) scores (n=28). PBMC senescence was assessed by estimating the telomerase activity (TA) and detecting the presence of senescent cells and endothelial dysfunction by estimating the concentration of nitrite and nitrate and of total antioxidant capacity (TAC). Statistical analysis was performed with SPSS version 16.0 (SPSS Inc., Chicago, IL). All p-values <0.05 were considered statistically significant.

Results: PBMC senescence 0.95 [p-value = 0.0001; 95% CI (0.874-1.026)] was a significant predictor of patients with higher IHR scores with a cut-off value of 21.65 with a sensitivity and specificity of 92% and 88% respectively. PBMC senescence, nitrite and nitrate and TA were found to be independently associated with high IHR scores.

Conclusion: PBMC senescence, TA and nitrite, and nitrate status are suitable measures to predict high cardiovascular risk in adults with CV risk. Nevertheless, long-term follow-up studies are needed to confirm these findings. (Arq Bras Cardiol. 2021; 116(1):37-47)

Keywords: Cardiovascular Diseases; Cell Aging; Endothelium; Biomarkers; Propensity Score; Risk Factors.

Introduction

Cardiovascular diseases (CVD) such as atherosclerosis and associated myocardial infarction (MI) are still one of the well-known and leading causes of mortality and morbidity worldwide, especially in India. Moreover, the social and economic costs incurred in the treatment of CVD are high. It has been estimated that more than 75% of the cardiovascular (CV) deaths occur in lower and middle-income countries.[1] Chronological aging is considered to be one of the strongest predictors for the occurrence of CV and cerebrovascular diseases, such as MI, heart failure (HF), atherosclerosis, and stroke; however, biological aging can be considered superior to chronological aging in the stratification of the CVD risk.[2] The process of biological aging particularly refers to the accumulation of endothelial damage, which occurs due to several mechanical, hemodynamic, and immunological mechanisms, and is determined by both social and environmental factors. Vascular Senescence (%) (VS), a kind of biological aging of the vascular system, is postulated to have prognostic and therapeutic relevance in atherosclerosis. Biological aging has been associated with the occurrence of adverse CV outcomes; however, the underlying mechanism of this process remains unknown.[3] Moreover, arterial aging is the primary reflection for biological aging.[4,5] The absence of telomerase activity (TA) leads to the shortening of telomeres, which is an important determinant of biological aging leading to several vascular diseases. The term endothelial dysfunction

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refers to a number of pathological conditions that include the altered anticoagulant and anti-inflammatory properties of the endothelium, dysregulation of vascular modelling and the impaired regulation of vascular growth. Endothelial dysfunction leads to attenuated production or the availability of nitric oxide (NO) and leads to the up-regulation of oxidative stress through the increased production of reactive oxygen species (ROS)[6]. Cell senescence has proven to be equivalent to endothelial senescence and thus vascular senescence.[7] In the current clinical practice, the risk of CVD is estimated and quantified on the basis of conventional risk factors such as age, diabetes, hypertension, smoking, hypercholesterolemia, and family history of CVD.[8] Nevertheless, individuals with CVD might have only one, or none of the traditional risk factors and there is a possibility that these risk factors might not fully account for the disease progression. Therefore, the evaluation of other non-traditional and uncommon risk factors might aid clinicians in predicting the future risk of CVD. In this light, we hypothesized that peripheral blood mononuclear cell (PBMC) senescence and endothelial biomarkers could influence the CV risk and could be suitable markers for the early detection of cardiovascular disease among adults with high CV risk.

Materials & Methods

Study Design and Setting

The study protocol was approved by the Institutional Ethics Committee (973/IEC/2016). All the study procedures were followed according to the Declaration of Helsinki. All the study participants of this cross-sectional study were screened and recruited between January 2017 and December 2017 from the General Medicine outpatient department (OPD) and hospital wards. Figure 1 provides the outline of the study.

Study Subjects

This study included all adults over 18 years of age, of both genders, who received medical care at the General Medicine OPD and hospital wards with no cardiac diseases. Patients with both higher and lower cardiovascular risk were included. Patients were classified on the basis of their Interheart Risk (IHR) score. The IHR score was calculated based on the presence or absence of known CV risk factors. Patients with any cardiac disease, active immune disease, and chronic liver or kidney diseases were excluded from the study.

Interheart Risk Score

After obtaining the informed consent form, the study participants were screened according to the inclusion/exclusion criteria, and the IHR score was measured. The IHR score was calculated using the version which did not include data on cholesterol levels. The IHR score consisted of information on medical history and data on the domains of age, gender, status with respect to diabetes, hypertension, smoking, family history of heart disease, waist-to-hip ratio, psychosocial factors, diet, and physical activity. The scores of the IHR ranged from 0 to 48, where higher and lower scores indicated higher IHR and lower IHR scores respectively. A high IHR score was defined as value16 units.[9]

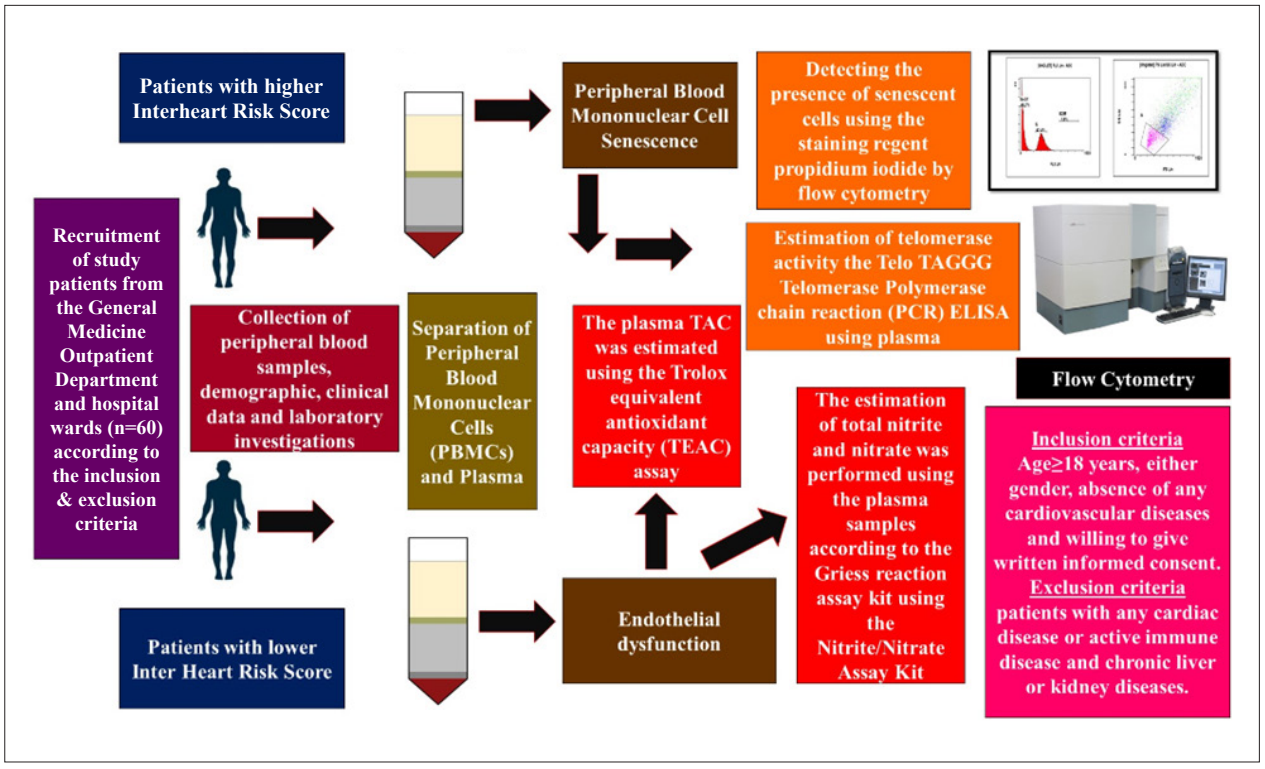


Figure 1 – *Flow chart representations of the research study.*

Sample Collection

Three ml of blood was obtained from the antecubital vein of the forearm in both heparin and Ethylenediaminetetraacetic acid (EDTA) vacutainers separately. The blood sample obtained in the EDTA vacutainers was subjected to centrifugation at 2,500 revolutions per minute (rpm) for 10 minutes and the isolated plasma was stored at -80°C. The blood sample collected in the heparin vacutainers were processed for the separation of Peripheral Blood Mononuclear Cell Senescence (PBMCs), using Ficoll-Histopaque reagent. The isolated PBMCs were those fixed with 70% ethanol and were stored at 4°C until further analysis.[10] Endothelial dysfunction was assessed by estimating the concentration of nitrite and of nitrate and antioxidant status.[11]

Quantification of Total Nitrite and Nitrate

The estimation of total nitrite and nitrate was performed according to the Griess reaction assay kit using the Nitrite/Nitrate Assay Kit (Sigma-Aldrich-Catalogue Number 23479, St. Louis, USA), so as to indirectly assess the bioavailability of nitric oxide (NO). Centrifugal filters, with a molecular weight 3,000 KDa cut-off, was used to filter the plasma samples (300μl each).The analysis of the flow through plasma samples was performed using a 96-well microtiter plate, and the absorbance was read at 540nm against the reference standards.

Estimation of Telomerase Activity

Plasma TA was estimated using the Telo TAGGG Telomerase Polymerase chain reaction (PCR) ELISA [Photometric enzyme immunoassay for the detection of telomerase activity, utilizing the Telomerase Repeat Amplification Protocol (TRAP), Roche Diagnostics GmbH, Roche Applied Science-Catalog Number 11854666910, Mannheim, Germany]. The assay was performed according to manufacturer's instructions.

Estimation of Total Antioxidant Capacity (TAC)

The plasma TAC was estimated using the Trolox equivalent antioxidant capacity (TEAC) assay. The analysis was performed according to manufacturer's instructions provided in the commercially available Antioxidant Assay Kit (Sigma-Aldrich-Catalog Number CS0790, St. Louis, USA). This assay was based on the ability to determine if the presence of low molecular weight antioxidants in the plasma will inhibit the production of ABTS+ produced by the oxidation of ABTS [2, 2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)]. The TAC was expressed in the form of Trolox equivalents (mM).

Fluorescence-activated Cell Sorting (FACS) Analysis

The PBMCs were isolated from the whole blood using Ficoll-Histopaque reagent. After the isolation of PBMCs, these were fixed with 70% ethanol and stored at 4°C overnight.[10] The isolated cells were then incubated for 10 minutes with RNase A (1mg/ml) for 10min at room temperature. PBMC senescence (%) was then detected using the staining regent propidium iodide by flow cytometry (FC 500 Beckmann Coulter).

Statistical Analysis

Statistical analysis for the study was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA); $p<0.05$ was considered statistically significant. The normality of data for continuous variables was checked using Q-Q plots. Continuous variables were summarized as the mean ± standard deviation (SD), and categorical data were expressed as the frequency (Percentages). Differences in the categorical variables between groups were evaluated with the chi-square test. Parametric tests were used based on the distribution of data. Differences in continuous variables between groups were analyzed using the Independent Samples t-Test. Pearson's correlation was performed to identify any association between the different variables. A Receiver operating curve (ROC) was plotted to identify the cut-off for all the laboratory assays so as to predict the high IHR score. A high IHR score was defined as value16 units.[9] All the necessary assumptions for performing the linear regression analysis were met. Multiple regression models were plotted to determine if the independent variable PBMC senescence, nitrite and nitrate, TAC, and TA could predict high IHR score.

Results

Baseline Characteristics of Study Patients

The baseline characteristics of the study patients have been illustrated (Table 1). The study patients (n=60) were classified into two groups of patients with lower (n=32) and higher IHR (n=28) scores. Patients with an IHR score≥16 were classified as higher IHR score patients and those with an IHR<16 were classified as lower IHR score patients. The mean age of study patients with lower and higher IHR scores was found to be 38.09±15.82 and 43.57±11.55 years, respectively. There was no significant difference in gender among the study groups. The mean IHR scores among patients with lower and higher IHR score patients were 8.5±4.27 units and 20.46±2.19 units, respectively. As expected, the presence of CV risk factors, such as diabetes and hypertension, were greater among patients with higher IHR scores than patients with lower IHR scores.

Peripheral Blood Mononuclear Cell Senescence

PBMC senescence was assessed among the study patients by estimating the TA and detecting the presence of senescent cells (Figure 2). For the mean PBMC senescence (%), the percentage of senescent cells was significantly lower among patients with lower IHR scores (12.41 ± 7.40) than patients with higher IHR scores (35.26 ± 10.02) [p=0.0001] ((Figure 3a). The mean TA (Units/3000cells) was significantly greater among patients with lower rather than higher IHR scores, [(1.80±0.53 Units/3000cells) versus (0.94±0.23 Units/3000cells) [p=0.0001] (Figure 3b). The presence of cardiac risk factors, such as diabetes, hypertension, and smoking, influenced the levels of PBMC senescence and TA (Table 2).

Table 1 – Demographics and Risk Factors of Study Participants

Sl No.	Characteristics	Subjects with lower IHR (n=32)	Subjects with higher IHR (n=28)	p-value
1.	Age, years	38.09±15.82	43.57±11.55	0.13
2.	Male Gender, n (%)	20 (62.5%)	14 (50%)	0.33
3.	Interheart Risk (IHR) Score	8.5±4.27	20.46±2.19	0.0001
4.	Smoking, n (%)	2 (6.2%)	5 (17.9%)	0.16
5.	Diabetes, n (%)	1 (3.1)	22 (78.6%)	0.0001
6.	Hypertension, n (%)	32 (100%)	7 (25%)	0.003
7.	Family history of Heart Disease, n (%)	2 (6.2%)	4 (14.3%)	0.30
8.	Sedentary Lifestyle, n (%)	8 (25%)	9 (32.1%)	0.54

Data was expressed as Mean ± Standard Deviation and Frequency (Percentage). The statistical tests used to compare continuous variables were the independent samples t-test, while for the categorical variables, the chi-square test was used; p-value less than 0.05 was considered statistically significant.

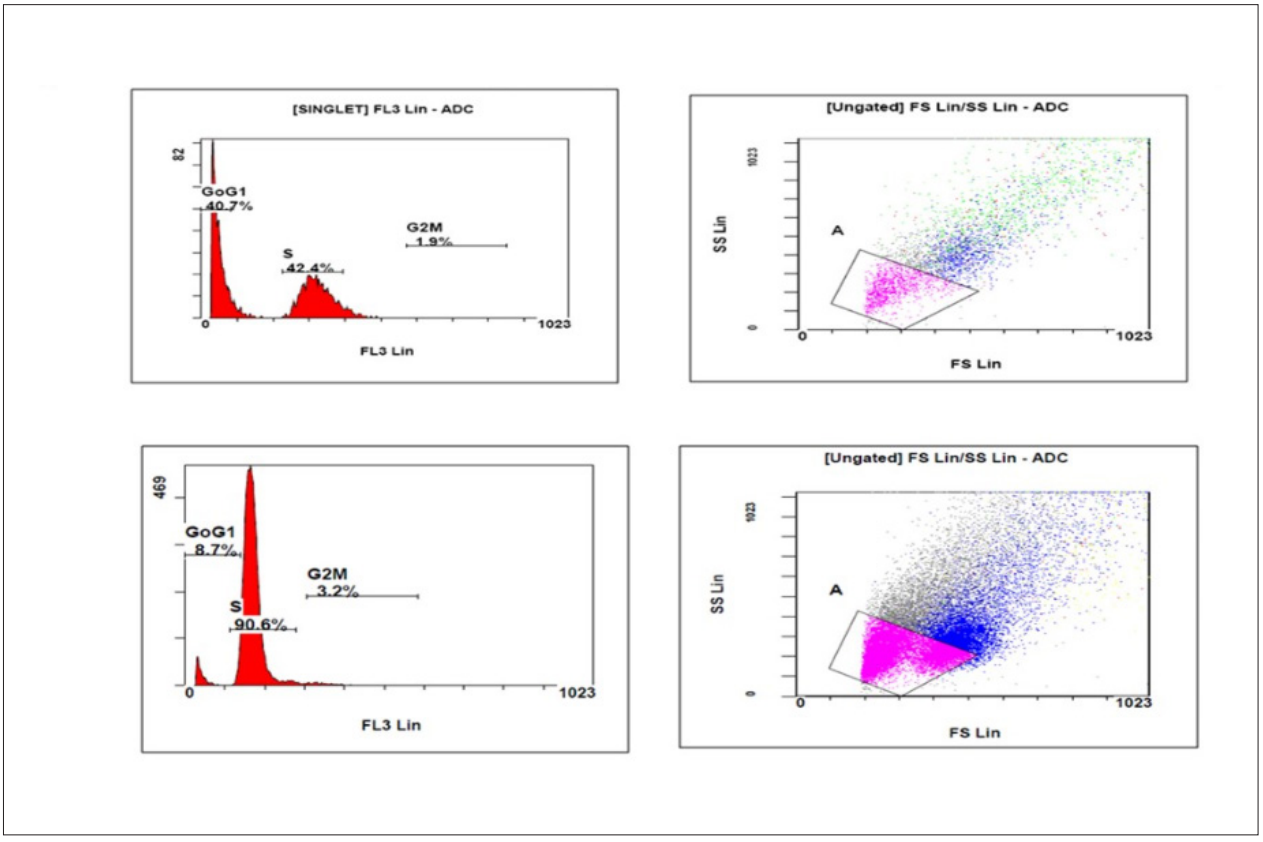


Figure 2 – *Identification and Quantification of Senescent Cells using Propidium Iodide.*

Table 2 – Quantification of Peripheral Blood Mononuclear Cell Senescence and Endothelial Dysfunction based on the presence and absence of risk factors

	PBMC Senescence		Nitrite & Nitrate		Telomerase Activity		TAC	
Risk Factors	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence
Diabetes	34.99±9.99*	15.67±11.44	204.22±42.39*	145.41±55.19	0.96±0.23*	1.67±0.60	0.52±0.08*	0.66±0.14
Hypertension	40.37±10.68*	20.79±13.26	224.71±28.01*	160.45±56.84	0.81±0.18*	1.47±0.59	0.40±0.09*	0.64±0.12
Smoking	36.05+12.38*	21.36+13.83	204.14±56.40	163.17±56.97	0.88±0.23*	1.46±0.60	0.51±0.12*	0.65±0.13
F/H/O Heart Disease	31.40±16.81	22.15±13.96	191.17±36.08	165.37±59.58	1.04±0.43	1.44±0.60	0.48±0.15	0.63±0.13
Sedentary Lifestyle	28.86±15.33	20.79±13.50	172.47±53.74	166.16±60.06	1.24±0.52	1.45±0.62	0.57±0.14	0.63±0.13

**p<0.05; PBMC: Peripheral Blood Mononuclear Cells; TAC: Total Antioxidant Capacity. Data was expressed as Mean ± Standard Deviation. The statistical tests used to compare the variables were independent samples t-tests; p-value less than 0.05 was considered statistically significant.*

Endothelial Dysfunction

The concentration of nitrite and nitrate was slightly higher among patients with higher IHR scores when compared to patients with lower IHR scores [205.14±43.60 μmole/l versus 135.41±48.95 μmole/l (p=0.0001)] (Figure 3c). The TAC was significantly higher among patients with lower IHR than with higher IHR scores [(0.71±0.08 mM/L) versus (0.50±0.09 mM/L) (p=0.0001] (Figure 3d). However, the TAC was estimated for only 30 subjects. A similar trend was observed among smokers, diabetics, and hypertensive patients (Table 2).

The Relationship Between PBMC Senescence and Endothelial Dysfunction

We observed a significant positive correlation between age and PBMC senescence (r=0.36, p=0.005), but a significant negative correlation was observed between age and TAC (r=-0.60, p=0.0001). IHR scores demonstrated significant positive correlations with PBMC senescence (r=0.75, p=0.0001) and nitrite & nitrate (r=0.56, p=0.0001), whereas significant negative correlations were observed with TA (r=-0.83, p=0.0001) and TAC (r=-0.92, p=0.0001). Additionally, PBMC senescence also showed significant correlations with the variables nitrite and nitrate, TAC, and telomerase activity (Figure 4).

ROC Curve Analysis for PBMC Senescence and Endothelial Dysfunction:

The ROC curve was plotted to check if PBMC senescence, nitrite and nitrate, antioxidant status, and TA could predict high IHR scores among the studied patients. The analysis demonstrated that PBMC senescence of 0.95 [p-value = 0.0001; 95% CI (0.874-1.026)] was a significant predictor of patients with higher IHR scores, with a cut-off value of 21.65, and with a sensitivity and specificity of 92% and 88%, respectively (Figure 5).

Multiple Regression Models for PBMC Senescence and Endothelial dysfunction

Multiple regression models were plotted to analyze the effect of the independent variables of PBMC senescence, nitrite and nitrate, and TA on the dependent variable IHR score (Table 3). It was observed that PBMC senescence, nitrate and nitrite, and TA were independently associated with high IHR scores (Table 3).

Discussion

The relationship between PBMC senescence and endothelial dysfunction, and the occurrence of CVD has been described in previous studies;[3,12] however, the information regarding the relationship between PBMC senescence, endothelial dysfunction, and CVD among subjects with no established CVD remains sparse. To the best of our understanding, this is the first clinical study conducted in the South Indian population that estimated PBMC senescence and determined its relationship with high CV risk using the IHR score. The main finding of our study was that PBMC senescence, nitrite and nitrate, and TA were independently associated with high IHR scores. The severity of PBMC senescence was greater among patients with higher CV risk when compared to patients with lower CV risk. PBMC senescence was estimated on the basis of TA and the percentage of senescent cells (%) among the studied patients.

Telomeres and telomerase play a significant role in the development and pathogenesis of CVD. It is well-known that, with each cell division, the length of telomeres shortens, whereas inflammation and oxidative stress, which are major mechanisms involved in the development and pathogenesis of CVD, are known to increase the rate of telomere shortening, leading to cell senescence.[13-15] Moreover, the presence of lower TA and shorter leukocyte telomere length (LTL) has been seen in the senescent endothelial cells, vascular smooth muscle cells (VSMCs), and atherosclerotic plaque, and these are also associated with plaque instability leading to CVD. The absence of TA, which maintains the telomere integrity and telomere length, makes the cell senescent and causes apoptosis.[16-18] Our study revealed that TA was significantly lower among patients with higher than lower IHR scores. In contrast to our findings, an earlier study, named coronary artery risk development in young adults (CARDIA), conducted among young patients with coronary artery risk development with prevalent coronary artery calcium (CAC), revealed that

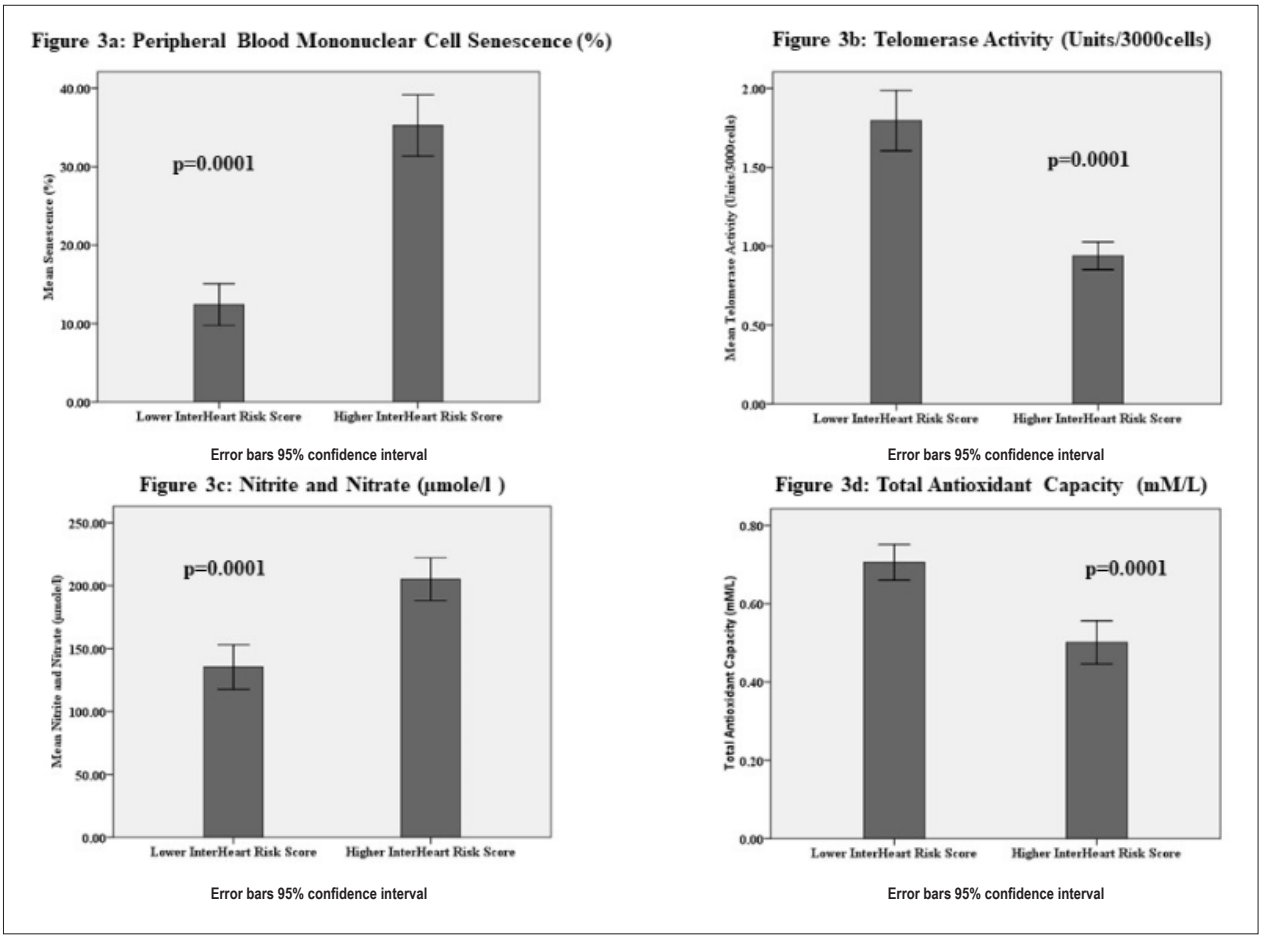


Figure 3 – *Comparision of peripheral blood mononuclear cell senescence, telomerase activity, nitrite/nitrate and total antioxidant capacity among patients with low and high interheart risk score. The statistics tests used to compare continuous variables were independent samples t-test; p-value less than 0.05 were consideres statistically significant.*

TA plays a vital role in the development of atherosclerosis. The findings of the study demonstrated that higher levels of telomerase predicted a higher prevalence of CAC among young to middle-aged men. However, patients with shorter telomere length presented a positive association between TA and CAC.[19] In an earlier cross-sectional study, the association between subclinical atherosclerosis burden and both average LTL and the abundance of short telomeres (%LTL<3 kb) was studied among 4,066 asymptomatic middle-aged subjects without the presence of any CVD. The study showed that the average LTL and short telomeres were not significant and independent predictors of subclinical atherosclerosis.[20] In one of the largest observational and genetic studies, conducted in 290,022 individuals from Copenhagen, it was revealed that the presence of short telomeres was associated with a higher risk of ischemic heart disease.[21]The differences in the study findings might be attributable to the heterogeneity observed in the study population and the sample size of the study. Moreover, a recent systematic review and meta-analysis of twenty-four studies revealed an inverse association between leukocyte telomere length and the risk of coronary heart disease (CHD), regardless of conventional vascular risk factors.[3] The systematic review included cardiovascular patients, whereas our study included patients free of CVD. Therefore, it can be suggested that measuring TA and LTL might be a useful marker for predicting the future risk of CVD. Presently, investigations are being carried out to gauge if statins could be used as potential therapeutic agents for telomerase activation and as effective geroprotectors.[22]

Lately, senescent cells have gained attention as a therapeutic target for several age-related diseases, such as CVD. Studies have shown that cell senescence has been equivalent to endothelial senescence, and thus vascular senescence as well. The present study then measured the percentage of senescent cells (%), which was significantly lower among patients with lower IHR scores, when compared to those with higher IHR scores. The transcriptional analysis of human VSMCs demonstrated that there was a suppression of the matrix Gla protein (MGP), an inhibitor of calcification, in the senescent VSMCs. Furthermore, there was also an up-regulation of transcript encoding bone morphogenic protein 2 (BMP2), which is a promoter of calcification.[23] Therefore, it can be suggested that the senescent VSMCs might play a prominent role in the development of age-related hardening

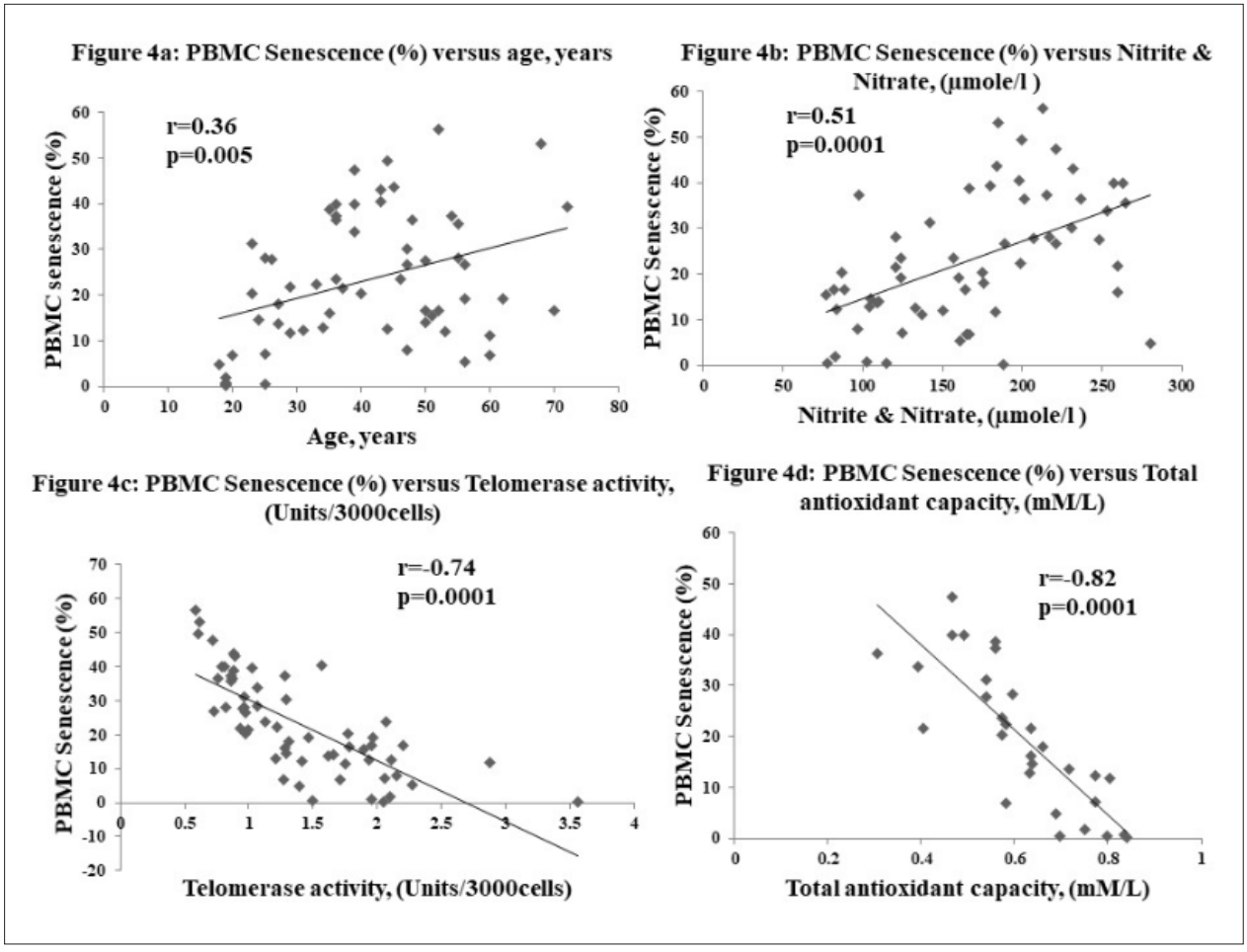
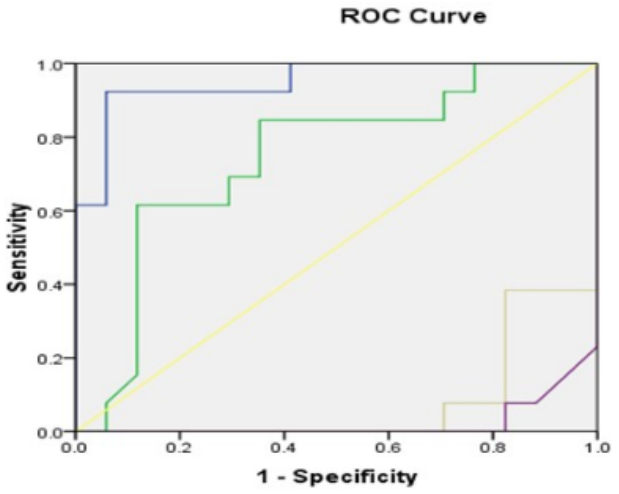


Figure 4 – *Correlation of PBMC senescence with age, nitrite/nitrate, telomerase activity and total antioxidant capacity.*

Table 3 – Multiple Regression Models to predict Interheart Risk Score

Model		Coefficients					
		Unstandardized Coefficients		Standardized Coefficients	t	Adjusted R Square	p-value
		B	Std. Error	Beta			
1	(Constant)	27.449	1.295		21.197	0.679	0.0001
	TelomeraseActivity	-9.575	.854	-0.827	-11.216		.000
2	(Constant)	21.160	2.470		8.567	.716	0.0001
	TelomeraseActivity	-8.357	.904	-0.722	-9.242		0.0001
	NitriteandNitrate	.027	.009	0.229	2.927		0.005
3	(Constant)	17.112	2.988		5.727	.735	0.000
	TelomeraseActivity	-6.608	1.169	-0.571	-5.652		0.000
	Nitrite and Nitrate	.021	.009	0.179	2.274		0.027
	Senescence	.113	.050	0.235	2.251		0.028

*B: Unstandardized Regression Coefficient; SEβ: Standard Error of Coefficient; β: Standardized Coefficient; p<0.05**



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Senescence	.950	.039	.000	.874	1.026
NitriteandNitrate	.744	.094	.024	.559	.929
TelomeraseActivity	.077	.047	.000	-.015	.169
AntioxidantStatus	.023	.021	.000	-.019	.064

Figure 5 – *Receiver operating characteristic curves for the prediction of High Interheart Risk Score.*

and stiffening through increased calcification. The stiffening and hardening of arteries lead to the development of high blood pressure, which is considered to be one of the major risk factors for the occurrence of coronary artery disease, HF, stroke, and MI.[24] Another study conducted to compare MGP expression in normal versus diseased aortic valve interstitial cells (AVICs) showed that the MGP expression was significantly decreased in the diseased AVICs relative to normal AVICs. These findings imply that the absence of an anti-calcification defense mechanism might contribute to the calcification of the aortic valve.[25] Therefore, estimating the percentage of senescent cells might be a potential and novel marker for predicting the development and progression of CVD. Novel therapeutic strategies that involve the prevention, removal, and replacement of the senescent cells are at their inception. Further understanding and more research are required to understand this biology so as to translate this knowledge into therapeutic applications.

The present study measured endothelial dysfunction by estimating the concentration of nitrite and nitrate and the TAC. TAC was found to be significantly lower among patients with higher IHR scores when compared to patients with lower IHR scores. Several epidemiological studies have demonstrated that people with a higher intake of antioxidant vitamins have a lower risk of developing MI and stroke.[26,27] However, a recent systematic review and meta-analysis of randomized controlled trials revealed that the current literature provided no evidence to support the use of vitamins and antioxidants for the prevention of CVD.[28] However, a recent systematic review of observational studies demonstrated a substantial association between higher levels of dietary total antioxidant capacity and risk factors of cardiovascular diseases.[29] Our study also showed that the nitrite and nitrate concentrations were higher among high-risk patients when compared to low-risk patients. In contrast, the Framingham offspring study demonstrated that a higher plasma nitrate concentration was associated with all-cause mortality but was not found to be associated with the incidence of CVD.[30] This might be due to the fact that the nitrite and nitrate concentrations present in the diet could be metabolized into NO, thereby promoting cytoprotection and cardiovascular benefits.[31]The results in our study might be contrasting due to the fact that certain diets, such as vegetables, fruits, and processed meats, are rich sources of nitrites and nitrates.[32] Hence, there are possibilities that high risk patients in our study had been exposed to such diets. The endothelial-dependent response to vasodilation is

regulated by the release of NO synthesized from the dietary nitrate, nitrite and amino acid L-arginine, via the endothelial nitric oxide synthase (eNOS) ,which leads to the production of intracellular cyclic GMP. However, endothelial dysfunction leads to the imbalance in the production of NO and ROS, in turn leading to the occurrence of several age-related diseases, such as CVD. The accumulation of ROS in the arterial plasma and intima leads to an increase in the low-density lipoprotein (LDL) oxidation; the uptake of this oxidized LDL by the arterial macrophages is one of the prominent factors for the formation and progression of atherosclerotic plaque. Therefore, the presence of antioxidants in the plasma, LDL particle, and cell wall can inhibit the LDL oxidation and can safeguard the vasoreactivity by increasing the release of endothelial NO and by reducing thrombogenicity.[12,33] Therefore, determining the TAC and the concentration of nitrite and nitrate can turn out to be a potential marker for the early prediction of CVD in the future.

Limitations

The main limitation of our study is with respect to the limited sample size. Another limitation is that our study did not have a prospective long-term follow-up with the confirmation of clinical events; instead, we calculated the risk based on the interheart risk score. Additionally, the blood samples were collected at different time points, which could have had an effect on the levels of laboratory assays.

Conclusions

Our study demonstrated that PBMC senescence, TA, and nitrite and nitrate are suitable measures to predict high cardiovascular risk in adults with CV risk. Therefore, measurements of the above markers might be used as an additional risk assessment tool to predict the risk of cardiovascular diseases among adults. Nevertheless, long-term prospective follow-up studies with the adjudication of clinical events are required to confirm these findings.

Author Contributions

Conception and design of the research: Emmanuel C, Mala K, Kumarasamy S, George M; Acquisition of data: Raj V, Charles S, Marimuthu C, Emmanuel C; Analysis and interpretation of the data: Raj V, Charles S, Goenka L, Emmanuel C,Ramamoorthy T, Marimuthu C, Mala K, Kumarasamy S, George M; Statistical analysis: Goenka L, Ramamoorthy T, George M; Obtaining financing: Mala K, George M; Writing of the manuscript: Goenka L, Ramamoorthy T, George M; Critical revision of the manuscript for intellectual content: Raj V, Charles S, Goenka L, Ramamoorthy T, Marimuthu C, Emmanuel C, Mala K, Kumarasamy S, George M.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

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Association of C-Reactive Protein to Albumin Ratio in Patients with Isolated Coronary Artery Ectasia

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Abstract

Background: Coronary artery ectasia (CAE) is defined as diffuse or localized dilatation of coronary artery lumen with a diameter of 1.5 to 2.0 times the adjacent normal coronary artery. The C-reactive protein to albumin ratio (CAR) is a useful inflammatory marker, which has been documented in coronary artery disease.

Objective: To analyze the association of CAE and CAR.

Methods: A case-control protocol was used in this study. We included 102 consecutive patients with isolated CAE without stenosis (56 men and 46 women; mean age 60.4 ± 8.8 years). The control subjects consisted of an equal number of sex and age matched patients with normal coronary arteries (55 men and 47 women; mean age 61.2 ± 9.1 years). Clinical features, laboratory findings, and medication use history were recorded. Student's t test, Mann-Whitney U test, chi-square test, and linear and logistic regression analysis were performed. A 2-sided $p < 0.05$ was statistically considered significant.

Results: The CAR was increased in patients with CAE compared to the controls (32 and 16; $p < 0.001$). In addition, the CAR was found to be an independent predictor of CAE (OR = 2.202; 95% CI 1.184 – 5.365; $p < 0.001$).

Conclusion: In the present study, we determined that CAR levels were significantly higher in the CAE group than in the control group, and the CAR was significantly correlated with CAE. (Arq Bras Cardiol. 2021; 116(1):48-54)

Keywords: C-Reactie Protein; Albumins; Coronary Artery Disease/complications; Inflammation; Coronary Aneurysm; Dilatation, Pathologic (ectasig).

Introduction

Coronary artery ectasia (CAE) is defined as diffuse or localized dilatation of coronary artery lumen with a diameter 1.5 to 2.0 times the adjacent normal coronary artery. Coronary aneurysms are defined as luminal dilatation with a > 2.0 fold increase.[1] With the rapid increase in applications of coronary angiography (CA), an increasing number of CAE have been detected. CAE has been shown to be a predictor of mortality. The mortality rates of patients with CAE are similar to those of patients with non-obstructive aneurysmal or 3-vessel disease.[2] The etiopathogenesis of this clinical entity is not fully understood. The most common cause of CAE in the Western population is atherosclerotic coronary artery disease (CAD). Kawasaki disease, collagen tissue diseases, and connective tissue diseases are the other causes of CAE. Percutaneous coronary invasive procedures and trauma rarely lead to CAE.[3-4] Chest pain is usually the primary symptom of CAE. However, arrhythmia, acute coronary syndrome, and sudden cardiac death are other observed clinical conditions of CAE.[5,6]

Previous studies have shown that inflammation may play a role in CAE.[7] CRP and albumin have been linked to CAD severity and to the presence of cardiovascular complications.[8-10] CRP, which is one of the most commonly used inflammatory biomarkers, is associated with endothelial dysfunction, prothrombotic status, remodeling, and destabilization of atherosclerotic plaques. Furthermore, elevated CRP levels in patients with atherosclerotic burden have been found to be associated with significant cardiovascular events.[11-14] On the other hand, inflammation causes hypoalbuminemia with disruption of albumin synthesis-catabolism balance. Serum albumin is the most important serum protein with vital functions in the human body, and it has anti-atherogenic properties, including antioxidant activities, inhibition of platelet activation, and modulation and aggregation of arachidonic acid metabolism.[15] Several previous studies have reported that hypoalbuminemia is associated with more frequent myocardial infarction and increased mortality in patients with acute coronary syndrome.[10,16,17] In comparison with CRP or albumin alone, the CRP to albumin ratio (CAR), a new inflammation-based risk index, has been shown to better reflect prognosis

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in patients with acute medical condition and malignancy.[18,19] However, the relationship between the CAR and CAE is not yet known. CAE is an inflammatory disease; thus, we hypothesized that the CAR could be associated with CAE. Our aim was to investigate the association between CAE and the CAR.

Methods

A case-control study approved by the Ethics Committee in Sanko University Hospital was performed. Patients with suspected coronary ischemia and typical chest pain following positive or equivalent results of noninvasive ischemic tests were included. All patients underwent CA. During CA, digital data of all patients were analyzed, and quantitative coronary measurements were performed. Catheter diameter was used as the reference to determine the actual coronary artery lumen diameter. Definition of the ectatic segment was determined by performing at least two measurements at the proximal, middle, and distal segments of the coronary arteries in patients with normal CA and in patients who were considered to have an ectatic coronary segment. CAE was defined as diffuse or localized dilatation of coronary artery lumen with a diameter 1.5 to 2.0 times the adjacent normal coronary artery, and these patients were included in the isolated CAE group. Patients without coronary plaque or ectasia were included in the normal coronary group.

The medical history of the study population was obtained from medical records and recorded in forms prepared for each patient. Hypertension (HT) was diagnosed when SBP was > 140 mmHg and/or when DBP > 90 mmHg, or by antihypertensive drug use. Diabetes mellitus (DM) was diagnosed when fasting blood glucose was ≥ 126 mg/dL or by antidiabetic drug use. Hyperlipidemia (HL) was defined as total cholesterol level > 200 mg/dL, history of dyslipidemia, and/or antilipidemic drug use. Patients who were smokers for 1 year or more were defined as smokers. BMI was determined using the standard formula. The LVEF was automatically calculated according to the modified Simpson's method, with the help of software on the echocardiography device.[20]

Laboratory Measurements

Blood glucose, creatinine, albumin, and CRP levels were determined as described. Serum total protein and albumin were measured by bromine cresol technique using a C8000 analyzer (Abbott Laboratories, IL, USA). CRP was measured by nephelometry (BN ProSpec System, Siemens). The estimated glomerular filtration rate was determined using the Cockcroft-Gault equation.

Coronary angiography

CA was performed using Judkins method, via femoral approach, using cranial and caudal angles in the right and the left inclined planes at 30 fps. Patients' CA images were analyzed by interventional cardiologists who were blinded to the study. CAE was defined by Falsetti and Carroll; [21] our study used the same method. Normal segments were defined as the absence of stenosis or ectasia determined by CA. Cases of CAE with coronary stenosis were excluded from the study.

Statistical analysis

Statistical analysis was carried out using SPSS v25 (SPSS Inc., USA). The normality of continuous variables was tested by the Kolmogorov-Smirnov test and presented as mean and standard deviation or median and interquartile range, according to data normality. Normally distributed continuous variables were compared by Student's t test, and Mann-Whitney U test was used for non-normal distribution. Student's t test for unpaired values was used. Categorical data were compared using chi-square test. In univariate linear regression analysis, variables with a significance level $p < 0.25$ were defined as potential risk markers and included as common variables in the whole variable model. Logistic regression analysis was performed to obtain independent determinants of CAE. A 2-sided $p < 0.05$ was statistically considered significant.

Results

Of 226 patients, 8 were excluded because of myocardial infarction and left ventricular dysfunction; 5 were excluded due to left ventricular hypertrophy and heart valve disease, and 6 were excluded due to HT and renal failure (n = 6). In addition, 3 patients were excluded because of other reasons such as cerebrovascular disease, liver dysfunction, autoimmune disease, neoplastic disease, and osteoporosis (n = 3). After these exclusions, 204 patients were enrolled. One hundred two patients with isolated CAE and no coronary artery stenosis (56 men and 46 women; mean age 60.4 ± 8.8 years) were enrolled as patient group, and the control group consisted of the same number of consecutive subjects with angiographically normal coronary arteries (55 men and 47 women; mean age 61.2 ± 9.1 years).

Patients' data are shown in Table 1. The demographic characteristics showed age and sex matched groups. CAD risk factors such as DM were also similar, but other risk factors (smoking, HT, HL, and family history) were significantly higher in the CAE group than in the control group ($p < 0.001$, $p < 0.001$, $p = 0.006$, and $p = 0.022$, respectively). No changes were observed in terms of treatment regimens.

Assessment of BMI, SBP, DBP, LVEF, heart rate, and fasting plasma glucose did not show any significant differences. Lipid panel parameters, triglycerides, and total cholesterol groups were similar; HDL was higher in the controls ($p = 0.012$), and LDL was higher in patients with CAE ($p < 0.001$). CRP, albumin, and the CAR differed significantly between the groups ($p < 0.001$). There was a similarity between the groups in terms of other laboratory parameters.

Applying a univariate logistic regression model, DM, smoking, HT, and the CAR correlated with CAE. Regression analysis revealed that smoking, HT, and the CAR were independent predictors of CAE (smoking: OR 1.812 [95% CI 1.124 – 2.655; $p = 0.024$], HT: OR 2.175 [95% CI 1.156 – 4.227; $p < 0.001$], CAR: OR 2.202 [95% CI 1.184 – 5.365; $p < 0.001$]) (Table 2).

Discussion

This reports shows that the CAR was significantly higher in the CAE group than in the control group. To our knowledge, we are the first to show that the CAR is closely associated with CAE.

Table 1 – Baseline characteristics of the study population

Variables	Patients with CAE (n = 102)	Control group (n = 102)	P value
Age, years	60.4 ± 8.8	61.2 ± 9.1	0.422
Sex (male), n, (%)	56 (54.9)	55 (53.9)	0.740
BMI (kg/m²)	27.4±3.1	25.2± 3.2	0.317
Diabetes mellitus, n (%)	15 (14.7)	12 (11.7)	0.422
Smoking, n, (%)	40 (39.2)	18 (17.6)	< 0.001
Hypertension, n (%)	38 (37.2)	27 (26.4)	< 0.001
Hyperlipidemia, n (%)	22 (21.5)	10 (9.8)	0.006
Family history, n, (%)	15 (14.7)	8 (7.8)	0.022
Previous medications, n, (%)			
Acetylsalicylic acid	20 (19.6)	16 (15.6)	0.224
Betablockers	22 (21.5)	17 (16.6)	0.314
ACEI/ARB	17 (16.6)	13 (12.7)	0.509
Statins	10 (9.8)	7 (7.1)	0.356
LVEF, (%)	60.2±3.4	61.3±3.9	0.533
SBP (mmHg)	122.0±9.1	118.6±7.5	0.424
DBP (mmHg)	88.0±7.2	85.6±4.1	0.358
Heart rate (beat/m)	75.2±9	73.8±7	0.411
Hemoglobin, g/dL	13.1+1.8	12.7+1.7	0.388
White blood cell, 10^3/mL	8.6±2.9	8.4±2.2	0.758
Platelet count, 10^3/mL	232.7±77.4	244.8±75.2	0.554
FPG (mg/dL)	127.1±42.8	123.1±45.2	0.146
Creatinine, mg/dL	0.86 (0.75-0.99)	0.85 (0.75-0.97)	0.785
eGFR, mL/min	92.3 (68.9-105.6)	93.8 (74.9-107.9)	0.656
Total cholesterol, mg/dL	168.0±36.0	158.2±39.8	0.411
HDL (mg/dL)	33.6±7.0	39.8±8.0	0.012
LDL (mg/dL)	123.2±32.6	98.4±29.4	< 0.001
Triglyceride, mg/dL	129 + 61	122 + 58	0.188
C-reactive protein, mg/dL	1.25 (0.50-2.84)	0.62 (0.27-1.16)	< 0.001
Albumin, g/dL	3.76 + 0.42	4.02 + 0.32	< 0.001
CAR, *100	32 (12-68)	16 (6-30)	< 0.001

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CAR: C-reactive protein to albumin ratio; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; FPG: fasting plasma glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; LVEF: left ventricular ejection fraction; SBP: systolic blood pressure

It is not clear which local or global factors are involved in CAE pathogenesis. It is reported that CAE is caused by the widespread abnormality in the vascular wall holding multiple segments and that it represents saccular ectasia rather than fusiform ectasia.[22] A relatively limited study on prognosis was performed for patients with CAE. Thirty years ago, the largest cohort study of CAE found that aneurysmal patients had a 5-year mortality rate of 26%.[23] Kajinami et al.[24] examined the autopsy of a patient with CAE and familial hypercholesterolemia, who died in the twentieth century due to acute myocardial infarction. Microscopic examination revealed a large amount of plasma cells, macrophages, and lymphocyte infiltration in the intimal/medial layers of the coronary arteries. Evidence of atherosclerotic reactions such as typical common hyalinization, focal calcification and fibrosis, lipid accumulation, intimal and medial damage, cholesterol, hemorrhage, and foreign body giant cell were observed during pathological examination of CAE.

Another potential factor leading to the development of CAE is nitric oxide (NO), which may cause coronary dilatation due to over-stimulation of the endothelium. Many patients have been given chronic glyceryl trinitrate for angina that

Table 2 – Factors associated with coronary artery ectasia

Linear regression analysis				Logistic regression analysis		
	Coefficients	95% CI	P value	OR	95% CI	P value
Age, years	0.052	0.013-0.107				
LVEF	0.002	−0.018-0.026				
BMI (kg/m²)	0.030	0.010-0.073				
Diabetes mellitus	0.168	0.011- 0.524	0.024*	1.277	0.811-1.613	0.102
Smoking	0.322	0.010-1.114	0.007*	1.812	1.124-2.655	0.024*
Hypertension	0.533	0.017-1.010	0.003*	2.175	1.156-4.227	< 0.001*
Hyperlipidemia	0.025	−0.020-0.056				
SBP (mmHg)	0.068	−0.017-0.122				
DBP (mmHg)	0.024	−0.002-0.048				
Heart rate (beat/m)	0.074	−0.024-0.172				
CAR, *100	0.618	0.119-1.496	< 0.001*	2.202	1.184-5.365	< 0.001*
HDL (mg/dL)	−0.076	−0.312-0.025				
LDL (mg/dL)	0.009	−0.057-0.020				

**P value < 0.05. Variables with p < 0.25 in univariate regression were included into multivariate regression. BMI: body mass index; CAR: C-reactive protein to albumin ratio; DBP: diastolic blood pressure; HDL: high density lipoprotein; LDL: low density lipoprotein; LVEF: Left ventricular ejection fraction; SBP: systolic blood pressure.*

may worsen ectasia via NO stimulation. These patients may have CAD, and atherosclerosis has been shown to cause inappropriate release of endothelial NO.[25] Quyyumi et al.[26] demonstrated the relationship between NO and atherosclerosis and reported that coronary vascular dilatation was caused by increased NO due to acetylcholine without angiographically proven atherosclerosis.

The underlying pathological mechanism of CAE is still not fully understood. Although a definite relationship between atherosclerosis and CAE has not been confirmed, CAE is considered to be a variant of CAD and the main cause of CAE is atherosclerosis.[23,27-29] The role of inflammation in the process of atherosclerosis is well known.[28-30] Atherosclerosis is associated with aneurysm formation that extends to tunica media during an inflammatory process, which eventually ends with degeneration of the cystic media. [31] Previous studies have shown that inflammatory markers such as plasma soluble adhesion molecules, leukocytes, adiponectin, lipoprotein-associated phospholipase-A2, CRP, plasminogen activator inhibitor-1, IL-1, TNF-alpha, and IL-10 have been significantly increased in patients with CAE.[32]

Many previous studies have shown that the CAR is associated with atherosclerosis and suggested that it should be considered as a marker of cardiovascular risk. This study found that the CAR was significantly higher in patients with CAE than in the control group, and it supports the hypothesis that atherosclerosis causes CAE.

Damaged ischemic or necrotic cells cause a systemic inflammatory response by releasing pro-inflammatory agents in tissue and plasma. The prognosis of the disease can change with the speed of inflammation.[33] Atherosclerosis has been shown to be strongly correlated with increased serum CRP.[34] In addition, CRP has been shown to be associated with endothelial dependent/independent coronary dysfunction in patients with CAD,[35] suggesting that increased CRP may predict dysfunction in STEMI patients and may be a strong predictor of no-reflow phenomenon.[36] In our study, elevated CRP levels showed a strong association between CAE and CRP.

Hypoalbuminemia is not only a risk factor; it also indicates poor prognosis in patients with STEMI.[37,38] Increased inflammation has been documented to contribute to albumin synthesis and breakdown.[39] Hypoalbuminemia leads to many complications including endothelial dysfunction as well as platelet aggregation and coronary artery stenosis induced by platelet dysfunction.[40-41] In a study of 1,303 subjects with acute coronary syndrome, serum albumin levels were shown to be associated with severity of CAD.[10] In our study, there was a negative correlation between serum albumin level and CAE.

It is believed that the CAR, as originally described by Fairclough et al.,[42] is better than CRP and albumin alone for prediction of medical complications.[42] Inflammation is one of the main characteristics of atherogenesis, and the CAR and demonstrates inflammatory conditions.

The CAR has recently been investigated as a potential biomarker for predicting the consequences of adverse cardiovascular events.[43] Cagdas et al.[44] showed that the CAR and the severity of CAD were associated. In malignant cancer patients, the CAR predicted prognosis and disease progression.[45,46] Therefore, the CAR is a more reliable biomarker for prediction of disease severity. Previous reports that evaluated the CAR in CAD showed promising outcomes. A study of STEMI showed that white blood cell count, neutrophil to lymphocyte ratio, and the CAR correlated with the no-reflow phenomenon.[43]

The results of our study showed that the association of the CAR with CAE was significant. This is the first study to show an association between CAE and higher CAR levels. Increased CAR was a prognostic marker of CAE. The results of a study on the relationship between familial hypercholesterolemia and CAE showed that dyslipidemia was one of the causes of CAE.[47] In our study, high LDL and low HDL levels were observed in patients with CAE. No significant change was found in triglyceride levels in patients with CAE compared to controls. A strong relationship has been shown between HT and CAE.[48] In our study, the prevalence of HT was higher in patients with CAE, and HT was independently associated with CAE.

We observed that the CAR was higher in patients than in control group. We assume that higher CAR may predict the risk of atherosclerosis in patients with CAE. A review of the literature shows that CAE is not an innocent clinical condition and that larger studies are needed in the future to create the best strategy for treatment and risk management.

Study limitations

More comprehensive and multicenter studies are needed to better explain the variability of inflammatory markers and the predictive role of serum CAR levels. The prognostic significance of the CAR was not evaluated, and it should be established in future investigations. This was a case-control study, and we were thus unable to obtain mortality data. Although the CAR is accepted as a new, sensitive myocardial marker, its specificity in determining the presence of CAE has been questioned, because many other conditions, especially other infections, may also increase CAR levels. CAR levels may be affected by other important factors, such as age, sex, and race. Finally, instead of using quantitative methods such as intravascular ultrasound, visual assessment was the only method used to diagnose and exclude patients.

Conclusion

Our study shows that CAR levels are higher in patients with CAE compared to subjects with normal coronary arteries. The high levels of CAR may support the hypothesis that the CAR could be related to the development of CAE. In our study, high CAR levels were significantly correlated with CAE.

Author Contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis and Critical revision of the manuscript for intellectual content: Sercelik A, Askin L, Turkmen S, Tanriverdi O; Writing of the manuscript: Askin L.

Potential Conflict of Interest

The authors report no conflict of interest concerning the materials and methods used in this study or the findings specified in this paper.

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Study Association

This study is not associated with any thesis or dissertation.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Sanko University Clinical Research under the protocol number 2019/06. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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The Relationship between CAR and CAE: Association of C-Reactive Protein to Albumin Ratio in Patients with Isolated Coronary Artery Ectasia

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Short Editorial related to the article: Association of C-Reactive Protein to Albumin Ratio in Patients with Isolated Coronary Artery Ectasia

Coronary artery ectasia (CAE), defined as an increase in coronary diameter 1.5 times the diameter of the normal adjacent bed,[1] is an uncommon finding in coronary angiography, with an incidence of 1.2 to 4.9%.[2] Most of the times, it is related to coronary atherosclerotic disease (CAD),[3] and they have several factors in common, such as lipoprotein accumulation f in the intimal layer, inflammatory cell infiltration, activation of the renin-angiotensin system and oxidative stress generation, with arterial expansion and remodeling. The high levels of nitric oxide cause vasodilation and excessive activation of extracellular matrix metalloproteinases, resulting in vascular dilation.[4] Less commonly, it can also be related to Kawasaky disease, connective tissue, infectious or autoimmune diseases.

Keywords

Coronary Artery Disease; Dilatation, Pathologic; Atherosclerosis; Lipoproteins; Oxidative Stress; Risk Factors

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The incidence is higher in men, hypertensive individuals and smokers. Cocaine users have a higher incidence of CAE and coronary aneurysms.[5] Interestingly, Diabetes Mellitus (DM) seems to be unrelated to CAE, and may even be a protective factor, a fact related to the inhibition of the expression of extracellular matrix metalloproteinases.[6]

The increase in C-reactive protein (CRP) is a factor largely related to increased inflammatory activity and cardiovascular risk,[7,8] as well as the reduction in serum albumin levels (A).[9]

In this recent publication[10] with 102 patients with and the same number without CAE, the authors demonstrated that patients with CAE had a high CRP/albumin (CAR) ratio compared to the control group, leading to the possibility of identifying CAE and its inflammatory association, implying prognosis and therapeutic management. This study is a pioneer in showing this association, and will certainly help in cardiology practice; however, to differentiate whether the high levels of this association are related to coronary ectasia or to the most prevalent risk factors in the group of cases, such as smoking, hypertension and dyslipidemia, and the consequent increase in the prevalence of CAD, prospective studies are still necessary, or perhaps, using patients with CAD and without coronary ectasia as a control group.

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Monocyte Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) Expression Correlates with cIMT in Mexican Hypertensive Patients

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Abstract

Background: Arterial hypertension (HTA) represents a major risk factor for cardiovascular morbidity and mortality. It is not yet known which specific molecular mechanisms are associated with the development of essential hypertension.

Objective: In this study, we analyzed the association between LRP1 monocyte mRNA expression, LRP1 protein expression, and carotid intima media thickness (cIMT) of patients with essential hypertension.

Methods: The LRP1 monocyte mRNA expression and protein levels and cIMT were quantified in 200 Mexican subjects, 91 normotensive (NT) and 109 hypertensive (HT). Statistical significance was defined as $p < 0.05$.

Results: HT patients group had highly significant greater cIMT as compared to NT patients (p=0.002) and this correlated with an increase in the expression of LRP1 mRNA expression (6.54 vs. 2.87) ($p = 0.002$) and LRP1 protein expression (17.83 vs. 6.25), respectively ($p = 0.001$). These differences were maintained even when we divided our study groups, taking into account only those who presented dyslipidemia in both, mRNA ($p = 0.041$) and proteins expression ($p < 0.001$). It was also found that Ang II mediated LRP1 induction on monocytes in a dose and time dependent manner with significant difference in NT vs. HT (0.195 ± 0.09 vs. 0.226 ± 0.12, $p = 0.046$).

Conclusion: An increase in cIMT was found in subjects with hypertension, associated with higher mRNA and LRP1 protein expressions in monocytes, irrespective of the presence of dyslipidemias in HT patients. These results suggest that LRP1 upregulation in monocytes from Mexican hypertensive patients could be involved in the increased cIMT. (Arq Bras Cardiol. 2021; 116(1):56-65)

Keywords: Monocytes; LRP1; mRNA; Hypertension/epidemiology; Mexico; Carotid Intima Media Thickness.

Introduction

Arterial hypertension (HTA) is a chronic and multifactorial disease that constitutes a serious public health problem.[1] Hypertension rarely causes symptoms in the early stages; it is a silent killer, causing accelerated atherosclerosis, damage to major organs, disability, and death from cardiovascular diseases.[2]

Atherosclerotic lesions include altered endothelial cells, circulating monocytes, vascular smooth muscle cells (VSMC) migration, and foam cell development.[3] The altered endothelium allows the entrance and retention of low density lipoprotein (LDL) into the intima layer.[4] Once LDL is trapped in the arterial intima, it undergoes changes, such as oxidation and aggregation, that facilitate its uptake by intimal monocytes-macrophages and VSMC through their recognition by non-classic LDL receptors.[5] These receptors are not regulated by cholesterol and allow a massive uptake of modified LDL, causing intracellular lipid accumulation.

The low-density lipoprotein receptor–related protein 1 (LRP1), which is a transmembrane multiligand receptor[6] belonging to the LDLR family, is expressed in different cells such as neurons, fibroblasts, tumoral cells, hepatocytes, vascular smooth muscle cells, and monocytes and macrophages.[7,8] It is known to participate in the uptake of modified LDL[9] and is over expressed in atherosclerotic plaques in both animal and human models.[10,11]

Furthermore, *LRP1* gene expression is increased in mononuclear cells from patients with coronary occlusion.[12,13] In monocytes and macrophages, *LRP1* contributes to the uptake of modified aggregated LDL.[14,15] Nevertheless, the effects of hypertension on *LRP1* expression in humans are not exactly known. Therefore, obtaining circulating monocytes made it possible to study the mechanisms of their participation in the formation of atherosclerotic plaque.[16] In another way, the cIMT is considered an excellent non-invasive marker for cardiovascular disease; it has been associated with atherosclerosis and cardiovascular risk factors[17,18] and the prevalence of cardiovascular disease, proving it is useful in the diagnosis of atherosclerosis.[19-21] Accordingly, the purpose of this

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paper was to study the *LRP1* mRNA levels and protein expression in monocytes from patients with essential arterial hypertension and their correlation with carotid intima media thickness.

Methods

Study Population and Design

A total of 200 unrelated Mexican subjects (109 patients diagnosed with essential hypertension and 91 normotensive subjects) were recruited at the Instituto Nacional de Cardiología "Ignacio Chávez". The inclusion criteria for both groups were: to be Mexican by birth with at least 3 previous generations, be older than 40 years, and to agree to participate in the study by signing an informed consent. Controls were apparently healthy, asymptomatic individuals, without a family history of hypertension or premature cardiovascular disease, with blood pressure ≤120/80 mmHg. For the hypertensive group, subjects had blood pressure ≥140/90 mmHg or had been previously diagnosed with essential hypertension. The exclusion criterion was suffering from a chronic degenerative disease. All participants answered standardized and validated questionnaires to obtain information on their family and medical history, alcohol and tobacco consumption, eating habits, and physical activity.

The ethics committee of the Instituto Nacional de Cardiología "Ignacio Chavez" approved the project; the patients gave written informed consent prior to the study. All procedures were in agreement with the Helsinki Declaration of 1975, as revised in 2013.

Anthropometric Measurement

The selected subjects underwent anthropometric measurements to determine their height in meters (m) and weight in kilograms (kg). Blood pressure was measured using a mercury sphygmomanometer, following the recommendations of the VII Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII).

Carotid Intima Media Thickness

A specialist in sonography resolution assessed the carotid intima media thickness (cIMT); all measurements were performed with A Sonosite Micromax ultrasound coupled to a 13 MHz multifrequency high-resolution linear transducer. Measurements were made on the common carotid after the examination of a 10-mm longitudinal section at a 2-cm distance from the bifurcation, the anterior or proximal wall, and the posterior or distal wall were measured on the lateral, anterior, and posterior projections, followed by an axis perpendicular to the artery to discriminate two lines: one for the intima-blood interface and the other to the media-adventitious interface. Five measurements were obtained of the right carotid and five of the left carotid, using average (average cIMT) and maximum values (maximum cIMT), automatically calculated by the software. cIMT was considered abnormal with values greater than or equal to 75 percentile by age and sex.[22]

Biochemical Determinations

Blood samples were collected after a 12-hour fasting period; glucose, total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) were measured in fresh samples (fasting plasma) using standardized enzymatic procedures in a Hitachi 902 analyzer (Hitachi Ltd,Tokio, Japan); low density lipoprotein cholesterol (LDL-C) was estimated using the DeLong et al. formula.[23] All assays were under an external quality control scheme (Lipid Standardization Program, Center for Disease Control in Atlanta, GA, USA).

Ang II serum concentrations were evaluated by capillary zone electrophoresis as previously described.[24] Total high-sensitivity C-reactive protein (hs-CRP) levels were determined by immunonephelometry on a BN Pro Spec nephelometer (Dade Behring Marburg GmbH, Germany). Inter-assay coefficient of variation (CV) values were <6 % for all of these assays. Non-HDL-cholesterol (non HDL-C) was calculated by subtracting HDL-C from total cholesterol. The dyslipidemia value was defined according to conventional cardiovascular risk factors: (TC) ≥200 mg/dL and/or HDL-C ≤ 40mg/dL and/or LDL-C ≥130 mg/dL and/or TG ≥150mg/dL.

Separation of Peripheral Blood Monocyte

Collected whole-blood in tubes with EDTA was diluted 1:1 with PBS 1×–1% heparin; Histopaque 1077 (10771, Sigma-Aldrich) was subsequently added. Peripheral blood mononuclear cells (PBMCs) were obtained from the central white band of the gradient after centrifugation. Next, monocytes were obtained by directly enriching for CD14+ cells by the magnetic sorting system (MACS; Miltenyi Biotec, Bergisch-Gladbach, Germany). 1 mL aliquot of TripureTM reagent (Roche Molecular Biochemicals) was then added for collecting the monocytes. Cells were stored at −80 °C.

Cell Line THP-I Culture

Human monocytic leukemia cells were maintained in a suspension culture of RPMI-1640 medium (Gibco-BRL) containing 2 mM glutamine, 25 mM HEPES, 1.5 g/L sodium bicarbonate, 50 U/mL penicillin, and 50 μg/mL streptomycin (Sigma), supplemented with 10% fetal bovine serum (FBS), at 37°C, in 5% CO2. Arrested THP-1 cells were pre-incubated with Ang II (1 μmol/L) for increasing periods of time to analyze the effect of Ang II on LRP1 expression in the monocytes. The dose of angiotensin II was selected on the basis of previous studies in our group and provides a plasma concentration of angiotensin II similar to that reported in patients with hypertension.[25]

RNA Extraction and cDNA Synthesis

Total RNA was extracted using monocyte Tripure™ Isolation Reagent (Roche Molecular Diagnostics, Indianapolis, USA), according to the manufacturer's instructions. RNA yield and quality were assessed by 1% agarose gel electrophoresis; RNA was stored at -80°C until analysis. Reverse transcription reaction was performed using 1 μg of total RNA for cDNA synthesis according to High Capacity cDNA Reverse Transcription kit (Applied Biosystems Foster City, CA, USA). The cDNA was stored at -80°C.

Gene Expression Assays

LRP1 gene expression (Hs00233899_m1) and *HPRT* (Hs99999909_m1) (endogenous gene) were performed via semi-quantitative real-time reverse-transcriptase polymerase chain reaction (RT-PCR), using a commercial kit. The "TaqMan Gene Expression" was performed using 1 μl reverse transcription products mixed with 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1 μl 20x assays and 8 μl nuclease-free water. After gentle mixing, the mixture was transferred to a real-time PCR microplate, using 7300 Real Time PCR System (Applied Biosystems) equipment.

The used conditions were: 50°C for 2 min and 10 min at 95°C, followed by 40 cycles at 95°C for 15s, and 60°C for 1min. Expression levels were measured in duplicate and the threshold cycle [Ct] values were determined and normalized using the endogenous gene expression (*HPRT*).

Western Blot analysis

Total protein was isolated from monocytes using TriPure™ Isolation Reagent (Roche Molecular Diagnostics), according to the manufacturer's instructions. The protein was quantified using Pierce BCA Protein Assay (Thermo Scientific, Waltham, MA, USA). Equivalent amounts of total protein (25 μg) were loaded onto 10% (v/v) SDS-polyacrylamide gels under reducing conditions. The samples were electrotransferred to nitrocellulose membranes, which were saturated at room temperature for 1 h in TTBS (20 mM Tris–HCl, pH 7.5, 500 mM NaCl, 0.01% Tween 20 and 5% non-fat milk). Western blot analyses were performed using specific monoclonal antibodies against human *LRP1* (85kDa -chain, clone 8B8 RDI 61067, dilution 1:40) and the corresponding secondary antibodies (1:10,000 dilution; Dako; Glostrup, Denmark). The QuantityOne software (Bio-Rad, Hercules, CA, USA) was used to quantify the bands present in the membranes via densitometry, and they were detected using ECL Prime Western Blotting Detection Reagent (Amersham). The expression levels were measured in duplicate and normalized by comparing them with the concentration of a loading protein control. The results were expressed as arbitrary units of intensity.

Statistical Analysis

Data were analyzed using the SPSS v19 software (SPSS Inc. Chicago USA). The results were expressed as the mean ± standard deviation (SD) in the continuous variables and percentages for categorical variables. The Shapiro-Wilk test used to assess normality. The comparison between groups was performed using the unpaired Student's t-test for continuous variables and chi square test for categorical variables. The correlation analysis was done according to the Pearson method. Multiple logistic regressions were used to explore the associations between cIMT and *LRP1* expression. Data is presented as odds ratios (OR) with a confidence interval of 95%. A p <0.05 value was considered as statistically significant. The sample size was calculated taking the reference of Schulz 2002,[13] according to proportions of independent samples, taking into account an incidence of the *LRP1* gene of approximately 0.08 in the cases and 0.02 in the controls with a Δ = 0.06, with a statistical power of 95%, p <0.05. According to the following formula our value of n was =79

$$n = \frac{po\,qo\left[z\alpha + z\beta\sqrt{\frac{pi\,qi}{po\,qo}}\right]^2}{(pi-po)^2}$$

po= Probability that *LRP1* expression occurs in cases

q0= Probability that *LRP1* expression doesn't occur in cases

pi= Probability that *LRP1* expression occurs in controls

qi= Probability that *LRP1* expression doesn't occur in controls

1.96= value <0.05

1.28= power (0.84)

$$n = \frac{(0.8)(0.92)\left[1.96+1.28\sqrt{\frac{p(0.02)\,q(0.98)}{(0.08)(0.92)}}\right]^2}{((0.08)-(0.02))^2}$$

Results

Characteristics of the Study Population

A population of 200 Mexican subjects was studied, of which 91 were normotensive (NT) and 109 were hypertensive (HT) subjects. The biochemical and anthropometric characteristics of the studied population are shown in Table 1. Out of the total population, 62.5% was female and 37.5% was male. Age, body mass index (BMI), cIMT, HDL-C, C-reactive protein, Ang II, and LDL-C/HDL-C, TC/HDL-C, TG/HDL-C indexes were statistically different between groups. These parameters were higher in the hypertensive group as compared to the normotensive group, except for HDL-C levels, which were lower in the hypertensive group. Obesity prevalence was 19.8% in normotensive and 44.1% in hypertensive subjects. No significant differences were found when the comparison was made between both genders of the same parameters. Also, we compared our groups according to dislipidemia levels according to ATP III; however, significant differences were only found in HDL-C (≤40 mg/dL), (NT= 16.5% vs HT= 32.7%, p=0.001) and triglycerides (≥150mg/dL) (NT= 42.7% vs HT= 57.3%, p=0.001) (data no shown).

Correlation Between Hypertension and Expression of LRP1 in Monocytes

With the purpose of ascertaining the levels of mRNA and protein expression, an LRP1 analysis was performed for both groups (Figure 1). Significant differences were found between NT versus HT groups in mRNA expression (P=0.002) and for protein expression (p=0.001). When men and women subjects were compared, the only signficiant difference found was in LRP1 mRNA in hypertensive subjects and there was an overexpression in women as compared to men

Table 1 – Anthropometric, clinical, and biochemical characteristics of study patients

Parameters	Normotensive (n=91)	Hypertensive (n=109)	p
Age (years)	46.0±11.35	50.36±11.57	0.007
Gender (W/M) (%)	61.5/37.5	64/36	0.313
Weight (kg)	71.44±14.30	75.21±12.71	0.056
Height (cm)	161.99±9.81	159.39±9.06	0.057
BMI (Kg/m2)	26.92±4.06	29.36±3.77	<0.001
SBP (mmHg)	110.23±9.07	142.78±10.82	<0.001
DBP (mmHg)	69.90±75.85	91.94±7.72	<0.001
cIMT mean (mm)	0.587±0.16	0.729±0.16	0.002
cIMT max (mm)	0.606±0.18	0.787±0.16	0.008
Total cholesterol (mg/dL)	197.32±40.41	198.91±37.42	0.772
Triglycerides (mg/dL)	166.56±94.45	192.95±98.43	0.001
Log TG	2.16±0.22	2.23±0.19	0.010
HDL-C (mg/dlL)	52.51±13.25	46.66±13.59	0.002
LDL-C (mg/dL)	117.02±33.20	122.23±31.74	0.258
LDL/HDL	2.36±0.84	2.76±0.91	0.001
Non HDL-C	144.80±41.28	152.67±36.54	0.154
CT/HDL	3.96±1.20	4.50±1.26	0.003
TG/HDL	3.62±2.73	4.65±3.21	0.017
Glucose (mg/dL)	89.36±7.78	89.18±8.91	0.877
C Reactive Protein (mg/dL)	2.37±2.06	3.87±2.85	0.011
Smoking	1.83±0.38	1.67±0.51	0.491

The values are expressed as mean ± SD or percentages for categorical values. Unpaired Student T Test and chi-square test for categorical values were used. BMI: body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; cIMT mean: carotid intima media thickness mean; IMT max: Intima-media thickness maximal; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol.

(p=0.044). Moreover, an increase in the LRP1 mRNA and protein expression was found in hypertensive dyslipidemic subjects as compared to normotensive dyslipidemic subjects (data no shown).

Conversely, to examine if others factors like cIMT and Ang II variable were analyzed to determine whether they could participate in the blood pressure values (Table 2). A significant difference was found between NT versus HT for cIMT (p=0.002) and Ang II (p=0.046), respectively. However, when subjects were broken down by gender, no differences were found in either of the two parameters.

Angiotensin II Effect on Monocyte *LRP1* Expression Levels

To study the effect of Ang II mediated *LRP1* induction on monocytes, the THP1 monocyte cell line was incubated with Ang II for 4h and 8h, with concentrations of 1 and 10 μM. In the THP1 monocyte cell line, Ang II increased *LPR1* mRNA expression in a dose and time dependent manner, being more evident at 8 hours of incubation (Figure 2).

Association between monocytes *LRP1* expression and carotid intima/media thickness from patients with hypertension.

To know if there was a relationship between the thickness of the cIMT and *LRP1* mRNA expression and/or *LRP1* protein expression, multiple logistic regressions adjusted by lipid profile, age, and gender were conducted (Table 3). A significant difference was found between cIMT and the *LRP1* mRNA expression levels (p=0.047) and *LRP1* protein levels (p=0.039) in hypertensive patients.

Therefore, an adjusted logistic regression for lipids was performed to analyze whether dyslipidemia could influence the association between *LRP1* and cIMT in hypertensive patients (Table 3, Models 1-4). An association between cIMT and *LRP1* mRNA expression with the entire set of lipid parameters was found: Model 1 (p=0.046), the association was maintained after adjusting each of the lipid parameters, Model 2 adjusted by total cholesterol (p= 0.053), Model 3 adjusted by triglycerides (p=0.049), Model 4 adjusted by HDL-C (p=0.038), and Model 5 adjusted by LDL-C (p=0.052).

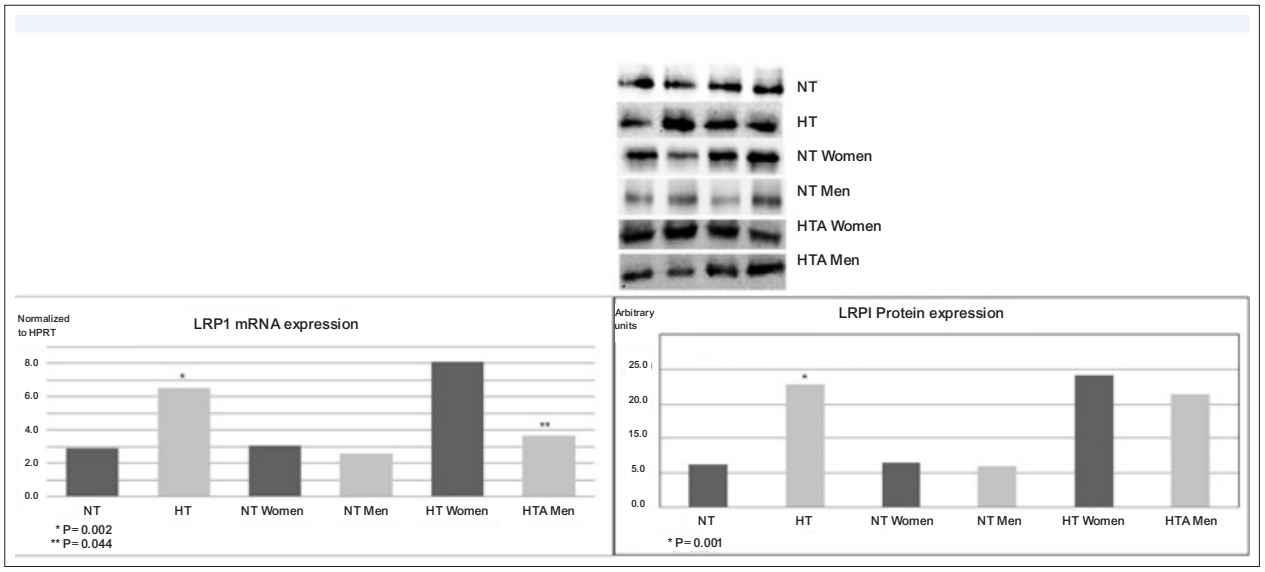

Figure 1 – *Quantification of LRP1 expression in total subjects and broken down by genders. (A) Comparison of LRP1 expression in monocytes from normotensive and hypertensive subjects. Real-time PCR analysis of LRP1 mRNA expression. Data were processed with a specially designed software, based on the Ct value of each sample, and normalized to HPRT1 (B) Western blot analysis showing LRP1 protein expression in monocytes.*

Table 2 – Values of cIMT and Ang II broken down by gender

	NT	HTA	p	NT Women	NT Men	p	HTA Women	HTA Men	p
IMT (mm)	0.568 ± 0.16	0.715 ± 0.16	**0.002**	0.553 ± 0.149	0.583 ± 0.178	0.303	0.692 ± 0.14	0.719 ± 0,19	0.643
Ang II (pmol/ml)	0.195± 0.09	0.226 ± 0.12	**0.046**	0.200 ±0.090	0.186 ± 0.090	0.468	0.220 ± 0.11	0.238 ± 0,14	0.482

NT: Normotensive; HTA: Hypertensive; IMT: mean intima-media thickness, Ang II: Angiotensin II. Unpaired Student T Test.

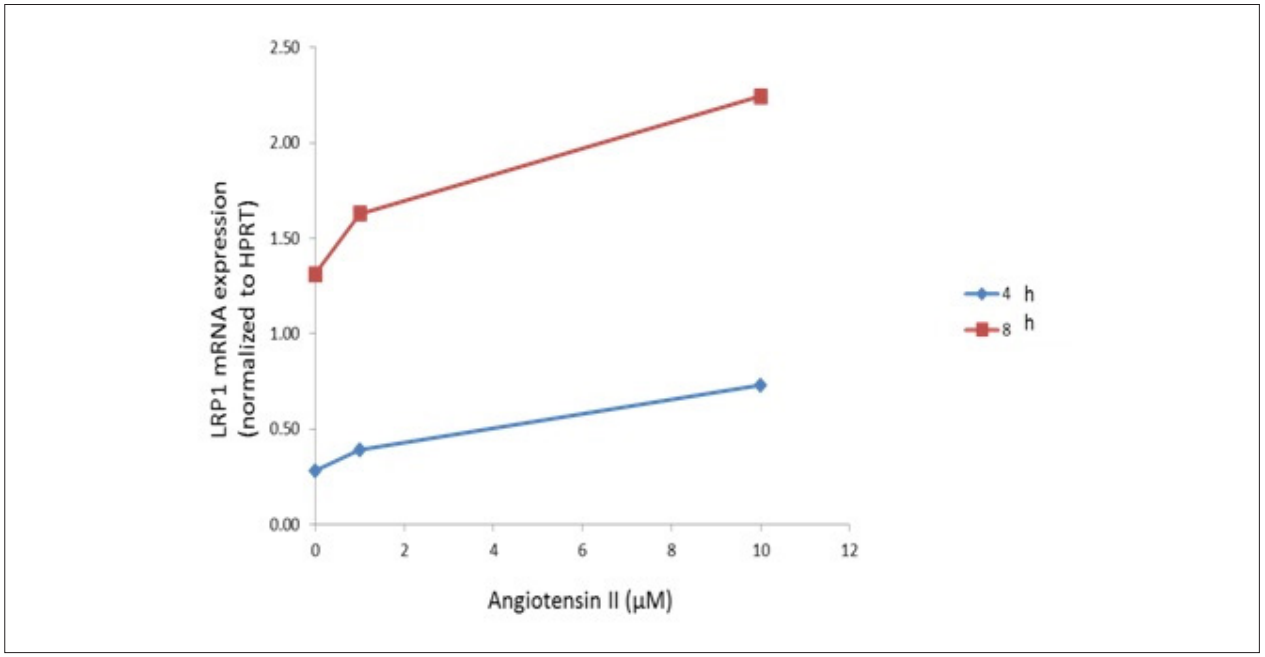

Figure 2 – *Effect of angiotensin II on the LRP1 expression in THP1 cells.*

Table 3 – Association between the expression of LRP1 and cIMT adjusted for lipid parameters in patients with hypertension

mRNA	OR [CI 95%]	p
Adjustment [-]	0.308 [0.230 – 38.650]	0.047
Model 1	0.310 [0.340 - 38.887]	0.046
Model 2	0.303 [-0.280 - 38.511]	0.053
Model 3	0.308 [0.131 – 38.832]	0.049
Model 4	0.312 [0.150 – 38.33]	0.038
Model 5	0.301 [-0.181 - 38.19]	0.052
Protein	**OR [CI 95%]**	**p**
adjustment [-]	0.312 [1.771 - 65.319]	0.039
Model 1	0.294 [-2.150 - 65.208]	0.066
Model 2	0.211 [1.544 - 65.637]	0.040
Model 3	0.313 [1.445 - 65.77]	0.041
Model 4	0.317 [2.020 - 66.015]	0.038
Model 5	0.313 [1.528 - 65.6689]	0.040

Model 1: adjusted by all lipid parameters. Model 2: adjusted by total Cholesterol. Model 3: adjusted by Triglycerides. Model 4: adjusted by HDL-C. Model 5: adjusted by LDL-C. Multiple logistic regressions analysis.

However, we did not observe an association between cIMT and LRP1 protein expression when adjusting the complete set of lipid parameters, Model 1 (p=0.066). Nevertheless, when we adjusted with each lipid parameter, an association was found: Model 2 adjusted by total cholesterol (p=0.040), Model 3 adjusted by Triglycerides (p=0.041), Model 4 adjusted by HDL-C (p=0.038), and Model 5 adjusted by LDL-C (p=0.040).

Afterwards, a linear regression was made between cIMT and expression levels of both *LRP1* mRNA and protein expression adjusted by lipid profile; a positive correlation between these variables was maintained (Figure 3).

Discussion

As expected, our results showed that the average cIMT was higher in hypertensive subjects than in normotensive subjects. However, this value was associated in an important way with the LRP1 overexpression in circulating monocytes.

cIMT is considered an atherosclerosis marker and an excellent predictor of death and cardiovascular events.[26] In hypertensive patients with coronary artery disease, increased cIMT is closely associated with atherosclerosis.[27] Our data showed a strong association between hypertension and cIMT. These results agree with previously published data in studies made in patients and animal models. In a study of

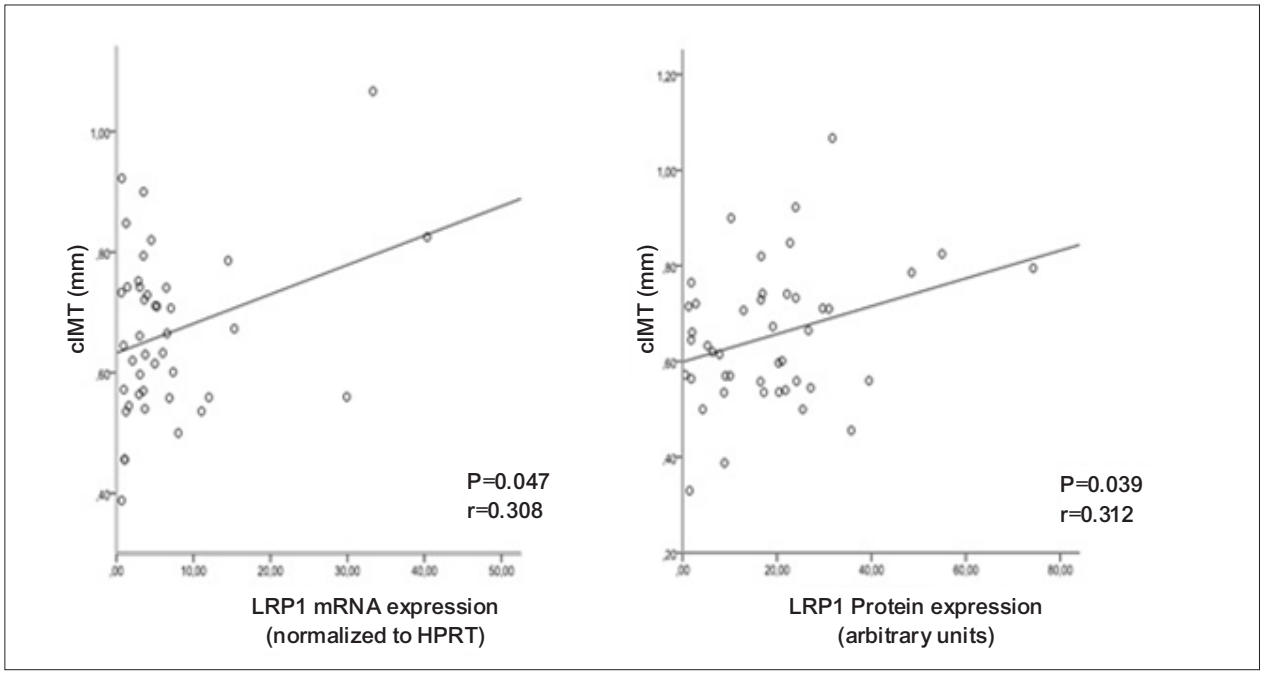

Figure 3 – *Correlation between cIMT and the expression levels of mRNA and LRP1 protein adjusted for CT, TG, HDL-C, and LDL-C. P<0.005 is considered as statistically significant.*

young people with borderline hypertension (130-140/85-89 mmHg), an increase in the cIMT in the brachial arteries was observed when patients were compared to normotensive subjects; an association between cIMT and ambulatory SBP of 24 hours was found.[27] In addition, hypertension, diabetes, and age are considered independent prognostic factors for intima hyperplasia in the radial artery.[28-30] In a hypertension animal model, a significant thickening of the intima-media was reported as the direct cause of the illness.[31,32]

Hypertension is among the main risk factors in the etiology of atherosclerotic vascular disease.[33,34] However, the mechanisms by which arterial pressure increases the incidence of atherosclerosis are not completely clear. Studies that focus on elucidating these mechanisms are critically important. There is a strong association between hypertension and the *LRP1* expression in the vascular wall of a rat model.[35] The upregulation of *LRP1* by hypertension has functional consequences as it promotes intracellular lipid accumulation and, thus, the formation of foam cells. Hypertension also has a high impact on vascular remodeling, chronic changes in hemodynamic forces, and structural alterations in the vascular wall.[36]

Our results show overexpression on both mRNA and protein expression of the *LRP1* receptor in monocytes from hypertensive patients. They also show that Ang II increased the expression of *LRP1* in cultures of THP-1 in a time and dose dependent manner. Therefore, the mechanism through which high blood pressure regulates the expression of *LRP1* could be mediated by the angiotensin II effect, which is considered one of the main hypertension mediators. It has also been reported that angiotensin induces the activity of Sp1/Sp3 transcription factors, which are involved in the recognition of *LRP1* promoter,[13] causing *LRP1* overexpression at a vascular level and favoring the formation of foam cell in human vascular smooth muscle cells.[33]

In addition to angiotensin II, blood flow acts on the function and structure of the endothelium through the modulation of the gene expression.[37] The functional changes that are experienced by monocytes due to continuous changes in blood flow might have a positive influence on *LRP1* expression, thus stimulating LDL uptake and causing an increase in cIMT.

In addition to a high prevalence of obesity, the Mexican population is facing a serious problem of dyslipidemia, which is explained by an interaction of genetic and environmental factors.[38]

In the analysis of dyslipidemia subjects according to conventional cardiovascular risk factors, we found an increase in the *LRP1* mRNA and protein expression in hypertensive dyslipidemic subjects as compared to normotensive dyslipidemic subjects, which could mean that *LRP1* is overexpressed by hypertension regardless of dyslipidemia.

Previous studies have shown that circulating soluble low-density lipoprotein receptor-related protein 1 (sLRP1) concentration may be intimately associated with hypercholesterolemia (LDL-C>200 mg/dL) and an upregulating effect of hypercholesterolemia on the expression of *LRP1* in cells of the vascular wall in *in vitro* and *in vivo* models.[39] Despite observing a high percentage of hypercholesterolemia in normotensive and hypertensive subjects, our results found no significant differences between both groups. A possible explanation for these differences could be: a) the association between sLPR1 and cholesterol was performed in hypercholesterolemic populations (severe hypercholesterolemia); b) the *LRP1* could be expressed in a wide range of tissues and the specificity could be different; in our case, the *LRP1* expression in monocytes was measured; c) the populations are very different; whereas our study was done using a mixture of indigenous American [65%], European [31%], and African [3%] subjects, the other study consisted solely of Caucasians.[40]

Our data indicate that the expression of *LRP1* in monocytes from hypertensive patients correlates with increased cIMT. Adjusted logistic regression shows that the correlation between cIMT and *LRP1* mRNA expression is maintained even after adjusting lipid parameters. However, this association was lost when the adjustment was done with *LRP1* protein. These results can be explained by the strong positive effect of modified LDL on the stability of *LRP1* protein.[41,42] Therefore, dyslipidemia probably contributes to maintaining a high *LRP1* protein expression in monocytes from hypertensive patients. This could justify why the association between cIMT and *LRP1* protein expression after adjustment for lipid profile is lost.

Conclusions

Our findings suggest that the effect of hypertension on atherosclerosis might occur through the overexpression of *LRP1* in circulating monocytes. Ang II induced monocyte *LRP1* upregulation, and it may play an important role in the increased cIMT associated with cardiovascular risk factor induction of atherosclerotic lesion progression. These results reinforce the high relevance of *LRP1* overexpression in the formation and progression of atherosclerotic plaques in humans.

Author Contributions

Conception and design of the research: Llorente-Cortés VC, Huesca-Gómez C; Acquisition of data: Gamboa R, Jaramillo-Estrella MJ, Martínez-Alvarado M del R, Torres-Paz YE, Gonzalo-Calvo D, Del Valle-Mondragón L, López-Marure R; Analysis and interpretation of the data: Gamboa R, Jaramillo-Estrella MJ, Soto ME, Huesca-Gómez C; Statistical analysis: Soto ME, Huesca-Gómez C; Obtaining financing: Huesca-Gómez C; Writing of the manuscript: Gamboa R, Jaramillo-Estrella MJ, Llorente-Cortés VC, Huesca-Gómez C; Critical revision of the manuscript for intellectual content: Gamboa R, Llorente-Cortés VC.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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New Markers of Carotid Thickening in Hypertension

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Short Editorial related to the article: Monocyte Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) Expression Correlates with cIMT in Mexican Hypertensive Patients

Arterial hypertension was considered an important cardiovascular risk factor only after the Framingham studies, as it was believed that it was a necessary "good" for good tissue perfusion.[1] These emblematic long-term cohort studies about the cardiovascular system brought data that allowed the evaluation of the interaction with several other diseases, such as dyslipidemias and diabetes, for the atheromatous plaque formation, which is the initial step for cardiovascular complications. A time when clinical examination was essential to detect markers of atherosclerotic disease.

However, with the evolution of knowledge, clinical biological markers were no longer sufficient to predict risk, as we increasingly need to articulate preventive measures as early as possible, for more effective treatment and better prevention. Additionally, the interaction between the environment, with all its risk factors and genetics proved to be interactive and of crucial importance in the development of the atherosclerotic plaque. Regarding hypertension, the genetic component with an estimated inheritance of 15-40% became clear, so much so that the brothers have a risk agreement rate for the disease ranging from 1.2 to 1.7.[2,3]

To understand this extremely complex mechanism, which involves several molecular and biochemical pathways, such as the renin-angiotensin-aldosterone system (RAAS), closely linked to hypertension, the analysis of biomolecular and/or genetic markers can add knowledge to reveal the several pathways that lead to atherosclerosis.

Ethnic and racial factors also contribute to it, predisposing to a higher prevalence of several diseases, including hypertension. An example of this fact are Afro-descendant and Latin populations, with greater disease prevalence and severity, in addition to more marked comorbidities related to hypertensive disease.[4,5]

The objective of the study by Gamboa et al.[6] was to evaluate the association of biomolecular and genetic markers with arterial hypertension, focusing mainly on the carotid intima-media thickness (CIMT) in Mexicans.[6]

The Mexican population has an ethnic mix of 65% American Indians, 31% Europeans, and 3% Africans, differing greatly from other countries with a predominance of Caucasians, where most studies are performed.[7] This diverse genetic load can lead to a specific behavior in terms of cardiovascular risk and marker expression.

The CIMT, which is a marker of atherosclerosis, correlates with an increase in deaths and cardiovascular events in adults and also with vascular abnormalities in hypertensive children and adolescents.[8] Lande et al. observed that children or adolescents with CIMT that was above normal values had more severe hypertension, irrespective of obesity, usually associated to hypertensive disease in this age group.[9,10]

Gamboa et al.[6] found higher CIMT values in the hypertensive group and associated it to an increase in LRP1 mRNA expression and the expression of LRP1 protein, which showed high and very evident values in hypertensive patients.

The mechanisms through which hypertension predisposes to atherosclerosis are not yet well understood, but it is known that they are multifactorial involving several causes, from endothelial aspects, to lipid and genetic ones. However, CIMT also increases as a physiological vascular reaction in adaptation to pressure increase and as the years progress, reflecting an adaptive response to aging and mechanical stress. These findings are interesting, demonstrating that these markers are higher in hypertensive patients with higher CIMT. This corroborates the multifactorial theory of hypertension and target-organ injury, where the genetic profile profoundly influences vascular injury.[11,12] A fact also found in experimental studies in animals that showed that LRP1 promotes the entry of lipids in monocytes that migrated to the vessel forming foam cells and, therefore, atherosclerosis.[13]

A curious finding was related to the division of groups by gender. The mRNA expression of LRP1 in hypertensive individuals was significantly higher in women and less significant in men, which was practically the same as in normotensive individuals. This makes understanding difficult, as it lacks an objective explanation of this difference. This did not occur in the expression of LRP1 protein, which increased in the hypertensive group in a similar manner in men and women. The mean age of hypertensive patients was 50.3 years and, possibly, hormonal factors related to the female gender may be involved.

In this study, angiotensin II (Ang II) was evaluated, considering the importance of RAAS in hypertension regulation. They found a positive relationship between Ang II and LRP1 expression, associating high pressure as a regulator of LRP1 expression mediated by Ang II.

The RAAS is very complex and knowledge about it has been increasingly expanding, as new data is added to the didactic biochemical cascade that begins with renin and ends

Keywords

Hypertension/epidemiology; Monocytes; mRNA, Carotid Intima-Media Thickness; Genetic Markers.

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with aldosterone. The complexity is such that the single block with angiotensin-converting enzyme inhibitors or Ang II AT1 blockers promotes fantastic clinical benefits; however, the double block has poor or even harmful results for the patient. Thus, obvious conclusions based on pathophysiological aspects are not fully applicable to RAAS.

The study, although complex regarding the data analysis, is a way for the development of new markers in arterial hypertension that can guide us in the search for early target-organ injuries that will be translated into more accurate therapy, with more optimized goals, benefiting the patient.

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Correlation between Cardiomegaly on Chest X-Ray and Left Ventricular Diameter on Echocardiography in Patients with Chagas Disease

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Abstract

Background: Cardiomegaly on chest X-ray is an independent predictor of death in individuals with chronic Chagas cardiomyopathy (CCC). However, the correlation between increased cardiothoracic ratio (CTR) on chest X-ray and left ventricular end-diastolic diameter (LVEDD) on echocardiography is not well established in this population.

Objectives: To assess the relationship between chest X-ray and LVEDD on echocardiography in patients with Chagas disease and its applicability to the Rassi score.

Methods: Retrospective study on 63 Chagas disease outpatients who underwent chest X-ray and echocardiography. Cardiomegaly on chest X-ray was defined as a CTR>0.5. LVEDD was analyzed as a continuous variable. ROC curve was used to evaluate the ability of LVEDD in detecting cardiomegaly by chest X-ray, with a cut-off point defined by the highest sum of sensitivity and specificity.

Results: Median age 61 years [interquartile range 48-68], 56% were women. CCC was detected in 58 patients, five patients had the indeterminate form of Chagas disease. Cardiomegaly was detected in 28 patients. The area under the ROC curve for LVEDD was 0.806 (95%CI: 0.692-0.919). The optimal cut-off for LVEDD was 60 mm (sensitivity = 64%, specificity = 89%). The use of LVEDD on echocardiography as a surrogate for CTR on chest X-ray changed the Rassi score values of 14 patients, with a reduction in the presumed risk in 10 of them.

Conclusion: LVEDD on echocardiography is an appropriate, highly specific parameter to distinguish between the presence and absence of cardiomegaly on chest X-ray in Chagas disease. (Arq Bras Cardiol. 2021; 116(1):68-74)

Keywords: Chagas Disease/physiopathology; Cardiomegaly; X-Rays; Chagasic, Cardiomyopathy; Heart Block.

Introduction

Chagas disease (CD) is caused by infection with the protozoan parasite *Trypanosoma cruzi* (*T.cruzi*), which is mainly transmitted to humans by insects in the subfamily Triatominae. Other modes of transmission include blood transfusion, bone marrow or solid organ transplantation from infected donors, vertical transmission from mother to fetus and oral ingestion of contaminated food.[1] The World Health Organization estimates that CD affects approximately 7 million individuals in the world, causing high morbidity and mortality, and significant social impact.[2]

Chagas cardiomyopathy is the most common and serious clinical form of CD, affecting 20-30% of chronically infected individuals.[3,4] The Rassi score is a validated score for mortality risk stratification of patients with chronic Chagas cardiomyopathy (CCC). Among the risk factors assessed by the score, cardiomegaly on chest X-ray stands out for its strong association with overall and cardiovascular mortality risk in patients with CCC.[5]

In the study by Rassi Jr. et al.,[5] although echocardiography was used to assess left ventricular end-diastolic diameter (LVEDD), this parameter was not shown to be an independent marker of mortality in CCC. However, in their study, LVEDD was analyzed in a categorical manner, using conventional cut-off points, which may not be the most appropriate for CCC patients, due to the segmental myocardial dysfunction, characteristic of this condition. Besides, calculation of the cardiothoracic ratio (CTR) by chest X-ray, in many cases, may encompass both atrial and ventricular dilatation, that are expressed linearly in this method. Despite widely available, the radiological study of the heart involves radiation, and echocardiography has become the most used method for cardiovascular evaluation. Therefore, there is a genuine interest in assessing left ventricular size and systolic function to estimate the risk of death using a single imaging test and the variables used in the original Rassi score.

The present study aimed to evaluate the relationship between cardiomegaly defined by the CTR on chest X-ray and the LVEDD determined by echocardiography in patients with CD.

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Methods

In this retrospective cross-sectional study, we studied patients of both sexes, adults (>18 years old), with diagnosis of CD, attending the outpatient clinic of the General Hospital of the University of Sao Paulo Medical School in Ribeirao Preto (HCRP-FMRP-USO), a tertiary referral hospital for CD. The diagnosis of CD was confirmed by two positive serological tests for detection of antibodies against *T. cruzi*, using different techniques.

Data were obtained by a systematic review of medical records of 158 patients who had participated in a previous clinical study,[6] which describes in detail the inclusion and exclusion criteria applied. Patients with CTR and complete evaluation of the independent predictors of the Rassi score by resting 12-lead electrocardiogram, echocardiography, CTR and 24-hour heart rhythm monitoring, and of the degree of dyspnea according to the New York Heart Association (NYHA) criteria were included. The maximum interval between the CTR (considered the reference method for comparison) and the echocardiography was of one year, and patients were clinically stable in this period. Patients who had changes in the clinical status in this period between the two tests were excluded from the study.

Chest x-ray

Cardiomegaly on chest X-ray was always evaluated in an anteroposterior view and defined as a CTR > 0.5. Eventual enlargement of the right ventricle was also evaluated by comparison with profile teleradiography, when this technique was available.

Echocardiography

Data of resting transthoracic echocardiography (the last before the chest X-ray) were used for analysis, considering the maximum interval of one year between the tests. The LVEDD was measured by the two-dimensional test, following recent echocardiography guidelines.[7] Other echocardiographic parameters related to left ventricular remodeling were assessed, including the left ventricular mass index (LVMI), left ventricular ejection fraction (LVEF), and the left atrial volume index (LAVI).

Statistical Analysis

Continuous variables with normal distribution were expressed as mean and standard deviation, and those without normal distribution were expressed as median and interquartile range (IQR). Analysis of the ROC (Receiver Operating Characteristic) was conducted to verify the ability of LVEDD, determined by echocardiography, to differ between presence and absence of cardiomegaly by the CTR. Finally, the impact of using cardiomegaly assessed by echocardiography, rather than the traditional CTR, on reclassification of patients with the cardiac form of CD by the Rassi score was evaluated. Individuals with the indeterminate form of CD were not classified by the Rassi score in the study, since patients with this form of the disease were not included in the original investigation of this instrument.

Ethics

The present study was approved by the research ethics committee (CAAE number 06415319.2.0000.5440; approval number 3.130.390) and conducted according to the Helsinki declaration and the Brazilian National Health Council resolution number 466/2012.

Results

Description of the Study Population Sample

Of the 158 patients with CD evaluated, 63 (40%) patients met the inclusion criteria and were included in this retrospective cross-sectional study. Demographic and clinical characteristics of participants are described in Table 1. Median age of participants was 61 (IQR 48-68) years, and many were women (56%). Only five (8%) patients had the indeterminate form of CD. Most patients (68%) had NYHA functional class I, followed by NYHA class II (21%) and III (115). The Rassi score of the 58 patients with CCC was 9 ± 5 points.

Table 1 – Characteristics of patients included in the study (n=63)

Demographic and anthropometric data	
Age (years)	61 [48-68]
Female sex	35 (56%)
Body mass index (Kg/m^2)	26.6 ± 4.7
Clinical data	
Functional class	
NYHA I	37 (59%)
NYHA II	17 (27%)
NYHA III	9 (14%)
Edema of lower limbs	12 (19%)
Swollen jugular vein	3 (5%)
ACEI or ARB	48 (76%)
Betablocker	34 (54%)
Spironolactone	16 (25%)
Diuretics	29 (46%)
Amiodarone	14 (22%)
Echocardiographic data	
Left ventricular end-diastolic diameter (mm)	54 [47-61]
Left atrial volume index (mL/m^2)	42 [26-59]
Left ventricular mass index (g/m^2)	123 [92-156]
Left ventricular ejection fraction (%)	51 [34-63]

Parametric and non-parametric continuous variables described as mean ± standard deviation and median [interquartile range], respectively. Categorical variables presented as absolute numbers and percentages NYHA: New York Heart Association; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker

Comparison between Chest Radiography and Echocardiogram

Cardiomegaly, assessed using the CTR, was detected in 28 (44%) patient. The mean interval between chest X-ray and echocardiography was 5 ± 174 days. LVEDD on chest X-ray was larger in the group of patients with cardiomegaly (61 IQR [53-70]) than in patients without cardiomegaly (49 IQR [46-55]), $p < 0.001$. The area under the ROC curve for LVEDD for detection of cardiomegaly on chest X-ray was 0.806 (95% confidence interval 0.692 – 0.919) (Figure 1). A LVEDD of 60 mm was defined as the cut-off with the highest accuracy, with sensitivity of 64% and specificity of 89%.

Patients with discordant test results regarding the detection of cardiomegaly did not show statistically significant differences in age, sex, LVMI, and LAVI compared with those patients with concordant test results.

Reclassification of the Rassi Score

In our study, the proportion of individuals at low, moderate and high risk by the Rassi score, using the CTR for detection of cardiomegaly was 36% (n = 21), 33% (n = 19) and 31% (n = 18), respectively. These proportions were 40% (n = 23), 28% (n = 16) and 32% (n = 19), respectively when cardiomegaly was detected by echocardiography using the most accurate cut-off point (Figure 2). In 44 (76%) patients, the Rassi score with echocardiography was the same as that estimated by the CTR. Among the 14 patients who showed a numerical change in the Rassi score, eight showed a reduction in the score and six showed an increase. Considering the risk categories (low, moderate, high), there was a change in category in 11 patients (19%); six showed a reduction and five showed an increase in the score (Figure 3).

Discussion

The present study demonstrated that there is a clear and significative relationship between detection of cardiomegaly by CTR and detection of left ventricular dilatation by echocardiography in a non-selected group of outpatients with diagnosis of CCC. These results open new perspectives to the use of LVEDD determined by resting transthoracic echocardiography in substitution for the estimation of CTR by chest X-ray, to determine the risk of death in patients with CCC using the Rassi score, whose results were eventually modified. This would prevent the use of radiological test, which requires radiation, despite low, and allow determining both left ventricular size and left ventricular systolic function by a single, non-invasive test that does not involve radiation. This perspective seems attractive, since the LVEDD determined by echocardiography was shown to be a highly specific parameter to distinguish between the presence and absence of cardiomegaly in patients with chronic CD.

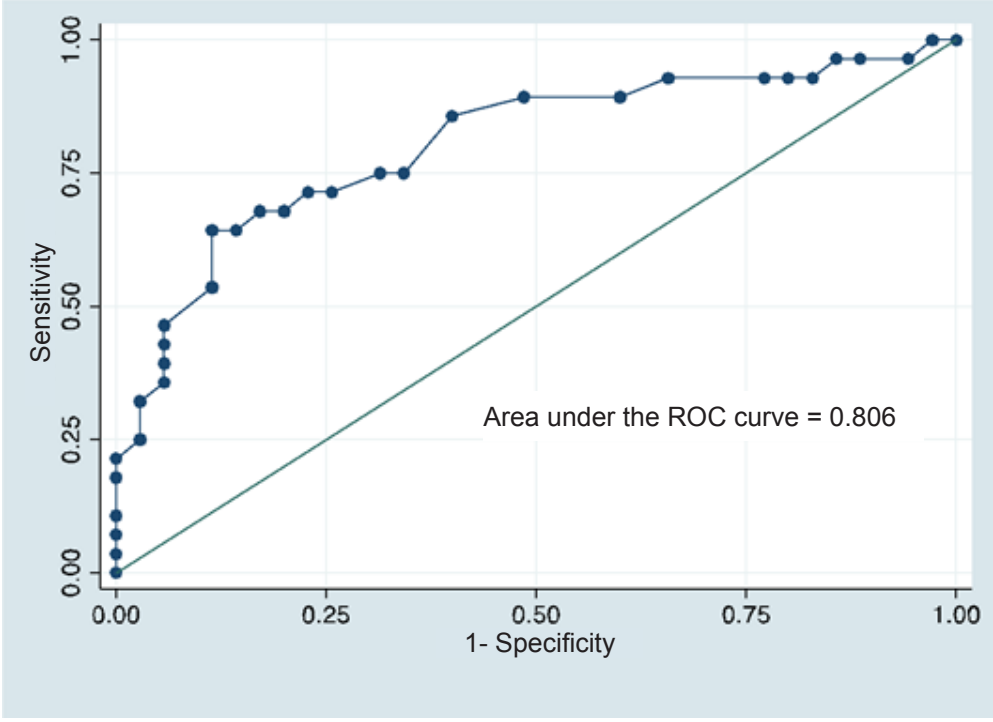

Figure 1 – *Area under the ROC curve of the left ventricular end-diastolic diameter for detection of cardiomegaly on chest radiography.*

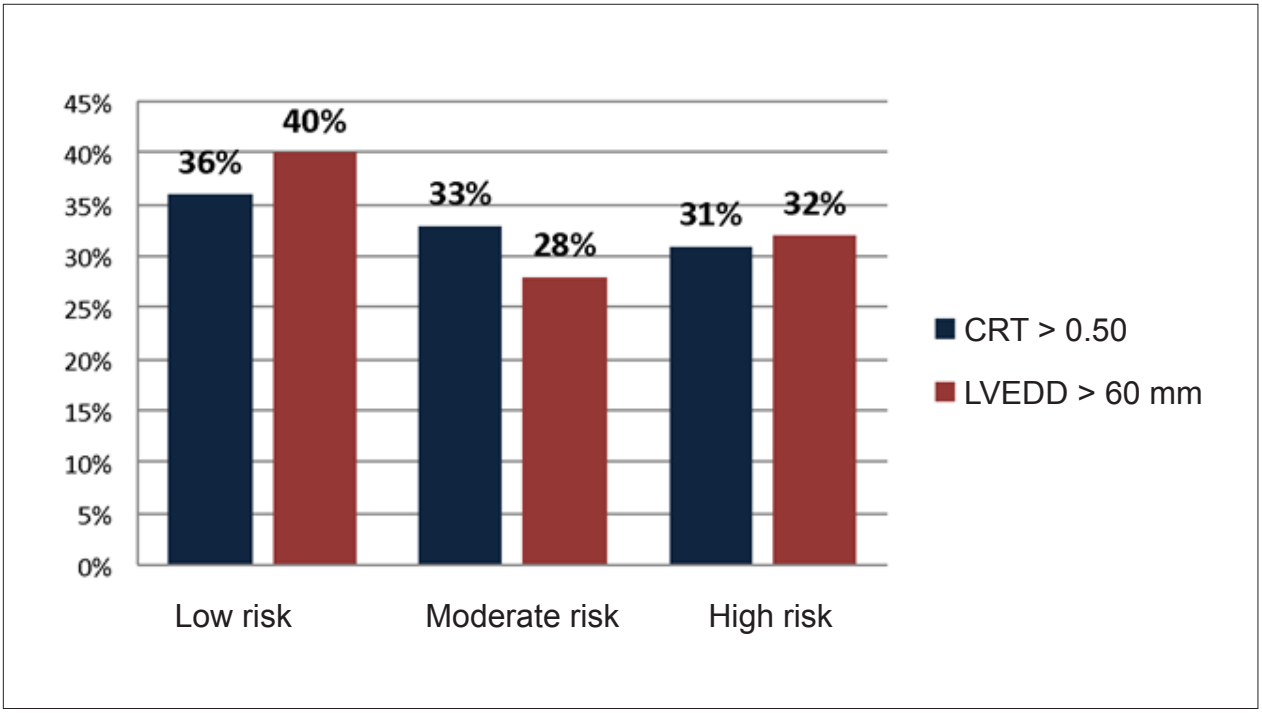

Figure 2 – *Risk of death according to the Rassi score on chest X-ray and echocardiography for definition of cardiomegaly in individuals with the cardiac form of Chagas disease; LVEDD: left ventricular end-diastolic diameter; CTR: cardiothoracic ratio.*

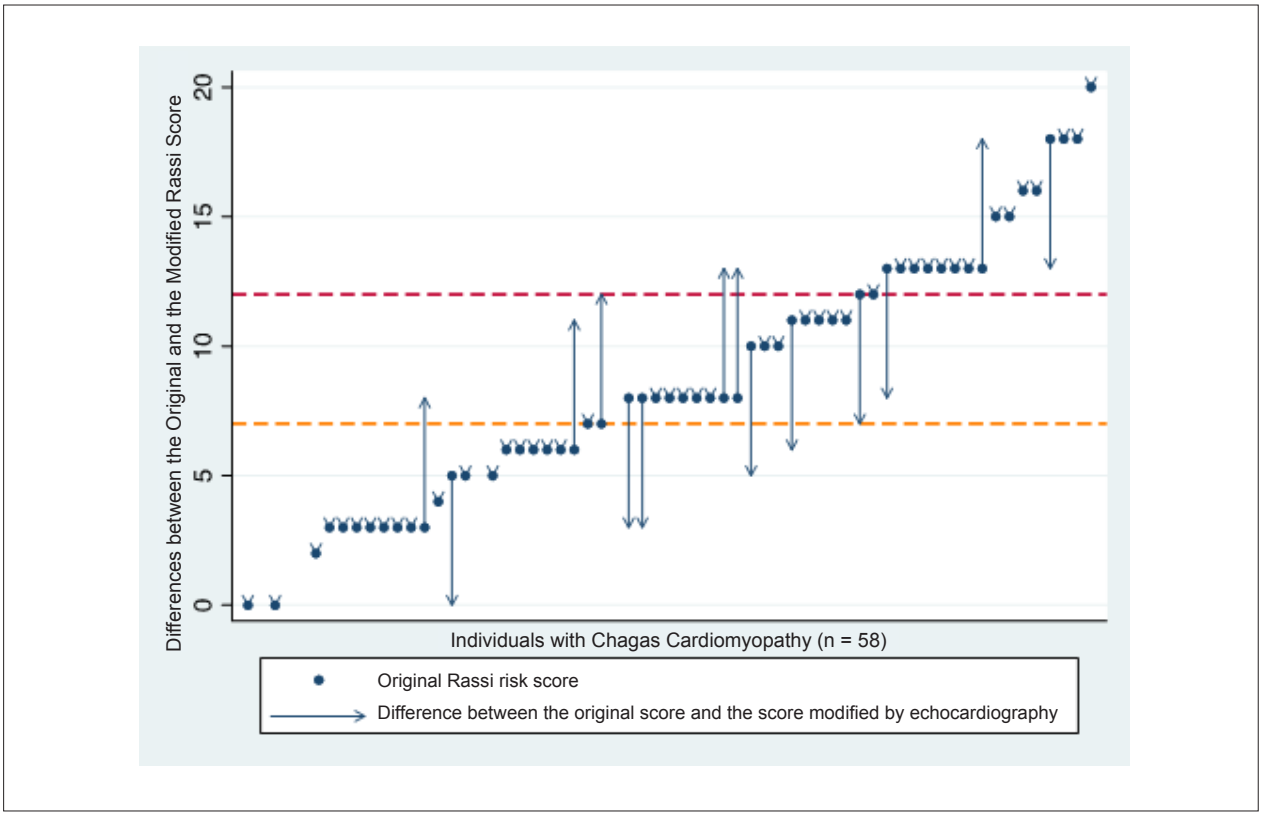

Figure 3 – *Individual risk of death according to the Rassi score on chest X-ray and echocardiography for definition of cardiomegaly in individuals with the cardiac form of Chagas disease; red dashed line and yellow dashed line correspond to a Rassi score of 12 (high risk) and 7 (moderate risk); LVEDD: left ventricular end-diastolic diameter; CTR: cardiothoracic ratio.*

Our findings corroborate previous observations reported by Pereira-Barreto et al.[8] in 1983, of chest X-ray and echocardiographic results of a smaller sample (n=22), showing a good correlation between CTR values and left ventricular function.[8] On the other hand, in a study published in 2003, Perez et al.,[9] conducted a comparative analysis between posteroanterior chest X-ray and resting echocardiographic results, and showed a poor correlation between the tests for LVEDD and LVEF results. Also, in this study,[9] CTR did not show high sensitivity or positive predictive value in detecting left ventricular dysfunction. Therefore, despite its high specificity in detecting left ventricular systolic dysfunction, a CTR > 0.5 would not be useful in assessing this condition in CD, due to its low sensitivity in detecting, on resting transthoracic echocardiography, left ventricular dilatation or left ventricular systolic dysfunction by altered LVEF.

The reasons of the discrepancies between these studies are not clear. In the study by Perez et al.[9] and in our study, although the population sample consisted of outpatients with CCC attending university hospital, there were differences between them. In our study, there were almost no exclusion criteria, except for those related to the availability of CTR and echocardiographic results within a one-year period. In contrast, the broad exclusion criteria used in consecutive patients in the study by Perez et al.[9] may have resulted in a highly selected sample that may not be representative of CD patients. Thus, in their study,[9] only 28% and 29% of patients had increased CTR and left ventricular dysfunction, respectively, and the mean LVEF was 61% (vs. 51% in our study). These aspects indicate that our population with CCC were more severely ill, especially considering that only 8% of them had the indeterminate form of CD. In addition, it is plausible that differences in the chest X-ray results regarding CTR values between the two studies are more apparent than real. In fact, in the study by Perez et al.,[9] patients were divided into two groups by CTR values (normal vs. abnormal, $p<0.05$), and results showed a significant association ($p<0.05$) of LVEF reduction with increase in ventricular diastolic dimension and left ventricular segmental dysfunction, as evidenced by the left ventricular wall motion index.

It is worth pointing out that the results of the present study indicate that the absence of cardiomegaly on chest X-ray does not discard cardiac involvement when patients are assessed by methods able to provide better anatomic and functional details, comparable with resting transthoracic echocardiography. Thus, despite specific, chest X-ray has low sensitivity in detecting cardiac involvement in CD patients, and its use as screening method or diagnostic criterion for the indeterminate form of CD may be questionable. In this context, although the indeterminate form of CD is still defined in current guidelines based on a normal CTR on chest X-ray,[3,10,11] substitution of this criterion with normal resting echocardiogram was already proposed in 2002.[12]

The Rassi score is universally regarded as the most valuable instrument for the establishment of the vital prognosis of patients with CCC. This is a robust score, developed by a multivariate analysis of many risk factors of death in CCC, externally validated in other independent cohorts. However, in the study by Perez et al.,[9] variables were analyzed in a dichotomous rather than continuous manner, which gives room for complementation. Among the variables analyzed in a non-continuous manner, there was the LVEDD, conventionally measured by echocardiography. However, when LVEDD was assessed in dichotomized categories, *i.e.*, presence or absence of increase, may be not appropriate for the evaluation of patients with CCC due to the typical segmental myocardial abnormality of the disease.[1]

Results of the present study are in line with the importance of echocardiography as an essential instrument for the follow-up of patients with chronic CD, especially of those with early myocardial deficits of CCC.[10] Echocardiography allows not only confirmation (or not) of cardiomegaly in case of questionable findings on chest X-ray, but also analysis of cardiomyopathy marked by regional changes of ventricular contractility that result from early and prominent disturbances in the natural course of the underlying disease.[13] Also, detection of these regional abnormalities at early stages of CCC has prognostic and therapeutic implications, in light of recent reports showing that even minimal changes in the left ventricular segmental wall motion index when left ventricular systolic function is preserved are determinant of severe outcomes including mortality.[13]

In the seminal study by Rassi Jr. et al.,[5] the cohort included in the multivariate analysis and development of the score consisted of 424 patients, most of them at low risk (61%), while 19% and 20% of the patients were at moderate and high risk, respectively. In the present study, we found a more balanced distribution of patients into the risk groups – 36%, 33% and 31% at low, intermediate, and high risk, respectively. Similarly, when LVEDD is substituted for CTR in the score, there was a small increase in the proportion of patients at low risk (40%) at the expense of a slight reduction in the percentage of patients at intermediate risk (28%). Therefore, further studies with clinical follow-up are warranted for validation of the Rassi score modified by substitution of the CTR as the radiological parameter of cardiac dilatation with the more specific echocardiographic parameter of left ventricular dilatation, and determination of the prognosis impact of such modification.

Limitations

The present study has some limitations. The tests compared (chest X-ray and echocardiography) were not conducted on the same day, similar to the other tests included in the Rassi score, which were performed on different days. In any case, efforts were made to minimize confounding factors and factors associated with clinical changes, to assure that no change in clinical status or in drug therapy of patients occurred in the interval between the tests. Right atrial dimensions were not measured, thereby limiting the assessment of dilation of this chamber as a discordant factor between echocardiography and chest X-ray. Finally, although patients were not followed longitudinally for the prognostic assessment of LEVDD by echocardiography, they have been followed-up at an outpatient clinic and will be reassessed for this parameter in the future.

Conclusions

LVEDD measured by echocardiography is an appropriate parameter to distinguish between the presence and absence of cardiomegaly on chest X-ray, with high specificity in patients with chronic CD. Substitution of echocardiographic LVEDD for the radiological CTR in the Rassi score was shown to be feasible and did not cause substantial change in the scores obtained. These results open new perspectives to avoid the use of a test that involves radiation and, rather, use a single test (echocardiography) to measure two components of the Rassi score – cardiac dimension and ventricular systolic function. The potential prognostic role of this modified Rassi score is a worthy subject for future studies.

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Author Contributions

Conception and design of the research: Rassi Junior A, Marin-Neto JA; Acquisition of data: Ramos MRF, Moreira HT, Volpe GJ, Romano M; Analysis and interpretation of the data: Ramos MRF, Moreira HT, Volpe GJ, Romano M, Maciel BC, Schmidt A, Marin-Neto JA; Statistical analysis: Moreira HT, Schmidt A; Obtaining financing: Marin-Neto JA; Writing of the manuscript: Ramos MRF, Moreira HT, Maciel BC, Schmidt A, Marin-Neto JA; Critical revision of the manuscript for intellectual content: Moreira HT, Volpe GJ, Romano M, Romano M, Maciel BC, Schmidt A, Rassi Junior A, Marin-Neto JA

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This article is part of the scientific research submitted by Matheus Rassi Fernandes Ramos, from Universidade de São Paulo Faculdade de Medicina de Ribeirão Preto.

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Can Transthoracic Echocardiography Replace Chest Radiography in the Evaluation of Cardiomegaly in Chagas Cardiomyopathy?

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Short Editorial related to the article: Correlation between Cardiomegaly on Chest X-Ray and Left Ventricular Diameter on Echocardiography in Patients with Chagas Disease

Chagas cardiomyopathy (CCM), first described in 1909 by Carlos Chagas,[1] is still associated with high morbidity and mortality and socioeconomic impact, especially in Latin American countries.

The search for epidemiological, clinical, laboratory, electrocardiographic, and image markers associated with its prognosis plays an important role in the risk stratification of these patients.

The cardiothoracic ratio (CR), originally described in 1919 and calculated from chest radiography imaging, is one of the variables associated with mortality in CCM.

Rodriguez-Salas et al.[2] identified CR>0.55 as an independent variable associated with mortality in a study with 960 patients. Notably, the left ventricular end diastolic diameter (LVDD) assessed by transthoracic echocardiogram was not associated with poor prognosis in this study.

Afterwards, Salles et al.[3] detected an association between CR>0.5 and all-cause mortality in a study including 738 patients, but the left ventricular end-systolic diameter measured by transthoracic echocardiogram was an independent variable most strongly associated with all-cause mortality, in addition CCM mortality and sudden death. This finding could be a more accurate reflection of the relationship between the left ventricular systolic dysfunction and poor evolution compared to the morphological aspect of this chamber.

On the other hand, Bestetti et al.[4] identified the LVDD upon transthoracic echocardiogram as an independent predictor of sudden death in a study with 74 patients and showed no association between altered CR and the outcome.

Finally, Rassi et al.[5] conducted a retrospective study with 424 patients and reported CR>0.5 as an independent variable associated with mortality, with a risk ratio of 3.43, higher than the risk ratio related to global or segmental change in left ventricular contractility of 2.46 identified by transthoracic echocardiogram. These findings contributed to the elaboration of the Rassi score, which attributes more points[5] to the cardiomegaly identified by chest radiography than the left ventricular dysfunction assessed by echocardiogram[3] and was validated in an independent cohort. Notably, despite being included as a variable in the study, the LVDD was not associated with the outcome and was not included in the score, which also considers the functional class of heart failure, non-sustained ventricular tachycardia (NSVT), low voltage and male gender.

In the same context, author Ramos et al.[6] evaluated the correlation between CR and LVDD in a group of 58 patients with CCM and their results are presented in the current edition of Arquivos Brasileiros de Cardiologia.

The authors found good accuracy (area under the ROC curve of 0.806) at the expense of high specificity (89%) and moderate sensitivity (64%) for a 60mm LVDD threshold in the detection of cardiomegaly identified by chest radiography.

The replacement of CR by LVDD to define the Rassi score resulted in a reclassification with a change in level in 24% of the patients, most of them with a reduction in risk range.

Although chest radiography exposes the patient to radiation, unlike the echocardiogram, the argument that the replacement of radiography with ultrasound may be beneficial to the patient does not seem justified, since the radiation dose in a radiography of thorax is minimal and its eventual carcinogenic potential in the population included in the study (average of 61 years) is questionable.

It is undeniable that every patient testing positive for Chagas disease and presenting signs/symptoms of cardiac involvement should undergo echocardiography as part of the investigation and risk stratification. A great correlation between CR and LVDD could discard chest radiography, with a practical advantage in routine but most likely without significant impact on cost.

The sensitivity of 64% of the LVDD for diagnosis of cardiomegaly in comparison with the chest radiography detected in the study is concerning, especially if we bear in mind that this variable assumes an importance in the Rassi score that is superior to the global/segmental left ventricular dysfunction, which may lead to a wrong reclassification in lower risk ranges.

The best way to confirm whether the new values of the Rassi score determined by replacing CR with LVDD improves risk stratification will be the follow-up of the cohort, already proposed by the authors.

Additional analyses such as linear regression between CR and LVDD values, Bland-Altmann analysis, kappa agreement coefficient and indexing of LVDD by the body surface can provide interesting data to elucidate the discrepancies between the methods.

Keywords

Chagas, Cardiomyopathy; Heart Failure; Cardiomegaly; Morbimortality; Epidemiology; Socioeconomic Factors; Echocardiography/methods; Radiography, Thoracic/methods.

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In the same scenario, the segmental involvement in CCM may be undersized upon evaluation of the morphology of a three-dimensional and complex structure like a ventricle by means of a simplified measurement such as the LVDD[7] instead of the end-diastolic volume. In fact, previous studies[8,9] identified the increased end-diastolic volume as a variable associated with mortality in CCM.

Even with its own limitations, the CR could possibly better represent the global cardiac involvement by this pathology compared to an isolated assessment of the left ventricle by a two-dimensional method. In CCM, we know that the right ventricle can be affected regardless of the left ventricle[10] and that the right ventricular systolic dysfunction is also associated with prognosis.[11] Thus, gathering the analysis of the right ventricle could improve the accuracy of the transthoracic echocardiogram when it comes to the identification of cardiomegaly in CCM and its association with the prognosis.

If, on one hand, chest radiography still seems to keep its place in the assessment of patients with CCM, further research in echocardiography is promising.

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Acute Hemodynamic Index Predicts In-Hospital Mortality in Acute Decompensated Heart Failure

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Abstract

Background: **The physical examination enables prognostic evaluation of patients with decompensated heart failure (HF), but lacks reliability and relies on the professional's clinical experience. Considering hemodynamic responses to "fight or flight" situations, such as the moment of admission to the emergency room, we proposed the calculation of the acute hemodynamic index (AHI) from values of heart rate and pulse pressure.**

Objective: **To evaluate the in-hospital prognostic ability of AHI in decompensated HF.**

Methods: **A prospective, multicenter, registry-based observational study including data from the BREATHE registry, with information from public and private hospitals in Brazil. The prognostic ability of the AHI was tested by receiver-operating characteristic (ROC) analyses, C-statistics, Akaike's information criteria, and multivariate regression analyses. p-values < 0.05 were considered statistically significant.**

Results: **We analyzed data from 463 patients with heart failure with low ejection fraction. In-hospital mortality was 9%. The median AHI value was used as cut-off (4 mmHg·bpm). A low AHI (≤ 4 mmHg·bpm) was found in 80% of deceased patients. The risk of in-hospital mortality in patients with low AHI was 2.5 times that in patients with AHI > 4 mmHg·bpm. AHI independently predicted in-hospital mortality in acute decompensated HF (sensitivity: 0.786; specificity: 0.429; AUC: 0.607 [0.540–0.674]; p = 0.010) even after adjusting for comorbidities and medication use [OR: 0.061 (0.007–0.114); p = 0.025).**

Conclusions: **The AHI independently predicts in-hospital mortality in acute decompensated HF. This simple bed-side index could be useful in an emergency setting. (Arq Bras Cardiol. 2021; 116(1):77-86)**

Keywords: **Heart Failure; Heart Rate; Blood Pressure; Prognosis; Mortality.**

Introduction

Heart failure (HF) is one of the main reasons for emergency admissions in the Western world.[1] Although previous studies have shown that treatment by a HF specialist can lead to better results, most cases of acute decompensated HF are originally evaluated and managed by emergency physicians[2,3] in facilities with different levels of resource availability.

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Despite recent advances in technology and medical devices, the physical examination remains the cornerstone of the evaluation of patients with HF.[4,5] Physicians evaluate congestion and perfusion from the patient's history and a physical examination, assigning hemodynamic profiles that guide therapy and provide prognostic information in an acute HF setting.[6] Although practical, the physician's assessment of perfusion lacks reliability[7] and depends on clinician experience,[8,9] providing subjective information.[10] Therefore, objective prognostic parameters that can be easily obtained in the emergency room would be useful in the management of acute HF.

Blood pressure and heart rate are parameters that can be easily obtained by any healthcare professional with good reproducibility and accuracy.[11,12] Systolic blood pressure is an independent predictor of in-hospital and post-discharge

outcomes in acute heart failure.[13,14] Additionally, low blood pressure and narrow proportional pulse pressure are markers of low perfusion.[4,6,9]

The relationship between admission resting heart rate and the prognosis of patients with HF is not as straightforward. In fact, the literature is controversial, showing that a high admission heart rate can be related to worse or better prognoses.[15-17] Although low resting heart rates reduce risk in patients with stable chronic HF with reduced ejection fraction (HFrEF),[18,19] the ability to increase heart rate during a "fight or flight" reaction certainly confers good prognosis,[20,21] regardless of the use of beta-blockers.

Acute admission to the emergency room is a stressful situation, expected to elicit autonomic responses that prepare the body to fight or flight.[22] Increases in pulse pressure and heart rate are thus expected in this scenario, augmenting perfusion in skeletal muscles and vital organs.

Based on the physiological hemodynamic responses inherent to "fight or flight" situations, we have proposed the calculation of the acute hemodynamic index (AHI) using heart rate and pulse pressure. Our main hypothesis was that AHI could be an objective in-hospital prognostic parameter to be used in patients with acute decompensated HFrEF. Therefore, we aimed to evaluate the in-hospital prognostic ability of AHI in acute decompensated HFrEF.

Methods

This analysis is based on the I Brazilian registry of HF (BREATHE Registry),[23,24] a cross-sectional, observational acute HF registry with longitudinal follow-up that happened from February 2011 to December 2012. For inclusion in the registry, patients should be over 18 years old and have been admitted with decompensated HF; patients should not have been submitted to a coronary artery bypass graft of percutaneous coronary intervention in the previous month or have been admitted with a sepsis diagnosis. Boston criteria were used for HF confirmation.[25] Participation in the registry did not require any special treatment regimen. Detailed methods, as well as exclusion and inclusion criteria, have been previously described.[24] Data on each patient are available online in individual registration forms.

This study includes the analysis of patients with acute decompensated HFrEF from hospital admission and follow-up until discharge, death, or transfer to another hospital (whichever happened first). The primary endpoint of the study was in-hospital mortality.

All patients in the registry with evidence of left ventricle ejection fraction < 40% were included in the present analysis, except those with missing information (admission heart rate, blood pressure, ejection fraction, or loss of follow-up due to transfer to another hospital). Individuals with pacemaker-controlled heart rhythm were also excluded, as their heart rate was not expected to be autonomic-driven (Figure 1).

Derived variables

Heart rate and systolic and diastolic blood pressure at admission were available from the registry database and were used for calculating derived variables as follows: pulse pressure = systolic blood presssure – diastolic blood pressure; proportional pulse pressure = pulse pressure / systolic blood pressure; AHI= (pulse pressure x heart rate) / 1000.

Ethics

This investigation conforms to the principles outlined in the Declaration of Helsinki. The study was approved by the Hospital do Coração, São Paulo (registry 144/2011) and the Institutional Review Board of each participating institution. All patients signed an informed consent form before enrollment.

Statistical analyses

Initially, a Shapiro-Wilk test was used to verify the normality of data distribution and validate the use of parametric statistics. Continuous variables were reported as means and standard deviations, while categorical variables were reported as proportions. Clinical and demographic data from patients who died during the hospitalization period (deceased) and those who were successfully discharged (alive) were compared using unpaired Student's t-tests or chi-squared tests. A two-sided p-value < 0.05 was considered significant.

After verifying a normal distribution, the 25th, 50th, and 75th percentiles of heart rate and systolic and diastolic blood pressure were used to construct receiver-operating characteristic (ROC) curves using in-hospital mortality as the main outcome. The cut-off value defined for the AHI was its 50th percentile. Sensitivity, specificity, and area under the ROC curve (AUC) were reported for each cut-off value. C-statistics were used to compare the prognostic ability of heart rate and blood pressure cut-off values to the AHI cut-off values.

Regression analyses were performed after verifying for linear relationships, multivariate normality, homoscedasticity, and the absence of multicollinearity and autocorrelation.

Multiple linear regression analyses were performed to test the independent prognostic ability of each significant cut-off value regarding heart rate, blood pressure, and AHI. This analysis included variables with statistical significance according to the previously cited unpaired Student's t-tests or chi-squared tests. As laboratory results were not available for all patients, they were not included in the regression analysis. Akaike's information criterion (AIC)[26] was used to compare multiple regression models. All statistical analyses and graphs were performed using STATA 14.2 (StataCorp, Texas, USA).

Results

The BREATHE registry included 463 patients with HFrEF admitted to emergency services in Brazil (Table 1), with an in-hospital mortality index of 9%. The main reason for decompensation was poor medication adherence (37% of discharged patients vs 31% of deceased patients, $p = 0.75$). Other important causes of decompensation were infection (21% of discharged patients vs 24% of decease patients, $p = 0.17$) and excessive salt or fluid intake (11% of discharhed patients vs 12% of deceased patients, $p = 0.9$).

Deceased patients presented more comorbidities and higher values of heart rate and systolic and diastolic blood

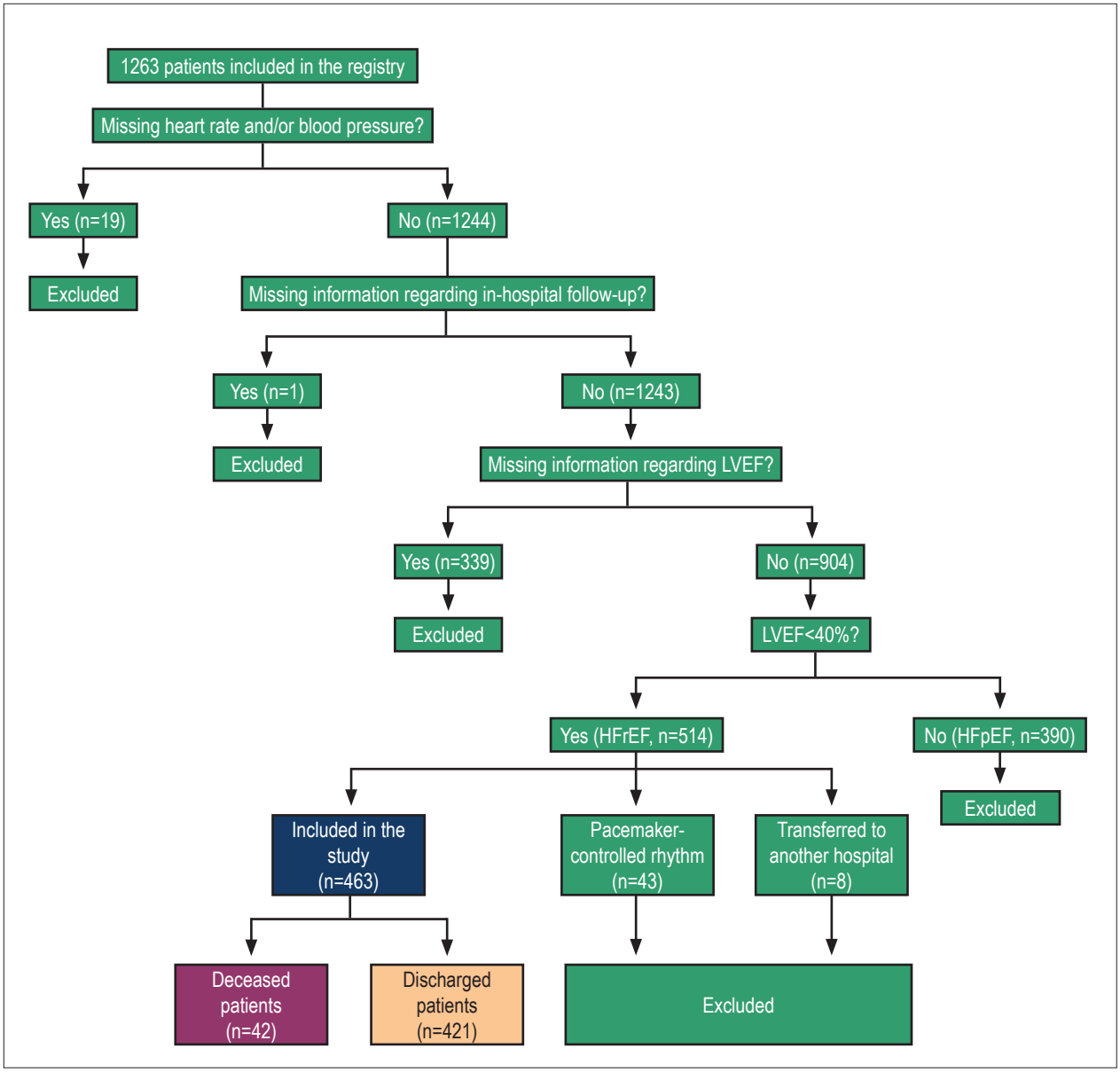

Figure 1 – *Patient selection flowchart. LVEF: left ventricular ejection fraction; HFrEF: heart failure with reduced ejection fraction; HFpEF: heart failure with preserved ejection fraction.*

pressure when compared to survivors. Considering the AHI's 50th percentile, its cut-off value was 4 mmHg·bpm; almost 80% of the deceased patients had a low AHI.

As the AHI calculation included heart rate and blood pressure values, we compared the AUC of AHI ≤ 4 mmHg·bpm as a cut-off value with the AUC of different cut-off values of heart rate and systolic and diastolic blood pressure (Table 2). AHI ≤ 4 mmHg·bpm was a better predictor of in-hospital mortality than heart rate ≤ 88 bpm, but had similar results when compared to prognostic cut-off values of blood pressure. When these hemodynamic prognostic factors were included in multivariate analyses, only AHI kept an independent prognostic ability (Table 3). The regression model including Chagas disease etiology, comorbidities, medications, and AHI showed a better predictive capacity for in-hospital mortality than the other proposed models (Model 0: without AHI; Models 1–4: with hemodynamic parameters added to model 0). Chronic kidney disease and a history of cancer or stroke remained as independent in-hospital mortality predictors in all proposed models. AHI ≤ 4 mmHg·bpm was independently related to in-hospital mortality in this registry even after adjusting for HF etiology, comorbidities, and medication use (Figure 2). Patients admitted with low AHI had a 12.1% chance of dying, which was 250% higher than that for patients with AHI > 4 mmHg·bpm (4.8%, p = 0.008, Figure 3). As this was a registry study, the research protocol did not intervene in the treatment received by patients. Inotropes

Table 1 – Demographic and clinical data of patients with acute decompensated heart failure with reduced ejection fraction

Characteristics	All patients (n = 463)	Discharged patients (n = 421)	Deceased patients (n = 42)	p-value
Demographic				
Age, years ± SD	61 ± 16	61 ± 15	58 ± 17	0.27
Male sex, n (%)	141 (30)	127 (30)	14 (33)	0.67
Heart failure etiology				
Ischemic, n (%)	155 (33)	141 (33)	14 (33)	0.98
Chagas disease, n (%)	53 (11)	43 (10)	10 (24)	0.008
Comorbidities				
Hypertension, n (%)	318 (69)	290 (69)	28 (67)	0.77
Atrial fibrillation, n (%)	109 (23)	101 (23)	8 (19)	0.51
Diabetes mellitus, n (%)	177 (38)	164 (39)	13 (31)	0.31
Chronic kidney failure, n (%)	98 (21)	81 (19)	17 (40)	0.001
Dyslipidemia, n (%)	162 (35)	150 (36)	12 (29)	0.36
Depression, n (%)	52 (11)	50 (12)	2 (5)	0.16
History of stroke, n (%)	56 (12)	46 (11)	10 (24)	0.015
History of cancer, n (%)	18 (4)	14 (3)	4 (9)	0.048
Treatment				
Beta-blocker, n (%)	273 (66)	241 (64)	32 (82)	0.023
ACEi/ARB, n (%)	274 (59)	251 (60)	23 (55)	0.50
Loop/thiazide diuretics, n (%)	311 (67)	277 (66)	34 (81)	0.046
Calcium channel blockers, n (%)	28 (7)	25 (7)	3 (8)	0.80
Digitalis, n (%)	121 (29)	102 (27)	19 (50)	0.005
Spironolactone, n (%)	182 (44)	156 (41)	26 (67)	0.002
Statins, n (%)	139 (33)	127 (34)	12 (31)	0.71
Hemodynamics				
Heart rate, bpm ± SD	90 ± 23	90 ± 23	82 ± 21	0.025
Systolic blood pressure, mmHg ± SD	121 ± 29	122 ± 30	112 ± 26	0.036
Diastolic blood pressure, mmHg ± SD	76 ± 19	77 ± 19	70 ± 14	0.020
Pulse pressure, mmHg ± SD	45 ± 18	45 ± 18	43 ± 18	0.30
Proportional pulse pressure, % ± SD	37 ± 9	37 ± 9	37 ± 8	0.75
AHI, mmHg·bpm ± SD	4 ± 2	4 ± 2	3 ± 2	0.08
AHI < 4 mmHg·bpm, n (%)	273 (60)	240 (57)	33 (79)	0.007
LVEF, % ± SD	27 ± 8	27 ± 8	25 ± 6	0.20
Hemodynamic profile				
A, %	49 (11)	45 (11)	4 (10)	0.81
B, %	311 (67)	288 (68)	23 (55)	0.07
C, %	81 (17)	68 (16)	13 (30)	0.02
L, %	22 (5)	20 (5)	2 (5)	0.99
Laboratory results*				
Hematocrit, % ± SD	40 ± 7	40 ± 6	38 ± 9	0.07
Hemoglobin, g/dL ± SD	13 ± 2	13 ± 2	13 ± 2	0.26
Creatinine, mg/dL ± SD	1.5 ± 0.9	1.5 ± 0.8	1.9 ± 0.9	0.001
Urea, mg/dL ± SD	68 ± 41	65 ± 38	100 ± 50	<0.001
Sodium, mEq/L ± SD	137 ± 13	138 ± 14	136 ± 6	0.51

*ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers; AHI: acute hemodynamic index; LVEF: left ventricular ejection fraction; SD: standard deviation. p-values were obtained in the univariate comparison between both groups. *N = 412.*

Table 2 – Sensitivity, specificity, AUC with 95% CI, and best cut-off values for in-hospital mortality in patients with acute decompensated heart failure with reduced ejection fraction

Proposed prognostic parameters	Univariate analysis				Comparison to AUC for AHI ≤ 4 mmHg·bpm
	Sensitivity	Specificity	AUC (95% CI)	p-value	p-value
AHI ≤ 4 mmHg·bpm	0.786	0.429	0.607 (0.540–0.674)	0.01	----
Heart rate					
≤ 74 bpm	0.309	0.750	0.530 (0.456–0.604)	0.39	----
≤ 88 bpm	0.667	0.513	0.590 (0.514–0.666)	0.03	0.048
≤ 104 bpm	0.857	0.254	0.556 (0.498–0.613)	0.58	----
Systolic blood pressure					
≤ 100	0.452	0.698	0.575 (0.496–0.654)	0.04	0.450
≤ 120	0.738	0.430	0.584 (0.513–0.655)	0.04	0.570
≤ 140	0.905	0.190	0.547 (0.498–0.596)	0.14	----
Diastolic blood pressure					
≤ 60	0.453	0.741	0.596 (0.518–0.676)	0.01	0.830
≤ 73	0.643	0.513	0.578 (0.500–0.655)	0.06	----
≤ 84	0.857	0.257	0.557 (0.499–0.614)	0.11	----

AUC: area under receiver-operating characteristic curves; CI: confidence interval; AHI: acute hemodynamic index.

were used in 11% of discharged patients and 28% of deceased patients ($p < 0.001$).

Discussion

The present study introduced the AHI and demonstrated that it is an independent predictor of in-hospital mortality in patients with acute decompensated HFrEF. In-hospital mortality in patients with acute decompensated HF is high, as shown by this Brazilian registry and by studies conducted in other countries.[27] Different reasons for this high short-term mortality include age, comorbidities, and the delay between symptom onset and hospital admission.[27] Since the management of patients with acute HF may include invasive and high-cost procedures such as circulatory support, it is critical to validate prognostic factors that can help guiding therapeutic decisions.[28]

Acute decompensated HF can be managed by HF specialists, general cardiologists, intensivists, emergency physicians, or internists; this can be performed in emergency departments, hospital wards, or intensive care units.[2] The physician's experience and the available resources can vary substantially. Together with the patients' diversity, these aspects hinder the production of widely applicable prognostic scores. Despite the recent attention received by biomarkers,[29] for example, their verification may not be available in remote or low-income health facilities. Nohria et al.[6] have introduced a practical clinical approach for categorizing patients with hemodynamic profiles, thus enabling prognosis prediction and guiding treatment in acute HF settings. This approach relies on clinician experience[8,9] and may be less useful when considering non-HF specialists. Our results corroborate the lack of accuracy of cardiovascular physical examinations,[9] since 11% of the patients were classified as hemodynamic profile A despite having acute decompensated HF.

Heart rate and blood pressure measurements are available in virtually any healthcare facility with good accuracy and requiring minimal training.[11,12] Previous studies have tried to use blood pressure and heart rate as prognostic factors in acute decompensated HF; the relationship between heart rate and prognosis in heart disease has been known for decades. Since the emergence of therapies using beta-blockers and more recently, ivabradine, low heart rates have been considered a target in the treatment of stable HF.[19] On the other hand, chronotropic incompetence is also a risk marker. Patients whose heart rates do not increase during exercise have worst prognoses than those with normal heart rate reserves, even with the use of beta-blockers.[20,21] Although previous studies have determined the expected increase in heart rate during an exercise test,[20,21] no normality values have been established for heart rate increases during "fight or flight" situations such as the admission to emergency rooms. Japanese patients with acute decompensated HF admitted with heart rates above 120 bpm presented lower mortality indices than those with lower heart rates.[15] Conversely, high heart rate was considered an independent predictor of short-term mortality in patients with acute decompensated HF in other studies.[16,30,31]

The OPTIMIZE-HF[14] registry found that systolic blood pressure values below 120 mmHg characterized patients with acute decompensated HF who had poor prognoses despite medical therapy. Low systolic blood pressure levels also indicated high short-term risk in a European cohort.[13] In our study, blood pressure below 120 mmHg was not independently related to mortality in a multivariate analysis. Patients in the BREATHE registry were younger,

Table 3 – Multivariate models for in-hospital mortality prediction including different non-invasive hemodynamic parameters

	Model 1		Model 2		Model 3		Model 4		Model 5	
AIC	**137.0**		**136.3**		**135.6**		**135.7**		**133.7**	
p-value vs Model 0	**0.294**		**0.183**		**0.113**		**0.116**		**0.035**	
Parameter	**OR 95% CI**	**p**	**OR 95% CI**	**p**	**OR 95% CI**	**p**	**OR 95% CI**	**p**	**OR 95% CI**	**p**
Chagas disease	0.089 0.006–0.171	0.035	0.784 -0.006–0.163	0.071	0.080 -0.003–0.164	0.060	0.777 -0.006–0.162	0.071	0.765 -0.007–0.160	0.072
CKD	0.104 0.041–0.167	0.001	0.104 0.040–0.167	0.001	0.107 0.044–0.170	0.001	0.100 0.037–0.164	0.002	0.112 0.048–0.175	0.001
History of stroke	0.840 0.054–0.163	0.036	0.089 0.011–0.168	0.025	0.093 0.014–0.170	0.021	0.858 0.007–0.164	0.032	0.092 0.013–0.169	0.022
History of cancer	0.143 0.011–0.276	0.033	0.148 0.016–0.281	0.028	0.139 0.007–0.271	0.039	0.139 0.007–0.272	0.038	0.140 0.009–0.273	0.037
Beta-blockers	0.168 -0.40–0.073	0.563	0.196 -0.037–0.076	0.497	0.180 -0.038–0.074	0.531	0.021 -0.035–0.077	0.463	0.172 -0.394–0.073	0.551
Loop and thiazide diuretics	-0.005 -0.066–0.057	0.887	-0.003 -0.065–0.058	0.918	-0.005 -0.066–0.057	0.884	-0.004 -0.065–0.058	0.909	-0.006 -0.068–0.056	0.850
Digitalis	0.053 -0.009–0.115	0.096	0.056 -0.005–0.117	0.073	0.538 -0.007–0.115	0.086	-0.003 -0.007–0.115	0.086	0.515 -0.009–0.113	0.100
Spironolactone	0.540 -0.004–0.112	0.068	0.527 -0.005–0.110	0.075	0.053 -0.004–0.111	0.071	0.053 -0.004–0.111	0.072	0.053 -0.005–0.110	0.074
Heart rate ≤ 88 bpm	0.277 -0.025–0.080	0.627								
Systolic blood pressure ≤ 100 mmHg			0.380 -0.018–0.094	0.188						
≤ 120 mmHg					0.042 -0.010–0.095	0.117				
Diastolic blood pressure ≤ 60 mmHg							0.046 -0.012–0.105	0.121		
AHI ≤ 4 mmHg*bpm									0.061 0.007–0.114	0.025

*AIC: Akaike's information criterion; OR: odds ratio; CI: confidence interval; CKD: chronic kidney disease; AHI: acute hemodynamic index. Model 0 included Chagas disease as heart failure etiology; chronic kidney disease; history of cancer; and home use of beta-blockers, loop and thiazide diuretics, digitalis, and spironolactone. Models 1 to 5 included all variables from model 0 plus another parameter and cut-off value, as follows: Model 1: heart rate ≤ 88 bpm; Model 2: systolic blood pressure ≤ 100 mmHg; Model 3: systolic blood pressure ≤ 120 mmHg; Model 4: diastolic blood pressure ≤ 60 mmHg; Model 5: AHI ≤ 4 mmHg*bpm.*

and treatment protocols were more updated when compared to those used in studies conducted almost a decade earlier. Furthermore, both studies[13,14] included patients with preserved and reduced ejection fraction, and the prognostic value of blood pressure is known to vary according to the left ventricular ejection fraction.[32] Low pulse pressure was defined as an independent predictor of mortality in acute decompensate HF by the VMAC-HF study.[33] Since the publication of this trial, HF therapy has evolved substantially, which may explain the lack of prognostic power of pulse pressure in our patients.

The intrinsic interaction between blood pressure and heart rates and how they are affected by HF medications may have influenced the results of previous investigations on each of these parameters. To our knowledge, this is the first study to introduce an index that analyzes both heart rate and pulse pressure in patients with acute decompensated HF; moreover, we have shown that the prognostic ability of the AHI is higher than that of heart rate or blood pressure alone.

Limitations

The present analysis has limitations. First, in-hospital mortality was based on investigator reports instead of being adjudicated. In fact, registries are observational studies and analyzing the treatment delivered to each patient was not within the scope of our study. As our main objective was to analyze the usefulness of an easily obtained index to be applied as soon as patients arrive in the emergency room, and considering the unavailability of troponin and brain natriuretic peptide (BNP) tests in some Brazilian health facilities, laboratory parameters were not included in the model.

Data in the registry was not obtained by any specific protocol, and blood pressure and heart rate measurements

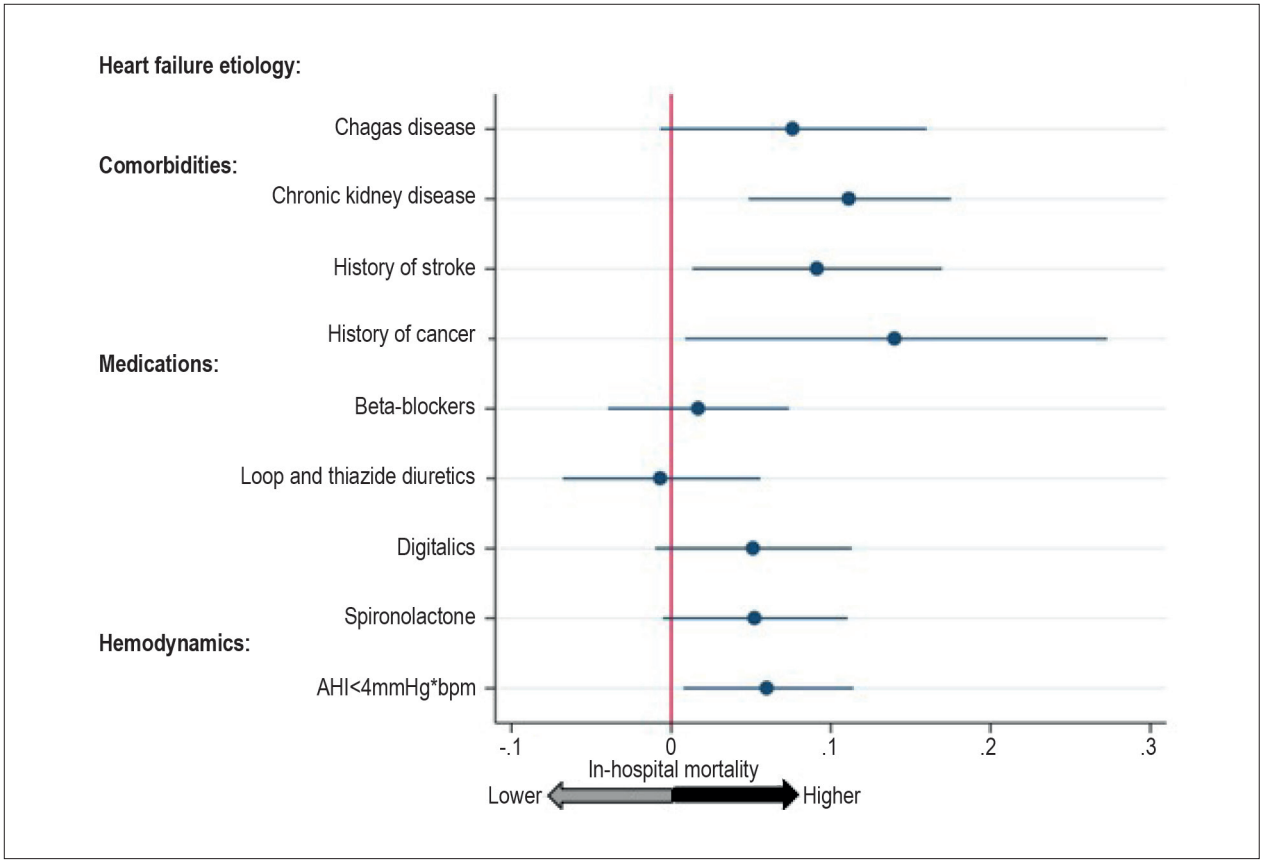

Figure 2 – *Odds ratios according to a multivariate regression model including heart failure etiology, comorbidities, medication use, and acute hemodynamic index (AHI) of patients admitted with acute decompensated heart failure with reduced ejection fraction (n = 463).*

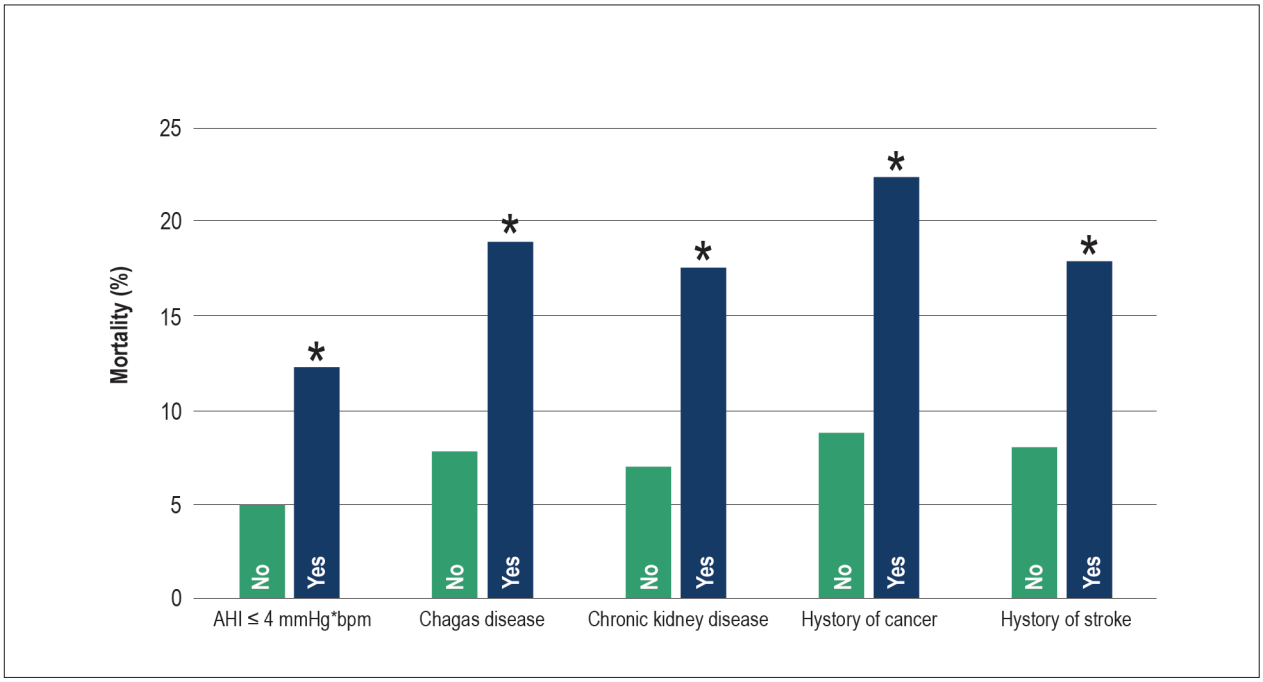

Figure 3 – *In-hospital mortality indices of patients with acute decompensated heart failure with reduced ejection fraction according to the presence of prognostic factors. *p < 0.05 in comparison to "No" within the same prognostic parameter.*

may have been performed using different equipment. Nevertheless, blood pressure and heart rate are vital signs that require minimal training for their measurement.[11,12] Additionally, the fact that the registry had no standardized assessment methods enhances the clinical applicability of our study, as it shows realistic results.

The present results are restricted to patients with HFrEF. The study was conducted from 2011 to 2012, before the approval of new HF medications as ivabradine and salcubitril-valsartan,[19] which could influence AHI values.

The Brazilian population is very diverse regarding ethnicity and access to health care facilities. The study included private and public hospitals in all regions of the country.[23] Although the generalization to other populations may be limited, we highlight that the demographical and clinical data of patients included in this registry are very similar to those of other cohorts.[14,16,30,31]

Finally, the AUC in the ROC analysis of the AHI was relatively low. Nevertheless, its sensitivity was quite good and this may be useful to guide emergency physicians while triaging patients.

Conclusion

Different prognostic factors have been proposed in acute decompensated HF but rely on biomarker measurement, medical staff training, and technology; these may not be widely available. The AHI is a practical, objective, and easily obtained prognostic factor for in-hospital mortality in patients with acute decompensated HF. Further prospective studies should evaluate the reproducibility of these results in other populations.

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Conception and design of the research: Albuquerque DC, Rohde LE, Almeida D, David J, Rassi S, Bacal F, Bocchi E, Moura L; Data acquisition: Lechnewski L, Homero A, Albuquerque DC, Rohde LE, Almeida D, David J, Rassi S, Bacal F, Bocchi E, Moura L; Analysis and interpretation of the data and Statistical analysis: Castro RRT; Writing of the manuscript: Castro RRT, Lechnewski L; Critical revision of the manuscript for intellectual content: Albuquerque DC, Rohde LE, Almeida D, David J, Rassi S, Bacal F, Bocchi E, Moura L.

Potential Conflict of Interest

The authors report no conflict of interest concerning the materials and methods used in this study or the findings specified in this paper.

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This study is not associated with any thesis or dissertation.

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Acute Hemodynamic Index Predicts In-Hospital Mortality in Acute Decompensated Heart Failure

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Short Editorial related to the article: Acute Hemodynamic Index Predicts In-Hospital Mortality in Acute Decompensated Heart Failure

Although acute heart failure (AHF) is associated with significant in-hospital mortality (around 9-11% in concordance with the mortality rate in the BREATHE registry) and high rates of rehospitalization after discharge, options for the management of these patients remain limited.[1]

Since overall survival is mainly determined by the initial management, accurate and early individual risk stratification can help physicians choose the intensity of care required and promote tailored medical decision-making with improvement of prognosis.[2]

The manuscript by Castro et al.[3] provides a simple, bedside tool, to stratify the population of patients with AHF with reduced ejection fraction, based on the calculation of the acute hemodynamic index (AHI) (AHI= $\frac{pulse\ pressure\ x\ heart\ rate}{1000}$) at admission. The authors report that patients with low AHI (≤ 4 mmHg bpm) had an in-hospital mortality that was 2.5 times higher than patients with an higher AHI.

In the present analysis from the BREATHE registry only patients with evidence of left ventricle ejection fraction below 40% were included, contrary to most of the previous publications. Although previous studies, generally based on outpatients with chronic heart failure (HF), have identified a number of variables that are associated with increased mortality, including etiology, patient age, peak oxygen consumption, left ventricular ejection fraction, serum sodium concentration, and B-type natriuretic peptide concentration, several factors have limited the development of similar models in patients with AHF, such as lack of a consistent definition of AHF, incomplete data in administrative data sets, and varying statistical methods. Consequently, unlike acute coronary syndromes, in which several systems have been developed for risk stratification, no clinically practical method of risk stratification exists for patients with AHF.[4]

Results from the American multicenter ADHERE HF Registry identified blood urea nitrogen level, systolic blood pressure (SBP), heart rate (HR), and age as the most significant predictors of mortality in patients with AHF.[1] Others studies have also shown that an increased HR predicts prognosis in patients presenting with HF.[5] Autonomic imbalance resulting from sympathetic overactivity and parasympathetic withdrawal is likely to be the underlying mechanism of increased HR in HF. Several pathophysiologic mechanisms, including increased myocardial oxygen consumption, reduced diastolic filling times, compromised coronary perfusion with induction of myocardial ischemia, and precipitation of rhythm disturbances have been proposed to explain the association between higher HR and worse outcomes.[2] However, it has also been demonstrated that chronotropic incompetence, especially in patients with chronic HF, is associated with reduced functional capacity and poor survival.[6] In the present study an higher HR was not associated with worse outcomes. In fact, patients who died had a mean HR of 82 bpm at admission while those who survived had 90 bpm. Nevertheless, in the multivariate analysis HR was not an independent predictor of mortality. The association between a lower HR and mortality was unexpected and we can speculate that this might be due to the higher prevalence of treatment with digitalis in patients who died, which some studies suggest to be associated with higher mortality, especially in patients with HF and atrial fibrillation.[7]

The finding that low SBP was associated with mortality is also consistent with other studies that have demonstrated the prognostic importance of this parameter, probably because low SBP and narrow proportional pulse pressure are markers of hypoperfusion.[7] The OPTIMIZE-HF[4] registry found that SBP values below 120 mmHg characterized patients with AHF who had poor prognosis despite medical therapy, but in the current study, blood pressure below 120 mmHg was not independently related to mortality in a multivariate analysis. It has been hypothesized that the elevated SBP at admission observed in the majority of AHF patients may be related to neurohormonal and cytokine activation resulting in increased afterload, but the pathophysiology may differ in patients presenting with low SBP and consequently low pulse pressure, who may be more likely to have advanced or end-stage disease with low cardiac output and signs of organ hypoperfusion. It is also reasonable to hypothesize that patients with an elevated SBP may respond more favorably to vasodilators and neurohormonal antagonists. Nevertheless, none of the pharmacologic agents studied in recent trials (vasodilators, inodilators, and calcium sensitizers) has improved clinical outcomes.[5,8]

In addition, most risk estimates have been derived from clinical trial datasets, which may not be representative of broad populations of patients admitted for HF.[1] Also, the number of variables and mathematical functions involved frequently require access to a computer or an electronic calculator to generate a score and to determine risk, making them

Keywords

Heart Failure; Atrial Fibrillation; Stroke Volume; Hemodynamic; Cardiac Output Low; Heart Rate.

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impractical for bedside assessment, and rely on biomarker measurement, medical staff training, and technology that may not be widely available.[4,9] In contrast, HR and BP measurements are available in virtually any healthcare facility with good accuracy and requiring minimal training, which makes AHI a practical, objective, and easily obtained prognostic marker.

Some limitations of this study should be acknowledged. It was an observational study including less than 500 patients, potentially not representative of the whole population of patients with AHF and its findings should be considered hypothesis-generating and subsequently validated in prospective studies in other populations.

The results of registry-based studies, like the BREATHE Registry, may additionally help to define models useful for the design of clinical trials to evaluate HF therapies, since they permit risk to be balanced across treatment groups and allow for selective inclusion criteria in order to enroll only patients at high risk for in-hospital mortality. They also contribute to the development of a clinical risk prediction model for AHF allowing clinicians to be better equipped to optimize in-hospital resource utilization based on patient-specific risk estimates, and additionally therapeutic decisions may eventually be guided by risk estimates as well. Patients estimated to be at a lower risk can be managed with less intensive monitoring and therapies available on a telemetry unit or hospital ward, whereas a patient estimated to be at a higher risk may require more intensive management in an intensive or coronary care unit.[2] Nevertheless, we should bear in mind that these models enhance, but don't replace, physician assessment.

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Cerebrovascular Disease Mortality Trend in Brazil (1996 To 2015) and Association with Human Development Index and Social Vulnerability

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Abstract

Background: **Cerebrovascular diseases (CBVD) are the second major cause of death in the world.**

Objective: **To analyze the mortality trend of CBVD in Brazil (1996 to 2015) and its association with Human Development Index (HDI) and the Social Vulnerability Index (SVI).**

Methods: **This is an ecological study. We analyzed the mortality rate standardized by CBVD. Death data were obtained from the Mortality Information System (SIM) and populational data from the Brazilian Institute of Geography and Statistics (IBGE). The model of regression by inflection points (Joinpoint regression) was used to perform the temporal analysis, calculating the Annual Percent Change (APC) and Average Annual Percent Change (AAPC), with 95% of confidence interval and a significance of 5%. Trends were classified as increasing, decreasing or stationary. A multivariate regression model was used to analyze the association between mortality by CBVD, HDI and SVI.**

Results: **During this period, 1,850,811 deaths by CBVD were recorded. We observed a reduction in the national mortality rate (APC -2.4; p = 0.001). Twenty federation units showed a significant trend, of which 13 showed reduction, including all states in the Midwest (n=4), Southeast (n=4) and South (n=3). The HDI was positively associated and the SVI was negatively associated with mortality (p = 0.046 and p = 0.026, respectively).**

Conclusion: **An unequal epidemiological course of mortality was observed between the regions, being higher in the Southeast and South states, with a significative tendency of reduction, and lower in the North and Northeast states, but with a significative tendency of increase. HDI and SVI showed an association with mortality. (Arq Bras Cardiol. 2021; 116(1):89-99)**

Keywords: **Brain Diseases/mortality; Epidemiology; Community Development; Social Vulnerability; Time Seies Studies; Morbimortality; Stroke/mortality; Emergency Medical Emergencies/organization and administration.**

Introduction

Chronic non-communicable diseases (CNCD) have occupied a prominent place in the epidemiological scenario, representing the biggest global health problem and causing about 38 million deaths annually (70% of all deaths), 16 million of which are considered premature (age < 70 years).[1] In Brazil, approximately 75% of deaths are caused by CNCD, which represents more than 1 million deaths each year.[2]

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The CNCD group consists of four subgroups: cardiovascular diseases (CVD), cancer, chronic respiratory disease and diabetes mellitus. Among the CVD, cerebrovascular diseases (CBVD) stand out, being the second leading cause of mortality in the world, behind ischemic heart diseases. Together, they were responsible for 15.2 million deaths in 2016.[1,3]

Of the Latin American countries, Brazil has one of the highest mortality rates due to CBVD. In the last decades, there was a significant increase in the number of deaths, from 104,000 in 1990 to 144,000 in 2015. On the other hand, the country has experienced a reduction in mortality rates, especially regarding early mortality, which decreased from 51.4% in 1990 to 35.1% in 2015.[4]

The impact of CBVD on morbidity and mortality is a challenge for the economic and social development of nations, especially in developing countries, which concentrate about 80% of all CBVD deaths.[1,5] Monitoring

the temporal behavior of indicators in Brazil, a country of continental dimensions and with important socio-spatial inequalities, is of fundamental importance for the definition of public policies that can impact the population's health situation.[6]

In this sense, this study aimed to analyze the trend of mortality from CBVD in Brazil (1996-2015) and its association with the Human Development Index (HDI) and the Social Vulnerability Index (SVI).

Methods

Study design, population and period

This is an ecological study involving all CBVD deaths that occurred in Brazil from 1996 to 2015 and the HDI and SVI. The entire country, the country regions and the federation units were adopted as the analysis units.

Variables

We analyzed the following sociodemographic variables: gender (male, female and unknown), age groups – in years (0-4, 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, 50- 59, 60-69, 70-79, 80 and over and unknown age), education - in years (illiterate, 1-3, 4-7, 8-11, 12 or more and unknown education) and marital status (single, married, widowed, divorced, other and unknown marital status). For the time series analysis, the variable mortality rate standardized by age and gender due to CBVD was included. For the association component, two social indices were selected: i) the HDI and its three dimensions (longevity, education and income) and ii) SVI and its three dimensions (urban infrastructure, human capital and income and work). These two indices measure, respectively, the degree of human development and the degree of social vulnerability to which a population is exposed.

Data source and data collection

Death data were collected from the Ministry of Health's Mortality Information System (SIM) (http://datasus.saude.gov.br/).[7] The International Disease Code (ICD-10) I60 to I69 was considered: I60- Subarachnoid hemorrhage; I61- Intracerebral hemorrhage; I62- other non-traumatic intracranial hemorrhages; I63- Cerebral infarction; I64- Stroke not specified as hemorrhagic or ischemic; I65- Occlusion / stenosis of pre-cerebral arteries that do not result in cerebral infarction; I66- Occlusion / stenosis of cerebral arteries that do not result in cerebral infarction; I67- Other cerebrovascular diseases; I68- Cerebrovascular disorders in diseases classified elsewhere; and I69- Sequelae of cerebrovascular diseases.[8] The population data necessary to calculate the indicators were obtained from the Brazilian Institute of Geography and Statistics (IBGE).[9]

To obtain the rates the following equations were used:

a) Annual mortality rate: number of deaths due to CBVD in the local and year /local population and year X 100,000 inhabitants;

b) Mortality rate for the period (1996-2015): mean number of deaths from CBVD of the time series (1996-2015)/population median of the time series (population mean of 2005 and 2006) x 100.000 inhabitants.

Finally, the HDI was obtained from the human development atlas (http://atlasbrasil.org.br/2013/) and the SVI from the social vulnerability atlas (http://ivs.ipea.gov.br/index.php/pt/), based on the year 2010. It should be noted that the HDI and SVI data are only calculated in the census years.

Standardization of mortality rates

In order to reduce the effects of the population-demographic structure, the crude rates were standardized by gender and age using the direct method, considering the Brazilian population in 2010 (census year) as the standard population and the following age groups: 0-4, 5 -9, 10-14, 15-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79 and 80 or more.

Statistical analysis

For the temporal analysis, the inflection point regression model (joinpoint regression model) was used. The model tests whether a line with multiple segments is more adequate to explain the temporal behavior of a data set when compared to a straight line or one with fewer segments. Therefore, the joinpoint allows identifying the trend of each indicator (whether stationary, increasing or decreasing), the points in time in which there is a change in this trend (joins), as well as the annual percentage variation (APC- Annual Percent Change) and the total period (AAPC- Average Annual Percent Change).[10] In the model configuration, the following parameters were adopted: minimum number of joins: zero; maximum number of joins: three; selection of the best model: Monte Carlo permutation test (n = 4499 permutations); error autocorrelation method: method based on date; confidence interval: 95% (95% CI); and significance level: 5%.

For the analysis of the association between social indicators and the standardized mortality rate, the multivariate regression model (OLS - Ordinary Least Square) was adopted.

For the analyses, the software Joinpoint Regression 4.5.0.1 (National Cancer Institute. USA), GeoDa 1.10.0.8 (University of Illinois at Urbana-Champaign, USA) and QGis 2.14.11 (Open Source Geospatial Foundation, USA) were used. The territorial meshes necessary for making the maps came from IBGE.

Ethical aspects

This study used secondary data in the public domain, in which it is not possible to identify the subjects. For this reason, the local Research Ethics Committee approval has been waived.

Results

Between 1996 and 2015, 1.850.811 deaths due to CBVD were recorded in Brazil, resulting in a mean of 92.540 cases/year. Of this total, 50.68% (n = 938.044) occurred in males and 77.80% (n = 1.440.170) in elderly people. The age group of 80 years or over was the only age group with a higher proportion of females than males. There was a high rate of low level of

education: 39.94% (n = 739.233) were illiterate or had up to three years of schooling. In this variable, a high proportion of unknown fields was observed (38.29% / n = 708.685) (Table 1).

When analyzing the time series, the mortality rate due to CBVD in the Brazilian population, considering both genders, showed a linear trend of reduction (APC -2.4%; 95% CI -2.7 to -2.0; p = 0.001), from 72.3/100,000 (1996) to 46.4/100,000 (2015). Similar behaviors were observed in the male population (APC -2.3%; 95% CI -2.6 to -1.9; p = 0.001) and in the female population (APC -2.4%; 95% CI -2.8 to -2.0; p = 0.001), of which rates decreased from 77.8 and 71.4/100.000 to 51.1 and 45.2/100.000, respectively (Figure 1).

Figure 1 shows that the spatial distribution of the mean rates is heterogeneous, being higher in the Southeast and South states and lower in the North states. The highest overall mean rates were observed in the states of Paraná (75/100,000) and Espírito Santo (71.3/100,000) and the lowest in the states of Rio Grande do Norte (40.9/100,000) and Bahia (48.0/100,000). The same scenario was observed for male mortality (Paraná with 83.4/100,000 and Espírito Santo with 79.8/100,000). In the female population, the highest rates were observed in Paraná (71.2/100,000) and Rio Grande do Sul (69.2/100,000) and the lowest in Rio Grande do Norte (40.7/100,000) and Bahia (49.1/100,000).

The trend in mortality rates was also analyzed considering the complete time series (1996-2015). The North region was the only one that showed a tendency towards an increase in mortality in the general population (APC 0.4%; 95% CI 0.1 to 0.8; p <0.001) and in the male population (APC 0.7%; 95% CI 0.3 to 1.1; p <0.001). The Midwest, Southeast and South regions showed a decreasing trend, both in the general population and in the male and female populations. The Southeast region showed the highest percentage of reduction in the time series (APC 3.8%) (Table 2).

In the stratified analysis by federation unit, 20 states showed significant trends, 7 showed increasing trends and 13 decreasing trends. All states in the Midwest, South and Southeast regions showed decreasing trends, with emphasis on Rio de Janeiro and Santa Catarina, with the highest reduction percentages. On the other hand, 5 of the 7 states with increasing trends are located in the northeast region (Maranhão, Piauí, Paraíba, Alagoas and Sergipe) and two in the north (Amazonas and Tocantins) (Table 2).

Only the Federal District was classified with very high HDI (HDI 0.824). All states in the Northeast and five in the North had medium HDI (between 0.600 and 0.699), with Alagoas and Maranhão standing out with the lower values (HDI 0.631 and 0.639, respectively). In parallel, these same states in the North and Northeast regions had the highest values in the SVI, especially Maranhão with very high SVI (SVI 0.521). All eight states classified as showing high social vulnerability are located in the North (n = 4) and Northeast (n = 4) regions (Figure 2).

The temporal regression model showed the states of the North and Northeast regions with the largest number of segments in the time series (joins), representing greater oscillation in rates over the years. The mortality rate in the Northeast showed four time segments: slight growth (1996-2003), stationary behavior (2003-2006), downward trend (2006-2010) and again a stationary behavior (2010-2015). Among the states in this region, only Bahia showed a linear behavior (Table 3).

Finally, the regression model showed a positive association between the mean mortality rate and the Municipal Human Development Index (p = 0.046), with the income dimension (p = 0.029), and a negative association with the general SVI (p = 0.026) and also in two dimensions: human capital (p = 0.046) and income and work (p = 0.018) (Table 4).

Discussion

Brazil has one of the highest mortality rates due to CBVD among the countries of Latin America and much higher than those observed in developed nations.[11] However, a temporal decline behavior has been observed over the last decades,[1] in the male and female populations, corroborating the national and international literature.[12-15]

Several authors have emphasized that such reduction in mortality can be explained by the expansion of access to health services and the adoption of prevention strategies.[14,15] In Brazil, the implementation of primary health care (PHC) stands out. The Family Health Strategy (FHS) develops actions to control risk factors, such as encouraging physical activity and adopting healthy eating habits, smoking control programs, diagnosis and systematic monitoring of chronic conditions (hypertension and diabetes, for example) and access to pharmaceutical assistance.[16,17] Between 1998 and 2017 there was a significant increase in the number of family health teams, going from approximately 2.000 to 41.000, reaching a coverage of 70% of the Brazilian population, which corresponds to approximately 143 million people.[17,18] Studies showed an association between the expansion of primary care and the reduction of mortality from diseases such as acute myocardial infarction and cerebrovascular diseases.[19]

In addition to PHC, Brazil has also advanced in the care of patients with CBVD. In 1997, the first stroke unit was implemented in Brazil, located in Joinville/SC. Based on this experience, in 2008, the Ministry of Health started the organization of the national stroke care network, resulting in Ordinance number 665/2012, with the purpose of implementing stroke referral services across the country.[20,21]

Another important action is the Strategic Action Plan for Confronting Chronic Noncommunicable Diseases (NCDs). Implemented in 2011 by the Ministry of Health, the plan established a set of goals for the country, such as the reduction of premature mortality due to NCDs, the prevalence of smoking and alcohol consumption in the population, an increase in the prevalence of physical activity and fruit consumption and containment of obesity increase.[22]

In the regional analysis, we found a heterogeneous behavior in the pattern of mortality from CBVD in the country, corroborating other studies.[4,23] Mortality rates were higher in the Southeast and South, but with a significant decreasing trend. In contrast, the North and Northeast regions had the lowest rates, but with a significant increasing trend over the historical series. This heterogeneous epidemiological-spatial context is the result of social, economic, demographic and epidemiological differences between the regions. Because of this, the results must be analyzed from the perspective of three dimensions: i) demographic and epidemiological transition; ii) social determinants of health and iii) quality of information systems.

Table 1 – Sociodemographic characterization of deaths due to Cerebrovascular diseases (CBVD), according to gender. Brazil, 1996-2015

Variables	Male n= 938044 (50.68%)		Female n= 912202 (49.29%)		Unknown n= 565 (0.03%)		Total of Deaths n= 1850811 (100%)	
	n	%	n	%	n	%	n	%
Age range								
0-4	1012	55.95	793	43.83	4	0.22	1809	1.00
5-9	565	53.25	496	46.75	0	0.00	1061	0.06
10-14	999	54.44	834	45.45	2	0.11	1835	0.10
15-19	1998	55.27	1616	44.70	1	0.03	3615	0.20
20-29	7158	52.66	6426	47.27	10	0.07	13594	0.73
30-39	21278	50.09	21186	49.87	17	0.04	42481	2.30
40-49	62652	50.98	60217	48.99	37	0.03	122906	6.64
50-59	124934	56.74	95185	43.23	65	0.03	220184	11.90
60-69	200551	57.92	145578	42.05	106	0.03	346235	18.71
70-79	268228	53.12	236627	46.85	135	0.03	504990	27.28
80 and over	246717	41.89	342104	58.09	124	0.02	588945	31.81
Unknown age	1952	61.85	1140	36.12	64	2.03	3156	0.17
Years of study								
Illiterate	162163	42.91	215672	57.07	90	0.02	377925	20.42
1-3 years	192038	53.15	169257	46.84	13	0.01	361308	19.52
4-7 years	126285	53.86	108156	46.13	11	0.01	234452	12.67
8-11 years	54461	54.17	46075	45.83	6	0.01	100542	5.43
12 years and more	29083	57.64	21369	42.35	4	0.01	50456	2.73
1-8 years *	4868	53.92	4158	46.06	2	0.02	9028	0.49
9-11 years *	4551	54.08	3860	45.87	4	0.05	8415	0.45
Unknown	364595	51.45	343655	48.49	435	0.06	708685	38.29
Marital status								
Single	163672	47.22	182828	52.75	87	0.03	346587	18.73
Married	499651	67.31	242565	32.67	143	0.02	742359	40.11
Widowed	152794	28.22	388484	71.76	124	0.02	541402	29.25
Divorced	40958	59.29	28116	40.70	6	0.01	69080	3.73
Other	12235	64.02	6875	35.97	1	0.01	19111	1.03
Unknown	68734	51.96	63334	47.88	204	0.16	132272	7.15

** Different grouping of years of study occurred because of changes in the death certificate in 2011.*

Since the 1940s, Brazil has going through important demographic changes: a reduction in the overall mortality rate and a decline in birth rates have resulted in major changes in the demographic regime and in the age structure of the population, with a significant increase in the number of elderly individuals.[24] In 2000, this population was just over 14.2 million, increasing to 19.6 million in 2010, and is expected to reach 41.5 million by 2030,[25] with a greater concentration in the Southeast and South regions. The impact of the population aging process on the pattern of morbidity and mortality is significant, since it implies an increase in chronic diseases,[26] among which CBVD stand out. In our study, 77.8% of deaths occurred among the elderly.

Studies indicate that the risk of mortality from CBVD in the elderly population is substantially higher than in other age groups. One reason is the accumulation of risk factors, such as hypertension, diabetes, dyslipidemia, alcoholism, smoking and inappropriate eating habits.[27,28] In Brazil, for example, the prevalence of hypertension can affect 68% of the elderly population.[29]

Furthermore, the demographic transition process occurs concurrently with a second transition, the epidemiological one, characterized by changes in the population's illness profile.[30] In the last decades, there has been a decline in infectious and parasitic diseases and an increase in the

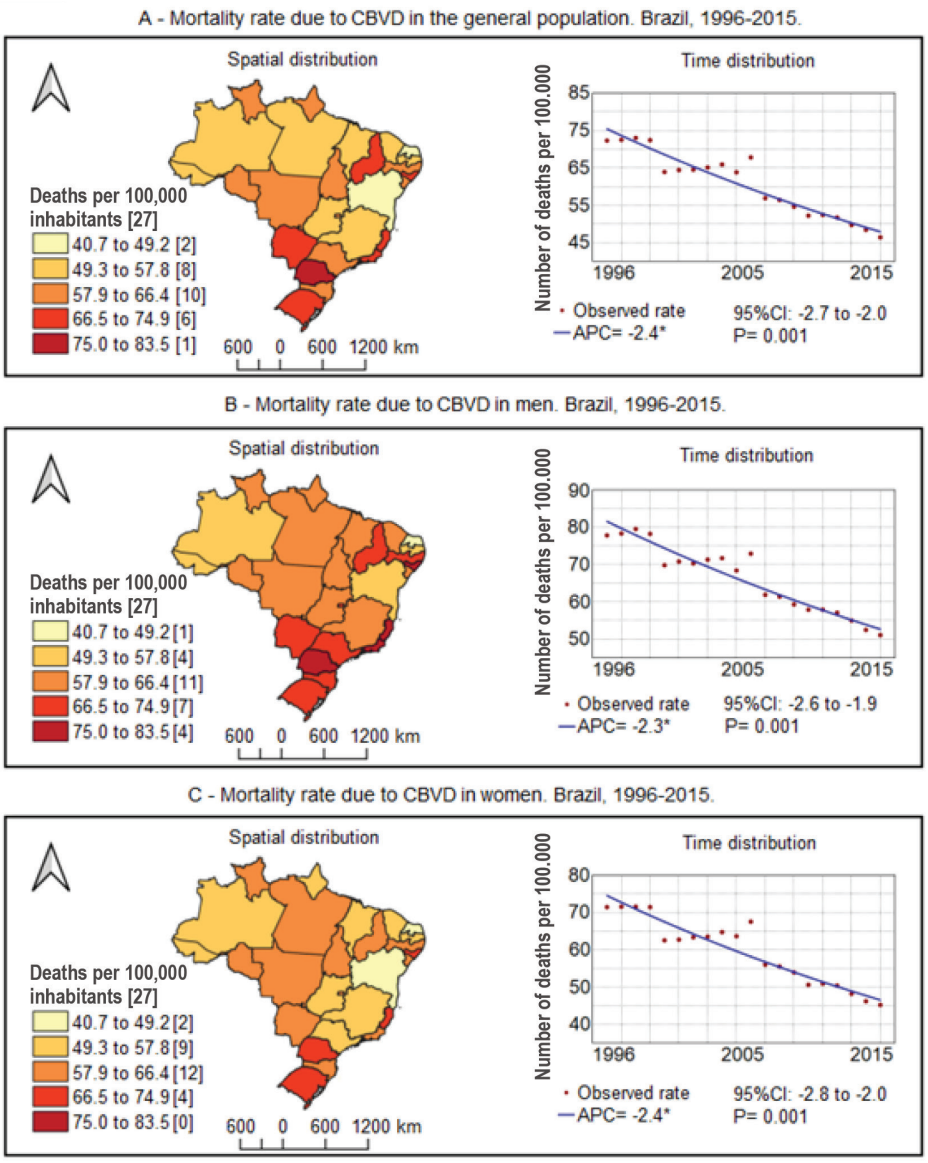

Figure 1 - *Spatial distribution and trend of mortality rates standardized by Cerebrovascular diseases (CBVD) in Brazilian states, all population and according to gender. Brazil, 1996-2015. APC: Annual Percent Change; hab.: inhabitant; nº: number; 95%CI: 95% Confidence Interval; CBVD: Cerebrovascular diseases.*

occurrence of chronic-degenerative diseases, many of which increase the risk of mortality from CBVD.[27] The North and Northeast regions are the most exposed to social vulnerability and show the lowest human development index, resulting in higher mortality from diseases related to an unfavorable social context and less from CBVD. In contrast to what was observed in the most developed regions of the country (Southeast and South). In this sense, the higher rates observed in the more developed states reflect social differences, and, consequently, greater participation of chronic conditions in the mortality profile. On the other hand, more vulnerable regions may have lower rates due to the persistence of mortality due to diseases related to poverty.[32]

The two transitions do not occur homogeneously in Brazil, with a mismatch between regions.[31] This phenomenon explains, in parts, the differences between Brazilian regions regarding CBVD mortality. This scenario justifies the positive association between CBVD mortality and human development and its negative association with social vulnerability, which represents the influence of the epidemiological and social context on the population's mortality profile.

However, the isolated analysis of the rates is not enough to understand the epidemiological dynamics of CBVD. In the North and Northeast regions, in general, the rates showed a temporal pattern of growth and, in the Southeast and South

Table 2 – Percentage of Average Annual Variation (PAAV) of mortality rates standardized by Cerebrovascular diseases (CBVD), according to gender, in Brazil, regions and federation units. 1996-2015

Spatial Unit	Both genders			Male			Female		
	Rate[1]		AAPC (CI 95%) p value	Rate[1]		AAPC (CI 95%) p value	Rate[1]		AAPC (CI 95%) p value
	1996	2015		1996	2015		1996	2015	
North	50.5	58.6	0.4* (0.1 to 0.8); p<0.001	51.0	61.6	0.7* (0.3 to 1.1); p<0.001	52.6	58.6	0.1 (-0.3 to 0.5); p=0.6
RO	68.2	51.4	-1.8* (-2.2 to -1.8); p<0.001	65.9	53.3	1.6* (-2.3 to -1.0); p<0.001	74.2	51.5	-1.9*(-2.3 to -1.4); p<0.001
AC	57.4	63.1	-0.1 (-1.9 to 1.7); p=0.9	59.5	62.7	0.4 (-2.3 to 3.1); p=0.8	58.7	66.9	0.4 (-4.3 to 5.4); p=0.9
AM	49.7	56.1	0.6* (0.2 to 1.1); p<0.001	49.7	58.0	0.9* (0.2 to 1.5); p<0.001	52.7	57.3	0.4 (-0.1 to 1.0); p=0.1
RR	75.5	46.8	-2.2* (-3.0 to -1.3); p<0.001	92.0	50.7	-2.0* (-3.6 to -0.4); p<0.001	56.4	44.2	-2.3*(-3.3 to -1.3); p<0.001
PA	46.3	61.8	1.2 (-0.8 to 3.4); p=0.2	45.7	66.0	1.9 (-0.3 to 4.1); p=0.1	49.5	60.8	0.1 (-2.0 to 2.2); p=0.9
AP	79.5	49.2	-1.7 (-7.8 to 4.9); p=0.6	77.1	54.3	-0.8 (-4.8 to 3.5); p=0.7	86.2	46.6	-1.2 (-3.8 to 1.5); p=0.4
TO	43.4	59.3	1.9* (0.9 to 2.9); p<0.001	48.0	60.1	1.4 (-1.3 to 4.3); p=0.3	40.0	60.7	2.3* (0.7 to 3.9); p<0.001
Northeast	45.4	54.4	0.9 (-0.7 to 2.4); p=0.3	46.8	60.7	1.3 (-0.3 to 2.9); p=0.1	46.7	52.7	0.6 (-1.1 to 2.3); p=0.5
MA	29.0	68.2	4.6* (2.0 to 7.4); p<0.001	31.7	76.6	4.7* (2.5 to 7.0); p<0.001	27.2	64.8	4.3* (1.6 to 7.0); p<0.001
PI	33.3	76.9	3.9* (2.9 to 4.8); p<0.001	35.0	90.4	4.2* (2.9 to 5.5); p<0.001	33.0	70.3	4.0* (3.0 to 4.9); p<0.001
CE	42.0	55.1	1.3 (-0.2 to 2.8); p=0.1	43.5	62.6	1.7* (0.2 to 3.2); p<0.001	42.8	52.1	0.7 (-0.2 to 1.6); p=0.1
RN	33.0	38.0	0.9 (-0.1 to 1.8); p=0.1	34.4	43.7	1.3* (0.2 to 2.5); p<0.001	33.0	35.6	-0.4 (-0.8 to 1.5); p=0.5
PB	37.5	48.5	1.7* (0.4 to 3.0); p<0.001	39.0	52.2	1.9* (0.3 to 3.4); p<0.001	38.4	48.6	1.4 (-0.1 to 2.8); p=0.1
PE	64.8	58.0	-0.8 (-2.2 to 0.6); p=0.3	68.1	66.6	-0.4 (-1.0 to 0.2); p=0.2	65.9	55.2	-1.1 (-2.6 to 0.4); p=0.1
AL	55.5	69.3	0.8* (0.2 to 1.5); p<0.001	57.8	77.7	1.2* (0.6 to 1.8); p<0.001	57.1	66.9	0.5 (-0.5 to 1.5); p=0.3
SE	41.8	57.6	1.7* (1.0 to 2.3); p<0.001	45.7	64.7	1.9* (1.0 to 2.9); p<0.001	40.2	55.6	1.5* (0.5 to 2.5); p<0.001
BA	47.7	45.2	-0.0 (-0.6 to 0.5); p=0.9	47.4	47.5	0.2 (-0.3 to 0.8); p=0.4	51.0	46.2	-0.2 (-0.8 to 0.3); p=0.4
Midwest	69.5	46.3	-2.8* (-3.4 to -2.2); p<0.001	72.2	49.2	-2.7* (-3.3 to -2.2); p<0.001	69.1	46.2	-2.3 (-4.8 to 0.2); p=0.1
MS	76.9	52.9	-2.4* (-2.9 to -2.0); p<0.001	83.9	54.5	-2.4* (-3.0 to -1.8); p<0.001	73.1	54.5	-2.4* (-2.8 to 1.9); p<0.001
MT	65.7	44.2	-1.9* (-3.0 to -0.8); p<0.001	66.3	45.6	-2.1* (-3.5 to -0.6); p<0.001	67.6	44.8	-2.5*(-3.2 to -1.9); p<0.001
GO	64.2	46.2	-2.2* (-2.6 to -1.8); p<0.001	66.0	49.7	-1.6* (-2.7 to -0.4); p<0.001	65.1	45.6	-2.2 (-2.6 to -1.8); p<0.001
DF	81.3	41.6	-4.0* (-4.6 to -3.5); p<0.001	91.6	46.4	-4.0* (-4.6 to -3.4); p<0.001	77.2	40.5	-3.4 (-5.4 to -1.3); p<0.001
Southeast	86.1	41.4	-3.8* (-4.1 to -3.4); p<0.001	96.3	45.8	-3.8* (-4.2 to -3.5); p<0.001	82.3	40.2	-3.8*(-4.2 to -3.4); p<0.001
MG	74.1	39.3	-3.2* (-3.5 to -2.9); p<0.001	81.0	41.5	-3.3* (-3.7 to -3.0); p<0.001	71.5	39.5	-3.0*(-3.3 to -2.8); p<0.001
ES	98.4	46.7	-3.6* (-4.3 to -2.9); p<0.001	108.7	51.7	-3.5* (-4.0 to -3.1); p<0.001	94.2	45.1	-3.5*(-4.2 to -2.8); p<0.001
RJ	101.6	42.3	-4.5* (-5.3 to -3.7); p<0.001	113.8	47.9	-4.3* (-4.9 to -3.7); p<0.001	97.7	40.7	-4.5*(-5.8 to -3.2); p<0.001
SP	84.3	41.7	-3.8* (-4.2 to -3.4); p<0.001	95.8	46.7	-3.8* (-4.0 to -3.5); p<0.001	79.4	40.1	-3.8*(-4.0 to -3.5); p<0.001
South	91.0	45.9	-3.7* (-4.1 to -3.2); p<0.001	96.9	49.5	-3.7* (-4.1 to -3.2); p<0.001	91.3	45.6	-3.6*(-4.0 to -3.2); p<0.001
PR	98.9	49.9	-3.8* (-4.1 to -3.6); p<0.001	108.0	55.3	-3.8* (-4.1 to -3.5); p<0.001	95.5	48.0	-3.8*(-4.1 to -3.5); p<0.001
SC	89.1	37.7	-4.4* (-4.8 to -4.0); p<0.001	94.4	39.7	-3.9* (-5.9 to -2.0); p<0.001	89.5	38.2	-4.3*(-4.7 to -3.9); p<0.001
RS	86.1	46.5	-3.1* (-4.2 to -1.9); p<0.001	88.9	49.4	-3.0* (-3.6 to -2.3); p<0.001	89.3	47.0	-3.4*(-4.1 to -2.6); p<0.001

**Statistical significance(p<0.05); [1] – Mortality rate/100.000 inhabitants; AAPC: Average Annual Percent Change; RO: Rondônia; AC: Acre; AM: Amazonas; RR: Roraima; PA: Pará; AP: Amapá; TO: Tocantins; MA: Maranhão; PI: Piauí; CE: Ceará; RN: Rio Grande do Norte; PB: Paraíba; PE: Pernambuco; AL: Alagoas; SE: Sergipe; BA: Bahia; MG: Minas Gerais; ES: Espírito Santo; RJ: Rio de Janeiro; SP: São Paulo; PR: Paraná; SC: Santa Catarina; RS: Rio Grande do Sul; MS: Mato Grosso do Sul; MT: Mato Grosso; GO: Goiás; and DF: Distrito Federal.*

regions, a decline was observed. These findings reflect the influence of social determinants of health on the pattern of mortality from CBVD. Socioeconomic conditions, including human development, income status and educational situation, have a significant influence on the risk of an individual dying from this group of diseases.[4,6,33-35]

A recent study of the Global Burden of Disease showed that Brazilian states located at the lower tertile of the Social Development Index showed lower reductions in mortality rates, when compared to states located in the upper tertile of development. The lower tertile comprised only states in the North and Northeast regions.[4] It is suggested that better

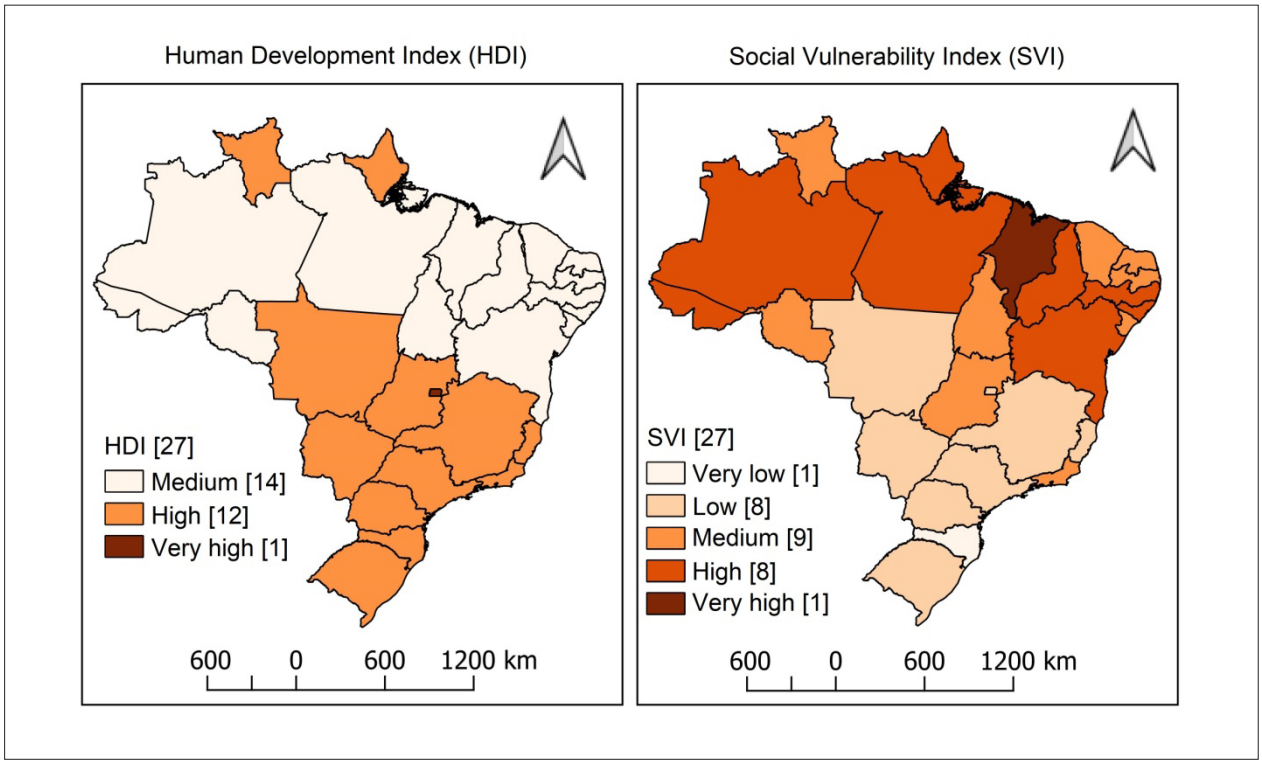

Figure 2 – *Spatial distribution of the Human Development Index (HDI) and the Social Vulnerability Index (SVI) in Brazilian states. Brazil, 2010.*

living conditions have a dual influence on the mortality trend: i) reduce risk factors for the occurrence of disease events; and ii) contribute to patient survival when such events occur, reducing the chance of death.

Finally, it is necessary to reflect on the quality of the mortality records. It is a challenge to adequately monitor the population's health conditions. The inadequate filling out of the death certificates, resulting in a high number of garbage codes, the difficulties in carrying out epidemiological investigations with undefined recorded deaths and the lack of trained human resources to act in the death surveillance services are common problems evidenced throughout the country, although the North and Northeast regions are the most affected by the problem.[36,37] The dubious quality of the information is an important limitation of this study.

Between 1996 and 2005, the percentage of deaths with undefined causes in these regions was higher than 20%, being even higher in the elderly population when compared to other age groups.[38] In this sense, mortality rates in the North and Northeast, for example, may be higher than the ones we disclosed in this study. On the other hand, it is necessary to highlight that in recent years, important advances in the quality of information have been observed in these regions.[13]

Conclusion

Mortality from CBVD in Brazil shows an irregular epidemiological behavior across the regions. The highest rates were observed in states with a better human development index and less social vulnerability, but with a decreasing trend over the time series. On the other hand, in less developed states and with greater vulnerability, the rates were lower, but with an upward trend. In this sense, we recommend that public policies should be developed considering the regional/local context.

Author contributions

Conception and design of the research: Souza CDF, Santos CD, Pereira MC, Paiva JPS, Leal TC, Silva LF, Araújo AKBF; Acquisition of data: Souza CDF, Silva LF, Mariano RS, Paiva JPS; Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Souza CDF, Santos CD, Pereira MC, Paiva JPS, Leal TC, Silva LF, Araújo AKBF, Baggio JAO, Oliveira DJ, Mariano RS; Statistical analysis: Souza CDF, Silva LF.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

Table 3 – Percentage of annual variation in mortality rates standardized by Cerebrovascular diseases (CBVD), according to gender. Brazil. 1996-2015

Spatial unit	Both genders		Male		Female	
	Period	AAPC (95% CI) p value	Period	AAPC (CI 95%) p value	Period	AAPC (95% CI) p value
North	1996-2015	0.4* (0.0 to 0.8); p<0.001	1996-2015	0.7* (0.3 to 1.1); p<0.001	1996-2015	0.1 (-0.3 to 0.5); p=0.6
RO	1996-2015	-1.8*(-2.2 to -1.8) ; p<0.001	1996-2015	1.6* (-2.3 to -1.0); p<0.001	1996-2015	-1.9* (-2.3 to -1.4); p<0.001
AC	1996-1999	-16.2 (-30.7 to 1.4); p=0.1	1996-2002	-5.6 *(-9.4 to -1.7); p<0.001	1996-1999	-16.2 (-30.7 to 1.4); p=0.1
	1999-2006	9.9* (3.1 to 17.2); p<0.001	2002-2006	13.2* (0.5 to 27.5); p<0.001	1999-2006	9.9* (3.1 to 17.2); p<0.001
	2006-2011	-6.4 (-17 to 5.6); p=0.2	2006-2015	-0.8 (-2.9 to 1.4); p=0.4	2006-2011	-6.4 (-17.0 to 5.6); p=0.2
	2011-2015	7.1 (-5.0 to 20.8); p=0.2			2011-2015	7.1 (-5.0 to 30.8); p=0.2
AM	1996-2015	0.6* (0.2 to 1.1); p<0.001	1996-2015	0.9* (0.2 to 1.5); p<0.001	1996-2015	0.4 (-0.1 to 1.0); p=0.1
RR	1996-2015	-2.2* (-3.0 to -1.3); p<0.001	1996-2015	-2.0* (-3.6 to -0.4); p<0.001	1996-2015	-2.3* (-3.3 to -1.3); p<0.001
PA	1996-2004	-0.8 (-2.9 to 1.2); p=0.9	1996-1998	11.6 (-1.1 to 25.9); p=0.1	1996-2004	-0.8 (-2.9 to 1.2); p=0.4
	2004-2008	6.3 (-3.3 to 16.8); p=1.4	1998-2001	-4.2 (-15.1 to 8.2); p=0.4		
	2008-2015	-2.3 (-4.7 to 0.2); p=-2.0	2001-2008	4.3* (2.2 to 6.4); p<0.001	2004-2008	6.3 (-3.3 to 16.8); p=0.2
			2008-2015	-0.5 (-2.1 to 1.2); p=0.5	2008-2015	-2.3 (-4.7 to 0.2); p=0.1
AP	1996-2007	-5.8* (-8.8 to -2.7); p=0.6	1996-2002	1.9 (-4.3 to 8.4); p=0.5	1996-2007	-5.8* (-8.8 to -2.7); p<0.001
	2007-2015	5.5* (0.1 to 11.3); p<0.001	2002-2006	-11.1 (-26.1 to 7.0); p=0.2	2007-2015	5.5* (0.1 to 11.3); p<0.001
			2006-2015	2.4 (-1.0 to 5.9); p=0.2		
TO	1996-2003	11.6* (7.5 to 15.8); p<0.001	1996-2000	3.1 (-2.5 to 8.9); p=0.3	1996-2003	11.6* (7.5 to 15.8); p<0.001
	2003-2015	-2.8* (-4.4 to -1.1); p<0.001	2000-2003	15.3 (-3.1 to 37.3); p=0.1	2003-2015	-2.8* (-4.4 to -1.1); p<0.001
			2003-2015	-2.3* (-3.3 to -1.3); p<0.001		
Northeast	1996-2003	1.7* (0.5 to 2.9); p=0.3	1996-2003	2.1* (-0.9 to 3.3); p=0.1	1996-2003	1.4* (0.1 to 2.7); p<0.001
	2003-2006	7.4 (-1.5 to 17.2); p=1.9	2003-2006	6.6 (-2.3 to 16.4); p=0.1	2003-2006	8.3 (-1.5 to 19.1); p=0.1
	2006-2010	-4.5* (-8.5 to -0.2); p=0.3	2006-2010	-3.7 (-7.8 to 0.6); p=0.1	2006-2010	-5.1* (-9.5 to -0.5); p<0.001
	2010-2015	0.3 (-1.7 to 2.2); p=0.3	2010-2015	1.1 (-0.9 to 3.1); p=0.2	2010-2015	-0.4 (-2.5 to 1.8); p=0.7
MA	1996-2006	4.5* (2.2 to 6.9); p<0.001	1996-2000	0.1 (-7.1 to 7.8); p=1.0	1996-2003	4.5* (2.2 to 6.9); p<0.001
	2003-2006	18.3 (-0.2 to 40.2); p=0.1	2000-2007	13.4* (8.9 to 18.0); p<0.001	2003-2006	18.3 (-0.2 to 40.2); p=0.1
	2006-2015	-0.2 (-1.7 to 1.4); p=0.8	2007-2015	-0.1 (-2.6 to 2.6); p=1.0	2006-2015	-0.2 (-1.7 to 1.4); p=0.8
PI	1996-2006	8.9* (7.6 to 10.3); p<0.001	1996-2007	8.4* (6.8 to 10.1); p<0.001	1996-2006	8.9* (7.6 to 10.3); p<0.001
	2006-2015	-1.3 (-2.7 to 0.2); p=0.1	2007-2015	-1.4 (-3.7 to 1.1); p=0.2	2006-2015	-1.3 (-2.7 to 0.2); p=0.1
CE	1996-2007	2.8* (1.7 to 3.9); p<0.001	1996-1998	11.5 (-2.2 to 27.0); p=0.1	1996-2007	2.8* (1.7 to 3.9); p<0.001
	2007-2015	-2.2* (-3.9 to -0.4); p<0.001	1998-2008	2.0* (0.8 to 3.2); p<0.001	2007-2015	-2.2* (-3.9 to -0.4); p<0.001
			2008-2015	-1.2 (-2.9 to 0.5); p=0.1		
RN	1996-2009	2.4* (1.4 to 3.5); p<0.001	1996-2008	4.2* (2.9 to 5.4); p<0.001	1996-2009	2.4* (1.4 to 3.5); p<0.001
	2009-2015	-3.9* (-7.0 to -0.6); p<0.001	2008-2015	-3.3* (-5.9 to -0.6); p<0.001	2009-2015	-3.9* (-7.0 to -0.6); p<0.001
PB	1996-1998	-11.8*(-22.1 to 0.0); p<0.001	1996-1999	-5.5 (-12.3 to 2.3); p=0.1	1996-1998	-11.8* (-22.1 to 0.0); p<0.001
	1998-2007	8.9* (7.4 to 10.4); p<0.001	1999-2007	10.6* (8.3 to 13.0); p<0.001	1998-2007	8.9* (7.4 to 10.4); p<0.001
	2007-2015	-3.1* (-4.4 to -1.8); p<0.001	2007-2015	-3.5* (-5.1 to -1.8); p<0.001	2007-2015	-3.1* (-4.4 to -1.8); p<0.001
PE	1996-1998	5.1 (-2.8 to 13.7); p=0.2	1996-2006	1.1* (0.3 to 2.0); p<0.001	1996-1998	5.1 (-2.8 to 13.7); p=0.2
	1998-2001	-6.0 (-13.1 to 1.7); p=0.1	2006-2015	-2.0* (-3.0 to -1.1); p<0.001	1998-2001	-6.0 (-13.1 to 1.7); p=0.1
	2001-2005	4.9* (0.9 to 9.1); p<0.001			2001-2005	4.9* (0.9 to 9.1); p<0.001
	2005-2015	-3.2* (-3.8 to -2.6); p<0.001			2005-2015	-3.2* (-3.8 to -2.6); p<0.001
AL	1996-2007	2.4* (1.2 to 3.7); p<0.001	1996-2007	3.2* (2.4 to 4.0); p<0.001	1996-2007	2.4* (1.2 to 3.7); p<0.001
	2007-2015	-2.1 (-4.0 to -0.1); p<0.001	2007-2015	-1.5* (-2.7 to -0.3); p<0.001	2007-2015	-2.1* (-4.0 to -0.1); p<0.001
SE	1996-2005	5.7* (4.0 to 7.5); p<0.001	1996-2005	5.8* (4.1 to 7.4); p<0.001	1996-2005	5.7* (4.0 to 7.5); p<0.001
	2005-2015	-2.1* (-3.5 to -0.7); p<0.001	2005-2015	-1.4* (-2.7 to -0.1); p<0.001	2005-2015	-2.1* (-3.5 to -0.7); p<0.001
BA	1996-2015	-0.0 (-0.6 to 0.5); p=0.9	1996-2015	0.2 (-0.3 to 0.8); p=0.4	1996-2015	-0.2 (-0.8 to 0.3); p=0.4

continuation

Midwest	1996-2015	-2.8* (-3.4 to -2.2); p<0.001	1996-2015	-2.7* (-3.3 to -2.2); p<0.001	1996-2005	-0.5 (-2.0 to 1.0); p=0.5
					2005-2008	-0.9 (-23.1 to 7.7); p=0.2
					2008-2015	-1.6 (-3.8 to 0.6); p=0.1
MS	1996-2015	-2.4* (-2.9 to -2.0); p<0.001	1996-2015	-2.4* (-3.0 to -1.8); p<0.001	1996-2015	-2.4* (-2.8 to 1.9); p<0.001
MT	1996-2015	-1.9* (-3.0 to -0.8); p<0.001	1996-1998	9.7 (-3.5 to 24.6); p=0.1	1996-2015	-2.5* (-3.2 to -1.9); p<0.001
			1998-2010	-2.3* (-3.1 to -1.4); p<0.001		
			2010-2015	-5.9* (-8.6 to -3.2); p<0.001		
GO	1996-2015	-2.2* (-2.6 to -1.8); p<0.001	1996-1999	2.9 (-3.0 to 9.1); p<0.001	1996-2015	-2.2 (-2.6 to -1.8); p<0.001
			1999-2007	-3.8* (-5.3 to -2.3); p<0.001		
			2007-2015	-0.9 (-2.1 to 0.4); p=0.2		
DF	1996-1998	5.7 (-14.4 to 30.6); p=0.6	1996-2015	-4.0* (-4.6 to -3.4); p<0.001	1996-1998	5.7 (-14.4 to 30.6); p=0.6
	1998-2015	-4.4* (-5.1 to -3.7); p<0.001			1998-2015	-4.4* (-5.1 to -3.7); p<0.001
Southeast	1996-2015	-3.8* (-4.1 to -3.4); p<0.001	1996-2015	-3.8* (-4.2 to -3.5); p<0.001	1996-2015	-3.8* (-4.2 to -3.4); p<0.001
MG	1996-2009	-2.6* (-3.2 to -1.9); p<0.001	1996-2015	-3.3* (-3.7 to -3.0); p<0.001	1996-2015	-3.0* (-3.3 to -2.8); p<0.001
	2009-2015	-5.5* (-7.4 to -3.5); p<0.001				
ES	1996-2015	-3.6 (-4.3 to -2.9); p<0.001	1996-2015	-3.5* (-4.0 to -3.1); p<0.001	1996-2009	-2.6* (-3.2 to -1.6); p<0.001
					2009-2015	-5.5* (-7.4 to -3.5); p<0.001
RJ	1996-2005	-5.1* (-5.9 to -4.4); p<0.001	1996-2010	-3.9* (-4.4 to -3.5); p<0.001	1996-2005	-5.1* (-5.9 to -4.4); p<0.001
	2005-2008	-0.6 (-8.9 to 8.4); p=0.9	2010-2015	-5.4* (-7.4 to -3.3); p<0.001	2005-2008	-0.6* (-8.9 to 8.4); p<0.001
	2008-2015	-5.4* (-6.5 to -4.3); p<0.001			2008-2015	-5.4* (-6.5 to -4.3); p<0.001
SP	1996-2015	-3.8* (-4.2 to -3.4); p<0.001	1996-2015	-3.8* (-4.0 to -3.5); p<0.001	1996-2015	-3.8* (-4.0 to -3.5); p<0.001
South	1996-2015	-3.7* (-4.1 to -3.2); p<0.001	1996-2015	-3.7* (-4.1 to -3.2); p<0.001	1996-2015	-3.6* (-4.0 to -3.2); p<0.001
PR	1996-2015	-3.8* (-4.1 to -3.6); p<0.001	1996-2015	-3.8* (-4.1 to -3.5); p<0.001	1996-2015	-3.8* (-4.1 to -3.5); p<0.001
SC	1996-2015	-4.4* (-4.8 to -4.0); p<0.001	1996-1998	5.2 (-9.2 to 21.9); p=0.5	1996-2015	-4.3* (-4.7 to -3.9); p<0.001
			1998-2002	-8.3*(-14.8 to -1.3); p<0.001		
			2002-2015	-3.9* (-4.7 to -3.2); p<0.001		
RS	1996-2012	-3.0* (-3.0 to -3.4); p<0.001	1996-1998	4.7 (-0.0 to 9.6); p<0.001	1996-2012	-3.0* (-3.4 to -2.6); p<0.001
	2012-2015	-5.4* (-10.0 to -0.6); p<0.001	1998-2006	-3.9* (-4.5 to -3.3); p<0.001		
			2006-2010	-1.6 (-3.8 to 0.7); p=0.1	2012-2015	-5.4* (-10.0 to -0.6); p<0.001
			2010-2015	-5.5* (-6.4 to -4.5); p<0.001		

**Statistical significance(p<0.05; AAPC: Average Annual Percent Change; RO: Rondônia; AC: Acre; AM: Amazonas; RR: Roraima; PA: Pará; AP: Amapá; TO: Tocantins; MA: Maranhão; PI: Piauí; CE: Ceará; RN: Rio Grande do Norte; PB: Paraíba; PE: Pernambuco; AL: Alagoas; SE: Sergipe; BA: Bahia; MG: Minas Gerais; ES: Espírito Santo; RJ: Rio de Janeiro; SP: São Paulo; PR: Paraná; SC: Santa Catarina; RS: Rio Grande do Sul; MS: Mato Grosso do Sul; MT: Mato Grosso; GO: Goiás; and DF: Distrito Federal.*

Table 4 – Regression model (OLS, Ordinary least square) between the mortality rate due to Cerebrovascular diseases (CBVD) and the Human Development Index (HDI) and Social Vulnerability Index (SVI). Brazil, 1996-2015

Variable	Coefficient	t Statistics	p value
Municipal Human Development Index (MHDI)	61.588	2.091	0.046*
MHDI Longevity	90.265	1.866	0.073
MHDI Education	47.075	1.861	0.074
MHDI Income	56.476	2.301	0.029*
Social Vulnerability Index (SVI)	-40.802	-2.353	0.026*
SVI Urban infrastructure	-15.998	-1.110	0.277
SVI Human capital	-31.883	-2.092	0.046*
SVI Income and work	-35.322	-2.528	0.018*

** significant association*

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Safety, Efficacy, and Dose Protocol of Metoprolol for Heart Rate Reduction in Pediatric Outpatients Undergoing Cardiac CT Angiography

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Abstract

Background: Image quality and radiation dose are optimized with a slow, steady heart rate (HR) when imaging the coronary arteries during cardiac computed tomography angiography (CCTA). The safety, efficacy, and protocol for HR reduction with beta blocker medication is not well described in a pediatric patient population.

Objective: Provide a safe and efficient metoprolol dose protocol to be used in pediatric outpatients undergoing CCTA.

Methods: We conducted a retrospective review of all pediatric outpatients who received metoprolol during CCTA. Demographic and clinical characteristics were summarized and the average reduction in HR was estimated using a multivariate linear regression model. Images were evaluated on a 1-4 scale (1= optimal).

Results: Seventy-eight pediatric outpatients underwent a CCTA scan with the use of metoprolol. The median age was 13 years, median weight of 46 kg, and 36 (46%) were male. The median doses of metoprolol were 1.5 (IQR 1.1, 1.8) mg/kg and 0.4 (IQR 0.2, 0.7) mg/kg for oral and intravenous administrations, respectively. Procedural dose-length product was 57 (IQR 30, 119) mGy*cm. The average reduction in HR was 19 (IQR 12, 26) beats per minute, or 23%. No complications or adverse events were reported.

Conclusion: Use of metoprolol in a pediatric outpatient setting for HR reduction prior to CCTA is safe and effective. A metoprolol dose protocol can be reproduced when a slower HR is needed, ensuring faster acquisition times, clear images, and associated reduction in radiation exposure in this population. (Arq Bras Cardiol. 2021; 116(1):100-105)

Keywords: Heart Defects, Congenital; Heart Rate; Metoprolol; Adrenergic, Antagonists; Computed, Tomography; Coronary Vessels.

Introduction

Cardiac computed tomography angiography (CCTA) is the imaging standard for non-invasive assessment of coronary arteries in patients of all ages.[1,2] To optimize image quality and radiation dose, a slower and steady HR is preferred.[3,4] A reduction in HR can be achieved by using beta blocker medication. Imaging coronary arteries in children presents unique challenges due to smaller vessel size and higher resting HR. The main diagnostic modality for coronary imaging in congenital heart disease (CHD) patients has historically been cardiac catheterization, requiring anesthesia, central vascular access, contrast administration, and significant radiation exposure. Cardiac magnetic resonance imaging is useful for coronary imaging in older children but has limited value in the youngest patients.[5] CCTA has been shown to be diagnostic in infants and children of all ages using latest generation scanner technology with appropriate spatial and temporal resolution.[6-8]

Radiation dose optimization techniques have significantly decreased radiation exposure as compared to earlier scanner technology. Coronary imaging can be reproducibly acquired in a single heart beat or in several heart beats during a single breath hold sequence in patients of all ages.[9] A slower, steady HR allows for the use of a narrow acquisition window for radiation exposure during systole or diastole depending on HR. The safety and efficacy of HR reduction with beta blocker medication is well described for coronary imaging in the adult population,[10-12] but is scarce in the pediatric setting.[6,13] The purpose of this retrospective study was to evaluate the safety and efficacy, and define a dosage protocol of metoprolol for HR reduction in an outpatient population of pediatric patients who underwent CCTA.

Methods

Patients

Patients between 6 and 18 years of age were included if they presented as an outpatient and received metoprolol prior to CCTA from January 1, 2007 to December 31, 2016. Patients

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were excluded if they underwent a CT scan for a non-CHD indication, underwent CCTA without metoprolol medication, or were referred for coronary imaging from the inpatient setting or presented as an outpatient but were scanned under anesthesia for cooperation with suspended respiration. The baseline HR was measured at presentation to the outpatient imaging center prior to administration of metoprolol medication and again during the scan. Metoprolol dose, image quality, and any adverse events were documented. The study was approved by the Institutional Review Board.

Scanner Platform, Scan Sequence, and Patient Preparation

CCTA were performed using a first, second, or third -generation dual-source CT scanner (Somatom Definition Flash, Siemens Healthcare, Forchheim, Germany) with gantry rotation time = 280ms, temporal resolution=66-83ms, and collimation=2×128×0.6 mm. A prospectively electrocardiogram-triggered high-pitch (3.4) scan was performed using automated online tube current modulation for slow and steady HR < 55 beats per minute (bpm) with the second or third generation dual source scanner. For higher HR or significant HR irregularity despite beta blocker, a retrospective electrocardiogram gated (Mindose) or sequential scan was done with the acquisition window adjusted for HR. Typically, a wider acquisition window that included systole was used for HR above 60 bpm. When coronary lesions were suspected in patients with symptoms of ischemia or Kawasaki disease, a retrospective electrocardiogram-gated (Mindose) or a sequential scan was used regardless of HR to allow evaluation of more than a single dataset. The tube potential was adjusted for all patients to a lower value based on the use of the automated software Care kV (Siemens, Forchheim) or on clinical judgement. In 2011, a 70 kV peak tube potential became available with a scanner upgrade. Scans were reconstructed using the Siemens second-generation iterative reconstruction algorithm, Safire, at a strength of 3. In 2014, a third- generation iterative reconstruction algorithm, Admire, began to be used, also with a strength of 3. Contrast dose was injected at the rate appropriate for age and intravenous gauge. Contrast was power-injected using a 20-24-gauge catheter based on patient size.

Image Quality Assessment

Images were retrospectively reviewed by two expert readers (KH and BC) qualitatively on a four-point scale: 1=fully acceptable with optimal visualization of all anatomical targets; 2=good image quality with diagnostic visualization of all anatomical targets; 3=marginal image quality with diagnostic visualization of most anatomical targets; and 4=poor image quality, non-diagnostic for evaluation of anatomical targets. Any discrepancies in the scoring of image quality were re-reviewed by KH and reconciled. Anatomic targets were defined as the ability to see clear definition of coronary ostia and origin from the great artery; clear definition of coronary course, including relationship to great arteries and sternum; and the ability to identify distal coronary vessel anatomy to determine coronary dominance. All scans with a score >1 were considered suboptimal. For these scans, the reason for the suboptimal image quality was determined.

Radiation Dose Estimation

Procedural dose length product in mGy*cm was used to estimate the radiation dose. A 32 cm phantom was used for dose length product estimates in all patients regardless of size. Radiation dose is reported as scan dose length product.

Metoprolol Administration Protocol

A standard metoprolol protocol was used for all patients included in this study. Children were screened for contraindications to beta blockade including severe aortic stenosis, moderate to severe pulmonary hypertension, or severe left or right ventricular systolic dysfunction. Patients with a history of any of these clinical entities were not given beta blocker medication. If the baseline HR was < 60 bpm, metoprolol was not administered. If the baseline HR was between 60-70 bpm, 1 mg/kg metoprolol to maximum oral dose of 100 mg was administered. If the baseline HR was > 70 bpm, 2 mg/kg metoprolol to maximum oral dose of 100 mg was administered. If the HR remained over 70 bpm one hour after oral dose, 0.2 mg/kg intravenous metoprolol was given to a maximal dose of 1 mg/kg for patients < 20 kg, or maximum of 20 mg total intravenous dose was given for those over 20 kg. If the HR in the scanner is > 70 bpm when baseline HR was acceptable, intravenous metoprolol only was given according to guidelines above.[14]

Statistical Methods

Patient demographic and clinical data were summarized using counts (%) for categorical variables, means ± standard deviations for symmetrically-distributed continuous variables, and medians (interquartile ranges) for skewed continuous variables. The change in HR following beta blocker administration was estimated using a multivariate linear regression model with difference in HR as a response variable and age, gender, dose length product, and metoprolol dose as covariates. Model assumptions were verified using residual analysis and the Shapiro-Wilk test for normality. The model estimates, their 95% confidence intervals (CI), and p-values are reported. The analysis was performed using R 3.5.2 in R-Studio 1.1.463 environment.[14,15] The significance level of 5% was used.

Results

Patient Demographics and Heart Rate Reduction

We identified 78 pediatric patients who underwent a CCTA scan with the use of metoprolol prior to image acquisition in the outpatient setting at our institution between January 2007 and December 2016. Fifty nine (75%) patients had the CCTA scan to assess coronaries and 19 (25%) had the study to assess another type of CHD. Patient demographics, HR, and beta blocker delivery mechanism are described in Table 1. The median age at scan was 13.33 (IQR 10, 16) years, 36 (46%) were male and the median weight of 46 (IQR 31, 61) kg. One patient received nitroglycerin with no adverse event.

Overall, the baseline HR was 77 (IQR 66, 90) bpm. The majority of patients, n = 51, (65%) received oral metoprolol

Table 1 – Patient demographics and heart rate reduction

Variable	All	Oral only	IV only	IV + Oral
Patient, n (%)	78 (100)	51 (65)	4 (5)	23 (29)
Age at scan, years	13.0±3.3	13.1±3.4	10.2±4.6	13.3±2.7
Male, n (%)	36 (46)	22 (43)	1 (25)	13 (57)
Weight, kg,*	46 (31, 61)	46 (29, 59)	32 (29, 58)	49 (36, 62)
HR initial, bpm,*	78±15	74±11	91±26	87±16
HR at scan, bpm,*	60±11	56±9	73±16	66±11
HR reduction, bpm,*	19±10	18±9	18±10	20±12
Relative reduction in HR, %,*	23 (16, 30)	24 (17, 30)	20 (17, 22)	23 (15, 33)

*N: number; IV: intravenous; kg: kilogram; HR: heart rate; bpm: beats per minute. * Continuous variables are reported as means ± standard deviations or as medians and interquartile (IQR, 25th, 75th percentile) ranges if skewed. Categorical variables summarized by counts (%).*

only and four patients (5%) received intravenous metoprolol only. The remainder of the patients received a combination of oral and intravenous metoprolol n = 23 (29%). Following the metoprolol administration, there was a 23% reduction in baseline HR that corresponds to 19 bpm, IQR (12-26). From the multivariate analysis, the estimated reduction in HR was 20 bpm 95% CI (17, 24) (Appendix 1).

Metoprolol Administration

Metoprolol dose is dependent on patient's weight as outlined in the Metoprolol Administration Protocol previously described. For those weighing less than ≤ 50 kg, the median oral and intravenous metoprolol dose was 1.6 mg/kg (IQR 1.3, 1.9) and 0.6 mg/kg (IQR 0.3, 0.8), respectively. For patients weighing over 50 kg, the median oral and intravenous metoprolol dose was 1.4 (IQR 1.0, 1.6) and 0.3 (IQR 0.1, 0.5) mg/kg, respectively (Table 2). The doses and amounts administered in practice are consistent with those specified in our clinical protocol.[14]

Radiation Dose and Imaging Details

Table 3 provides scan radiation dose and imaging details. The median procedural dose-length product was 57 (IQR 30, 119) mGy*cm. The mean image quality score was 1.2. Out of 78 scans, 11 (14%) were of suboptimal quality with 10 cases scored as a "2" due to poor contrast and/or noise and one case ranked "3" due to patient motion. The representation of the imaging sequences was uniform, with approximately a third of patients included in each sequence type. No complications were reported during CCTA imaging procedures or after the procedure until the time of discharge from the outpatient setting.[15]

Discussion

In adult patients undergoing CCTA, beta blocker use with adequate HR control has been shown to improve image quality.[16] Oral pre-medication has been shown to be effective in the adult population, although variation in efficacy is affected by dosing.[16] It is well documented that risks of repeated exposure to anesthesia and ionizing radiation for all CHD patients should be avoided.[17-21] Therefore, a slower HR allows for the use of prospective electrocardiogram triggering, which has been shown to significantly reduce radiation dose for coronary angiography.[22] In our experience, intravenous metoprolol after an oral dose did not have an additional effect on reducing HR. Therefore, we have discontinued administration of intravenous metoprolol after oral dose in our pediatric patient population since 2013. Of note, three patients did receive IV metoprolol after 2013 due to elevated HR during topogram acquisition due to anxiety. HR reduction in pediatric populations can be safely and effectively achieved with a standardized metoprolol delivery protocol for patients undergoing CCTA assessment in the outpatient setting. With careful screening for contraindications, we found no complications or side effects with the use of beta blockers in pediatric patients.

Table 2 – Beta Blocker Protocol- Dose and Delivery by Weight

Variable	All	Weight ≤ 50 kg	Weight > 50 kg
Dose oral, mg/kg (n=74*)	1.5±0.5	1.7±0.5	1.3±0.4
Dose IV, mg/kg (n=27*)	0.5±0.3	0.6±0.3	0.4±0.2
Amount oral, mg	50 (50, 100)	50 (50, 75)	100 (62, 100)
Amount IV, mg	20 (15, 28)	20 (13, 23)	20 (18, 35)

*Mg: milligrams; kg: kilogram; IV: intravenous; *includes those that received both IV and oral. Continuous variables are reported as means ± standard deviations or as medians and interquartile (IQR, 25th, 75th percentile) ranges if skewed.*

Table 3 – Scan Radiation Dose and Imaging Details

DLP, mGy*cm*	57 (30, 119)
Scan image quality*	
1, n (%)	67 (86)
2, n (%)	10 (13)
3, n (%)	1 (1)
4, n (%)	0 (0)
Imaging Sequence	
Anatomic-Prospective ECG triggered with high pitch (Flash), n (%)	26 (33)
Anatomic-Prospective ECG triggered (Prospective), n (%)	25 (32)
Functional-Retrospective ECG gated (Spiral), n (%)	27 (35)

*DLP: dose-length product; mGy*cm. *categorical variables summarized by counts (%); continuous variables reported as median and interquartile (IQR, 25th, 75th percentile) ranges. Description of image quality: 1=fully acceptable with optimal visualization of all anatomical targets; 2=good image quality with diagnostic visualization of all anatomical targets; 3=marginal image quality with diagnostic visualization of most anatomical targets; and 4=poor image quality, non-diagnostic for evaluation of anatomical targets*

Limitations

This report is limited to findings regarding HR and metoprolol use and does not have a comparison group. The authors agree that a prospective design would have been more robust; however, this was a retrospective review that analyzed our clinical practice. Furthermore, the readers for this study were not blinded, which could introduce bias.

Conclusion

A metoprolol dose protocol in the outpatient pediatric population with CHD before the acquisition of cardiac CTA showed safety and efficacy in heart rate reduction in patients between 6 and 18 years of age. An adequate heart rate control in pediatric population with metoprolol can provide clearer images due to reduction in motion and artifact, ensure faster acquisition times, and reduce radiation exposure.

Author Contributions

Conception and design of the research: Casey SA, Chu BJ, Lesser JR, Han BK; Data acquisition: Casey SA, Caye DJ, Chu BJ, Lindberg BJ, Lesser JR, Han BK; Analysis and interpretation of the data: Casey SA, Chu BJ, Han BK; Statistical analysis: Casey SA, Stanberry LI, Han BK; Obtaining financing: Han BK; Writing of the manuscript and Critical revision of the manuscript for intellectual content: Nunes MO, Witt DR, Casey SA, Chu BJ, Han BK.

Potential Conflict of Interest

The authors report no conflict of interest concerning the materials and methods used in this study or the findings specified in this paper.

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Study Association

This study is not associated with any thesis or dissertation.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Alina Health IRB under the protocol number 1036442-1. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013.

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In Search for Optimal Image Quality in Pediatric Cardiac CT Angiogram

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Short Editorial related to the article: Safety, Efficacy, and Dose Protocol of Metoprolol for Heart Rate Reduction in Pediatric Outpatients Undergoing Cardiac CT Angiography

"There can be no keener revelation of a society's soul than the way in which it treats its children". Years after his death, these Nelson Mandela's prolific words still resonate universally with our moral and ethical foundations and, as researchers ourselves, we are very happy to know that Science walks on the right path of history.

There are a number of methodological and ethical challenges of performing research in children. However, there can be no progress in pediatric clinical care without research in this population, whose findings may also otherwise be relevant to adult medicine. Given that approximately 1% of born children will have some kind of significant heart disease,[1] it is of crucial importance to maximize the safety and efficacy profile of diagnostic and therapeutic interventions. Computed tomography cardiac angiography (CTCA) is being increasingly used, but its diagnostic accuracy in children is highly dependent on optimal image quality while minimizing radiation exposure as much as possible.

Even with modern scanners, image quality in CTCA is still highly dependent on a stable and relatively slow heart rate (HR).[2] To achieve optimal pre-scan conditions, beta-blocker administration is often advocated and a number of societal documents have been published providing guidance for patient selection and administration.[3,4] Nevertheless, the different pharmacokinetic behavior of beta-blockers in pediatric patients (in addition to the higher baseline HR, body movement and smaller coronary arteries) cast a shadow regarding the optimal strategy and dosage to obtain high quality images without incurring in the risk of bradyarrhythmias.[5] Beta-blockers should be given at an appropriate dose given the potential side effects, but doses and protocols typically vary among facilities.

De Oliveira Nunes et al.[6] share an elegant study that sheds a much needed and awaited light on this uncertainty. The aim of this study was to clarify the safety and efficacy of a metoprolol protocol in a series of pediatric outpatients referred for CTCA. We have summarized the protocol used in the Figure 1. Briefly, if a patient's HR is below 60bpm, then no HR reduction is necessary. For those with a HR of at least 60bpm, in the absence of contraindications to beta-blocker use (e.g. severe aortic stenosis or significant pulmonary hypertension), a protocol using metoprolol was employed (Figure 1). The average image quality that resulted was close to optimal in most cases, with only 14% effectively being suboptimal as deemed by the researchers. The authors should be commended for the protocol adaptations that came with time and experience. They first started by treating patients with oral with oral metoprolol followed by IV metoprolol if the HR was persistently elevated (above 70bpm). However, they elegantly noted that there was no significant additional reduction in HR with IV metoprolol, so they stopped its use from 2013 onwards. Although some protocols still advocate its use in clinical practice, particularly in adults,[4] this calls for dedicated prospective studies to answer this question, particularly in high-risk children, in whom the use of IV beta-blockers can increase risk with little benefit.

CT technology is continuously evolving and allows better image quality, with increasing detector width, shorter CT acquisition times and optimal ECG-gating. One particularly interesting feature of this study is the wide period of time it encompassed, from 2007 to 2016. This means that technological advances took place: different generation scanners and different tube potentials were used, thus more likely representative of the real world.

Some gaps in evidence, however, still exist. Different beta-blockers can be used (with potential differences in efficacy), patients younger than 6 years were not included in this study (who are more likely to contribute to poorer image quality) and a prospective study design with a control group would increase the evidence level. Additional investigation in these areas are thus desirable.

Even high-end modalities can struggle with appropriate attainment of optimal cardiac images, particularly in the pediatric population. It is thus important to focus on the points that can still be improved in our clinical practice, and here specifically on HR. This aspect should be well established among teams of pediatric cardiologists and radiology technicians, before attempting (potentially unnecessary) acquisition schemes that could result in more radiation exposure or even require sedation. Elegant solutions such as the ones presented in *Arquivos Brasileiros de Cardiologia* by De Oliveira Nunes et al.[6] are always welcome and allow us to support decision-making in our clinical practice.

Keywords

Heart Defects, Congenital; Diagnostic Imaging; Adrenergic Agents; Metropolol; Heart Rate; Angiotomography.

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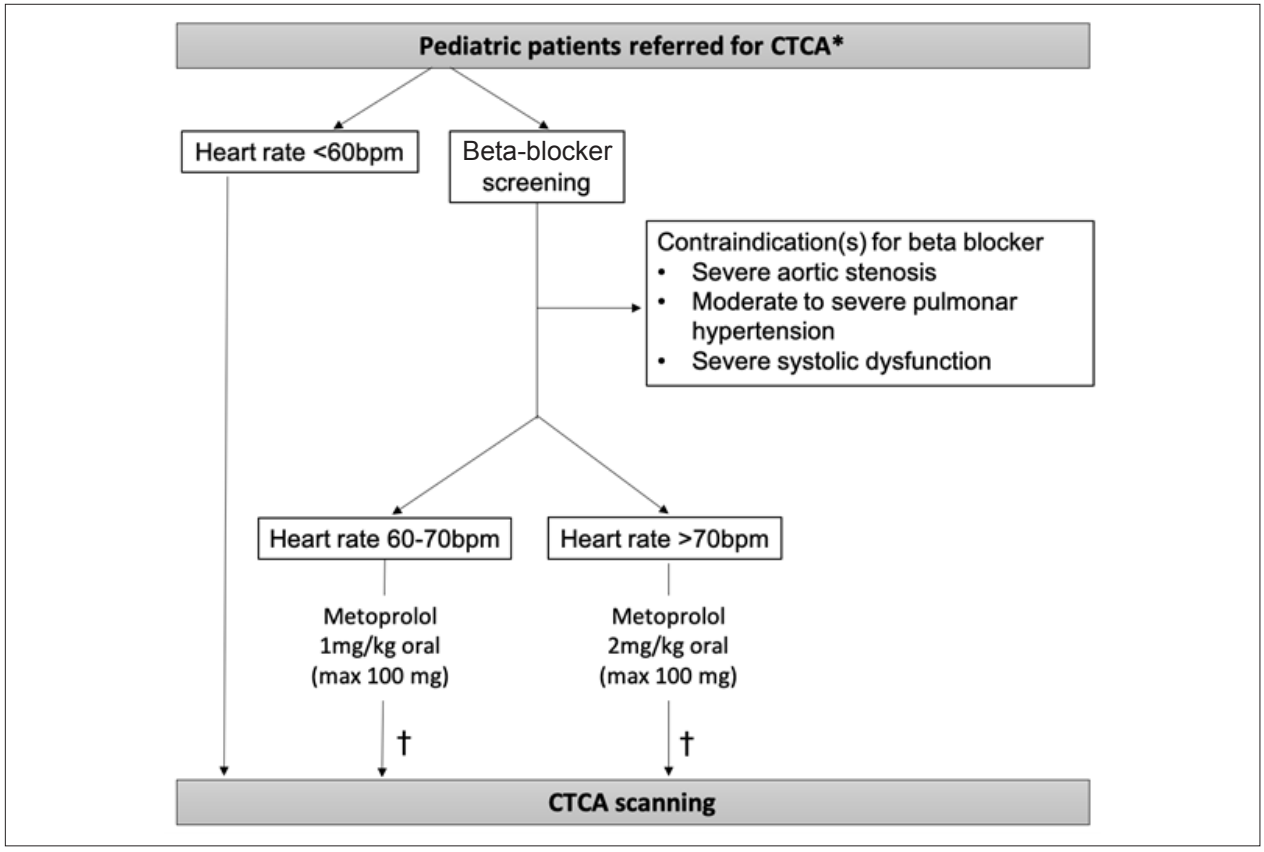

Figure 1 – *Summary of the study protocol used by De Oliveira Nunes et al.6 CTCA: computed tomography cardiac angiography. * Patients between 6 and 18 years old. † The investigators stopped giving an additional IV dose of metoprolol (from 2013 onwards) in the case of persistent heart rate >70bpm one hour after oral dose as no significant additional heart rate reduction was appreciated.*

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Evaluation of 1-Year Follow-up of Patients Included in the Registry of Clinical Practice in Patients at High Cardiovascular Risk (REACT)

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Abstract

Background: **In clinical practice, there is evidence of failure to prescribe evidence-based therapies for patients at high cardiovascular risk. However, in Brazil, data on 1-year outcomes of these patients remain insufficient.**

Objectives: **To describe the use of evidence-based therapies and the occurrence of major cardiovascular outcomes and their major predictors in a 12-month follow-up of a Brazilian multicenter registry of patients at high cardiovascular risk.**

Methods: **This prospective observational study documented the outpatient clinical practice of managing patients over 45 years of age and of high cardiovascular risk in both primary and secondary prevention. Patients were followed-up for 1 year, and the prescription of evidence-based therapies and the occurrence of major cardiovascular events (myocardial infarction, stroke, cardiac arrest, and cardiovascular death) were assessed. P-values < 0.05 were considered statistically significant.**

Results: **From July 2010 to August 2014, a total of 5076 individuals were enrolled in 48 centers, 91% of the 4975 eligible patients were followed-up in cardiology centers, and 68.6% were in secondary prevention. At 1 year, the concomitant use of antiplatelet agents, statins, and angiotensin-converting enzyme inhibitors reduced from 28.3% to 24.2% ($p < 0.001$). Major cardiovascular event rate was 5.46%, and the identified predictors were age, patients in secondary prevention, and diabetic nephropathy.**

Conclusions: **In this large national registry of patients at high cardiovascular risk, risk predictors similar to those of international registries were identified, but medical prescription adherence to evidence-based therapies was inferior and significantly worsened at 1 year. (Arq Bras Cardiol. 2021; 116(1):108-116)**

Keywords: **Cardiovascular Diseases; Risk Factors; Prescription Drugs; Multicenter Studies as Topic; Medical Record Linkage.**

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Introduction

Cardiovascular diseases are usually manifestations arising from an arterial atherosclerotic substrate.[1-4] Together, they affect more than 4% of the global population and their acute complications, known as cardiovascular events, are the leading cause of death and disability in both men and women worldwide.[2-4] In Brazil, as in other developing countries, the frequency of those diseases continues to increase over the years, which reinforces the need for a better understanding of the outcomes of those patients in clinical practice.[2-7]

Despite the high morbidity and mortality, several strategies to reduce the risk of complications in those patients have been developed.[8-12] Among the options, patients at high cardiovascular risk may benefit from antithrombotic (antiplatelet) therapies, statins, and angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs).[8-12] However, the use of those therapies in clinical practice has proved to be insufficient, especially in developing countries.[13-15] In Brazil, previously reported partial data from the Registry of Clinical Practice in Patients at High Cardiovascular Risk (REACT) showed that the combined use of antiplatelet agents, statins, and ACEIs was identified in only 34% of this population.[15] Despite the relevance of those data, there are limitations in the analysis because information on medical prescription adherence to evidence-based therapies was collected in a cross-sectional fashion and changes in prospective follow-up have not been reported yet. Furthermore, there remains the need to identify the actual expected event rate and the predictors associated with such events in a Brazilian population of individuals at high cardiovascular risk.

The present study aimed to assess, in patients at high cardiovascular risk treated at Brazilian centers over 12 months, the proportion of those continuously receiving interventions with proven benefit and the factors associated with late clinical outcomes, particularly major cardiovascular event rate during follow-up.

Methods

The REACT registry is a project to document the actual care of patients at high cardiovascular risk in centers across all Brazilian regions, including both public and private hospitals as well as primary health care units.

Study Design and Implementation

The REACT registry is a Brazilian Society of Cardiology (SBC) project whose operation was conducted by the HCor Research Institute (IP-HCor) and whose methods were reported elsewhere.[15,16] Briefly, this is an observational, prospective, multicenter study whose inclusion of patients occurred voluntarily from July 2010 to August 2014 in 48 health care facilities that included both public and private hospitals as well as primary health care units. All 5 Brazilian regions were covered with the following distribution of participating centers: Southeast (45.8%), North (6.3%), Northeast (14.6%), South (29.2%), and Midwest (4.2%). For the selection of participating centers, open invitations were sent to interested centers by the SBC and the coordinating center (IP-HCor). The study was initiated after approval by the relevant Research Ethics Committee, and data were collected after individual patient consent was obtained. Nationwide data from the cross-sectional analysis that documented the clinical practice of managing patients at high cardiovascular risk have been reported elsewhere.[15] Additionally, longitudinal follow-up of these patients at 6 and 12 months had the following objectives: to measure medical prescription adherence to recommended evidence-based therapies, to evaluate the occurrence of major cardiovascular events, and to identify their respective predictors.

Study Participants

Briefly, study participants should be over 45 years of age and have at least one of the following factors:[15,16] 1) any clinical evidence of arterial disease (coronary artery, cerebrovascular, or peripheral artery disease); 2) diabetes mellitus (DM); 3) 3 cardiovascular risk factors, except DM: hypertension, smoking, dyslipidemia, age over 70 years, diabetic nephropathy, family history of coronary artery disease, asymptomatic (subclinical) carotid artery disease. The first group had known arterial disease and consisted of patients considered to be in a stage of secondary prevention regardless of having other inclusion criteria. Other participants were considered as primary prevention with DM (second inclusion criterion) or without DM (those included only by the third inclusion criterion). Because this was a clinical practice study with pragmatic criteria, the exclusion criteria were refusal to provide informed consent, a psychiatric or neurocognitive condition that prevented obtaining reliable clinical data (at the investigators' discretion), and life expectancy less than 6 months.

Study Procedures and Analyzed Variables[15,16]

Data were collected at admission for baseline data (index visit) and also at two follow-up visits at 6 and 12 months to measure medical prescription adherence to recommended evidence-based therapies and to assess occurrence of major cardiovascular events. These follow-up visits could be conducted in person at the centers or by telephone. Because this was a pragmatic study, the identification of comorbidities (e.g., hypertension, dyslipidemia) could be performed as follows: report by patient, use of (antihypertensive, lipid-lowering) drugs, or at the investigators' discretion (in the latter, the centers were advised to follow the recommended diagnostic criteria adopted in the current SBC guidelines). Data on drug prescriptions were collected to assess medical prescription adherence to evidence-based recommendations. The evidence-based therapy regimen that was considered in the REACT registry was consistent with current guidelines.[8-12] No data were collected on the effective use of drugs by patients.

Study Outcomes

As described in previously reported REACT methods,[16] the primary outcome was related to prescription of interventions with proven benefit (e.g., aspirin, statins, ACEIs) and impact on late clinical outcomes. Late clinical outcomes included

myocardial infarction, stroke, cardiac arrest, and overall and cardiovascular mortality.[15,16] These outcomes were reported by the investigator, with no participation of an independent event adjudication committee.

Statistical Analysis

The distribution of continuous variables was assessed for normality using histograms. Normally distributed continuous variables were described as mean ± standard deviation. Categorical variables were described as absolute and relative frequencies, and proportions were compared by the chi-square test or the Fisher-Freeman-Halton exact test. Independent predictors of combined events (death, myocardial infarction, cardiac arrest, or stroke) were identified using Cox proportional hazards models, as data on the dates of the events were collected. This predictor analysis was initially performed in a univariate fashion to assess the following factors: age, sex, history of coronary artery disease, previous acute myocardial infarction, history of stroke/transient ischemic attack, history of peripheral artery disease, DM, hypertension, diabetic nephropathy, smoking, asymptomatic carotid artery disease, and combined use of antiplatelet agent, statin, and ACEI at baseline. Variables with p-value < 0.15 were included in a multivariate analysis. Reported p-values are two-tailed, and $p < 0.05$ was considered statistically significant in the final analyses. The assumptions of proportionality for each predictor and global variable were assessed using standardized Schoenfeld residuals.[17] Generalized estimating equation (GEE) models were used to assess drug therapy over time. All analyses were conducted using the software R, version 3.6.1.

Results

Between July 2010 and August 2014, 5076 patients were recruited in this national registry; however, excluding patients without eligibility and baseline data, 4975 patients remained for analysis, 91% of whom were followed-up at cardiology centers (Table 1). For 407 patients (8.2%), obtaining 12-month follow-up data was not possible (loss to follow-up).

Baseline Characteristics

The patients' clinical profile showed that mean age was 65.4 (± 10), 52.5% were male, and 68.6% were patients in secondary prevention (Table 1). Coronary artery disease was the most common diagnosis of established cardiovascular disease and was found in almost 60% of the sample (Table 1).

Medical Prescription Adherence to Evidence-based Therapies

Among the patients included in the study, 74.6% used antiplatelet agents, 72.2% used statins, and 42.5% used ACEIs (Table 2). The percentage varied according to the inclusion criterion and was higher in the secondary prevention group, in which the use of antiplatelet agents and the use of statins was close to 80% (Table 2). Among the patients with history of myocardial infarction, 73.8% received beta-blockers at baseline. At follow-up, the concomitant use of antiplatelet agents, statins, and ACEIs reduced from 28.3% to 24.2% ($p < 0.001$), and the most evident reduction was found in ACEI users (Figure 1).

Control of Risk Factors

Overall, 16.7% of patients had blood pressure ≥ 140 x 90 mm Hg. In baseline laboratory assessment, glycated hemoglobin was < 7% in 47.5% of diabetic patients, with control being more frequent in primary prevention patients. Low-density lipoprotein (LDL)-cholesterol level was > 70 mg/dL in 76.6% of patients, and > 90% of secondary prevention patients had LDL-cholesterol > 50 mg/dL. Among the patients without previous diagnosis of hypertension and/or DM, 17.9% (94/524) had blood pressure ≥ 140 x 90 mm Hg, 3.6% (77/2161) had fasting blood glucose ≥ 126 mg/dL, and 4.1% (88/2161) had glycated hemoglobin ≥ 6.5%. In a combined fashion, 10.3% (247/2392) of the patients without previous diagnosis of hypertension or DM had pathological levels of blood pressure or blood glucose.

Guidance for nonpharmacological measures was reported in about 80% of prescriptions, being similar in both primary and secondary prevention groups for smoking cessation, but higher in primary prevention group for physical activity and cardioprotective diet.

Clinical Outcomes

Overall (either cardiovascular or not) mortality at 12 months was 4.92%; this was higher in the Northeast region (9.33%; 95% CI 6.1%-12.6%) followed by the Midwest (8.6%; 95% CI 3.0%-14.1%), South (4.9%; 95% CI 3.7%-6.1%), and Southeast (4.3%; 95% CI 3.5%-5.1%) regions. The analysis of the North region was compromised by low inclusion (99 patients) with 30% loss to follow-up, with report of only 1 death (1.5%; 95% CI 0.0%-4.3%).

Major cardiovascular event rate in the total population was 5.46 per 100 patient-years in the secondary prevention group (Figure 2), and the predictors identified for cardiovascular events were age, secondary prevention, and diabetic nephropathy (Table 3).

Discussion

The REACT registry followed-up for 1 year approximately 5000 patients at high cardiovascular risk, almost 70% of whom were in secondary prevention. The patients' profile shows a balance between male and female, and hypertension and dyslipidemia were the most common risk factors (found in > 70% of patients). Antiplatelet prescription was not identified in about 20% of secondary prevention patients, and the combined use of antiplatelet agent, statin, and ACEI in the entire high-risk population ranged from 28.3% at baseline to 24.2% at 1 year. The risk of major cardiovascular events at 1 year was 5.46 per 100 patient-years, and the three most important factors associated with such events were inherent to patient clinical status: age, secondary prevention, and diabetic nephropathy.

Although heterogeneous, the group of patients included in the REACT registry is in line with the current concept of

Table 1 – Baseline characteristics

Baseline characteristics	Total (n = 4975)
Age; mean ± SD	65.4 ± 10 (n = 4975)
Sex (male)	2614/4975 (52.5%)
Ethnicity	
White	3422/4975 (68.8%)
Black	571/4975 (11.5%)
Yellow (Asian)	76/4975 (1.5%)
Brown	900/4975 (18.1%)
Red (native Brazilian)	6/4975 (0.1%)
Type of center	
Cardiology	4505/4950 (91%)
Neurology	7/4950 (0.1%)
Vascular surgery	3/4950 (0.1%)
Endocrinology	114/4950 (2.3%)
Internal medicine	99/4950 (2%)
Primary care	222/4950 (4.5%)
Prevention	
Primary	428/4975 (8.6%)
Primary with DM	1135/4975 (22.8%)
Secondary	3412/4975 (68.6%)
BMI; mean ± SD	28.5 ± 5.2 (n = 4959)
BMI ≥ 25	3660/4959 (73.8%)
CAD	2867/4975 (57.6%)
Previous acute myocardial infarction	1510/4975 (30.4%)
Stroke	710/4975 (14.3%)
Peripheral artery disease	799/4975 (16.1%)
DM	2814/4975 (56.6%)
Multiple risk factors (at least 3)	3057/4975 (61.4%)
Hypertension	4451/4975 (89.5%)
Dyslipidemia	3638/4975 (73.1%)
Diabetic nephropathy	406/4975 (8.2%)
Age > 70 years	1700/4975 (34.2%)
Current smoking	515/4975 (10.4%)
Family history of CAD	2478/4975 (49.8%)
Asymptomatic carotid artery disease	605/4975 (12.2%)
Blood pressure	
Systolic	132.3 ± 19.7 (n = 4975)
Diastolic	79.5 ± 11.4 (n = 4975)
Laboratory tests	
Total cholesterol (mg/dL)	178 ± 58.5 (n = 3041)
LDL-cholesterol (mg/dL)	99.6 ± 39 (n = 2834)
HDL-cholesterol (mg/dL)	45.4 ± 14.4 (n = 2996)
Triglycerides (mg/dL)	159.8 ± 116.3 (n = 3049)
Blood glucose (mg/dL)	126.7 ± 55.2 (n = 3327)
Glycated hemoglobin (%)	7.2 ± 2.1 (n = 1953)
Creatinine (mg/dL)	1.1 ± 0.8 (n = 3305)

BMI: body mass index; CAD: coronary artery disease; DM: diabetes mellitus; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SD: standard deviation.

cardiovascular prevention, in which characterizing individuals in terms of cardiovascular risk is more important than classifying them as having DM, hypertension, or dyslipidemia. Previously reported partial results of the REACT registry from 2013[15] had included 2403 patients and analyzed data from 2364 after baseline data quality analysis. In the present analysis, 2673 patients were added to the previous sample, leading to a total of 5076 participants at the end of the study (4975 patients eligible for analysis). In the current report, in addition to the sample being more than double the previously reported sample, prospective data on 12-month follow-up were included.[15] Thus, in addition to allowing greater precision in the assessment of baseline data, this report included data on patient outcomes. There were limited data on 12-month follow-up from a large contemporary population of patients at high cardiovascular risk because, even in large international studies that included Latin America such as the REACH trial,[18] the sample size of patients from our continent in this study,[18] represented less than half of the cases included in the REACT study.

Regarding prescribed evidence-based therapies to reduce cardiovascular risk, this study found that well-established therapies such as antiplatelet agents for secondary prevention were not prescribed for a significant portion of the high-risk population. In international registries of high-risk patients,[19-21] there was great variability in adherence to therapy and control of risk factors. In the REACT study, even with 90% of patients being followed-up at cardiology centers, important gaps in the control of cardiovascular risk were identified. Regarding medical prescription, in addition to a significant proportion of nonadherence at baseline, there was an absolute reduction of approximately 4% in the combined prescription of antiplatelet agent, statin, and ACEI at 12-month follow-up. These differences demonstrate the need to develop strategies for a better control of risk factors with greater prescription of evidence-based therapies in the Brazilian population.[22]

Twelve-month follow-up in the REACT study allowed an analysis of the rate of major cardiovascular events and their major predictors. The factors with stronger association were related to patient status, such as age, secondary prevention, and nephropathy, and are consistent with previously established concepts in international studies.[21,23,24] In view

Table 2 – Use of therapies for cardiovascular prevention and control of risk factors according to population characteristics

	Primary (n = 428)	Primary with DM (n = 1135)	Secondary (n = 1733)	Secondary and DM (n = 1679)	Total (n = 4975)	p
Drug (baseline)						
Antiplatelet agent	225/428 (52.6%)	731/1135 (64.4%)	1403/1733 (81%)	1354/1679 (80.6%)	3713/4975 (74.6%)	< 0.001
Statin	276/428 (64.5%)	720/1135 (63.4%)	1347/1733 (77.7%)	1249/1679 (74.4%)	3592/4975 (72.2%)	< 0.001
ACEI	171/428 (40%)	519/1135 (45.7%)	758/1733 (43.7%)	787/1679 (46.9%)	2235/4975 (44.9%)	0.043
Combination	64/428 (15%)	263/1135 (23.2%)	527/1733 (30.4%)	554/1679 (33%)	1408/4975 (28.3%)	< 0.001
Beta-blocker (patient with AMI)			607/816 (74.4%)	507/694 (73.1%)	1114/1510 (73.8%)	-
Thiazide diuretic (patients with hypertension)	174/387 (45%)	555/1038 (53.5%)	496/1481 (33.5%)	642/1545 (41.6%)	1867/4451 (41.9%)	< 0.001
Control of risk factors (baseline)						
Glycated hemoglobin						
< 7%	146/150 (97.3%)	321/655 (49%)	361/408 (88.5%)	292/740 (39.5%)	1120/1953 (57.3%)	< 0.001
7% to 8%	1/150 (0.7%)	144/655 (22%)	22/408 (5.4%)	150/740 (20.3%)	317/1953 (16.2%)	
≥ 8%	3/150 (2%)	190/655 (29%)	25/408 (6.1%)	298/740 (40.3%)	516/1953 (26.4%)	
Blood glucose						
< 100 mg/dL	185/284 (65.1%)	137/838 (16.3%)	664/1074 (61.8%)	236/1131 (20.9%)	1222/3327 (36.7%)	< 0.001
100 to 125 mg/dL	90/284 (31.7%)	268/838 (32%)	342/1074 (31.8%)	310/1131 (27.4%)	1010/3327 (30.4%)	
≥ 126 mg/dL	9/284 (3.2%)	433/838 (51.7%)	68/1074 (6.3%)	585/1131 (51.7%)	1095/3327 (32.9%)	
Blood pressure						
< 130/80 mm Hg	274/428 (64%)	582/1135 (51.3%)	1066/1733 (61.5%)	904/1679 (53.8%)	2826/4975 (56.8%)	< 0.001
130/80 to 139/89 mm Hg	97/428 (22.7%)	322/1135 (28.4%)	432/1733 (24.9%)	466/1679 (27.8%)	1317/4975 (26.5%)	
≥ 140/90 mm Hg	57/428 (13.3%)	231/1135 (20.4%)	235/1733 (13.6%)	309/1679 (18.4%)	832/4975 (16.7%)	
LDL-cholesterol						
< 50 mg/dL	1/269 (0.4%)	40/712 (5.6%)	53/939 (5.6%)	93/914 (10.2%)	187/2834 (6.6%)	< 0.001
50 to 69 mg/dL	25/269 (9.3%)	97/712 (13.6%)	145/939 (15.4%)	173/914 (18.9%)	440/2834 (15.5%)	
≥ 70 mg/dL	243/269 (90.3%)	575/712 (80.8%)	741/939 (78.9%)	648/914 (70.9%)	2207/2834 (77.9%)	

P-value (chi-square test) < 0.05 indicates that preventive therapy/risk factor are dependent on the population characteristic. ACEI: angiotensin-converting enzyme inhibitor; AMI: acute myocardial infarction; DM: diabetes mellitus; LDL: low-density lipoprotein.

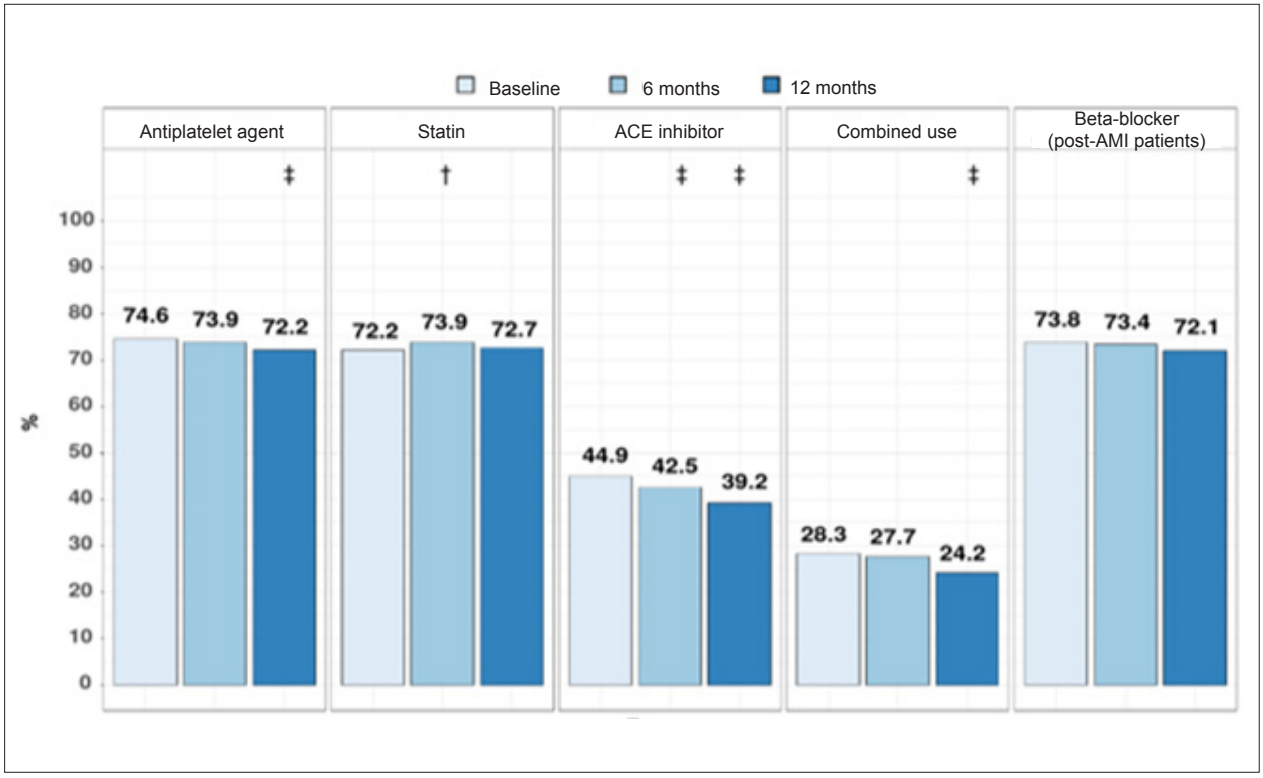

Figure 1 – *Prescription of cardiovascular prevention therapies according to follow-up time. To compare the continuity of drug prescription between follow-ups and baseline, a generalized estimating equation (EEG) model was adjusted for binary data to account for dependence between observations. ‡ p-value < 0.001; comparison between follow-up and baseline. † p-value < 0.01; comparison between follow-up and baseline. * p-value < 0.05; comparison between follow-up and baseline. ACE: angiotensin-converting enzyme; AMI: acute myocardial infarction.*

of such findings, having primary cardiovascular and kidney disease prevention as a priority in public health policies is required. Adequate screening and control of risk factors such as hypertension, dyslipidemia, and DM are crucial in this primary cardiovascular disease prevention strategy. In the REACT registry, 10.3% of patients without previous diagnosis of hypertension or DM had blood pressure and/or blood glucose levels within pathological parameters. Thus, in addition to the search for efficient models to improve adherence to evidence-based recommendations,[22] there is a need to improve the identification of these risk conditions in the population and work together to control them. This is because, although evidence-based therapy reduces the risk of events, event rate will remain higher in the secondary prevention group regardless of other variables. This joint systematic approach reinforces the concept that preventive efforts are not related only to the risks attributable to the elevation of isolated factors, such as blood pressure or serum cholesterol, but also to the action of multiple factors, affecting the overall absolute risk of each individual.

Study Limitations

Although the invitation was open to interested centers across Brazil, the North, Northeast, and Midwest regions had a proportionally low representation. Additionally, the participating centers were mostly cardiology centers and had a structure for clinical research, and the participants were included voluntarily. Thus, the results may not be applicable to populations that do not fit these characteristics (e.g., health care facilities with fewer resources, especially in the North, Northeast, and Midwest regions). Nonetheless, even in facilities with more favorable conditions, relevant gaps were identified in the application of evidence-based practices. Another limitation is related to possible factors associated with cardiovascular events, as patient socioeconomic and cultural variables were not collected and clinical outcome data were not adjudicated, with missing 12-month data from 407 patients. However, clinical outcome review in pragmatic observational studies is usually conducted by investigator's report, without any specific adjudication committee, and the REACT registry represents a scenario closer to the identification of events in actual clinical practice. Regarding the 12-month follow-up, considering that data losses occurred at different time points, analyses were performed using the Cox model and, therefore, patients were censored at the last recorded contact to minimize variations in follow-up duration. Finally, adherence to therapy was assessed based on medical prescriptions and no data were collected on eligibility, on the actual use of prescribed therapies, and on the main barriers to the prescription and use of therapies. Thus, the REACT results reflect physicians' overall adherence in terms of prescribing evidence-based therapies, but without data on the actual use of these therapies.

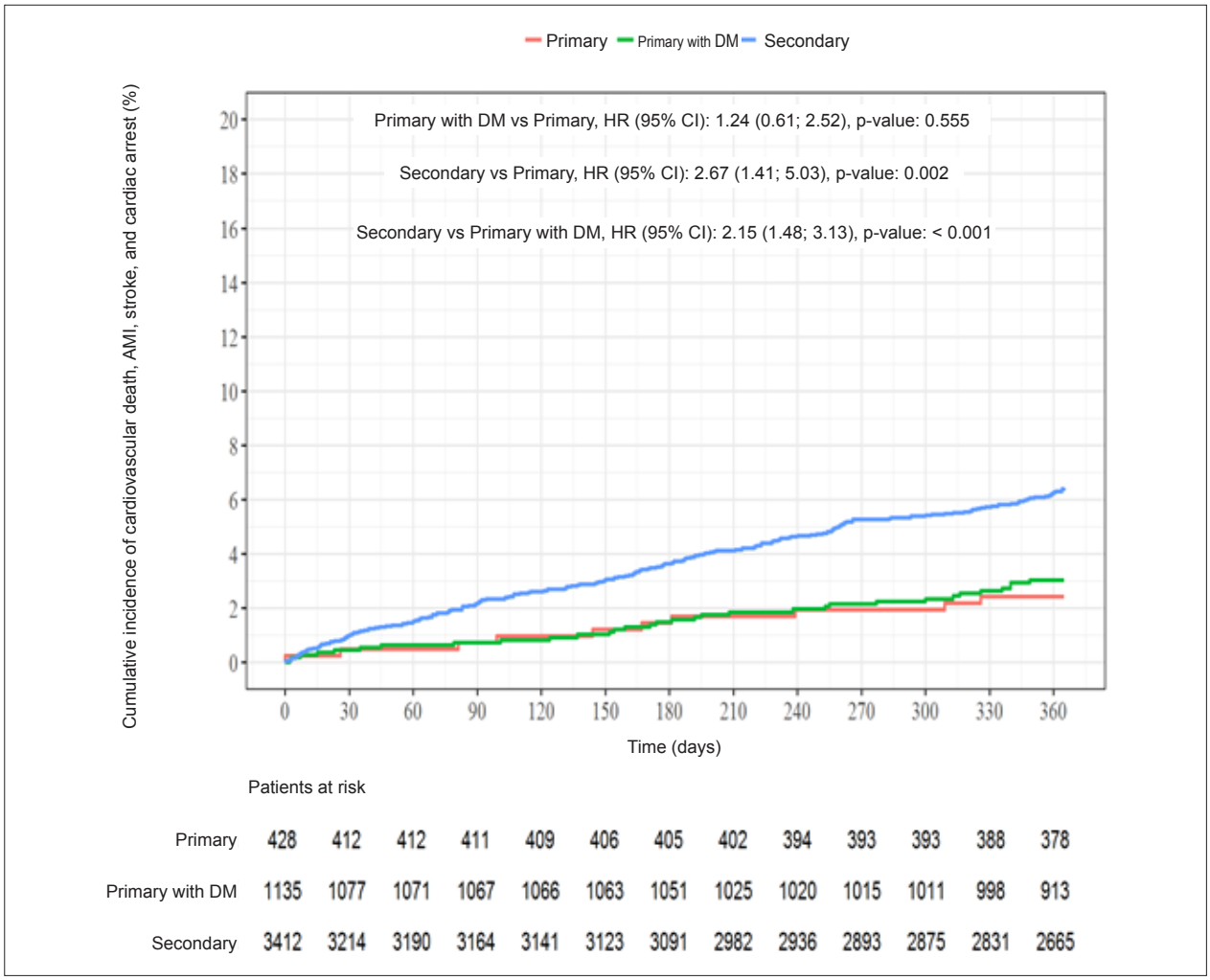

Figure 2 – *One-year event rate according to inclusion criterion. AMI: acute myocardial infarction; DM: diabetes mellitus; HR: hazard ratio.*

Conclusion

In a large prospective study of patients at high cardiovascular risk, failures in the prescription of evidence-based therapies were higher than what is expected in international registries, and these failures increased during the 1-year follow-up. A cardiovascular event rate > 5% per year was also identified in patients included as secondary prevention, which was an independent predictor of risk, as well as age and nephropathy. These findings can be used in the development of projects to improve quality of care and other health care policies in order to reduce the risk of cardiovascular events in the Brazilian population.

Author Contributions

Conception and design of the research: Barros e Silva PGM, Berwanger O, Lopes RD, Guimarães JI, Andrade JP, Paola AAV, Malachias MVB, Mattos LAP, Bacal F, Dutra OP; Acquisition of data: Barros e Silva PGM, Precoma DB, Cavalcante MA, Vilela-Martin JF, Figueiredo EL, Lopes RD, Bodanese LC; Analysis and interpretation of the data and Writing of the manuscript: Barros e Silva PGM; Obtaining financing: Berwanger O, Guimarães JI, Andrade JP, Paola AAV, Malachias MVB, Mattos LAP, Bacal F, Dutra OP; Critical revision of the manuscript for intellectual content: Berwanger O, Precoma DB, Cavalcante MA, Vilela-Martin JF, Figueiredo EL, Lopes RD, Bodanese LC, Guimarães JI, Andrade JP, Paola AAV, Malachias MVB, Mattos LAP, Bacal F, Dutra OP.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Table 3 – Predictive factors for cardiovascular risk. Univariate and multivariate analyses

Variables	Univariate analysis		Multivariate analysis	
	HR [95% CI]	p-value	HR [95% CI]	p-value
Age (1-year increment)	1.036 [1.025; 1.047]	< 0.001	1.035 [1.024; 1.046]	< 0.001
Sex (male)	1.123 [0.900; 1.401]	0.303	-	-
History of CAD (yes)	1.686 [1.329; 2.139]	< 0.001	1.324 [0.989; 1.772]	0.059
Previous AMI (yes)	1.672 [1.338; 2.090]	< 0.001	1.515 [1.155; 1.988]	0.003
History of stroke/TIA (yes)	1.738 [1.335; 2.263]	< 0.001	1.481 [1.132; 1.938]	0.004
History of PAD (yes)	1.951 [1.520; 2.503]	< 0.001	1.651 [1.271; 2.143]	< 0.001
DM (yes)	1.191 [0.951; 1.492]	0.127	1.227 [0.967; 1.557]	0.093
Hypertension (yes)	0.829 [0.593; 1.159]	0.272	-	-
Diabetic nephropathy (yes)	1.826 [1.324; 2.518]	< 0.001	1.438 [1.021; 2.025]	0.037
Smoker (yes)	0.950 [0.656; 1.376]	0.785	-	-
Asymptomatic carotid artery disease (yes)	1.008 [0.724; 1.404]	0.963	-	-
Combined drugs (yes)*	1.083 [0.852; 1.377]	0.513	-	-

Combined drugs: combined use of antiplatelet agent, statin, and angiotensin-converting enzyme inhibitor at baseline. AMI: acute myocardial infarction; CAD: coronary artery disease; HR: hazard ratio; PAD: peripheral artery disease; TIA: transient ischemic attack; DM: diabetes mellitus.

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Rediscovering Brazil: How We Prevent and Treat Cardiovascular Disease

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Short Editorial related to the article: Evaluation of 1-Year Follow-up of Patients Included in the Registry of Clinical Practice in Patients at High Cardiovascular Risk (REACT)

In Brazil, cardiovascular diseases (CVD) represent 27% of total deaths. These are mainly due to coronary heart disease (32%), stroke (28%) and heart failure (18%).[1,2] Although CVD are the leading cause of death in all five Brazilian regions, the percentage of deaths from CVD is higher in the more developed regions, i.e., South and Southeast.[2]

Preventing cardiovascular disease is preventing deaths from heart attack, stroke and heart failure. In addition to non-pharmacological measures, pharmacological measures are effective and should be applied following the stratification of cardiovascular risk and use of evidence-based drugs. Among subjects with high cardiovascular risk, i.e., those with the greatest chance of cardiovascular events in the next ten years, using pharmacological therapies saves lives. Optimized medical therapy promotes a 36% reduction in mortality, 27% reduction of death/myocardial infarction/stroke and improves the quality of life of patients with heart disease. However, despite the efficacy established and proven in clinical trials, in real life the adherence to therapy is low, even in developed countries, with about 40% of patients receiving optimized therapy after 5 years of diagnosis.[3]

The REACT study brings new data and important messages both for researchers and clinical practice Brazilian doctors. The purpose of the study was to document the national outpatient clinical practice in the treatment of individuals with high cardiovascular risk and to document it both in the baseline and in the 12-month follow-up, also bringing data on adherence to optimized therapy, factors related to adherence and occurrence of cardiovascular events.[4]

The study included about 5,000 individuals, 70% of whom already had cardiovascular disease. The authors included subjects from all the five regions of the country. However, the data is proportionally smaller in the poorest and most distant regions (North: 6.3% and Northeast: 14.6% of the total sample of the study), because in these parts of Brazil capturing information is hard task, as well as follow up subjects is also difficult, making it more difficult to inform and maintain high adherence to the evidence-based medicine practice. Therefore, the first message that the REACT study shows us is the national disparities in the occurrence of CVD and in the appropriate treatment (or not) at the front where primary care doctors are practicing. This must be perceived by health program managers and medical societies to implement programs adjusted to the disparities between regions in Brazil.

Using evidence-based therapies are the most powerful predictor of longer survival free from adverse cardiac events.[5] A Brazilian study in patients with coronary disease showed that optimized drug treatment is cost-effective.[6] In the REACT study, after 12-months follow up only 24% of subjects used concomitantly antiplatelet drugs, statins and ACE inhibitors, showing that the vast majority of patients were not receiving treatment that would increase survival and save money from public coffers and from Brazilian families. Therefore, another message this study brings us is precisely to say that although science has advanced and brought us certainty about the treatment of CVD, the information has not yet reached the doctor at the front.

Finally, the REACT study also found relevant data related to the control of cardiovascular risk factors and comorbidities. Approximately 10% of patients who had diabetes mellitus and hypertension had not been diagnosed even with diagnostic markers with values within the pathological range. In addition, just over 20% of the subjects had LDL cholesterol in the therapeutic target for high cardiovascular risk. As patients were in specialized cardiological centers, greater control of risk factors and comorbidities was expected; this alert us to an even greater problem, since among subjects being monitored by primary care physicians, there may be greater inertia in the detection and diagnosis of risk factors and in the institution of appropriate therapy. It is likely that real-life Brazil has even worse numbers in the diagnosis and treatment of CVD.

In conclusion, disease-modifying therapies reduce death among those at high cardiovascular risk. Clinical practice improvement programs under the coordination of the Brazilian Society of Cardiology, including professional training with the involvement of a non-specialist physician (the primary care physician) must be implemented to ensure that information on the topic is disseminated and reaches the five corners of Brazil, increasing the use of optimized medical therapy and reducing the number of deaths from CVD.

Keywords

Cardiovascular Diseases/complications; Cardiovascular Diseases/epidemiology; Cardiovascular Diseases/mortality; Morbimortality; Myocardial Infarction; Stroke; Prevention and Control; Risk Factors.

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Catheter Ablation of Focal Atrial Tachycardia with Early Activation Close to the His-Bundle from the Non Coronary Aortic Cusp

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Abstract

Background: Atrial tachycardia (AT) ablation with earliest activation site close to the His-Bundle is a challenge due to the risk of complete AV block by its proximity to His-Purkinje system (HPS). An alternative to minimize this risk is to position the catheter on the non-coronary cusp (NCC), which is anatomically contiguous to the para-Hisian region.

Objectives: The aim of this study was to perform a literature review and evaluate the electrophysiological characteristics, safety, and success rate of catheter-based radiofrequency (RF) delivery in the NCC for the treatment of para-Hisian AT in a case series.

Methods: This study performed a retrospective evaluation of ten patients (Age: 36±10 y-o) who had been referred for SVT ablation and presented a diagnosis of para-Hisian focal AT confirmed by classical electrophysiological maneuvers. For statistical analysis, a p-value of <0.05 was considered statistically significant.

Results: The earliest atrial activation at the His position was 28±12ms from the P wave and at the NCC was 3±2ms earlier than His position, without evidence of His potential in all patients. RF was applied on the NCC (4-mm-tip catheter; 30W, 55ºC), and the tachycardia was interrupted in 5±3s with no increase in the PR interval or evidence of junctional rhythm. Electrophysiological tests did not reinduce tachycardia in 9/10 of patients. There were no complications in all procedures. During the 30 ± 12 months follow-up, no patient presented tachycardia recurrence.

Conclusion: The percutaneous treatment of para-Hisian AT through the NCC is an effective and safe strategy, which represents an interesting option for the treatment of this complex arrhythmia. (Arq Bras Cardiol. 2021; 116(1):119-126)

Keywords: Arrhythmias, Cardiac; Tachycardia, Atrial; Catheter, Ablation/methods; Bundle of His; Electrophysiologic, Techniques/methods; Electrocardiography/methods.

Introduction

Focal atrial tachycardias (AT) usually originate from certain structures comprised of atrial tissue, such as the *crista terminalis*, pulmonary veins, atrial appendages, and coronary sinus ostium. Radiofrequency (RF) catheter ablation has been established as the method of choice for the treatment of such arrhythmias. Although *foci* originating in the para-Hisian region are rare, they are a therapeutic challenge due to close proximity to the His-Purkinje system (HPS). As one attempts ablation via the right atrium, risk of affecting the AV node and HPS, thus causing atrioventricular (AV) block, would rise. However, the use of the retroaortic access to explore the non-coronary cusp (NCC), which is anatomically adjacent to the aforementioned region, is a well-known alternative strategy.[1] Experience with the efficacy and safety of this type of ablation remains limited. This study reports on a case series of para-Hisian atrial tachycardia that were mapped and ablated by the NCC. Electrophysiological characteristics and results with this approach were analyzed. Additionally, anatomy of the region and procedural strategies are discussed.

Method

Records of 10 patients (8 women and 2 men; mean age: 36±10 years), from two Brazilian institutions (Heart Institute/InCor, University of São Paulo Medical School; Antonio Prudente Hospital, Fortaleza), subjected to catheter ablation between January 2014 and March 2017,

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were analyzed. Antiarrhythmic drugs were discontinued for at least five half-lives before the procedure. They were evaluated by physical examination, chest X-ray, and echocardiogram, and none of them showed structural heart disease.

The patients underwent electrophysiological study after 8-hours of fasting, under continuous monitoring and with a sedation level controlled by an anesthesiologist. Triple puncture was performed in the femoral vein, and standard catheters (3) were introduced in the coronary sinus (decapolar; 6F), His bundle region (quadrupole, 7F), and base of the right ventricle (quadripolar; 7F).

Programmed atrial stimulation or atrial burst was made with an EP-recording system (EP tracer; Netherlands) to induce tachycardia in two patients; spontaneous onset of tachycardia was observed in one patient; and isoproterenol (10-20mcg; IV infusion) was necessary in seven patients. In one case, an electroanatomic mapping system (Carto 3; Biosense) was available.

Diagnosis of AT was confirmed by using the following electrophysiological observations and maneuvers: changes in the A-A interval before changes in the V-V interval, ventricular entrainment during tachycardia with V-A-A-V-type response, or even changes in the V-A interval during tachycardia (absence of the V-A linking). In all cases, atrial activation with less than 50% of the tachycardia cycle length was observed, indicating a focal pattern of activation.

When the earliest atrial activation was in the right atrial septum and was followed by detectable His potential on the same site, the AT was defined as para-Hisian. Finally, the femoral artery was punctured in order to allow retrograde aortic valve region mapping in detail.

A 4-mm-tip therapeutic catheter was used for radiofrequency (RF) delivery (30W/55ºC during 60 seconds), taking the right and left oblique fluoroscopic incidences as references for anatomical location (Figure 1). In one patient, an electroanatomic mapping system was used (Figure 2). In all cases, the earliest activation site was identified by the NCC, regarding the onset of the peripheral P wave, similar to that detected by the catheter placed in the right interatrial septum, but with the advantageous absence of His bundle potential in the former (Figure 3). Procedural success was defined as the termination of tachycardia during RF application, and non-induction of tachycardia after multiple attempts to induce it with atrial burst or after isoproterenol infusion.

Statistical Analysis

Continuous data are given as mean ± standard deviation (SD) if normally distributed and as median plus interquartile range if not. Otherwise, counts and percentages (%) will be used for categorical data. The Shapiro-Wilk test was used to determine the normality of distribution. The Mann-Whitney U test was employed to compare differences between groups for non-parametric continuous values. Finally, the Fisher's exact test was applied to assess the categorical data in a 2x2 contingency table. For all tests, a p-value of <0.05 was considered statistically significant (2-sided). SPSS software version 19.0 (SPSS, Inc, Chicago, Illinois) was used for statistical analysis.

Results

The Clinical and electrophysiological characteristics of patients can be seen in Tables 1 and 2. All continuous variables, except for P-wave duration during sinus rhythm and tachycardia, displayed normal distribution (Table 2).

None of the patients had previously undergone catheter ablation. The mean atrial tachycardia cycle length was 362±43 ms. Earliest atrial activation recorded on His catheter was 28±12 ms in relation to the peripheral P wave. The atrial

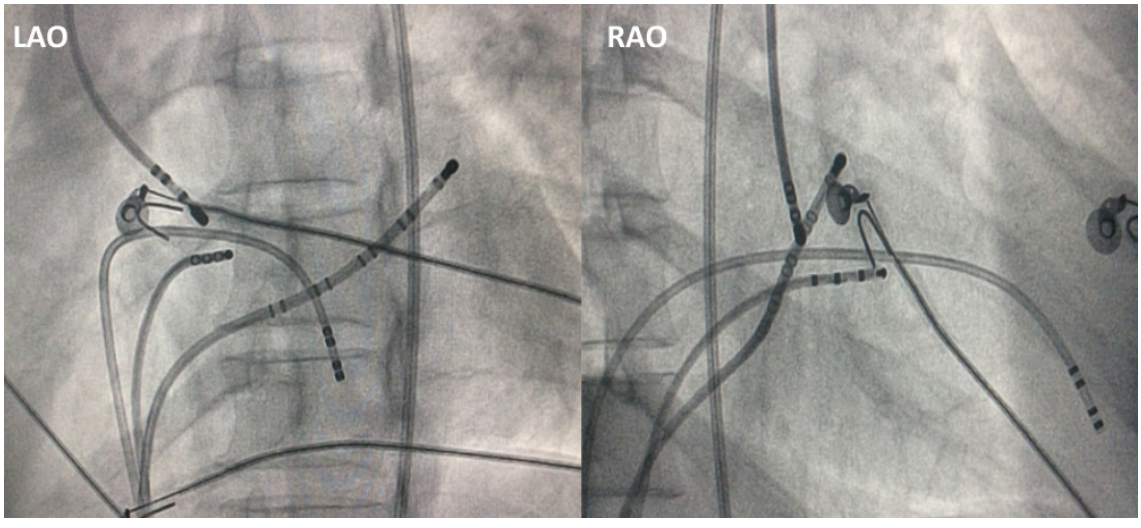

Figure 1 - *The positioning of ablation catheter on the NCC in right and left oblique projection.*

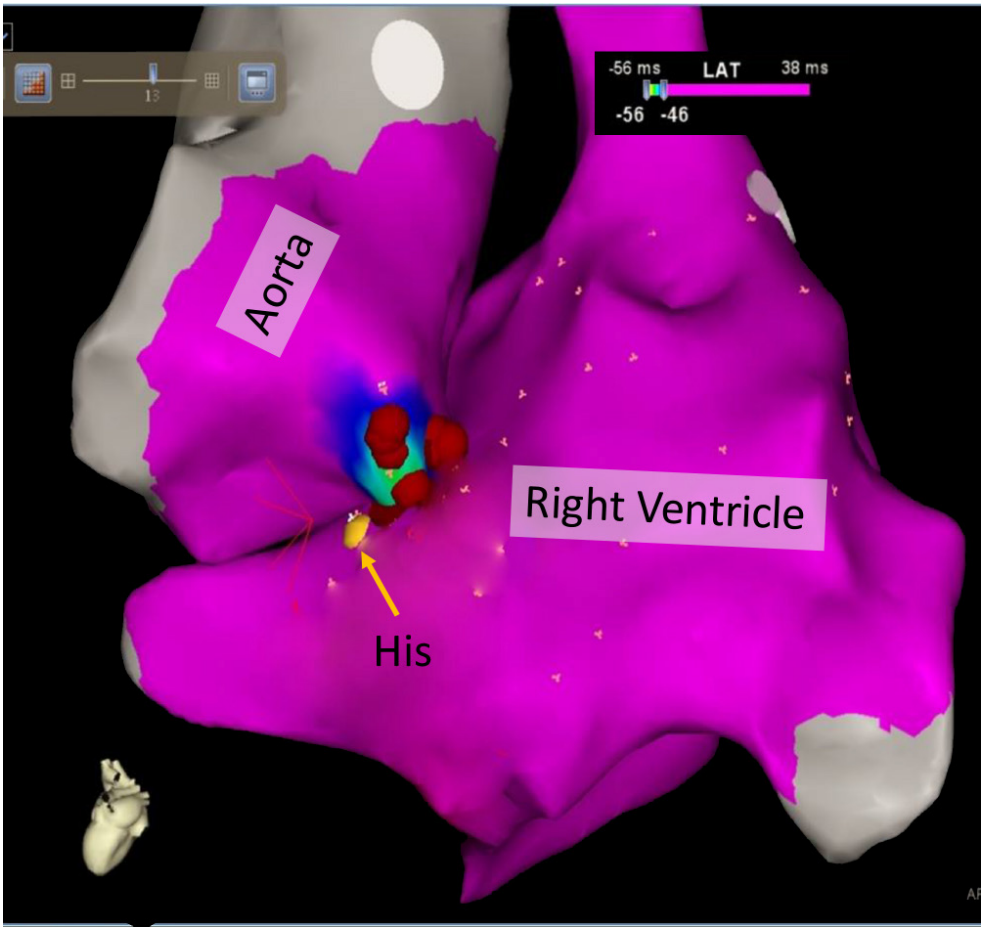

Figure 2 – *Electroanatomic mapping showing RF application points on the NCC. Note the intimate relationship of the non-coronary cusp with the para-Hisian region.*

local activation time recorded by the catheter on the NCC cusp was 3±2 ms earlier compared to the His catheter.

In all cases, the initial ablation site was the NCC, and was successful in 9 of 10 cases. The remaining case required mapping and attempt of ablation in the para-Hisian region with low power (20w), which was unsuccessful as well.

The mean time to atrial tachycardia interruption after RF delivery was 5 seconds. Junctional rhythm and an increase in the PR interval were not observed during application of RF in all cases. All procedures were well tolerated and without complications.

Over a follow-up of 30 ± 12 months, no patient presented a recurrence of atrial tachycardia and remained asymptomatic in clinical evaluation and with dynamic continuous ECG Holter monitoring.

Regarding the surface electrocardiogram, it was observed that the morphology of the P wave demonstrated a biphasic or triphasic pattern in 6 of 10 patients in inferior leads and were significantly shorter in duration compared to the sinus rhythm in all cases 93 ± 17 vs. 112 ± 20 ms ($p<0.05$). (Figure 4).

Discussion

Morphological relations

The aorta occupies a central position at the base of the heart, deeply wedged between the right and left atrioventricular junctions. The spatial relations of the sinuses of Valsalva thus demonstrate proximity with the atrial walls and the adipose tissue interposing between them at the base of the heart. Considering the semilunar pattern of attachment of the aortic leaflets, it is evident that the topographical relations vary according to the deepness inside the sinus. Figure 5A and 5B shows the anatomical relations of the non-coronary aortic sinus relative to the right atrial structures. In particular, the deepest portion of the non-coronary (also called the non-adjacent) aortic sinus is very closely related to the atrioventricular component of the cardiac septum. Figure 5C shows the sinus wall and the landmarks of the junctional area and atrioventricular node

Thus, the NCC becomes an alternative target within a therapeutic strategy in which failure in intervention occurs when ablation is attempted on both sides of the atrial septum

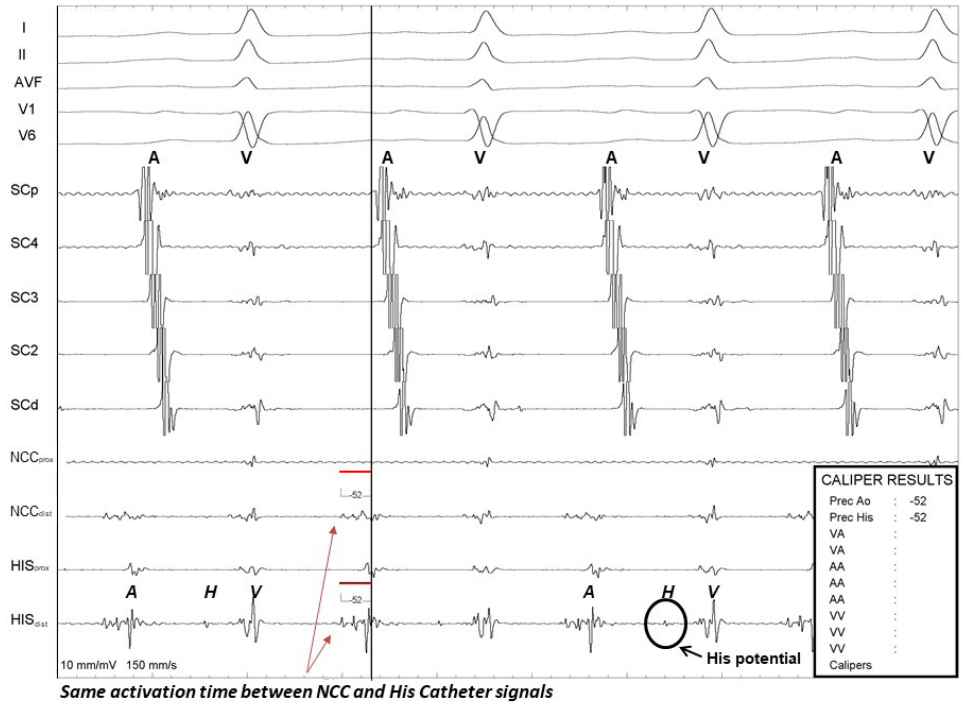

Figure 3 – *Sequentially from top to bottom: peripheral derivations, coronary sinus from proximal to distal electrodes, and recording catheters close to the aortic valve and His bundle region. Similar earliest local atrial activation time is observed in relation to the onset of the P wave from the septal para-hissian region and NCC, but with no His potential seen in the later.*

Table 1 – Clinical characteristics of the evaluated patients

Patient	Age	Sex	Structural heart disease	Duration of symptoms (months)	Ineffective AADs	Past Failed Ablation
1	38	F	None	≤12	0	NO
2	49	M	None	12-24	2	No
3	22	F	None	12-24	1	No
4	28	F	None	12-24	0	No
5	31	F	None	≥48	3	No
6	33	F	None	≥48	1	No
7	46	F	None	≤12	1	No
8	58	F	None	12-24	2	No
9	25	M	None	≤12	0	No
10	30	F	None	≤12	1	No

AADs: antiarrhythmic drugs.

or risk of atrioventricular block occurs as a result of the electrogram record of His bundle close to the ablation target.[2]

From the embryological point of view, neural crest cells contribute to form the aortopulmonary septum, endocardial cushion in the outflow tract, and isolation of the His bundle from the surrounding myocardium. Remnants of these cells on the perinodal region can justify the substrate, which gives rise to maintains arrhythmia.[3] The NCC originates from atrial myocardium, while the right and left coronary cusp originate from ventricular myocardium. This fact explains the frequency of atrial arrhythmias in the NCC and ventricular arrhythmias in the right and left cusps.[3]

Prevalence of tachycardias originating in the perinodal region is about 7-10% in different series with several series, and case reports have shown that para-Hisian tachycardias can be adequately treated with a low complication rate.[4]

Table 2 – The electrophysiological characteristics of the evaluated patients

Variable	Value
Mean age (years)	36 ± 10*
Duration of the p wave during tachycardia (ms)	93 ± 17§
Duration of the p wave during sinus rhythm (ms)	112 ± 20§
Ablation success rate	9/10 (90%)
Mean tachycardia cycle length (ms)	362±43*
Earliest atrial activation recorded on His catheter (ms)	28±12*
Earliest activation site to his-bundle region (ms)	3±2*
Mean fluoroscopy time (minutes)	14 (10 – 18)
Time from ablation start to tachycardia interruption (seconds)	5 (2 – 8)
Use of 3-D mapping system	1/10
Coronary angiography	0/10
Major complications	0/10

**Data is presented as mean + standard deviation (SD). §Value is displayed as median + interquartile range (IQR).*

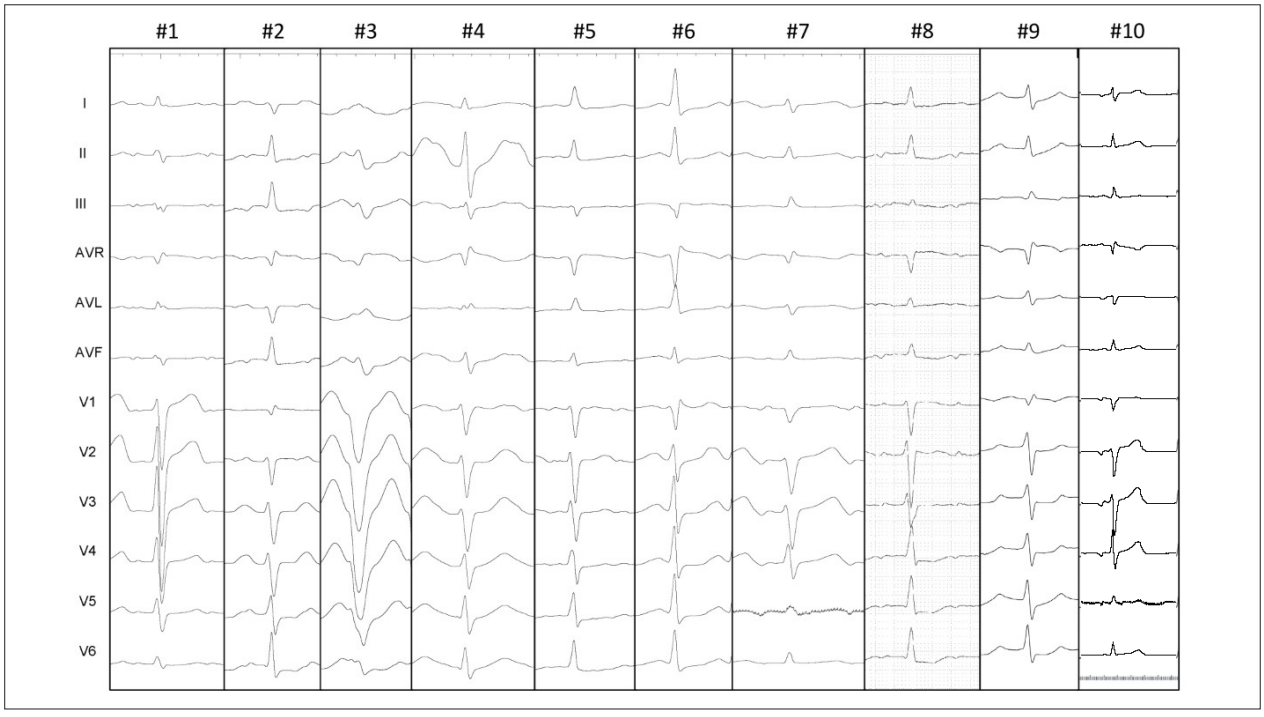

Figure 4 – *P-wave morphology of all cases. The morphology of the P wave, demonstrating biphasic or triphasic patterns in 6 of 10 patients in inferior leads.*

Approach of these tachycardias through the NCC reduces the risk of damage to the conduction system, providing a greater stability to the catheter during RF application, as well as good contact with the tissue. Targeting the right atrial extensions at the NCC, farther from the His Purkinje system, which is situated in the endocardium, is the likely explanation for ablation being effective at this site.[5]

With regard to complications, RF application can cause damage to the heart valves, although this complication has not been reported up to the limits of power (30w) and temperature (55°C) in several series.[6] Coronary angiography was not routinely performed before applying RF because, in our practice, the presence of a local electrogram with atrium greater than the ventricle (A/V ratio >1), anatomically close to a catheter used as a reference in the right atrium, parallel to the conduction system, marks a safe ablation site. Regarding the mapping technique, a ratio greater than or equal to 1 between the amplitudes of the atrial and ventricular electrograms was observed in all patients in the ablation target. This electrophysiological

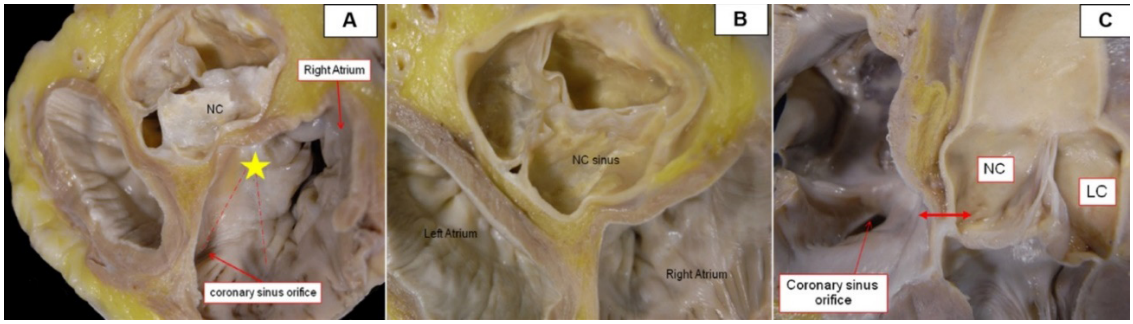

Figure 5 – *An intimate relationship between the NCC and the His bundle region can be observed. A) Oblique view of a short axis section at the base of the heart, showing the non-coronary sinus of Valsalva (NC) and the landmarks of the triangle of Koch (dotted lines) and membranous septum (star). B) Short axis section at the base of the heart showing the spatial relations of the aortic sinuses and the adipose tissue present between the aorta and the atrial walls. C) Longitudinal section of the aortic root showing the short distance between the deep portion of the non-coronary sinus of Valsalva (NCC) and the area corresponding to the apex of the triangle of Koch, located antero-superiorly to the coronary sinus orifice (double-headed arrow).*

feature is of great value because inversion of this A/V relationship suggests that the limit of the NCC is being crossed and the catheter is then supported over the right cusp. This leads to a greater risk of injury to the conduction system, thus serving as an aid and anatomical reference when only fluoroscopy is used.[7]

Electroanatomical mapping (EAM) was used in only one case. The reason for this is that most of our patients came from the public health system, where the above procedure is not available. However, in our sample, as described by Toniolo et al.,[8] it was possible to achieve high success rates despite not using it.

On the other hand, there are situations in which EAM is essential. Bitaraes et al. recently published a case of a pregnant woman with a focal AT refractory to pharmacological treatment, in which the Catheter ablation was successfully performed by the non-coronary aortic cusp with zero fluoroscopy, using only EAM.[9]

Our findings disagree with those of Ouyang et al.,[10] who observed -/+ P wave in the V1 lead associated with P+ in D1 and AVL suggested NCC origin. According to this author, the relationship between the presence of the -/+ P wave, with its most prominent portion being positive, and origin in the left atrium is a relevant fact. Recently, Madaffari et al.[11] published data of P-wave morphology, where a characteristic narrow and biphasic (-/+) or triphasic (+/-/+) P wave in the inferior and precordial leads reliably identifies the group of AT arising from the para-Hisian region. The present study found that the morphology of the P wave, demonstrating a biphasic or triphasic pattern in 6 of 10 patients in inferior leads and a significantly shorter P wave when compared to the sinus rhythm, was variable at the precordial leads.

In our study, an unsuccessful attempt to ablate tachycardia by the NCC occurred in only one of the ten cases, which was ineffective from the right atrium as well. It was assumed that a more aggressive strategy on the right side of the septum could have resulted in both damage to the conduction system and atrioventricular block, justifying the low power output tested (20w). Tachycardia stopped during the applications, but it could be induced again during the infusion of isoproterenol. A deeper target in the septal region could explain the difficulty in eliminating the substrate. Another limitation is that the operator did not explore the left septal region in this case. An irrigated catheter was also not used because, in our opinion, the aortic root is a high blood flow region and unless power delivery was repeatedly limited by high temperature cutoffs, irrigation should not make a significant difference. In the clinical follow-up, the patient was asymptomatic, under the use of betablockers. Thus, no new ablation attempt was performed.

Recently Lyan et al.[12] evaluated different strategies for catheter ablation of focal atrial tachycardia originating near the His bundle region in 68 patients and found that the acute success rate of para-Hisian AT ablation at the NCC was higher than that of ablation at the LA septum and at the RA septum ($p<0.05$). For this reason, they sustain that NCC must be the first and preferred approach to these tachycardias regardless of the local activation time, which rins in line with findings from Bohora et al.[13] By contrast, Madaffari et al.[11] sustain that NCC is only one of three possible approaches to achieve success, and the choice should be based on the local activation time.

Our findings are in agreement with Lyan et al.[12] and Bohora et al.[13] as the NCC approach should be the first choice to perform ablation in this scenario with high success rates.

Conclusion

This study confirms previous observations that the mapping and ablation of focal atrial tachycardia with early activation close to the His-Bundle from the non-coronary aortic cusp (NCC) is an effective and apparently safe procedure. It can therefore be concluded that retroaortic exploration should be mandatory in such cases. A surface electrocardiogram can suggest the suitable target near the His-Bundle region but not in all cases. The knowledge of the relations of the NCC with the conduction system is crucial in the ablation of these tachycardias. These findings should be considered in the therapeutic strategy of this complex arrhythmia.

Author Contributions

Conception and design of the research: Chokr M, Moura LG, Sousa IBS, Scanavacca M; Acquisition of data: Chokr M, Moura LG, Sousa IBS, Pisani CF, Hardy CA, Melo SL, Ponte Filho AD, Costa IP, Tavora RV, Sacilotto L, Wu TC, Darrieux FCC, Hachul DT, Aiello V, Scanavacca M; Analysis and interpretation of the data: Chokr M, Moura LG, Sousa IBS, Pisani CF, Ponte Filho AD, Costa IP, Sacilotto L, Wu TC, Darrieux FCC, Hachul DT, Aiello V, Scanavacca M; Statistical analysis and Obtaining financing: Chokr M, Moura LG, Sousa IBS; Writing of the manuscript: Chokr M, Moura LG, Sousa IBS, Aiello V, Scanavacca M; Critical revision of the manuscript for intellectual content: Chokr M, Moura LG, Sousa IBS, Pisani CF, Scanavacca M.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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Para-Hisian Atrial Tachycardia and Atrioventricular Nodal Reentry Tachycardia: After 25 Years The Same History?

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Short Editorial related to the article: Catheter Ablation of Focal Atrial Tachycardia with Early Activation Close to the His-Bundle from the Non Coronary Aortic Cusp

The recent history of the para-hisian atrial tachycardia (PHAT) looks a lot like the older history of the atrioventricular (AV) nodal reentry tachycardia (AVNRT). The debate about precise anatomic boundaries of AV nodal reentry continues today again in the AVNRT,[1] even 75 odd years after the first suspect that some mechanisms of supraventricular tachycardia could involve the region of the AV node.[2] In this type of arrhythmia, the first target for the catheter ablative therapy was the fast pathway of the AV node:[3] this approach showed a high success rate, but induction of AV block was found in more than one every five patients.[4] After a few years, an approach was proposed from the slow pathway,[5] that proved to be more effective and safer than the approach from the fast pathway.[4] For some years, this new approach generated some debate in the electrophysiology (EP) community. Some authors even suggested to cross over from one technique to the other as long as AVNRT persisted.[6] Nowadays, the approach from fast pathway is definitely abandoned and when we think of catheter ablation of AVNRT, we think only about ablation of the slow pathway.

PHAT are a group of atrial tachycardia (ATs) originating near the His-bundle (HB) region. Their prevalence is quite high in some casuistries,[7] therefore it is important learning to recognize and to treat them. Nowadays, in the same manner of AVNRT, also for PHAT a debate is present for both the anatomic site of origin of this type of arrhythmia,[8] both about the mechanism,[9,10] both about the best catheter ablation approach.[11-14] Some authors hypothesized the presence of a small re-entrant circuit adjacent to the tricuspid annulus;[10] in contrast, other authors described PHAT as focal ATs arising from various location around the tricuspid or mitral annuli.[9] PHAT are amenable to catheter ablation from multiple approaches including right inter-atrial septum, left inter-atrial septum by a transeptal puncture and non coronary sinus (NCS) of Valsalva of the aortic root by a transaortic approach via the femoral artery.

In this issue of *Arquivos Brasileiros de Cardiologia*, Chokr et al.[15] importantly, describe a case-series of patients ablated from the NCS of Valsalva. One of the most relevant findings of their work is the fact the this type of ablation is feasible with a relatively low radiological exposure also without a 3-D electroanatomic mapping system and without the intracardiac echocardiography, of which the current EP community seems to be unable to do without. For this reason, this method can be feasible in every EP lab. However, even if it was already proved that an ablative approach from the NCS of Valsalva is more effective and safer than an approach from the HB region in the right and/or left inter-atrial septum,[12,14] regardless of the earliest atrial activation site, there are authors that in some situations suggest as first choice the ablation of the right or left inter-atrial septum,[11,13] despite the risk of creating damages to the AV conduction system. Probably, as in AVNRT history, in another 25 years, when we will think about PHAT ablation, we will think only about the NCS of Valsalva approach, and the approach from the HB region will be definitely forgotten!

Keywords

Non Coronary Sinus of Valsalva; Atrial Tachycardia; Atrioventricular Nodal Reentry Tachycardia

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Atrial Fibrillation (Part 1): Pathophysiology, Risk Factors, and Therapeutic Basis

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Abstract

Atrial fibrillation is the most common sustained arrhythmia in clinical practice, with a preference for older age groups. Considering population ageing, the projections for the next decades are alarming. In addition to its epidemiological importance, atrial fibrillation is evidenced by its clinical repercussions, including thromboembolic phenomena, hospitalizations, and a higher mortality rate. Its pathophysiological mechanism is complex and involves an association of hemodynamic, structural, electrophysiological, and autonomic factors.

Since the 1990s, the Framingham study of multivariate analyses has demonstrated that hypertension, diabetes, heart failure, and valvular disease are independent predictors of this rhythm abnormality along with age. However, various other risk factors have been recently implicated in an increase of atrial fibrillation cases, such as sedentary behavior, obesity, sleep disorders, tobacco use, and excessive alcohol use. Moreover, changes in quality of life indicate a reduction in atrial fibrillation recurrence, thus representing a new strategy for excellence in the treatment of this cardiac arrhythmia.

Therapeutic management involves a broad knowledge of the patient's health state and habits, comprehending 4 main pillars: lifestyle changes and rigorous treatment of risk factors; prevention of thromboembolic events; rate control; and rhythm control. Due to the dimension of factors involved in the care of patients with atrial fibrillation, integrated actions performed by interprofessional teams are associated with the best clinical results.

Introduction

Atrial fibrillation (AF) is characterized by the complete disorganization of atrial electric activity and consequent loss of atrial systole with a characteristic and easily recognizable electrocardiographic pattern. However, its diagnosis is challenging since many patients are asymptomatic or have fleeting symptoms, thus hindering its record. AF is the most common sustained arrhythmia in clinical practice, affecting 3% of the adult population and preferentially affecting older adults.[1] With population ageing, the projections for the next decades are alarming. The number of patients with AF aged over 55 years in 2060 is estimated to be more than twice that of 2010, which will demand enormous amounts of public resources.[2] In addition to its epidemiological importance, AF is evidenced by its clinical repercussions, including thromboembolic phenomena, increasing the chances of a stroke by 4 times; it is also associated with a higher risk of all-cause mortality and other important conditions such as heart failure.[3,4]

While the age-adjusted incidence and prevalence of AF is lower in women than in men, the same is not true for morbidity and mortality. AF is associated with a higher relative risk for all-cause mortality, stroke, mortality from cardiovascular causes, cardiac events, and heart failure in women.[5]

Patients with this rhythm abnormality are also more vulnerable to hospitalizations. A recent meta-analysis including 35 studies and 311 314 patients reported a hospitalization rate of 43.7/100 people per year. Cardiovascular diseases represented the biggest causes of hospitalization, but non-cardiovascular causes such as cancer and lung diseases were also frequent in this group of patients.[6]

This article aims to review pathophysiological aspects, risk factors, and basis for treatment of AF. Guidelines for preventing thromboembolic events and performing catheter ablation will be addressed in other manuscripts.

Pathophysiological Mechanisms

Various pathophysiological alterations lead to fibrillation, including hemodynamic, electrophysiological, structural, and autonomic (modulatory) factors, as well as triggering factors represented by extrasystoles and atrial tachycardias (Figure 1). These vary from genetic polymorphisms to macroscopic changes in atrial structure, interfering with the electrical activity of cells and resulting in disorganized atrial electrical activity.

The electrical properties of the myocardium are controlled by ionic channels present on the cell membrane. Cell activation relies basically on sodium, calcium, and potassium channels. The cells' refractory period roughly depends on the time between cell activation and the return of the action potential to its initial level. An increase in ionic influx (calcium and sodium) prolongs the refractory period, while an increase in potassium efflux results in a shortening of this period. Another important component of the normal electrophysiology of the heart are connexins: These are proteins present in the junctions between cardiomyocytes

Keywords

Atrial Fibrillation/physiopathology; Arrhythmias Cardiac/physiopathology; Risk Factors; Obesity; Sedentarism; Combined Modality Therapy.

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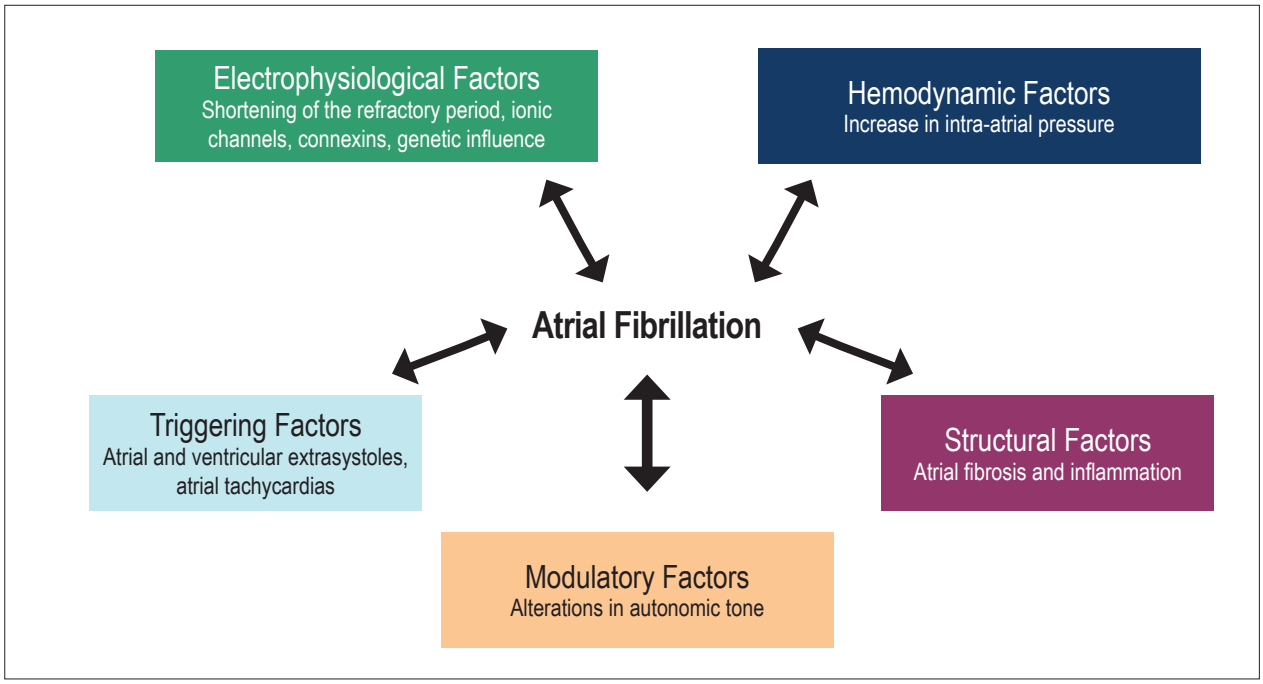

Figure 1 – *Pathophysiological factors implicated in the genesis of atrial fibrillation.*

which are responsible for the ionic permeability between cells, allowing normal propagation of the electrical impulse.[7] In AF, there are alterations in these components of normal cell electrophysiology and these are named electrical remodeling. The most common form of electrical remodeling results from an acute entry of calcium into the cells, which depolarize with an increased frequency. This leads to the inactivation of calcium currents and to an increase in potassium currents, resulting in a shortened duration of the action potential and in increased vulnerability to AF, in addition to favoring early recurrence after cardioversion and the progression of paroxysmal forms to more persistent forms of arrhythmia.[8] Genetic factors can be related to defects in ionic channels and a predisposition for AF. Familial forms of arrhythmia, albeit rare and heterogeneous, are well-described in the literature.[9,10] The role of genetics in AF is being studied and represents a promising path in the increasingly modern search for methods of personalized treatment.

Currently, the most widely accepted theories for the initiation and maintenance of arrhythmia are the presence of ectopic foci as triggers and reentry as a maintenance factor. Initial studies already indicated that the topical application of stimulating substances such as aconitine (an alkaloid able to cause bradycardia and hypotension) in the atrium promoted rapid atrial tachycardia, which in turn induced AF.[11] The crucial study for understanding the focal origin of AF was conducted by Haïssaguerre et al.[12] the authors mapped atrial electrical activity in patients with AF and observed early ectopic foci that preceded the occurrence of arrhythmia and mainly originated inside the pulmonary veins (Figure 2).

Whereas focal activity is necessary for the initiation of AF, an atrial substrate favorable for AF maintenance is equally important. Structural, anatomical, and electrophysiological characteristics are essential for the occurrence and maintenance of reentry circuits, which are currently considered fundamental in the maintenance of arrhythmia. Reentry can be anatomical (with obstacles that create slow conduction zones, such as fibrosis) or functional (homogeneous refractoriness resulting from the erratic propagation of the atrial electrical activation wavefront). These conditions increase the probability of multiple simultaneous reentry waves, contributing to the perpetuation of AF.[13]

Autonomic activity also plays an important role in the initiation and maintenance of AF.[14] Vagal activation can alter acetylcholine-activated potassium currents, with consequent reduction of action potential duration; this may stabilize reentry circuits.[15] Moreover, adrenergic activation can cause intracellular calcium accumulation, which could trigger arrhythmia.

Changes in the atrial myocardium structure, particularly fibrosis, separate muscle fibers and interfere in the continuity of electrical impulse conduction, resulting in a reduced conduction speed fundamental for reentry.[16] Fibrosis leads to AF progression, potentially representing a therapeutic target[17] and a predictor of treatment response.[18] Although electrophysiological factors, such as electrical remodeling, and morphological factors, such as fibrosis and atrial dilation (structural remodeling), are considered the main factors involved in AF pathophysiology, increasing evidence has reported that infectious or inflammatory processes can permeate and unite these two situations. A case-control study with 56 870 participants evaluated the association between influenza virus infection, vaccination, and risk of AF. The authors demonstrated that infection increased the

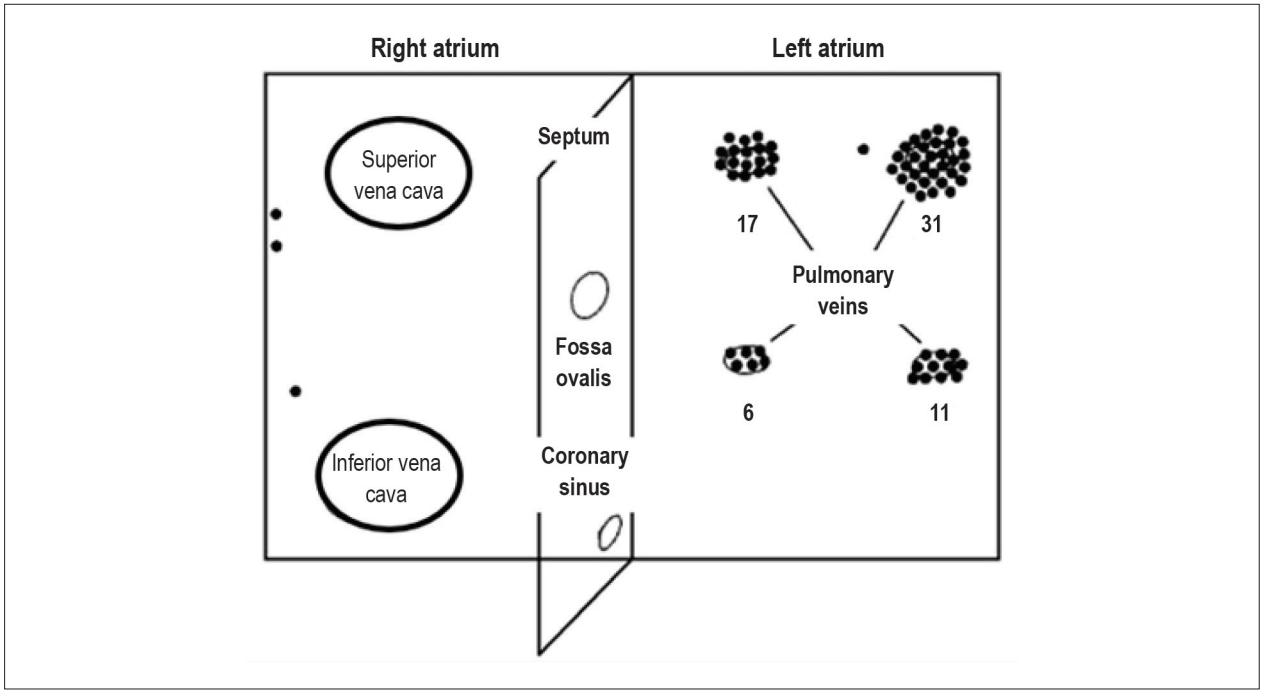

Figure 2 – *Triggering foci of atrial fibrillation in various points of the atria (dark spots) predominantly originated in the pulmonary veins. Adapted from Haïssaguerre M, Jaïs P, Shah DC, et al.12 Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med. 1998;339(10):659–66.*

risk for developing arrhythmia, while vaccination presented a protective effect in different groups of patients.[19] The presence of inflammatory infiltrate, cellular necrosis, and interstitial fibrosis was higher in patients with AF with no register of structural cardiac disease when compared to patients without arrhythmia.[20] These studies have demonstrated a higher concentration of mediators or markers of inflammatory activity such as interleukin-6 or C-reactive protein (high sensitivity) in patients with AF.[21]

Risk Factors for Atrial Fibrillation

The high number of AF cases observed in clinical practice is not only justified by the patients' age; other factors also contribute to this outcome. Since the 1990s, the Framingham study of multivariate analyses has demonstrated that hypertension, diabetes, heart failure, and valvular disease, in addition to age, are independent predictors of this rhythm abnormality.[22]However, various other risk factors have recently been implicated and changes in quality of life indicate a reduction in AF cases, thus becoming a new pillar for excellence in the treatment of AF.[23]

Obesity and Atrial Fibrillation

Obesity, defined as a body mass index (BMI) of over 30 kg/m^2, shows clear association with the occurrence of AF. An important meta-analysis including 51 studies and 626 603 individuals demonstrated a 29% increase in the risk of AF for each 5-unit increase in BMI. In addition, risks for postoperative and post-ablation AF considering the same weight increment were also 10% and 13% higher, respectively.[24] Progression of the disease from the paroxysmal to the permanent form is also more significant in obese patients, as reported by a longitudinal cohort study with a 21-year follow-up.[25] Genetics also seems to justify this association. A study with over 50 000 individuals demonstrated that genetic variants associated with high BMI were correlated with the incidence of AF, suggesting a causal relationship between the two conditions.[26]

From this knowledge, many prospective studies have been conducted for demonstrating the impact of weight reduction in AF recurrence.[27-32] The LEGACY study (Long-Term Effect of Goal-Directed Weight Management in an Atrial Fibrillation Cohort) included 355 patients followed up for 4 years and divided into 3 groups according to the weight loss at the end of the study. Researchers observed a 6-fold higher probability of being free of rhythm abnormalities in participants who lost (and maintained) more than 10% of body weight when compared to those who lost less than 3% or gained weight in the same period.[28] Another prospective and observational study evaluated 149 patients with BMI values over 27 kg/m^2 who were subjected to AF ablation and to an in-person weight reduction program; these patients presented longer arrhythmia-free survival when compared to the control group.[27] Similar results were observed in a prospective study with 4021 obese patients in sinus rhythm and with no previous history of arrhythmia. Groups underwent to bariatric surgery or to conventional treatment. The weight loss observed in the intervention group was associated with a significant reduction in the risk of AF.[33]

On the other hand, a secondary analysis of the Look AHEAD study (Action for Health in Diabetes), which analyzed patients with diabetes, did not observe a reduction in AF occurrence with the implementation of a weight loss and physical activity

program.[34] Another population-based study demonstrated that low lean body mass was also related to the presence of AF.[35] Therefore, the real role of body fat distribution in arrhythmogenesis still requires further clarifications; however, obesity should be recognized as a potentially modifiable risk factor, since a 10% minimum reduction in body weight could decrease the risk of AF in obese and overweight patients.

Obstructive Sleep Apnea

Obstructive Sleep Apnea (OSA) is characterized by the complete or partial recurrent obstruction of the upper airway, resulting in periods of apnea, oxyhemoglobin desaturation, and frequent nocturnal awakenings. The recognition of this sleep disorder by cardiologists has become fundamental after publications showed an increase in mortality from cardiovascular causes in patients with untreated OSA.[36] Many factors contribute to cardiovascular damage in these patients, and numerous mechanisms may possibly be involved. However, 3 main factors deserve attention: intermittent hypoxia, frequent awakenings, and alterations in intrathoracic pressure. These alterations trigger sympathetic nervous system hyperactivity, endothelial dysfunction, and inflammation.[37-40] The sympathetic activation observed in these patients is an important factor that partially justifies the high prevalence of cardiac arrhythmias in this population, including AF. Moreover, OSA can damage left atrial function. Studies with three-dimensional echocardiography demonstrated left atrial dysfunction and remodeling, which were reversed after effective treatment with positive pressure.[41,42]

In an epidemiological study, the occurrence of nocturnal cardiac arrhythmias was more frequent in patients with severe OSA, which was defined as an apnea/hypopnea index (AHI) of over 30 events per hour. Atrial fibrillation occurred in 1.65% of cases with severe OSA and in 0.2% of controls ($p = 0.03$).[43] Another analysis of outpatients followed up for chronic AF in a tertiary hospital and subjected to basal polysomnography discovered that 81.6% presented OSA.[44] OSA and AF are conditions that share risk factors such as age, sex, obesity, hypertension, and heart failure, hence a causal demonstration is challenging in the scientific literature.

In a prospective study[45] with patients referred for electrical cardioversion of AF/atrial flutter, 82% of patients with OSA who received no or inadequate treatment presented recurrence, while this number was 42% in patients who received treatment ($p = 0.013$). In addition, within the group of patients who did not receive treatment, those who presented a higher drop in oxygen saturation during apnea events had even higher recurrence ($p = 0.034$). Treatment of OSA reduces the risk of AF recurrence not only in patients subjected to electrical cardioversion, but also in those who go through catheter ablation. In a study with 426 patients subjected to pulmonary vein isolation, 62 patients presented OSA confirmed by polysomnography, of which 32 were continuous positive airway pressure (CPAP) machine users and 30 were untreated. CPAP therapy was associated with a higher AF-free survival rate when compared with patients who did not use the machine (71.9% vs 36.7%; $p = 0.01$). The authors concluded that CPAP therapy in patients with OSA subjected to percutaneous treatment of AF improved arrhythmia recurrence rates, and in cases of OSA without adequate treatment, electrical isolation had low therapeutic potential.[46] A meta-analysis was then performed for determining the role of OSA in patients with AF subjected to catheter ablation; the study concluded that OSA is associated with a higher risk of AF recurrence after ablation (risk ratio [RR] 1.25, 95% confidence interval [CI] 1.08 to 1.45, $p = 0.003$).[47]

In conclusion, OSA occurrence is high in patients with AF and current data suggest a dose-response relationship between OSA severity and AF recurrence. Adequate treatment of this sleep abnormality reduces clinical AF recurrence even in patients subjected to catheter ablation. Therefore, adequate investigation and treatment (if necessary) are important measures in the clinical management of these patients.

Physical Activity and Atrial Fibrillation

Physical inactivity is a public health problem associated with the increase in cardiovascular diseases, heart failure, stroke, cancer, obesity, type 2 diabetes, and hypertension.[48] It thus promotes various risk factors for AF, whereas the literature has recently suggested physical inactivity as an independent risk factor for AF. Five population-based studies have demonstrated a clear relationship between physical inactivity and increased risk for AF.[49-53] The CARDIO-FIT study (Cardiorespiratory Fitness on Arrhythmia Recurrence in Obese Individuals With Atrial Fibrillation) evaluated the impact of cardiorespiratory fitness gain in the occurrence of AF in obese and overweight patients.[32] Each peak metabolic equivalent gained during follow-up was associated with a 9% reduction in arrhythmia recurrence, even after correction for weight and risk factors. In a study with patients with permanent AF, 12 weeks of moderate to intense exercise were related to a significant increase in quality of life when compared to controls.[54] These findings were reproducible by other randomized controlled studies and the resulting meta-analysis demonstrated that exercise training improves exercise capacity, quality of life, and left ventricular ejection fraction.[55]

On the other hand, the relationship between physical activity and AF appears to be not linear, but a U-shaped curve; that is, its extremes (whether it be sedentary behavior of strenuous exercise) increase the risk of AF.[56] Notably, the strenuous exercise being referred to here relates to exercises performed in extreme doses that exceed recommendations and correspond to a very small percentage of the population. Interestingly, the effect of intense exercise seems to be influenced by sex. A meta-analysis on the subject demonstrated that vigorous physical activity is associated with a significant increase in risk in men (odds ratio [OR]: 3.30; 95% CI 1.97 to 4.63; $p = 0.0002$); conversely, intense physical activity was even more significant for a decrease in the risk of AF in women.[57] The mechanisms involved in this difference are still not completely elucidated, but the fact is that moderate physical activity should be encouraged as prevention and treatment, and for improving quality of life in patients with AF.

Other potential modifiable risk factors

The effects of alcohol in atrial remodeling and in the autonomic nervous system can partially justify the higher AF

recurrence observed in individuals who use alcohol.[58] A population-based study with 109 230 healthy participants whose alcohol consumption was quantified through questionnaires demonstrated that, in men, the risk of AF increased along with the quartiles for weekly use of alcohol, suggesting a dose-response association. The same was not verified in women.[59] Even more interestingly, alcohol abstinence has recently been reported to be related to a reduction in the recurrence of arrhythmia in patients with AF. A multicenter, prospective, randomized study performed in Australian hospitals selected patients with an alcohol consumption higher than 10 weekly doses who had paroxysmal or permanent AF and who were in sinus rhythm at baseline evaluation. The group was divided 1:1 between continuing usual alcohol consumption and practicing alcohol abstinence. A total of 140 patients were included; AF recurrence occurred in 53% of patients in the abstinence group, while 73% of patients in the control group presented recurrence. Time to first recurrence was longer in the abstinence group, and the total number of events after a 6-month follow-up was significantly smaller in those who interrupted alcohol use in comparison with controls.[60]

Studies that evaluated the relationship between tobacco use and AF initially presented conflicting results; however, a meta-analysis including 16 prospective studies and 286 217 participants demonstrated a higher prevalence of AF among tobacco users, while habit cessation was associated with risk reduction.[61] Tobacco use also negatively influenced the results of interventional AF treatment.[62]

It is worth noting that the use of high doses of corticosteroids has also been related with an increased risk of AF.[63] To the present moment, no convincing data have related the use of caffeine with an increased risk of AF; some studies suggest a modest protective effect.[64] The same happens with anxiety disorders: In a recent population-based study with 37 402 adults, no relationship was observed between anxiety or depression symptoms and AF.[65]

Figure 3 summarizes the main modifiable risk factors related to quality of life.

Therapeutic Basis for Atrial Fibrillation

Therapeutic management of AF involves a broad knowledge of the patient's health state and habits and comprehends 4 main pillars: lifestyle changes and rigorous treatment of risk factors; prevention of thromboembolic events; rate control; and rhythm control[66] (Figure 4). We will discuss the therapeutic basis related to long-term treatment.

Lifestyle Change and Rigorous Control of Risk Factors

This pillar aims to reduce the modifiable risk factors associated with quality of life and to rigorously treat cardiovascular comorbidities. Therefore, in addition to controlling body weight, treating tobacco use, tackling sedentary behavior, reducing alcohol use, and optimizing sleep quality, a rigorous control of arterial hypertension, diabetes, and dyslipidemia should also be implemented.

Arterial hypertension is deleterious for patients with AF; not only it constitutes a risk factor for thromboembolic events, but it is also associated with a higher probability of bleeding and recurrence of this arrhythmia. A meta-analysis of AF prevention through the use of renin-angiotensin-aldosterone system inhibitors included 87 048 patients from 23 randomized controlled trials and demonstrated that the use of these drugs reduces the probability of arrhythmia in approximately 33%.[67]

A sub-analysis of the SPRINT study (Systolic Blood Pressure Intervention Trial) evaluated strategies of intensive blood pressure control (systolic blood pressure [SBP] > 120 mmHg) or standard treatment (SBP < 140 mmHg) in AF occurrence. After 5.2 years of follow-up, the risk of AF was 26% lower in the intensive control group when compared to standard control.[68]

Studies demonstrating benefits of arterial pressure control in reducing the risk of AF have been reproducible in the literature, including patients with reduced left ventricle ejection fraction;[69,70] however, some contradictory results have also been published.[71,72] Other factors may possibly influence primary and secondary AF prevention in patients with hypertension and studies are still necessary for better understanding this relationship.

A meta-analysis involving 7 prospective cohort studies and 4 case-control studies, including 108 703 patients with AF, demonstrated that diabetes is associated with a 34% increase in risk for this type of arrythmia, even after adjusting for confounding factors.[73] The pathophysiological mechanisms of this relationship are still being investigated, but could be multiple, including the impacts of diabetes in the autonomic nervous system observed in diabetic neuropathy. Moreover, hyperglycemia is capable of independently increasing sympathetic tone and reducing parasympathetic tone, which could favor the occurrence of arrhythmia. The atrial electrical and structural remodeling associated with oxidative stress also contributes to AF.[74] However, the relationship between diabetes and AF has become even more important with the report that a rigorous glycemic control was associated with a better control of AF. In an analysis with 12 606 patients, 5-year diabetes treatment was associated with a reduction of approximately 30% in AF cases.[75]

Diabetes can also hinder the progression of patients with AF subjected to catheter ablation. A recent multicenter study including 7 high-volume centers in Europe demonstrated a higher AF recurrence within 1 year in the group of patients with diabetes.[76] Glycemic control also appears to favorably influence the progression of patients subjected to ablation. An observational analysis of patients after ablation demonstrated that the use of pioglitazone was associated with a lower need for a second ablation procedure.[77]

The relationship between dyslipidemia and AF is still under investigation: An observational analysis including 2 large databases (MESA and Framingham) demonstrated that high HDL levels were associated with lower risk of AF, whereas high triglyceride levels were associated with a higher risk. No relationship with LDL was observed.[78] Conversely, a prospective population-based study did not find an association between HDL and triglyceride levels and AF, while low LDL levels were associated with a higher

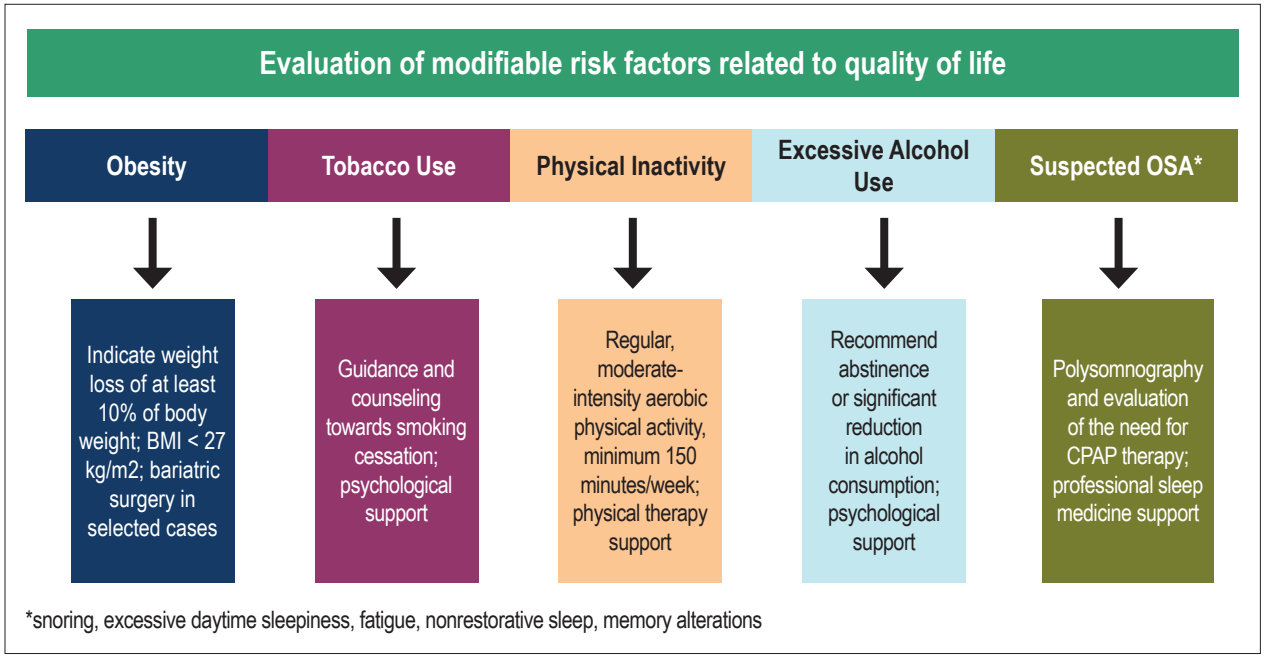

Figure 3 – *Risk factors for atrial fibrillation related to quality of life and their respective guidelines. BMI: body mass index; OSA: obstructive sleep apnea; CPAP: continuous positive airway pressure.*

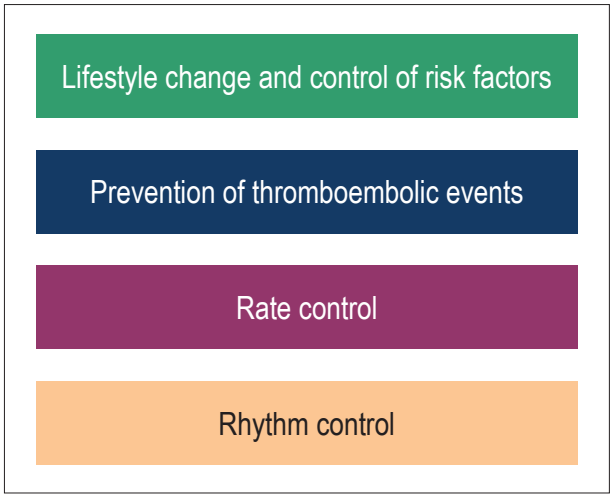

Figure 4 - *Pillars of the therapeutic management of a patient with atrial fibrillation.*

risk of AF. Moreover, the use of hypolipidemic drugs did not influence the occurrence of AF.[79]

Actually, these specific analyses aimed at a single risk factor fail to demonstrate combined actions that are usually employed in clinical practice. For evaluating this effect, 281 consecutive patients who had undergone catheter ablation were selected; they had multiple risk factors and were offered an aggressive program for addressing them. Patients who participated in the program presented significantly higher weight reduction and control of arterial pressure, glycemia, and dyslipidemia. As a consequence, these participants presented higher reductions in AF frequency, duration, and symptoms when compared to the control group ($p < 0.001$).[80]

Prevention of Thromboembolic Events

AF is a form of arrhythmia where evaluating eligibility for the prevention of thromboembolic events is mandatory. The use of anticoagulants is superior to treatment with aspirin alone or associated with clopidogrel. It should be indicated for all patients with AF, except when these are classified as very low risk or during the validity of contraindications to the use of this drug class.[81] Left atrial appendage occlusion represents a second alternative for preventing thromboembolic events in patients with restrictions to anticoagulant use.

Heart Rate Control in Arterial Fibrillation

Heart rate (HR) control is an integral part of the treatment of patients with AF and is normally sufficient for reducing symptoms. The therapeutic target of HR has not yet been established in the literature. The RACE study (Rate Control Efficacy in Permanent Atrial Fibrillation) selected 614 patients with permanent AF who were eligible for rate control; patients were randomized into a lenient strategy (resting HR < 110 bpm) or strict strategy (resting HR < 80 bpm and < 110 bpm during moderate exercise). The objective was to evaluate both strategies regarding a composite outcome including death from cardiovascular causes, hospitalization due to heart failure, stroke, systemic embolism, bleeding, and severe arrhythmias. After a 2-year follow-up, no significant changes were observed between the two approaches, and the frequency of symptoms and adverse events was similar between groups.[82] In a subsequent analysis, the lenient strategy was also not associated with adverse cardiac remodeling.[83]

Drugs used for this purpose include beta blockers, calcium channel blockers (diltiazem, verapamil), digoxin, or a combination thereof.[84] It is worth mentioning that amiodarone can be used in selected cases.

Beta blockers are considered first-line drugs for heart rate control in patients with AF owing to their good tolerability, symptom reduction, and functional improvement. Their therapeutic options, doses, and most common adverse effects are demonstrated in Table 1. It is worth noting that, in case of therapeutic failure, a combination of drugs can be used. In patients with ventricular dysfunction, beta blockers remain the first-choice drug class due to their benefits in this population, and an association with digoxin can be used when necessary. Calcium channel blockers should not be used in patients with heart failure with reduced ejection fraction due to their negative inotropic effect.[84] Finally, atrioventricular node ablation followed by artificial cardiac stimulation represents a therapeutic option in case of failure of the medication-based approach.

Rhythm Control in Patients with Atrial Fibrillation

Acute restoration of sinus rhythm and therapy for maintenance of sinus rhythm are important strategies in the management of patients with AF. Although the maintenance of sinus rhythm appears to be intuitively superior when compared to the rate control strategy , there is no strong scientific literature supporting this claim. The multicenter AFFIRM study randomized patients with AF to these two treatment strategies; they evaluated 4060 patients with a mean age of 69.7 years, 70.8% of which presented arterial hypertension and 38.2%, coronary artery disease. The study reported 310 deaths among patients in the rate control group and 356 among those performing rhythm control after a mean follow-up of 3.5 years (maximum 10 years) ($p = 0.08$). Moreover, the group subjected to rhythm control presented more adverse effects to medications and a higher number of hospitalizations.[85] A similar result was observed in the RACE study, where the primary outcome (death and cardiovascular morbidity) occurred in 17.2% of patients following the rate control strategy and in 22.6% of those performing rhythm control after a 2.3-year follow-up ($p = 0.11$).[86]

Although these studies did not present advantages of rhythm control for survival, some aspects are worth mentioning. A sub-analysis of the AFFIRM study using models for determining relationships between survival, baseline clinical variables, and time-dependent variables demonstrated that the presence of sinus rhythm and anticoagulant use were associated with a lower risk of death. On the other hand, the use of antiarrhythmic drugs was associated with higher mortality after adjusting for sinus rhythm. These data suggest that the benefit of sinus rhythm may have been overlooked and alternative methods for maintaining sinus rhythm with less adverse effects could be promising.[87] Another criticism of these results refers to the short follow-up period. In fact, in a population-based analysis with a follow-up period of more than 5 years, mortality was 41.7% in the group subjected to a rhythm control strategy and 46.3% in the rate control group.[88] Therefore, one should consider that the choice between controlling rhythm or rate should be individualized and this is frequently a dynamic process. In a certain moment, the rhythm control strategy may be attractive, but in older patients with less pronounced symptoms, rate control may constitute an alternative.

Acute restoration of sinus rhythm is performed through chemical or electrical cardioversion according to the current protocols. For the subsequent maintenance of sinus rhythm, long-term use of antiarrhythmic drugs, catheter ablation, or the association of strategies are possibilities that should be discussed with the patient. The use of antiarrhythmic drugs for maintaining sinus rhythm is common in the clinical management of patients. Table 2 shows the available drugs used with this objective in Brazil, with their respective doses and adverse effects. It is important to mention that the adverse effects of antiarrhythmic drugs used in the long term are countless, and Table 2 displays the most common or severe ones. In fact, the choice of antiarrhythmic drugs is established more for their safety profiles than for their efficacy. A classic example is amiodarone: Despite presenting a superior rhythm control effect in comparison with other antiarrhythmic drugs, its use is restricted to patients with heart failure due to important toxic effects of its long-term use.[81] Propafenone and sotalol are predominantly used in patients with no structural heart disease; notably, sotalol can cause QT interval prolongation and electrocardiographic monitoring is recommended when employing these medications.

Table 1 – Drugs used for heart rate control in patients with atrial fibrillation. Adapted from ESC Scientific Document Group.84 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J. 2016;37(38):2893-2962

Drugs most frequently used for heart rate control in patients with atrial fibrillation			
		Dose	Adverse effects
Beta blockers	Metoprolol	100 to 200 mg/day	Lethargy, headache, edema, respiratory symptoms, gastrointestinal alterations, dizziness, atrioventricular block, hypotension
	Nebivolol	2.5 to 10 mg/day	
	Bisoprolol	1.25 to 20 mg/day	
	Carvedilol	3.125 to 50 mg, twice a day	
Calcium channel blockers	Diltiazem	60 mg, three times a day (maximum dose 360 mg/day)	Dizziness, malaise, lethargy, headache, edema, gastrointestinal alterations, atrioventricular block, hypotension
	Verapamil	40 to 120 mg, three times a day (maximum dose 480 mg/day)	
Digoxin		0.0625 to 0.25 mg/day	Gastrointestinal alterations, dizziness, blurred vision, headache, proarrhythmic effects in toxic doses

Table 2 – Antiarrhythmic drugs used for the maintenance of sinus rhythm

Drugs used for the maintenance of sinus rhythm		
	Dose	**Adverse effects**
Propafenone	150 to 300 mg, three times a day	Vertigo, heart palpitations, cardiac conduction disorders, bradycardia, tachycardia, anxiety, sleep disorders, headache
Sotalol	80 to 160 mg, twice a day	Bradycardia, dyspnea, chest pain, heart palpitations, syncope, dizziness, diarrhea, nausea, vomiting, fatigue, rash, torsade de pointes
Amiodarone	100 to 200 mg/day	Neutropenia, agranulocytosis, bradycardia, tachycardia, torsade de pointes, hypo and hyperthyroidism, optic neuropathy, neuritis, pancreatitis, elevated transaminase levels, acute liver injury, confusional state, interstitial pneumonitis, bronchospasm, eczema, urticaria, hypotension

Catheter ablation aiming at the electrical isolation of pulmonary veins is an interventional procedure widely used for the prevention of AF recurrence. Overall, catheter ablation is superior to antiarrhythmic drugs for maintaining sinus rhythm;[89] it is currently indicated in symptomatic patients with paroxysmal or persistent AF refractory or intolerant to at least one antiarrhythmic drug, or as first-line treatment of symptomatic paroxysmal AF according to patient preferences. Other individualized indications may also occur. The CABANA study compared catheter ablation and optimized drug therapy in patients with paroxysmal and persistent AF according to the composite outcome of total mortality, stroke, major bleeding, and cardiac arrest. After a follow-up of 5 years, no significant differences were observed between both strategies,[90] but quality of life analyses demonstrated significant clinical improvement and a superior quality of life in patients subjected to ablation.[91]

Integrated Care of Patients with Atrial Fibrillation

Offering the complex necessary actions for achieving excellence in the care of patients with AF is challenging in clinical practice. The institution of lifestyle changes, rigorous control of risk factors, and promotion of adequate anticoagulation, on top of decisions related to different therapeutic strategies, when centered around a single professional, could produce unsatisfactory results. In this sense, organizing health care services with interprofessional teams when treating patients with AF is fundamental for ensuring the best care. In fact, a randomized study comparing usual care with multidisciplinary care demonstrated a reduction of 35% in relative risk for the composite outcome of hospitalization and mortality.[92] Another important aspect lies on the fact that the complete absence of AF events is often utopic, and treatment should aim to provide improvements in quality of life, promote cardiovascular prevention, and mitigate clinical recurrences.

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Author contributions

Conception and design of the research, Writing of the manuscript and Critical revision of the manuscript for intellectual content: Cintra FD, Figueiredo MJO.

Potential Conflict of Interest

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Changes in the Profile of Emergency Room Patients during the COVID-19 Outbreak in a General Hospital Specialized in Cardiovascular Care in Brazil

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Introduction

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] On March 11, 2020, COVID-19 was declared a pandemic by the World Health Organization, and the first case was reported in Brazil by the end of February.[2]

Given the absence of specific treatment and the high morbidity and mortality of COVID-19, particularly in high-risk groups, extraordinary public health measures have been implemented worldwide.[1] Considering public health, the traditional outbreak response strategy of isolation, quarantine, social distancing, and community containment has been implemented in multiple countries and has played an important role in preventing disease spread.[3]

Since the first COVID-19 case was reported in Brazil, in addition to social distancing measures, a massive campaign has been implemented to prevent patients from seeking medical care at emergency rooms (ER) unless extremely necessary. Most campaign actions took place on social media, traditional media, and government reports.[4,5] These actions were justified by the worrisome COVID-19 spread in ERs and the habit of the Brazilian population of seeking ER care as an alternative to regular care with primary care physicians.[6]

The number of patients around the country seeking medical assistance in ERs for reasons other than acute respiratory syndromes has decreased significantly, particularly after the implementation of social distancing measures.[7,8] Despite these changes, there is a lack of scientific data on the real impact of the COVID-19 outbreak on ERs in Brazil. Aiming to address this knowledge gap, we compared the sociodemographic and clinical characteristics of patients seeking ERs before and after the onset of the COVID-19 outbreak in Brazil.

Keywords

Cardiovascular Diseases; COVID-19; SARS-CoV-2; Coronavirus, Pandemics; Acute Respiratory Syndrome; Hospitalization; Public Hospital; Epidemiology.

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Methods

We conducted a retrospective single-center study assessing the medical records of all consecutive patients who sought medical care in an ER of a private general hospital specialized in cardiovascular care. This facility is located in a state capital of Brazil's Central-West region. We compared data of patients treated before the implementation of quarantine measures in the city and those treated afterwards. The study was approved by the institution's Ethics Committee and as no patient identification data were to be used, a consent form was not required.

The mean number of patients treated monthly in the institution's ER in 2019 was 1500. Since social distancing measures were officially implemented on March 16, 2020 by a state resolution, we decided to compare data referring to the 2 months after quarantine implementation (March 16, 2020 to May 16, 2020) with the same period of the previous year (March 16, 2019 to May 16, 2019).

The assessed variables were: number of patients, age, sex, city of residency, health insurance, reason for seeking medical assistance, and time spent in the ER; we also evaluated whether the patient was a hospital employee, required sick leave, received medication, underwent any laboratory or imaging tests, underwent an electrocardiogram (EKG), was discharged from the ER, required hospital admission, or required admission to an intensive care unit (ICU).

Detailed descriptions of the methods are provided on the Supplemental Material.

Results

During the 2 assessed months of 2019 (pre-COVID-19), the total number of patients treated at the ER was 2934. This number decreased to 1380 in the same months of 2020 (during COVID-19), which translates into a 57% reduction in the total number of treated patients. The number of patients treated per month during the studied time frame is shown on Figure 1.

The sociodemographic characteristics of patients treated at the ER pre- and during the COVID-19 crisis are shown on Table S1 (Supplemental Material). Their mean age was decreased,

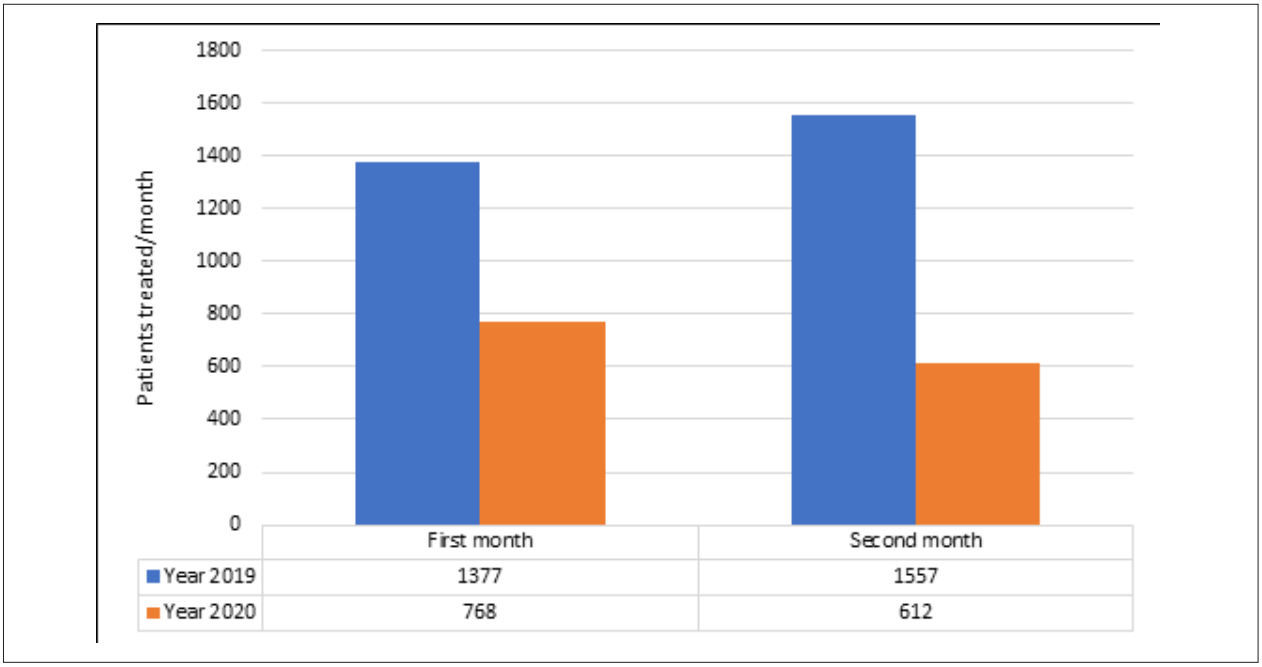

Figure 1 – *Emergency room patients treated per month in the same time frame of the previous year and during COVID-19 social distancing. First month – from March 16 to April 15. Second month – from April 16 to May 16.*

as well as the percentage of patients aged ≥ 60 years and coming from cities other than Goiânia. The proportion of hospital employees and of patients with no health insurance increased during the COVID-19 outbreak.

When comparing the clinical characteristics of patients and treatments pre- and during the COVID-19 outbreak, we observed that almost all variables changed significantly. The number of urgent triage classifications increased, and so did the time spent by patients at the ER. The number of diagnostic procedures performed at the ER (electrocardiographies, laboratory and image tests) increased, while medication use decreased. Patients requiring hospital admission increased, particularly those requiring ICU admission. When comparing the most common diagnoses, there was a decrease in infectious gastroenteritis and dengue fever cases. Conversely, the number of patients with anxiety disorders and respiratory viral syndromes increased. No changes were seen on the proportion of cardiovascular diseases in relation to other diagnoses, although a 49.6% absolute reduction in their cases was observed. A summary of these findings is presented on Table 1.

Additionally, on Table S2 (Supplemental Material), the sociodemographic and clinical differences between patients with or without respiratory viral syndromes were compared. The most significant differences towards patients without respiratory viral syndromes were in the proportion of patients aged ≥ 60 years, triaged as urgent, who required medication, or underwent electrocardiography at the ER. On the other hand, the percentages of patients who were hospital employees, underwent imaging tests, or required sick leaves were the most significantly different and higher in those with respiratory viral syndromes.

Discussion

A significant change in the number of patients treated at ERs worldwide during the COVID-19 outbreak has been reported by letters to editors, points of view, and non-scientific documents. Nevertheless, to our knowledge, this is the first scientific study presenting real-life results of these changes. In our study, we observed a significant reduction in the number of patients cared for at the ER, reaching a 57% decrease. Changes in frequencies of different diagnoses also happened, as well as in the care given to the patients.

The comparison between the 2 months following official COVID-19 social distancing measures and the same period of the previous year was based on seasonal differences observed in patients treated at ERs. In the Brazilian region where the study was conducted, arboviruses, particularly dengue fever, have a high prevalence during the assessed months.[9] Therefore, we believe that our method of comparison is the most reliable and effective for avoiding bias.

We observed a 49.6% absolute reduction in the number of patients with cardiovascular diseases treated at the ER. An Italian study found similar results when assessing only hospital admissions for acute myocardial infarction over a period of one week in comparison with the same week of 2019.[10] Another study, conducted in the USA, found that weekly hospitalization rates for acute myocardial infarction decreased by up to 48% during the COVID-19 period.[11] Although the absolute reduction found in our study was similar to other international data, we found no changes in the relative percentage of patients with cardiovascular diseases treated at the ER during the COVID-19 outbreak.

Table 1 – Clinical aspects of patients and treatments before and during the COVID-19 outbreak in an emergency room of a Brazilian private tertiary hospital

Variables	pre-COVID-19	During COVID-19	p-value
n	2934	1380	
Triaged as urgent	491 (16.7%)	276 (20.0%)	0.009
Time spent at ER* (minutes)	277.8 (222.6)	194.7 (140.0)	< 0.001
Required sick leave	146 (5.0%)	177 (12.8%)	< 0.001
Received medication on ER*	1958 (66.7%)	846 (61.3%)	< 0.001
Laboratory test on ER*	311 (10.6%)	612 (44.3%)	< 0.001
Electrocardiography on ER*	897 (30.6%)	533 (38.6%)	< 0.001
Image examination on ER*	812 (27.7%)	502 (36.4%)	< 0.001
Discharged from ER*	2617 (89.2%)	1132 (82.0%)	< 0.001
Hospital admission	236 (8.0%)	138 (10.0%)	0.033
ICU† admission	81 (2.8%)	110 (8.0%)	< 0.001
Cardiovascular disease	474 (16.2%)	235 (17.0%)	0.470
Infectious gastroenteritis / colitis	160 (5.5%)	22 (1.6%)	< 0.001
Dengue fever	240 (8.2%)	18 (1.3%)	< 0.001
Anxiety disorders	115 (3.9%)	110 (8.0%)	< 0.001
Genitourinary diseases	92 (3.1%)	36 (2.6%)	0.340
Gastrointestinal diseases	62 (2.1%)	34 (2.5%)	0.470
Musculoskeletal and connective tissue diseases	102 (3.5%)	56 (4.1%)	0.340
Respiratory viral syndromes	21 (0.7%)	203 (14.7%)	<0.001

*Values given as means (± standard deviation) or n (%). *ER: emergency room; †ICU: intensive care unit.*

An interesting aspect of the results presented here is the increase in the percentage of patients with anxiety disorders being treated at the ER during the COVID-19 pandemic.[12] This finding is supported by various publications that assessed COVID-19, social distancing measures, and the impact on the population's mental health.[13-15]

Clinical features of suspected/confirmed COVID-19 cases can be seen in our results when comparing patients with and without respiratory viral syndromes. Firstly, the treatment of these patients is time-consuming, which was indicated by a significant increase in time spent at the ER. Since this is a highly contagious disease, patients required more sick leaves. The number of treated patients who were hospital workers also increased, suggesting a high prevalence of COVID-19 in health care professionals, as previously reported.[16] Finally, the higher number of patients requiring ICU admission indicated disease severity.[17]

Potential limitations of this study need to be acknowledged. This was a single-center study conducted in the capital of a state in which the number of COVID-19 cases was low when compared to other state capitals in Brazil. Secondly, we selected only the most common diagnoses defined by the attending ER physician, which left some diseases uninvestigated. Finally, the patients' comorbidities were not reported, since this information was not available on the database used in this study.

It is important to highlight that data collection during a public health emergency is extremely challenging. All efforts were targeted at the pandemic; not only on patient care, but also on the worrisome possibility of health care providers being infected. As more scientific data becomes available, health care teams will be able to provide better care for patients with COVID-19 and other diseases in these difficult times. Another important aspect is the fact that this is an observational study that described changes on patients' features, thus not being accurate for establishing cause-effect relationships.

Author Contributions

Conception and design of the research, Obtaining financing and Statistical analysis: Jardim TSV, Jardim FV, Coragem JT, Jardim PCBV; Analysis and interpretation of the data, Writing of the manuscript and Critical revision of the manuscript for intellectual content Acquisition of data: Jardim TSV, Jardim FV, Coragem JT, Castro CF, Firmino GM, Jardim PCBV

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Hospital do Coração de Goiás under the protocol number 01/2020. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013.

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*Supplemental Materials

For additional information, please click here.

COVID-19 in Early Postoperative Heart Transplantation - Initial Experience

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Introduction

The coronavirus disease (COVID-19) pandemic is rapidly increasing worldwide. Brazil is the country with the second highest number of cases, and it is considered South America's epicenter.[1]

Cardiovascular disease is known to be an important risk factor for infection susceptibility, illness severity, and poor prognosis in COVID-19. Heart transplantation (HT) recipients may have an increased risk due to their comorbidities; however, it has been theorized that immunosuppression might protect them from the cytokine storm responsible for worse outcomes.[2,3] On the other hand, infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported in HT patients with disease presentation similar to general population, questioning the theorized immunosuppression protective mechanism.[4-6]

Herein, we present four cases of COVID-19 during the early postoperative (PO) HT period, with different short-term outcomes, including one death due to respiratory complications.

Case Reports

Case 1

A 51-year-old male patient, on PO day 50, presented chest pain with pleuritic characteristics. His chest computed tomography (CT) showed a ground glass pattern (Figure 1A), and was diagnosed with COVID-19 (Table 1). No specific treatment was required. Transthoracic echocardiography (TTE) showed normal (67%) left ventricle ejection fraction (LVEF). He was discharged home after receiving treatment for minor infectious complications related to immunosuppressive status.

Keywords

Covid-19/complications; Pandemics; Risk Factors; Severe Acute Respiratory Syndrome; Heart Transplantation; Imunossupression.

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Case 2

A 22-year-old female patient had primary graft dysfunction requiring extracorporeal membrane oxygenation (ECMO) for recovery. After weaning from ECMO on PO day 7, she presented fever that lead to COVID-19 diagnosis (Table 1). She required oxygen therapy, without mechanical ventilation or specific treatment. CT scan (Figure 1B) showed a ground glass pattern. The patient was discharged after anticoagulation due to minor pulmonary embolism. Last TTE showed 60% LVEF.

Case 3

A 48-year-old male patient, during hospitalization for decompensated heart failure, presented respiratory symptoms and chest CT suggestive of COVID-19; however, this was excluded after 3 negatives tests. Early PO was uneventful until PO day 21 (Figure 1C), when he presented fever and was diagnosed with COVID-19 (Table 1). Supplementary oxygen therapy was required, but not mechanical ventilation. The patient received azithromycin during his COVID-19 treatment. He was discharged with normal LVEF assessed by TTE (63%).

Case 4

A 31-year-old male patient, on PO day 5, presented cough and delirium. Chest CT showed ground glass images in both lungs (Figure 1C) and he tested positive for COVID-19 (Table 1). Supplementary oxygen therapy was needed, and he progressively got worse, requiring mechanical ventilation. The patient received azithromycin during his COVID-19 treatment. Last LVEF assessed by TTE was normal (65%). The patient died on PO day 12 due to acute respiratory failure.

Discussion

The pandemic of SARS-CoV-2 infection is dramatically increasing worldwide.[1] Elective surgeries have been cancelled and ward/ICU beds dedicated to pre- and postoperative care have been designated for patients with COVID-19. Cardiac surgeons and cardiologists are facing serious issues in making decisions to treat surgical patients in this period, since it is necessary to balance the risk of cardiovascular death due to delayed intervention, the risk of operating a patient in incubation or asymptomatic period of COVID-19 infection, and the risk of being infected during hospitalization after cardiac surgery.[7]

Concerning patients with heart failure, the challenge is even greater, because, due to cardiac decompensation, these patients frequently require long hospitalizations, which increase the risk of COVID-19. From 2010 to 2018, 44% of patients were hospitalized at the time of HT.[8] During the

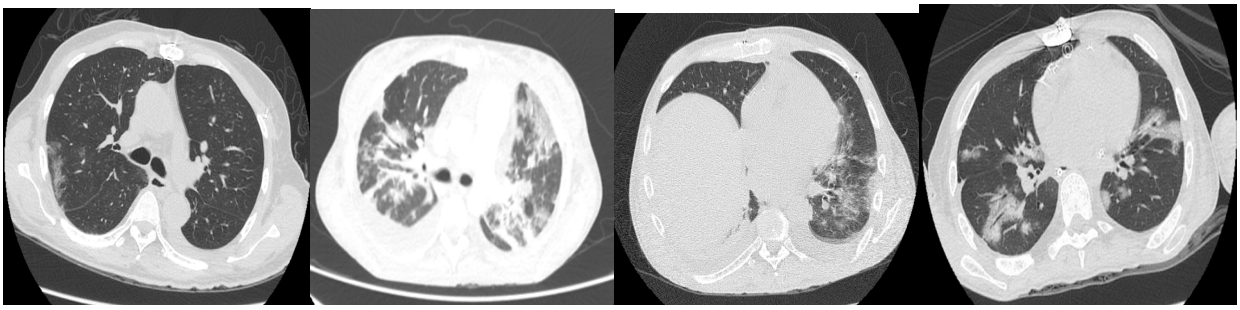

Figure 1 – *Chest computed tomography revealing peripheral ground glass opacities in case 1 (A), case 2 (B), case 3 (C) and case 4 (D).*

Table 1 – Baseline characteristics and laboratory tests at the time of COVID-19 diagnosis

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	55	22	48	31
Sex (male/female)	Male	Female	Male	Male
Heart disease etiology	Chagasic	Dilated	Chagasic	ARVC
INTERMACS	1	2	2	3
Preoperative condition	Inotropic + ECMO	Inotropic + IABP	Inotropic + IABP	Inotropic
Immunosuppression during COVID-19	Corticosteroids + Mycophenolate + Cyclosporine	Corticosteroids + Cyclosporine	Corticosteroids	Corticosteroids + Mycophenolate
LOS pre-HT (days)	14	80	58	143
Cold ischemia time (minutes)	212	261	146	161
LVEF PO HT (%)	67	60	63	65
PO COVID-19 diagnosis (days)	50	45	24	5
COVID-19 presentation	Mild	Moderate	Moderate	Severe

ARVC: arrhythmogenic right ventricular cardiomyopathy; COVID-19: coronavirus disease 2019; ECMO: extracorporeal membrane oxygenation; HT: heart transplant; IABP: intra-aortic balloon pump; INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support; LOS: length of stay; LVEF: left ventricular ejection fraction; PO: postoperative.

pandemic, many HT centers are reassessing their waiting lists, prioritizing patients with lower life expectancy or hospitalized patients who have contraindications for durable left ventricular assist device (LVAD).[9] Unfortunately, this strategy is not feasible for all centers due to a lack of resources, especially during the pandemic.

Our HT recipients include mostly hospitalized and prioritized patients, and durable LVAD was not possible. Most of our patients who underwent HT during the last 10 years were hospitalized at the time of HT. Despite all the preventive measures taken during hospitalization according to institutional protocols, these patients are at high risk of being infected by SARS-CoV-2.

According to the staging classification proposed by Siddiqi and Mehra, only one of our patients had severe COVID-19.[10] The first three patients presented with mild and moderate forms, not requiring specific or intensive care treatment. Only two patients received azithromycin. The last patient died due to acute respiratory failure. Based on our limited experience and other published reports, COVID-19 may have similar presentation in HT recipients during the early PO phase (from mild to severe forms), whether compared to HT recipients in the late PO period or to the general population.[4-6]

To our knowledge, this case series is the first to report results in HT recipients developing COVID-19 during the early PO period, and our experience has shown similar disease presentations compared to non-HT recipients previously reported. Larger series are required to better understand this hypothesis. It currently seems that HT should be considered for patients who cannot be discharged home in centers where durable LVAD are not available, considering individual risks and benefits, weighed for each patient and local situation.

Author Contributions

Conception and design of the research, Analysis and interpretation of the data and Writing of the manuscript: Guerreiro GP, Silveira LMV, Manuel V, Steffen SP; Data acquisition: Guerreiro GP, Silveira LMV; Critical revision of the manuscript for intellectual content: Guerreiro GP, Silveira LMV, Manuel V, Steffen SP, Bacal F, Gaiotto FA, Jatene FB.

Potential Conflict of Interest

The authors report no conflict of interest concerning the materials and methods used in this study or the findings specified in this paper.

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Study Association

This study is not associated with any thesis or dissertation.

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Statins and COVID-19: To Suspend or Not to Suspend? That is the Question!

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Introduction

In the midst of so many uncertainties that permeate the new coronavirus disease 2019 (COVID-19), the evidence relating the presence of dyslipidemia to disease severity and consequent prognostic implications are still scarce. In May 2020, a retrospective Chinese study investigated the association between changes in cholesterol levels and prognosis in approximately 600 patients with COVID-19, who were paired by age and sex with healthy controls. First, it was observed that low-density lipoprotein cholesterol (LDL-C) and total cholesterol levels were significantly lower in patients with COVID-19. Second, there was a trend for LDL-C and total cholesterol levels to decrease as the severity of infection increased (mild, severe, and critical, respectively).[1] In that study, high-density lipoprotein cholesterol (HDL-C) levels were also decreased in severe cases. Similar data were observed by Fan et al.,[2] where levels of LDL-C were inversely associated with the severity of COVID-19. These data suggested a possible relation between low cholesterol levels and worsening of COVID-19 infection. In addition, experimental studies have shown that statins might increase the abundance of the angiotensin-converting enzyme 2 (ACE2), which could in part contribute to the entry of the virus into the cell and increase the risk of infectivity.[3]

Based on these previous findings, it was hypothesized that use of lipid-lowering therapies like statins could aggravate COVID-19 infection. However, it is known that serum cholesterol levels may drop in patients with active viral or bacterial infections,[4,5] since LDL and HDL have a role in the immune system.[6] On the other hand, hyperlipidemia can compromise the immune response and further exacerbate the inflammatory status of COVID-19 patients, increasing cardiovascular risk.[7] So, the question that ensues is, should statins be suspended or not in patients with COVID-19?

Keywords

COVID-19; Coronavirus; Betacoronavirus; Pandemics; Cholesterol; Dyslipidemias; Infection; Dydroxymethylglutaryl-CoA Reductase Inhibitors; Lipoproteins.

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COVID-19, Infections, Thrombosis, and Statins

Evidence of Potential Benefit

In addition to lowering pro-atherogenic lipoproteins, statins have other well-documented systemic effects, such as improvement in endothelial dysfunction, as well as anti-inflammatory and anti-thrombotic properties that lead to stabilization of atherosclerotic plaques.[8] Meta-analyses of randomized clinical trials have shown that statins can significantly reduce concentrations of C-reactive protein,[9] von Willebrand factor antigen,[10] and endothelin-1.[11]

An observational study with 3,043 patients hospitalized for the influenza virus found a lower risk of mortality in those using statins, before or during hospitalization (adjusted odds ratio [OR] 0.59).[12] Benefit from statins was also observed in hospitalized patients with viral pneumonia, resulting in lower mortality and need for intubation (OR 0.26).[13]

Given the pro-inflammatory and pro-thrombotic status observed in patients with more severe COVID-19, the characteristics of these drugs may be important for these patients.

Table 1 shows details of some studies examining the effects of statins in patients with viral infections and COVID-19.

In a retrospective cohort study from Belgium, De Spiegeleer et al.[14] evaluated 154 elderly people (mean age: 86 years) who contracted COVID-19, and observed a significant trend for absence of symptoms in those previously taking statins (OR 2.91; 95% confidence interval (CI), 1.27 to 6.71). This remained statistically significant even after adjusting for covariates (OR 2.65; 95% CI, 1.13 to 6.68).

Another retrospective study of approximately 14,000 patients with COVID-19 found a lower risk of mortality with previous use of statins. In this study, 1,219 patients were receiving statins, and the all-cause mortality at 28 days in this group was 5.2%, while in the non-statin group it was 9.4% (adjusted hazard ratio [HR] 0.58; 95% CI, 0.43 to 0.80; p = 0.001).[15] In another study with 87 patients with COVID-19 admitted to the intensive care unit, a slower progression to death was found in those receiving atorvastatin.[16]

Daniels et al.,[17] through a retrospective single-center study, found a reduced risk of severe COVID-19 in patients who were using statins prior to admission (adjusted OR 0.29), and a faster time to recovery among those without severe disease

Table 1 – Evidence of Possible Benefits of Statins in the Viral Disease Scenario, as well as in COVID-19

Study	Study Design	Patients and Disease	Total (N) Mean Age	Adjustment for Covariates	Results
Vandermeer et al. 2011[12]	Multistate	Patients hospitalized with influenza virus infections	3,043 70 years	Age, race, CVD, lung and renal disease, influenza vaccination, and antiviral administration	Statins prior or during hospitalization versus no statin were associated with a protective odds of death within 30 days Adjusted OR 0.59; 95% CI, 0.30 to 0.92
Henry et al. 2018[13]	Retrospective	Patients with viral pneumonia	539 64 years	NA	Statins continued in hospital versus discontinuation reduced death and/or need of intubation throughout the hospital stay OR 0.26; 95% CI, 0.08 to 0.81; P = 0.02
De Spiegeleer et al. 2020[14]	Retrospective multicenter cohort	COVID-19–positive subjects	154 86 years	Age, sex, functional status, hypertension, and diabetes mellitus	The use of statins was related to the absence of symptoms during COVID-19 OR 2.91; 95% CI, 1.27 to 6.71; P = 0.011 Adjusted OR 2.65; 95% CI, 1.13 to 6.68; P = 0.028
Zhang et al. 2020[15]	Retrospective	Patients hospitalized for COVID-19	13,981 58 years	Age, sex, and SpO2 at admission	Use of statins versus no statin was correlated to the reduction in the risk for 28-day all-cause mortality Adjusted HR 0.58; 95% CI, 0.43 to 0.80; P = 0.001
Rodriguez-Nava et al. 2020[16]	Retrospective cohort	Patients with COVID-19 admitted to intensive care unit	87 68 years	Age, hypertension, CVD, invasive mechanical ventilation, respiratory rate > 30, SpO2 < 94%, PaO2/FiO2 < 300 mmHg or lung infiltrates > 50%, number of comorbidities, and other adjuvant therapies (including hydroxychloroquine, intravenous steroids, azithromycin, tocilizumab, colchicine, and antibiotics)	The use of statin (specifically atorvastatin) has reduced the progression to death Adjusted HR 0.38, 95% CI, 0.18 to 0.77; P = 0.008
Daniels et al. 2020[17]	Retrospective single-center	Patients hospitalized for COVID-19	170 59 years	Age, sex, obesity, hypertension, diabetes, chronic kidney disease and CVD	Use of statins prior to admission reduced development of severe disease Adjusted OR 0.29; 95% CI, 0.11 to 0.71; p = 0.009 Statin use increased rate of recovery from COVID-19 among subjects who had not yet experienced severe disease Cause-specific adjusted HR for recovery 2.69; 95% CI, 1.36 to 5.33; p = 0.004
Song et al. 2020[18]	Retrospective cohort	Patients hospitalized for COVID-19	249 62 years	Age, sex, race, CVD, chronic pulmonary disease, diabetes, and obesity	Statin use decreased risk for invasive mechanical ventilation Adjusted OR 0.45; 95% CI, 0.20 to 0.99; p = 0.048

OR: odds ratio; HR: hazard ratio; CI: confidence interval; SpO_2: peripheral oxygen saturation; CVD: cardiovascular disease; NA: not applicable.

(HR adjusted for recovery 2.69). In addition, in a retrospective cohort study of patients hospitalized with COVID-19 (N = 249) in the United States, the use of statins correlated with decreased risk for invasive mechanical ventilation (adjusted OR 0.45).[18]

Of course, the quoted studies are severely limited by their retrospective design; these data, despite being favorable to use of statins in viral infections, are only hypothesis generating, and they may be subject to a selection bias of individuals receiving better care. The question that ensues is, would there be any evidence that statins may prevent infectious diseases? In a post hoc analysis of patients included in the JUPITER trial,[19] which randomized 17,802 individuals with LDL-C < 130 mg/dL and high-sensitivity C-reactive protein ≥2.0 mg/L to receive rosuvastatin 20 mg/day or placebo followed for a median of 1.9 years, Novack et al.[20] observed that the use of statins reduced, albeit modestly, the incidence of pneumonia (HR 0.83, 95% CI, 0.69 to 1.00). These results, which deserve to be proven in an adequately designed trial, suggest that statins may reduce pneumonia risk due to possible beneficial mild anti-inflammatory, antioxidant, immunomodulatory, anti-apoptotic, and endothelial effects according to the authors.[18] Whether this would benefit patients with COVID-19 is uncertain.

In addition to pulmonary complications, SARS-CoV-2 may also induce thrombosis.[21] Would statins have beneficial effects in these cases? In a pre-specified analysis of the same JUPITER trial,[19] the impact of rosuvastatin on the first occurrence of pulmonary embolism or venous thromboembolism was analyzed. Although there were no differences in the rates of pulmonary embolism between the groups (rosuvastatin and placebo), the group that received the statin showed a 43% reduction in the rates of venous thromboembolism (HR 0.57; 95% CI, 0.37 to 0.86; p = 0.007).[22] Furthermore, a study-level meta-analysis of 13 observational cohort studies (N = 3,148,259) and 23 randomized clinical trials (N = 118,464) showed that, in both observational cohort studies and randomized clinical trials, there was a reduction in risk of deep venous thromboembolism but not of pulmonary embolism, when statin use was compared with controls (relative risk [RR] 0.75; 95% CI, 0.65 to 0.87; p < 0.0001; 0.85; 95% CI, 0.73 to 0.99; p = 0.038). A greater benefit was also found for the risk of venous thromboembolism with the use of rosuvastatin compared to other statins (RR 0.57; 95% CI, 0.22 to 0.75; p = 0.015).[23] Possible mechanisms to explain these results include the effects of statins on pro-thrombotic factors, such as reduced D-dimer, factor VIII,[24] plasminogen activator inhibitor 1, and tissue factor levels, as well as decreased platelet aggregation and increased expression of thrombomodulin.[25] Figure 1 presents some proposed mechanisms where statins may act as antithrombotic and anti-inflammatory agents and could exert favorable effects in patients with COVID-19.

Since a non-negligible portion of patients infected by SARS-CoV-2 (especially the more severe patients) may present alterations in the coagulation system and a high rate of venous thromboembolism,[26] the maintenance of statins may improve these individuals' prognosis. However, similarly to the possible anti-infectious properties, this also needs to be confirmed in randomized clinical trials.

Statin Suspension and Increased Risk of Cardiovascular Events?

The concern that low cholesterol levels could be deleterious to patients with COVID-19 may lead to inappropriate suspension of lipid lowering medications in patients at high risk of cardiovascular disease. Statins are the cornerstone for lipid lowering therapy with the aim of reducing the risk of coronary artery disease (CAD); as a group, statins are one of the most prescribed drugs in the world. The Cholesterol Treatment Trialists Meta-analysis (CTT)[27] showed that for each 1.0 mmol/L (~ 40 mg/dL) reduction of LDL-C, all-cause mortality was reduced by 10% (RR 0.90, 95% CI, 0.87 to 0.93; p < 0.0001), in addition to a 20% reduction in CAD deaths (RR 0.80; 99% CI, 0.74 to 0.87; p < 0.0001).

An important scenario where statin suspension could be deleterious is during the early period after an acute coronary syndrome event. In this scenario, the addition and maintenance of statins are fundamental, and drug suspension may increase patients' risks. In this sense, a Brazilian observational study with 249 patients observed a rebound inflammatory effect in the acute phase of myocardial infarction (MI) after statin withdrawal. Sposito et al.[28] found that, at the beginning of the study, those who were receiving statins had lower C-reactive protein values when compared to those who were not, before the onset of MI. On the fifth day after MI, median C-reactive protein was significantly higher in the group where statins had been suspended.[28] In addition, in an analysis of patients presenting with CAD and chest pain within the last 24 hours in the PRISM study[29] (N = 1,616), Heeschen et al.[30] reported that the use of statins reduced the rate of events after 30 days, compared to patients without those medications (adjusted HR 0.49, 95% CI, 0.21 to 0.86). When statins were suspended after admission, cardiac risk increased (OR 2.93; 95% CI, 1.64 to 6.27; p = 0,005), and, although it was not statistically significant, there was a trend to greater risk compared to patients who had never received statins (OR 1.69; 95% CI, 0.92 to 3.56).[29] Therefore, the withdrawal of these drugs should be viewed with extreme caution, especially after an acute coronary event, since this may lead to appearance of complications, worsening patients' prognosis.

In short, the use of statins is based on solid and robust literature, and their discontinuation, except for medical indication, may lead to acute events, further increasing the risk of patients infected by COVID-19, especially of those in secondary prevention and those who have had a recent acute coronary event. Physicians and patients should keep this knowledge in mind.

When Should We Consider Suspending the Statins in Patients with COVID-19?

According to European Society of Cardiology guidelines, in rare cases where patients with COVID-19 develop severe rhabdomyolysis or increased liver enzymes, temporary suspension of statin therapy is prudent.[31,32] Furthermore, if the patient is at imminent risk of life, suspension should be carried out, at least until recovery from the infection.[33]

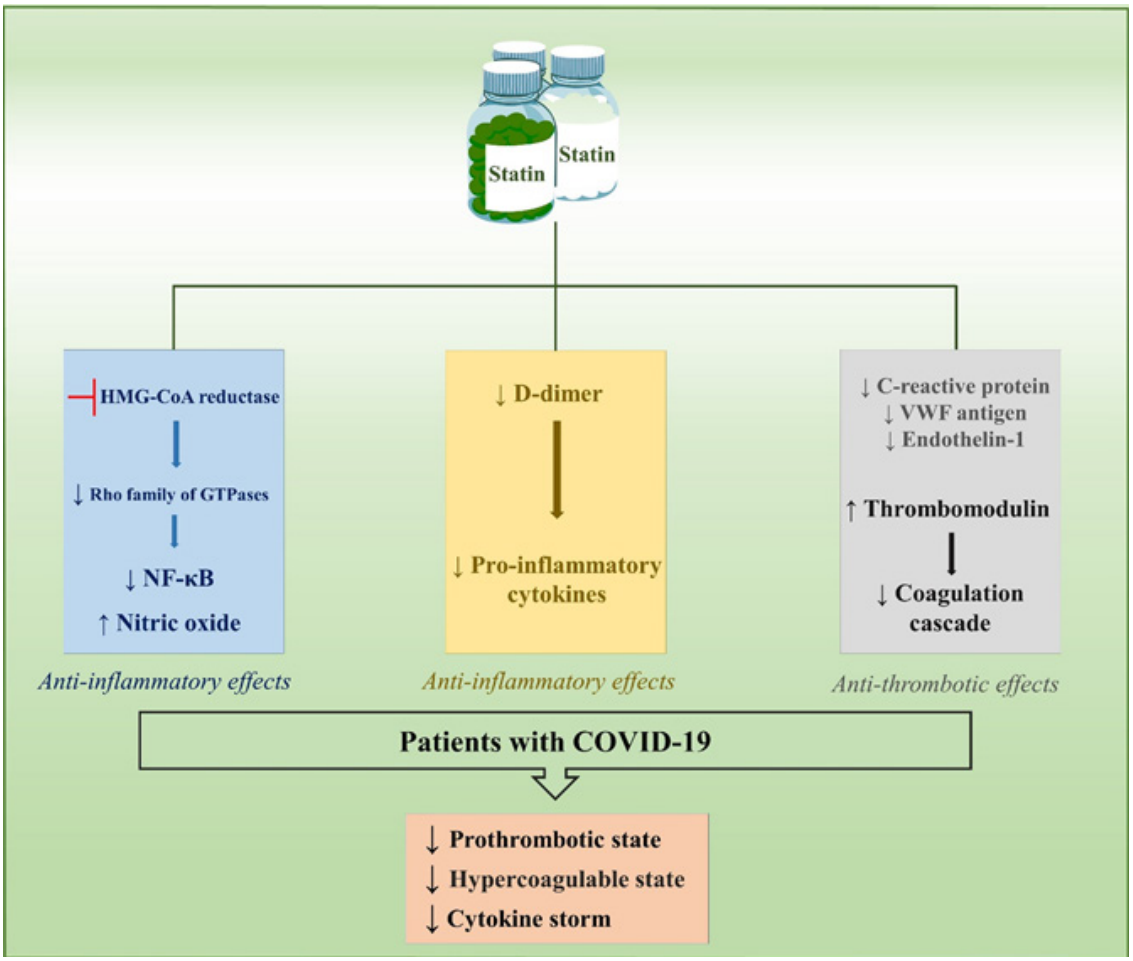

Figure 1 – *Some proposed mechanisms for statins to reduce pro-inflammatory and prothrombotic state in patients with COVID-19.[8-11,24,25] HMG-CoA reductase: 3-hydroxy-3-methylglutaryl-CoA reductase; NF-κB: nuclear factor kappa B; VWF: von Willebrand factor.*

Conclusions

The use of statins is supported by solid literature, with unquestionable cardiovascular benefits. Despite evidence that lower cholesterol concentrations are associated with more severe course of COVID-19, there is, however, no evidence that statins may worsen prognosis. On the contrary, these drugs may reduce the pro-inflammatory and pro-thrombotic mechanisms that characterize more severe cases of COVID-19. Currently, there is no evidence to support discontinuation of statins in patients with COVID-19, except when important elevations of hepatic enzymes, rhabdomyolysis, or drug-attributed risk of life occur. On the other hand, there is no indication for the use of these drugs specifically to prevent complications of SARS-CoV-2 infection.

Author Contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data and Writing of the manuscript: Ferrari F, Santos RD; Critical revision of the manuscript for intellectual content: Santos RD.

Potential Conflict of Interest

RDS has received honoraria related to consulting, research and/or speaker activities from: Aché, Amgen, AstraZeneca, Esperion, Kowa, Novo Nordisk, Merck, MSD, Pfizer, PTC and Sanofi/Regeneron.

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Study Association

This study is not associated with any thesis or dissertation work.

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Telemedicine in Cardiology for Outpatient Follow-Up of Patients at High Cardiovascular Risk in Response to the COVID-19 Pandemic

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Introduction

COVID-19, an infectious disease caused by the new type of coronavirus (SARS-Cov-2), usually shows a benign clinical course, although it can lead to acute respiratory distress syndrome. The main risk factors for the severe form of COVID-19 include older age and presence of comorbidities, such as diabetes, hypertension and other cardiovascular diseases.[1]

In response to the COVID-19 pandemic, elective medical appointments have been reduced.[2] Even though an increase in cardiovascular events as an adverse effect of this healthcare system reorganization would be expected, some reports have suggested a possible reduction of such outcomes in countries with high prevalence of SARS-CoV-2 infection.[3] However, the mechanisms related to this decline are not well understood.

In this context, telemedicine has been used as a strategy for remote assistance and management of patient care, hence allowing for the identification of those in need of a priority medical appointment, as well as remote guidance.[4]

Therefore, this study aimed at assessing the short-term results of measures adopted in response to COVID-19 pandemic by using telemedicine in the following-up of patients at high cardiovascular risk.

Methods

Study Population

This cross-sectional study retrospectively assessed data from patient medical records of teleorientation services performed by cardiologists in the Hospital das Clínicas of the Medical School of Ribeirão Preto, University of São Paulo (HCFMRP-USP), between May 4 and 8, 2020, of patients treated in the ischemic heart disease outpatient clinic who had not attended a scheduled medical appointment since the COVID-19 outbreak, which was declared a pandemic by the World Health Organization (WHO) on March 11, 2020.

Teleorientation

Teleorientation is a modality of telemedicine adopted by the HCFMRP-USP as a strategy during the COVID-19 pandemic, in accordance with the Brazilian Federal Council of Medicine (CFM) Official Letter No. 1,756/2020 and the Ordinance No. 467 of the Ministry of Health (MoH), issued on March 20, 2020.

At the HCFMRP-USP, the use of teleorientation follows institutional rules (HCFMRP-USP Ordinance 96/2020), and can be performed by telephone call, using a standardized questionnaire from the institutional electronic medical record system. The patient (or a representative person) is always informed about the reasons for the contact and asked about consent to be recorded. As a routine if necessary, at least two telephone call attempts were made in different days.

Clinical Data and Management of Outpatient Care

During teleorientation, physicians actively asked whether in the last two weeks from the phone call the patient had any symptoms suggestive of COVID-19 and whether the patient was subjected to laboratory test for SARS-CoV-2. Moreover, the patient was asked about emergence or worsening of chest pain or discomfort, seeking for emergency room, need of hospitalization, treatments received, main reason for not showing up in the outpatient clinic return appointment, and need for renewing medical prescriptions. Finally, the patient or a representative was asked whether the consultation rescheduling had been better or worse for the patient's health.

Statistical analysis

Continuous variables are reported as mean and standard deviation, if normally distributed. Data normality was assessed by the Shapiro-Wilk test. Categorical variables are presented as absolute numbers and percentages. The significance level adopted was lower than 0.05. STATA software was used to perform statistical analysis.

Ethics

This study was approved by the local HCFMRP-USP Research Ethics Committee (protocol nº 4.078.545), conducted under the ethical principles of the Declaration of

Keywords

Betacoronavirus/infection; COVID-19; Pandemics; Telemedicine; Coronary Artery Disease/complications; Ambulatory Care.

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Helsinki, and developed in accordance with the Resolution no. 466/2012 of the National Health Council.

Results

The study included 240 patients, as shown in the flowchart of patient enrollment process (Figure 1). Data were provided by the patient in 70% of the cases (n = 169), whereas in 30% of the cases (n = 71) data were provided by a patient representative.

Patients mean age was 65 ± 10 years, 62% men (n = 148) (Table 1). All patients had coronary artery disease or myocardial ischemia, 60% of them had prior myocardial infarction.

Clinical Course

Symptoms suggestive of COVID-19 were reported by 32 (13%) patients. Rhinorrhea and nasal congestion were the most frequent symptoms, described by 13 individuals, followed by fever (n = 10), odynophagia (n = 9), worsening or onset of dyspnea (n = 5), and anosmia (n = 2). No patient reported hospitalization by COVID-19 or testing for SARS-CoV-2 infection.

New onset or worsening chest pain was reported by 14 (6%) and 12 (5%) patients, respectively. Of these 26 patients, 13 individuals were admitted to emergency rooms, and 3 of them were hospitalized, 1 due to myocardial infarction and 1 due to acute coronary syndrome. Both patients were treated with percutaneous coronary intervention (PCI). A third patient was unable to report the diagnosis that led to the hospitalization. One death was reported: a woman aged 80 years, with reduced left ventricular ejection fraction. Unfortunately, we did not have access to the death certificate to assert the cause of death.

Outpatient Follow-up

The majority of patients (80%) rescheduled the medical appointment, following the recommendations of the HCFMRP-USP, while 13% of patients reported non-attendance due to fear of nosocomial infection with SARS-Cov-2, 3% of the patients had no means of transport to get to the appointment, and 4% of the patients reported other reasons (supplementary table). High, intermediate and low priority medical appointments were scheduled for 15%, 22% and 63% of patients, respectively.

The need for renewal of prescriptions was reported by 8% of the patients. Half of the patients contacted considered that the rescheduling was better for their health, while this strategy was considered neutral or worse by 30% and 20% of the patients, respectively.

Discussion

This study assessed the short-term results of strategies to the following-up of outpatients at high cardiovascular risk by means of telemedicine in response to the COVID-19 pandemic. As the main findings, 11% of the contacted patients had worsening of their cardiovascular condition in the first months of the pandemic, but only half of those patients sought medical evaluation for that reason. Moreover, an important proportion of patients reported fear of attending health facilities due to the potential risk of in-hospital contamination by SARS-CoV-2. In this scenario, teleorientation was highly feasible, of good

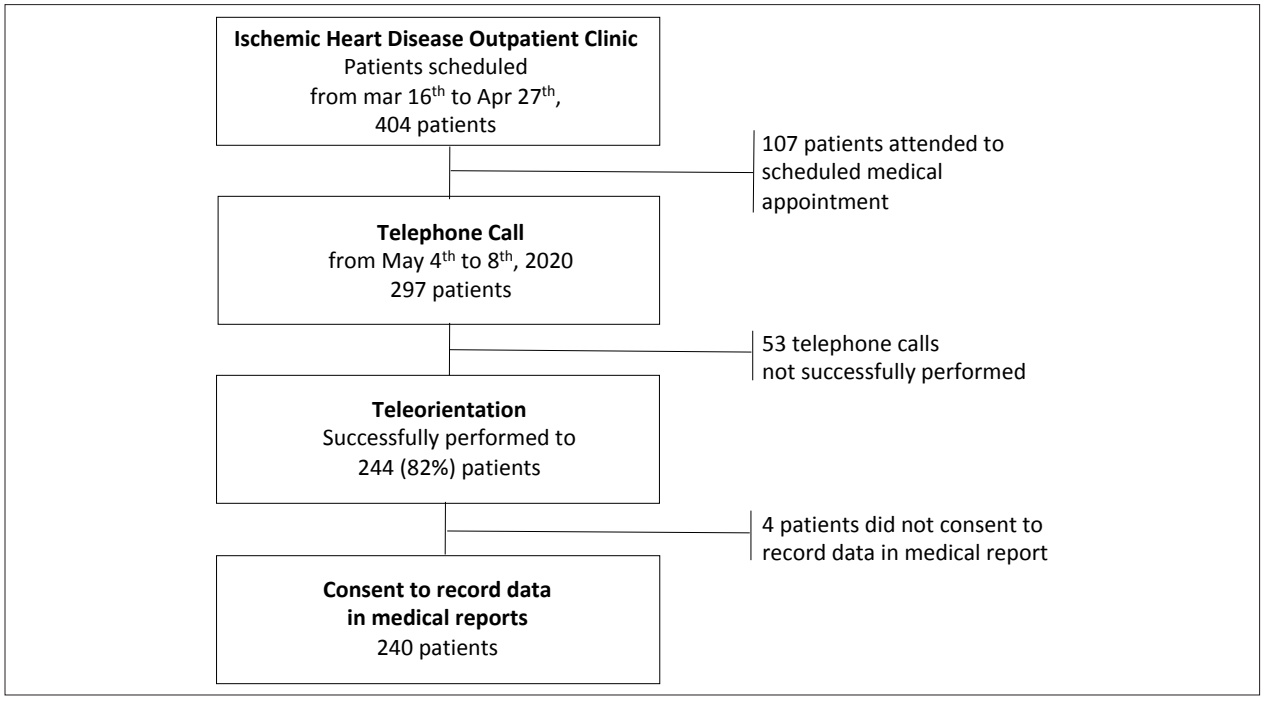

Figure 1 – *Patient enrollment.*

Table 1 – Clinical characteristics of the 240 patients assessed in the study

Demographic	
Age (years)	65 ± 10
Men	148 (62%)
State of residence	
State of Sao Paulo	235 (98%)
Others	5 (2%)
City	
Ribeirao Preto	68 (28%)
Other	172 (72%)
Clinical data	
Hypertension	197 (82%)
Diabetes	136 (57%)
Smoking	
Current	49 (20%)
Former	79 (33%)
Medication in use	
ACE or ARB	194 (81%)
Statins	230 (96%)
Coronary artery disease	
With Previous myocardial infarction	143 (60%)
No previous myocardial infarction	97 (40%)
Percutaneous coronary intervention	141 (59%)
Coronary artery bypass graft	61 (25%)
Left ventricular ejection fraction*	
Normal	129 (54%)
Mid-range	56 (24%)
Reduced	54 (23%)

*ACE: angiotensin converting enzyme; ARB: angiotensin receptor blocker. *Left ventricular ejection fraction was not assessed in one patient.*

acceptance by patients and very useful in the management of medical appointments based on clinical priorities.

Since the first cases of COVID-19, some investigations have reported a decrease in the medical care demand due to cardiovascular events.[5] Data from catheterization laboratories from the US have shown an estimated 38% reduction in emergency ST-segment-elevation myocardial infarction (STEMI) activations at the beginning of the pandemic breakout in that country.[3] Similarly, a more recent study involving 141 countries has indicated that in about two thirds of them there was a decrease of 40% or more in hospital admissions due to STEMI during the first months of the pandemic.[6]

A frequent hypothesis to those findings has been fear of SARS-CoV-2 infection in medical facilities, as has been demonstrated recently in a Brazilian case report.[7] In this study, 13% of patients reported fear of in-hospital infection as the main reason for not attending the previously scheduled medical appointment.

In addition, this study contributes to advance the current knowledge of the telemedicine field, by showing its high feasibility and good acceptance by the patients. The telemedicine-based strategy used in this study allowed for efficient management of medical appointments, scheduled as priority for 15% of contacted patients, while other 85% of patients could postpone their medical visit and hence remain in social distancing. In addition, other needs could be fulfilled, such as medical prescription renewal, which was required for 8% of the contacted patients.

Teleorientation was not successfully completed in 18% of the cases, thus it is not possible to rule out that the proportion of patients who had clinical worsening, and even death rate, was greater than the observed.

Conclusions

Telemedicine in cardiology in response to the COVID-19 pandemic was highly feasible, very effective and widely accepted by patients, allowing for the screening of priority cases and the management of outpatient return appointments.

Author Contributions

Conception and design of the research: Moreira HT, Volpe GJ, Pazin Filho A, Schmidt A; Acquisition of data: Moreira HT, Volpe GJ, Rezek UC, Mendonça PC, Teixeira GCA, Santos BM, Olivieri APG, Chierice AJA, Monteiro HZ, Araújo NM; Analysis and interpretation of the data: Moreira HT, Volpe GJ, Schmidt A; Statistical analysis: Moreira HT; Writing of the manuscript: Moreira HT; Critical revision of the manuscript for intellectual content: Volpe GJ, Rezek UC, Mendonça PC, Teixeira GCA, Santos BM, Olivieri APG, Chierice AJA, Monteiro HZ, Araújo NM, Maciel BC, Pazin Filho A, Schmidt A.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

There were no external funding sources for this study.

Study Association

This study is not associated with any thesis or dissertation work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Hospital das Clínicas da FMRP-USP under the protocol number 4.078.545. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013.

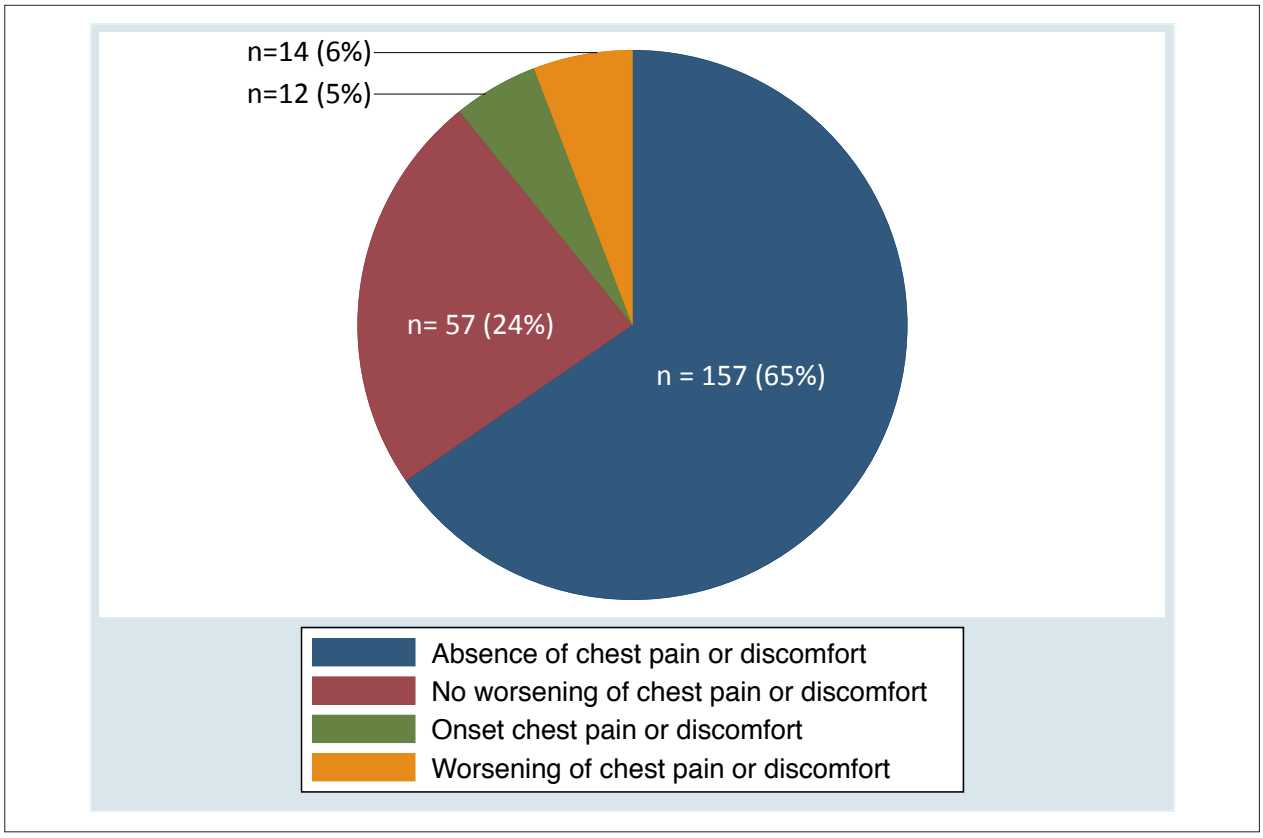

Figure 2 – *Presence of warning symptoms reported by outpatients with chronic coronary artery disease.*

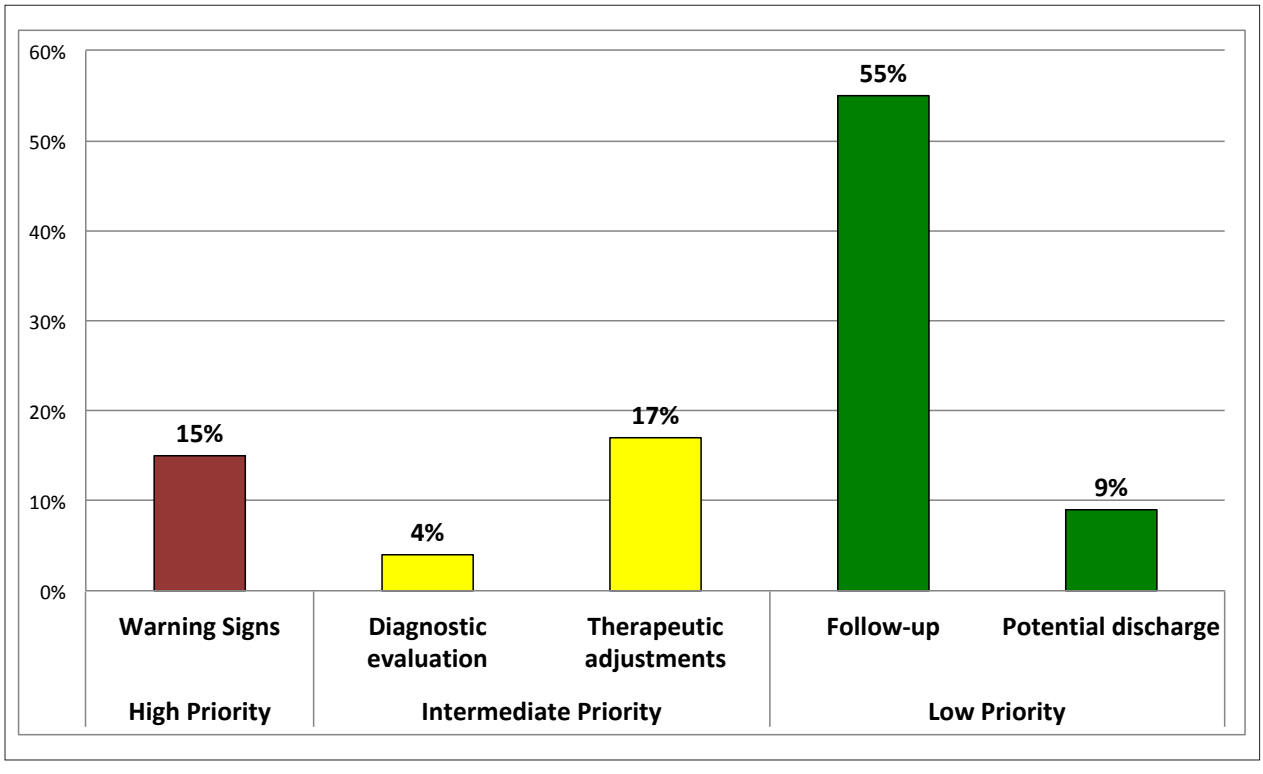

Figure 3 – *Medical visits screened by teleorientation.*

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Confounding Factors in the Analysis of the Relationship between Aortic Arch Calcification with a Non-Dipper Blood Pressure Pattern

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Dear Editor,

We read the following article with great interest: "Aortic Arch Calcification on Routine Chest Radiography is Strongly and Independently Associated with Non-Dipper Blood Pressure Pattern". In this study, the aim was to evaluate a possible relationship between aortic arch calcification on chest radiographs and the non-dipper blood pressure pattern. Altogether, 406 patients were analyzed and divided into two groups: dipper and non-dipper. Approximately 261 (64%) patients presented the pattern of non-dipper blood pressure, against 145 (36%) with dipper blood pressure. It was found that the non-dipper group presented a higher prevalence of aortic arch calcification (70% vs. 33%, $p<0.0001$).

In the multivariate analysis of the study, the outcome of interest is whether the participant belongs to the non-dipper group from a dichotomous variable. As this is a cross-sectional design and not a case-control design, since the independent variables are not retrospective and there is no pairing of the groups, the most indicated analysis strategy would be Poisson or Cox regression. Unlike logistic regression that has Odds Ratio (OR) as a measure of effect, Poisson and Cox regression estimate the Prevalence Ratio (PR), whose application is more appropriate to the design. OR and PR will only be similar when the outcomes are infrequent (<10%).[1]

Keywords

Aorta, Thoracic; Calcification; Blood Pressure; Prevalence Ratio; Thorax/radiography.

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The use of OR in this study brings potential bias due to the high prevalence of the outcome (PADV), making the point estimate over dimensional and its confidence interval more dilated. This condition of analysis brings doubts for variables such as age, body mass index, left ventricular mass index, triglycerides and the glomerular filtration rate which has very borderline confidence intervals (OR~1). It is very likely that calcification is associated with PADV, but not alone and/or to a lesser extent.[2]

Nearly 59 (22.6%) of the patients in the non-dipper group and 25 (17.2%) in the dipper group were diabetics. The researchers did not indicate what kind of diabetes the patients had in the non-dipper group and whether or not these patients were insulin resistant (IR). It is known that IR leads to high plasma levels of insulin and that it acts at the level of hypothalamic receptors of the central nervous system (CNS), leading to an increase in sympathomimetic flow.[3,4] This way, there is a predominance of sympathetic activity. Several studies have shown that sympathetic activation is directly proportional to the severity of hypertension. Thus, in the most severe forms of hypertension, the sympathomimetic flow is more prominent.[3] The authors could have evaluated the real influence of IR on diabetics of the non-dipper group in order to identify the real action of diabetes, avoiding a confounding factor, because it is not possible to state whether such patients had the pattern of non-dipper pressure due to IR or other factors.

Another important consideration is that the study does not mention some limitations of Ambulatory Blood Pressure Monitoring (ABPM), considering that the patients' sleep quality was not evaluated. It is known that low quality of sleep associated with the level of discomfort related to the method can significantly interfere with nightly blood pressure drop. Besides, patients with arrhythmia were not excluded from the study, such as atrial fibrillation, atrial flutter and frequent ventricular extrasystoles.[5] Thus, the correlation between calcification in the aortic arch and the non-dipper pattern assessed by ABPM could also be important confounding factors.

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Reply

Dear Editor,

Thank you for giving us the opportunity to respond to the comments and valid points raised by the authors. We would also like to thank the authors for their interest in and constructive comments on our paper.[1]

In this cross-sectional study, we investigated the potential relationship between aortic arch calcification (AAC) and non-dipper blood pressure (NDBP) pattern. We agree that this relationship could be evaluated with Cox regression analysis. However, we think that logistic regression analysis is also a suitable statistical analysis method for the study.[2,3] When Cox regression analysis was used instead of multiple regression analysis, and age, gender, hypertension, glomerular filtration rate, serum triglyceride level, left ventricular mass index, body mass index and AAC were taken as confounders, presence of AAC on chest radiography was again the only independent predictor of NDBP pattern ($p \leq 0.001$, HR=1.633 CI=1.215–2.194). These results were also confirmed with linear regression analysis. In linear regression analysis, the presence of AAC on chest radiography was associated with a lower systolic blood pressure drop at night.

Diabetes mellitus (DM) is known to be associated with NDBP pattern and insulin resistance is likely one of the most important etiopathogenetic pathways underlying this association.[4,5] In our study, DM was defined as being treated with insulin or oral hypoglycemic agents. Although there was no relationship between DM and NDBP pattern ($p=0.201$), we found a significant relationship between DM and AAC in this study ($p=0.006$). Since our main focus in this study was to investigate the potential relationship between AAC and NDBP pattern, we did not prioritize studying insulin resistance.

We agree that sleep quality may significantly affect nighttime blood pressure.[6] Night-shift workers were excluded from the study; however, we did not use any scale to quantify sleep quality in the study participants. Although we did not receive any negative feedback about sleep quality from any of the participants, we agree that the lack of evaluation of sleep quality is a limitation of the study.

Although all patients were in sinus rhythm at the time of enrollment, we cannot exclude the possibility of short atrial fibrillation/flutter episodes. Long-term rhythm monitorization is needed to detect paroxysmal arrhythmic episodes and to quantify the frequency of ventricular extrasystoles, which were beyond the scope of this study.

Once again, we would like to thank the authors for their thoughtful comments.

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Position Statement on Fat Consumption and Cardiovascular Health – 2021

Development: Atherosclerosis Department (Departamento de Aterosclerose – DA) of the Brazilian Society of Cardiology (Sociedade Brasileira de Cardiologia – SBC)

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Note: These statements are for information purposes and should not replace the clinical judgment of a physician, who must ultimately determine the appropriate treatment for each patient.

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Position Statement on Fat Consumption and Cardiovascular Health – 2021	
The report below lists declarations of interest as reported to the SBC by the experts during the period of the development of these update, 2020.	
Expert	**Type of relationship with industry**
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List of Abbreviations

ABCA1 - ATP binding cassette transporters A1
ABCG1 - ATP binding cassette transporters G1
ACC - American College of Cardiology
Acetyl-CoA - acetyl-coenzyme A
AHA - American Heart Association
Akt - protein kinase B
ALA - alpha-linolenic acid
AMI - acute myocardial infarction
AMPK – AMP-activated protein kinase
APoA-I - apolipoprotein AI
APoB - apolipoprotein B
AST - aspartate transaminase
BMI – body mass index
CAD - coronary artery disease
CE - cholesteryl ester
CETP - cholesteryl ester transfer protein
CHS - Cardiovascular Health Study
CRP - C-reactive protein
CVD - cardiovascular disease
DASH - Dietary Approaches to Stop Hypertension
DHA - docosahexaenoic acid
DIVAS - Dietary Intervention and VAScular function
DPA - docosapentaenoic acid
DRI - Dietary Reference Intakes
EAS - European Atherosclerosis Society
eNOS - endothelial nitric oxide synthase
EPA - eicosapentaenoic acid
EPIC - European Prospective Investigation into Cancer and Nutrition
ER - endoplasmic reticulum
ESC - European Society of Cardiology
FCS - familial chylomicronemia syndrome
FFA - free fatty acid
HbA1c - glycated hemoglobin
HF - heart failure
HMG-CoA - 3-hydroxy-3-methylglutaryl coenzyme A
HOMA-IR - homeostasis model assessment of insulin resistance
HPFS - Health Professionals Follow-up Study
ICAM - intercellular adhesion molecule
IKK - IκB kinase
IL - interleukin
iNOS - inducible nitric oxide synthase
IRS-1 - insulin receptor substrate-1
JACC - Japan Collaborative Cohort Study for Evaluation of Cancer Risk
JNK - c-Jun N-terminal kinase
LOX-1 - oxidized LDL receptor-1
LPL - lipoprotein lipase
LPS - lipopolysaccharide
MCP - monocyte chemoattractant protein
MRI - magnetic resonance imaging
MUFA - monounsaturated fatty acid
NAFLD - nonalcoholic fatty liver disease
NASH - nonalcoholic steatohepatitis
NCD - noncommunicable disease
NF-kB - nuclear factor kappa B
NHANES - National Health and Nutrition Examination Survey
NHS - Nurses' Health Study
NO - nitric oxide
NYHA - New York Heart Association
ORIGIN - Outcome Reduction with an Initial Glargine Intervention
PGC - peroxisome proliferator-activated receptor gamma coactivator
PGE2 - prostaglandin E2
PKC – protein kinase C
PPARγ-2 - peroxisome proliferator-activated receptor gamma
PREDIMED - *Prevención con Dieta Mediterránea*/Prevention with Mediterranean Diet
PUFA - polyunsaturated fatty acid
PURE - Prospective Urban Rural Epidemiology
ROS - reactive oxygen species
SFA – saturated fatty acid
SBC - *Sociedade Brasileira de Cardiologia*/Brazilian Society of Cardiology
SCD1 - stearoyl-CoA desaturase-1
SCFA - short-chain fatty acid
SMC - smooth muscle cell
SREBP - sterol regulatory element-binding protein
T2D - type 2 diabetes
TC - total cholesterol
TG - triglyceride
TLR - toll-like receptor
TMA - trimethylamine
TMAO - trimethylamine N-oxide
TNF - tumor necrosis factor
UFA - unsaturated fatty acid
VCAM - vascular cell adhesion molecule
WHI - Women's Health Initiative
WHO - World Health Organization
ω3 - omega-3
ω6 - omega-6
ω9 - omega-9

Definition of Grades of Recommendation and Levels of Evidence

Classes (grades) of recommendation:

Class I: conditions for which there is conclusive evidence, or, in the absence of conclusive evidence, there is general agreement that a given procedure is safe and useful/effective.

Class II: conditions for which there is conflicting evidence and/or a divergence of opinion about the safety and usefulness/efficacy of a procedure.

Class IIA: weight of evidence/opinion is in favor of the procedure; the majority agrees.

Class IIB: the safety and usefulness/efficacy are less well established, with no prevailing opinions in favor of the procedure.

Class III: conditions for which there is evidence and/or general agreement that the procedure is not useful/effective and in some cases may be harmful.

Levels of evidence:

Level A: data were derived from multiple randomized clinical trials that involved large numbers of patients with similar outcomes and/or robust meta-analyses of randomized clinical trials.

Level B: data were derived from less robust meta-analyses, a single randomized clinical trial, or non-randomized (observational) studies.

Level C: data were derived from consensus of expert opinion.

Statement

Cover Letter

Nutrition plays a key role in the genesis of noncommunicable diseases, which are currently considered one of the most important public health problems worldwide. The quality and quantity of food, in particular dietary sources of fats, can influence both the pathogenesis and prevention of cardiovascular diseases (CVDs). Experts all over the world have developed evidence-based guidelines on fat consumption and suggested an adequate amount of dietary fat, as well as limited consumption of saturated and trans fats. Priority has been given to assessing and proposing healthier eating patterns instead of valuing individual foods, with a much more rational approach to cardiovascular prevention by ensuring an adequate energy intake with the dietary inclusion of grains, fruits and vegetables, restriction of refined carbohydrates and ultra-processed foods, and intake of healthier fats rather than saturated and trans fats.

This position statement aims to guide health professionals in understanding the effects of different fatty acids and to propose appropriate dietary measures targeted at CVD prevention and control.

The Department of Atherosclerosis of the Brazilian Society of Cardiology brought together the country's leading experts to prepare this document in a clear and objective manner in order to provide the best information available to improve clinical practice in our country for the prevention and treatment of CVD.

Yours sincerely,
Prof. Maria Cristina de Oliveira Izar, PhD

Content

1. Introduction

The relevance of diet in the genesis of noncommunicable diseases (NCDs) is well documented in the literature.[1] This set of diseases is currently considered one of the most important public health problems and accounts for approximately 71% of mortality worldwide.[2] In Brazil, in 2016, NCDs were associated with 74% of total deaths, especially cardiovascular diseases (CVDs).[3] The quality and quantity of food, in particular dietary sources of fats, can influence both the pathogenesis and prevention of CVD.

Guidelines and statements on fat consumption have been developed for over 50 years, first published by the American Heart Association (AHA).[4] In the last decades, government agencies and international medical societies, such as the World Health Organization (WHO), United States Government, Institute of Medicine, and European Food Safety Authority, among others, have been engaged in the development of scientific reports based on high-quality evidence.[5] In Brazil, the first guideline on fat consumption was published in 2013 by the Brazilian Society of Cardiology (SBC).[6]

The first studies, published in the 1950s, showed that increased fat intake was significantly associated with an increased prevalence of atherosclerosis.[4] Preliminary studies were based on the analysis of population-based data obtained from dietary surveys, which evaluated the effects of the amount and types of saturated (SFA) and unsaturated (UFA) fatty acids on mortality and CVD. Therefore, the first recommendation regarding fat consumption established a maximum limit of 30% of total energy intake from fat and recommended a reduction in the intake of SFAs.[4] Subsequent guidelines published by the AHA[5] and the 2015-2020 Dietary Guidelines for Americans[7] followed the same line of recommendation for CVD prevention, establishing a maximum limit of 35% of energy from fat, varying according to the lipid profile of each individual. In addition, recommendations included a maximum SFA intake of 10% of energy, promotion of UFA intake, and exclusion of trans fatty acids from the diet.

Thus, the AHA recommendation of a low-fat diet has, in fact, the aim to suggest an adequate amount of fat intake. This recommendation was based on the very high intake of fat by the American population (36-46% of energy), which was associated with increased cardiovascular risk. In addition, only for hypercholesterolemic individuals, the American College of Cardiology (ACC) and AHA[8,9] recommend a limit of 5 to 6% of calories from SFAs. Likewise, the 2019 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines for the management of dyslipidemias: lipid modification to reduce cardiovascular risk[10] recommend limiting the intake of SFAs (<7% of energy) and total fat (<35% of energy).

Controversial results are common in the field of nutrition research, due to inconsistency in protocols regarding study period, study population, sample size, and type of nutrient used in the comparison group.[10] The replacement of calories from SFAs with polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids reduces cardiovascular risk,[11] whereas the replacement of SFAs with refined carbohydrates, such as sugar, elicits the opposite effect.[12] In addition, SFAs can be found in a wide variety of foods, with different structure and composition, such as meat, milk, oils, and processed foods. The fact that SFAs are present in fat emulsions, such as milk, and in solid matrices of palm oil with sugar, such as store-bought cookies, induce different effects on plasma lipids.[13]

Another important factor that can interfere in the analysis of the role of fatty acids is the dietary pattern in which they are consumed. From a cardiovascular point of view, SFAs can be associated with deleterious effects when consumed in a context of a diet high in sugar and low in fiber, whereas their negative impact may be attenuated in a context of healthy eating patterns.[14]

Although, in general, SFAs are associated with increased cardiovascular risk, it is important to note that not all SFAs increase plasma cholesterol levels and cardiovascular risk.[11] In addition, several studies have shown that increased SFA intake can induce an increase in HDLc.[11] However, this information should be interpreted with caution, because these HDL particles are enriched with pro-inflammatory proteins, which may reduce their functionality and affect some stage of reverse cholesterol transport.[15]

International guidelines point out the importance of following healthy eating patterns, such as a Mediterranean diet[16-18] and the Dietary Approaches to Stop Hypertension (DASH) diet.[19] The Dietary Guidelines for the Brazilian Population[20] also highlight the importance of following healthy eating patterns and emphasize that the study of isolated nutrients does not fully clarify the influence of diet on health. These guidelines also explain that benefits should be attributed less to an individual food and more to the combination of foods that characterize the dietary pattern.

As a common point, all these guidelines emphasize the importance of an adequate energy intake, inclusion of grains, fruits, and vegetables in the diet, and reduction of refined carbohydrates, especially sugars. Regarding fat intake, priority should be given to the consumption of MUFAs and PUFAs, with limited intake of SFAs, which is consistent with the AHA guidelines[9] on the recommended healthy profile of fat intake.

According to recent data from the National Health and Nutrition Examination Survey (NHANES) study, there has been a reduction in the intake of refined carbohydrates and SFAs by the American population. Nevertheless, this population still exceeds the recommended amount of these nutrients.[21]

In Brazil, no study has provided sufficient data for a detailed over-time analysis of the percentage consumption of fats. However, important results from the 2019 Brazilian Household Budget Survey (POF/IBGE),[22] which compared the period from 2017-2018 to 2002-2003, showed a significant decrease in household expenses with oils and fats. In addition, this survey showed a reduction in the intake of legumes (grains). The survey also showed that almost one-third of the population eats out, which increases the likelihood of eating in snack bars, where people often choose foods of low nutritional quality, that is, with a low content of fibers and vitamins and a high concentration of fats and refined carbohydrates. Although there was a small increase in household expenses with fruits, data from the 2018 Brazilian Telephone Survey for Surveillance of Risk and Protective Factors for Chronic Diseases (VIGITEL) show that only 24.4% of the population consumes fruits and vegetables within the amounts recommended by the Brazilian Ministry of Health[3] and that 32% of the population eats high-fat meat daily. Moreover, ultra-processed foods that have low nutritional value, such as sandwich cookies, are those that most contribute to the consumption of SFAs and sugar.[22]

Brazil was one of the 195 countries included in the Global Burden of Disease Study 2017,[1] whose main objective was to evaluate the impact of diet on NCD morbidity and mortality. The main causes of cardiovascular mortality attributable to diet included high intakes of sodium and trans fats and low intakes of fruits, vegetables, whole grains, and foods that are sources of PUFAs. The study also showed that, in Brazil, the main dietary risk factor associated with cardiovascular mortality and morbidity was low intake of grains, which, in our population, are mainly represented by beans. In fact, data collected by both Brazilian surveys, VIGITEL and POF, emphasize that there was a reduction in the consumption of beans, which, in addition to being part of the Brazilian food culture, are part of a healthy dietary pattern due to their low fat content and significant amount of fiber.

Despite the deleterious impact of trans fats on cardiovascular risk, a recent study conducted in Brazil revealed that one-fifth of packaged foods are still prepared with this fatty acid.[23] In addition, other commonly consumed snack foods, such as fried or baked snacks, puff pastry, and pies, among others, are often prepared with trans fats. In this respect, the diet currently consumed by some Brazilians contrasts with current international recommendations on healthy eating.

The present position statement developed by SBC aims to describe recent advances regarding the effects of different fatty acids, ranging from their influence on the gut microbiota, liver lipid metabolism, and adipose tissue to the main aspects related to CVD risk and control.

2. Fatty Acid Classification and Sources

2.1. Monounsaturated Fatty Acids

MUFAs are characterized by the presence of a single double bond in the carbon chain. Oleic acid (omega-9) is the most abundant MUFA in nature, accounting for 90% of all MUFAs,[24] with olive and canola oils as the main oil sources. MUFAs also play a prominent role in the composition of fatty acids in several nuts, such as macadamia nuts (59%), hazelnuts (46%), peanuts (41%), almonds (31%), cashews (27%), and pistachios (24%).[25] Another oil rich in MUFAs is high oleic acid, which has been used in some countries and can be prepared from sunflower, canola, or soybean oils.[26,27] With due attention to the high SFA content, meat products are also considered important sources of MUFAs, accounting in some cases for 40

to 50% of the composition of foods such as beef, chicken,[28] and pork.[29]

2.2. Polyunsaturated Fatty Acids

PUFAs are part of a broad group of fats with two or more double bonds in the carbon chain. This characteristic confers widely different biological functions and, therefore, their impact on cardiovascular health is also distinct depending on the type of PUFA consumed. They are part of the omega-6 (ω6) or omega-3 (ω3) series depending on the position of the first double bond counted from the methyl end of the carbon chain. The ω6 fatty acids are classified as linoleic acid (18:2), whose main sources are oils (sunflower, corn, and soybean), walnuts, and Brazil nuts, and arachidonic acid (20:4), obtained from endogenous conversion of linoleic acid. The main ω3 fatty acids are alpha-linolenic acid (ALA [C18:3]) of plant origin, whose main sources are soybean, canola, flaxseed, and chia seeds,[30,31] and eicosapentaenoic acid (EPA [C20:5]) and docosahexaenoic acid (DHA [C22:6]), found in fish and cold-water crustaceans from the Pacific and Artic oceans. Linoleic and linolenic fatty acids are considered essential for humans, and must be obtained from food. However, according to the Dietary Reference Intakes (DRI), supplementation is not necessary since a moderate intake of soybean or canola oil (about 15 mL/day) ensures an adequate consumption.[32] EPA and DHA, on the other hand, can be produced endogenously by the enzymatic action of ALA desaturases and elongases, but this conversion is limited and affected by physiological and external factors.[33-35] Another source of EPA and DHA is krill oil, a shrimp-like crustacean found in the South Seas. Krill oil is a unique source of EPA and DHA, since most ω3 fatty acids are found in phospholipids, with greater bioavailability of krill ω3 compared to marine ω3.[36]

2.3. Saturated Fatty Acids

SFAs have a simple molecular structure and are characterized by the absence of double bonds in the straight carbon chain. They are classified as short-chain (acetic acid [C2:0], propionic acid [C3:0], and butyrate [C4:0]), medium-chain (caproic [C6:0], caprylic [C8:0], and capric [C10:0] acids), and long-chain (lauric [C12:0], myristic [C14:0], palmitic [C16:0], and stearic [C18:0] acids).[37] In addition, they are also classified according to the melting point, a key feature to determine the absorption mechanism. Short- and medium-chain fatty acids (C2-C10), which have a low melting point, are absorbed via the portal system, whereas long-chain fatty acids (C14-C18) are absorbed via the lymphatic system by chylomicrons. Lauric acid is absorbed mostly by chylomicrons, but also via the portal system.[38]

This structural difference allows SFAs to have different biological and metabolic actions,[39] acting as signaling agents to modulate the protein-protein and protein-plasma membrane interactions through processes known as myristoylation and protein palmitoylation.[40]

SFAs can be synthesized endogenously in most cells from acetyl-coenzyme A (acetyl-CoA) derived from the metabolism of carbohydrates, amino acids, and fats.[41] The most abundant source is palmitic acid (meat and palm oil), followed by stearic acid (cocoa), myristic acid (milk and coconut), and, in a small amount, lauric acid (coconut). The main dietary sources of palmitic acid are meat and palm oil.[42,43]

2.4. Trans Fats

The main dietary source of trans fats is elaidic acid (18:1, n-9t), present in vegetable fats prepared from the partial hydrogenation of vegetable oils, which are widely used in the food industry.[44] Trans fat is also found, in small amounts, in meat and milk in the form of vaccenic acid (18:1, n-11t), which is synthesized by the biohydrogenation of fats under microbial action in ruminant animals.[44]

3. Plasma Concentration of Total Cholesterol and Lipoproteins

Reduced SFA intake is recommended because SFAs increase plasma LDLc concentrations.[45] SFA intake has been shown to have a linear correlation with plasma lipid concentrations and to increase total cholesterol (TC), LDLc, and HDLc concentrations, as demonstrated in the WHO study.[11] One of the publications of the Prospective Urban Rural Epidemiology (PURE) study, which investigated the association between diet and plasma lipids in more than 100 000 participants, also revealed an increased plasma concentration of TC, LDLc, and HDLc.[46] The authors also showed a linear association between SFA intake and increased plasma lipids when comparing the highest quintile of intake (>11.2% of energy) to the lowest quintile (<4.03% of energy).

It is important to note that SFAs increase all lipoprotein classes, but the elevation observed in HDL may not be sufficient to overcome the deleterious effects of LDL on cardiovascular risk.[47] The different SFAs exert different effects on the lipid profile and, therefore, on cardiovascular risk. Compared to carbohydrate, myristic acid (C14:0) produces the largest increases in the concentrations of TC and LDLc, followed by palmitic acid (C16:0) and lauric acid (C12:0), an effect not observed with stearic acid.[11] The explanation is that stearic acid is rapidly converted to oleic acid in the liver by stearoyl-CoA desaturase-1 (SCD1).[48] Regarding HDLc, myristic, lauric, and palmitic acids increase HDLc concentrations when isocalorically replacing carbohydrates.[11]

SFAs act on plasma cholesterol by different mechanisms. In 1969, Spritz and Mishkel[49] demonstrated that, due to the straight carbon chain, SFAs can be packed in the core of lipoproteins, allowing them to carry a larger amount of cholesterol.[49] Later, it was demonstrated that SFAs, in combination with cholesterol, are able to reduce LDL receptor activity, protein, and mRNA,[50,51] thus impairing LDL clearance.[52,53] In addition, SFA intake increases the RNAm of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, phosphomevalonate kinase, and lanosterol synthase—important enzymes in the cholesterol synthesis pathway.[54]

A study performed in the cohorts of the Nurses' Health Study (NHS) (1984-2012) and Health Professionals Follow-up Study (HPFS) (1986-2010) showed that the isocaloric replacement of 1% energy from lauric, palmitic, or stearic acids with PUFAs or MUFAs reduced the risk of coronary heart

disease.[55] This effect is associated with the impact of UFAs on plasma lipids, which reduces LDLc concentrations and may also reduce HDLc concentrations.[56] A meta-analysis showed that, for each replacement of 1% energy from SFAs with PUFAs, there is a reduction in plasma concentrations of TC, LDLc, HDLc, apolipoprotein AI (ApoA-I), and apolipoprotein B (ApoB).[56] When SFAs are isocalorically replaced with MUFAs, more modest reductions, although significant, are observed in plasma lipids, including TC, LDLc, HDLc, and ApoB.[11]

A review of observational and intervention studies concluded that the replacement of SFAs with PUFAs reduces LDLc levels and, subsequently, CVD risk.[57] A prospective cohort study involving 84 628 women and 42 908 men showed that the isocaloric replacement of SFAs (5% energy) with complex carbohydrates was associated with an 11% reduction in the risk of coronary heart disease.[58] Conversely, the Women's Health Initiative (WHI) intervention trial, which investigated the effect of reducing fat intake and increasing vegetable, fruit, and grain intakes on cardiovascular outcomes, reported that the dietary intervention had no effect on reducing cardiovascular risk.[59] However, the intervention had only a mild effect on reducing LDLc levels and decreasing SFA intake (only 2.9% compared to controls). The reduction in total fat intake also reduced MUFA and PUFA intake.[59] Moreover, the isocaloric replacement of SFAs with carbohydrates reduces TC, LDLc, HDLc, ApoA-I, and ApoB.[11]

Two important meta-analyses of clinical trials showed a neutral effect of MUFAs on plasma lipid concentration.[60,61] A recent systematic review and regression analysis of intervention studies showed that the replacement of 1% energy from SFAs with an equivalent amount of MUFAs significantly reduced the plasma concentrations of TC, LDLc, and HDLc.[11] Conversely, the isocaloric replacement of carbohydrates with MUFAs increased HDLc levels, an effect that decreases with increasing unsaturation of fatty acids.[62] Also, a diet rich in MUFAs (20% of energy) was shown to reduce the plasma concentration of TC, LDLc, small LDL particles, oxidized LDL, and HDLc.[63] In a study of overweight individuals, increased MUFA intake (from 7 to 13% of energy) also contributed to a reduction in TC and LDLc levels, but with no changes in HDLc.[64] Overall, adequate MUFA intake has shown a positive effect on lipid metabolism, with effects opposite to those of SFAs.

The replacement of 1% energy from SFAs with ω6 was shown to reduce TC by 2 mg/dL, with minimal impact on HDLc.[56] An important meta-analysis of observational epidemiological studies points to the cholesterol-lowering effect of ω6 when replacing SFAs and trans fats in humans.[56] The replacement of 10% energy from SFAs with ω6 was associated with a reduction of 18 mg/dL in LDLc levels, a greater impact than that observed with the isocaloric replacement of carbohydrates. In addition, the high plasma concentration of ω6 was associated with a reduction in the TC/HDLc ratio.[56]

Increased ω6 intake was associated with a small reduction in plasma TC concentration, and only minimal or no effect was observed in HDLc and LDLc concentrations. Therefore, current evidence is insufficient to propose ω6 supplementation for the primary and secondary prevention of CVD.[65]

With regard to ω3 fatty acids, the results of a systematic review showed inconsistent data on the effect of ALA on plasma cholesterol.[66] A meta-analysis of randomized trials found no significant influence of ALA supplementation on TC and LDLc levels, with minimal effect on HDLc (reduction of 0.4 mg/dL).[67] DHA, however, was associated with elevated LDLc,[66] and the same result was observed with fish-oil supplementation.[68] This increase in cholesterol is probably attributable to the decreased expression of sterol regulatory element-binding protein 2 (SREBP-2), which regulates the LDL receptor synthesis,[69,70] induced in a dose-dependent manner by DHA.

Another study showed that ALA-rich or EPA/DHA-rich diets did not promote changes in the lipid profile compared to a MUFA-rich diet.[71] A similar result was obtained with oils enriched with EPA, DHA, and ALA.[72] In this study, a beneficial effect on plasma lipids was observed only in the wash-in period, when the participants who had a SFA-rich diet received a MUFA-rich diet.[72] It is important to note that, when analyzing the effects of ω3 fatty acids on cholesterolemia, the type of comparison made in the study should be considered, because UFAs, when used as a substitute in SFA-rich diets, promote beneficial effects; supplementation, however, shows different results.

Trans fatty acids have a greater atherogenic effect, due to their strong impact on cholesterolemia.[73] An important meta-analysis of randomized controlled trials showed the deleterious actions of these fatty acids on the plasma concentrations of TC, LDLc, and VLDLc.[56] Furthermore, trans fatty acids exert an additional adverse effect by reducing plasma HDLc concentrations compared to SFAs.[74-77] The reduction in HDLc results from the increased catabolism of ApoA-I.[74,75] Also, trans fatty acids increase the activity of cholesteryl ester transfer protein (CETP), a protein involved in the transfer of cholesteryl esters (CEs) and triglycerides (TGs) among plasma lipoproteins, thus enriching ApoB-rich particles with CEs. On the other hand, HDL particles become richer in TGs, favoring their catabolism.[78] Trans fat also acts deleteriously by reducing the clearance of ApoB100-containing particles, thus increasing its concentration in plasma,[75] which contributes to the formation of small, dense LDL particles that are more atherogenic.[79] A meta-analysis of randomized controlled trials showed that, each 1% energy replacement of TRANS fat with SFAs, MUFAs or PUFAs, decreased the total cholesterol/ HDL-C and the ApoB/ApoAI ratio.[80] Therefore, given the recognized negative impact of trans fats on the lipid profile, national and international guidelines recommend their exclusion from the diet.[7,8,20]

4. Plasma Concentration of Triglycerides

Fatty acids act differently on triglyceridemia by modulating transcription factors that participate in the synthesis of lipogenic enzymes involved in fatty acid production.

SFAs are able to modulate genes involved in lipid synthesis. SFAs have been shown to induce the hepatic expression of peroxisome proliferator-activated receptor gamma coactivator 1β (PGC-1β), which in turn activates SREBP, a transcription factor involved in gene transcription of lipogenic enzymes such as acetyl-CoA carboxylase-1 and fatty acid synthase,[81] related

to fatty acid synthesis, favoring greater TG production.[54] In addition, SFAs increase SREBP processing and its translocation to the cell nucleus, inducing the transcription of target genes.[82]

A systematic review published by the WHO[11] showed that, for each replacement of 1% energy from SFAs with PUFAs or MUFAs, there was a reduction in plasma TG concentration (0.88 mg/dL and 0.35 mg/dL, respectively). The replacement of SFAs with carbohydrates, however, increased plasma TG concentration by 0.97 mg/dL.[11] Conversely, it is known that PUFAs are involved in the reduction of plasma TG concentration by blocking SREBP, with a more pronounced effect exerted by ω3 fatty acids.[83]

Regarding the action of ALA on triglyceridemia, an experimental study in animals observed a null to mild effect with the use of flaxseed.[84] In humans, a systematic review showed that the TG-lowering effect results from the intake of large amounts of flaxseed oil.[66] A meta-analysis of 14 randomized controlled trials observed no significant effect of ALA supplementation on plasma TG concentrations.[67] Similarly, increased ω6 intake was not associated with decreased plasma TG concentrations.[65]

Clinical studies show that supplementation with 2 to 4 g/day of EPA and DHA can reduce plasma TG concentration by 25 to 30%.[66,85,86] A 4-week EPA or DHA supplementation in healthy subjects reduced the postprandial concentrations of TG, ApoB48, and ApoB100 (16%, 28%, and 24%, respectively), possibly due to the increased activity of lipoprotein lipase (LPL).[87]

The triglyceride-lowering effect of PUFAs is related to their ability to reduce SREBP1 expression and activity.[81] In animal models and in vitro studies, both EPA and DHA decreased SREBP1, reducing the expression of lipogenic enzymes.[88,89,90]

The ability of ω3 fatty acids to reduce TGs appears to be dose-dependent, with reductions of about 5 to 10% for each 1 g of EPA/DHA consumed daily, being greater in individuals with higher baseline TG concentrations.[91-93] A study of individuals with borderline or high TG values who received 1 to 4 g/day of krill oil for 6 weeks showed a reduction in plasma TG concentrations (18.6 to 19.9 mg/dL). With a supplementation of 0.5 g/day of krill oil, the reduction in TG levels was 13.3 mg/dL.[36]

5. Cardiovascular and Coronary Heart Disease

5.1. Saturated Fatty Acids

Despite the important biological activities of SFAs, high SFA intake has a deleterious effect on lipid metabolism and cardiovascular risk,[94,95] as they increase plasma LDLc concentrations, which is one of the main risk factors for the development of atherosclerosis and, consequently, CVD.[11] A comprehensive systematic review conducted by the Cochrane Library, in 2015, showed that decreased SFA intake was able to reduce cardiovascular events by 17%, compared to usual diet.[96] In addition, in the same meta-analysis subgrouping the studies that replaced SFAs with PUFAs showed a 27% reduction in cardiovascular events. For this reason, nutritional recommendations to reduce cardiovascular risk include reducing SFA intake.

However, in recent years, meta-analyses and observational studies have drawn conflicting conclusions about the relationship between SFA intake and cardiovascular risk.[12,94,96,97-99] This discrepancy is due, in part, to the macronutrient used for SFA replacement, since a reduction in one dietary macronutrient leads to an increase in another.[100] Meta-analyses of prospective observational studies assessing the effect of SFAs on the occurrence of cardiovascular events, without considering the type of macronutrient used for SFA replacement, observed no effect of SFA intake on cardiovascular risk.[98,101] Conversely, the replacement of SFAs with PUFAs or complex carbohydrates from whole grains proved to be beneficial and was associated with a lower risk of coronary heart disease. The replacement of SFAs with simple carbohydrates, however, had no impact on the risk of cardiovascular events,[97,99] since high sugar intake has a detrimental effect on cardiovascular health.

The PURE study, conducted in 18 countries, evaluated the association of dietary components with total mortality and cardiovascular events and showed that the risk of total mortality and non-CVD mortality was positively associated with higher carbohydrate intake and negatively associated with higher intakes of fat (PUFAs, MUFAs, and SFAs) and proteins (% of energy). It is worth noting that the highest fat and SFA intake was 35% and 13% of energy, respectively, and the highest carbohydrate intake median reached 77% of energy. In addition, increased SFA intake was associated with a lower risk of stroke. Total fat intake, as well as SFA and UFA intake, was not associated with myocardial infarction risk or CVD mortality.[102] The type of carbohydrate consumed was not analyzed separately, but it was observed that, in low-income and middle-income countries, people consumed carbohydrates mainly from refined sources. Further analysis showed that total fat and SFA intake correlated with increased plasma concentrations of TC and LDLc.[46] In 2018, in that same cohort, dairy intake was shown to be negatively associated with total and CVD mortality, CVD, and stroke.[103]

Randomized studies have evaluated the effects of dietary interventions on the occurrence of cardiovascular events; however, the differences in total fat intake between the intervention and control groups were not substantial in most studies.[59,104,105] The WHI trial followed, for about 8 years, 48 835 women who were randomly assigned to either dietary modification (reducing fat intake to 20% of energy and increasing vegetable and grain intakes) or to a control group (guidance through diet-related education materials). After 6 years of follow-up, the dietary intervention did not reduce the occurrence of coronary artery disease (CAD) or stroke, despite the significant reduction in total fat intake.[59]

A prospective cohort study showed that higher SFA intake was associated with a lower risk of ischemic heart disease, but not with the risk of coronary heart disease.[106] In another cohort, the intake of palmitic acid, but not of total SFAs, was positively associated with the risk of CAD.[107]

Recent studies have shown that different types of SFAs have heterogeneous cardiometabolic effects and correlate differently with cardiovascular risk, coronary heart disease, and the incidence of type 2 diabetes (T2D). In this context, lauric, myristic, palmitic, and stearic acids are associated with

an increased risk of coronary heart disease[55,108] and T2D,[14,109] whereas pentadecanoid acid (15:0)[110] and margaric acid (c17:0) are associated with the intake of dairy products, and long-chain SFAs (20:0 to 24:0) correlate inversely with the incidence of CVD and T2D.[14,110]

5.2. Replacement of Saturated with Unsaturated Fatty Acids

A prospective cohort study that investigated 83 349 women and 42 884 men, from 1986 to 2012, showed that the isocaloric replacement of 5% energy from SFAs with MUFAs or PUFAs was associated with an estimated decrease in total mortality by 13% and 27%, respectively. In addition, the replacement of SFAs with PUFAs reduced the risk of death from CVD, cancer, and neurodegenerative diseases.[93] Intervention studies have shown that the isocaloric replacement of 10% energy from SFAs with PUFAs reduces the risk of cardiovascular events by 27%,[111] and 5% replacement reduces the risk of CAD by 10%.[94] The isocaloric replacement (1% of energy) of SFAs (12:0 to 18:0) with complex carbohydrates reduced the risk of coronary heart disease, as demonstrated in the analysis of the HPFS and NHS studies.[55]

5.3. Replacement of Saturated Fatty Acids with Carbohydrates

A prospective cohort study involving 84 628 women and 42 908 men showed that the isocaloric replacement of SFAs (5% energy) with complex carbohydrates was associated with an 11% reduction in the risk of coronary heart disease.[58] Likewise, the isocaloric replacement of only 1% energy in the form of SFAs (12:0 to 18:0) with complex carbohydrates reduced the risk of coronary heart disease.[55]

Conversely, an intervention study evaluating the effect of reducing fat intake and increasing vegetable, fruit, and grain intakes on cardiovascular outcomes observed no effect of diet on reducing cardiovascular risk.[59] However, the intervention had only a mild effect on reducing LDLc levels (2.7 mg/dL) and decreasing SFA intake (only 2.9% compared to controls). It is worth noting that the reduction in total fat intake also reduced MUFA and PUFA intakes, which are associated with a favorable lipid profile from a cardiovascular point of view.[59]

Regarding plasma lipids, isocaloric replacement of SFAs with carbohydrates reduces TC (1.58 mg/dL), LDLc (1.27 mg/dL), HDLc (0.38 mg/dL), ApoA-I (7.0 mg/dL), and ApoB (3.6 mg/dL), whereas it increases TG concentrations (0.97 mg/dL).[11]

With regard to MUFAs, several studies based on a Mediterranean diet have shown positive effects in the prevention of cardiovascular risk factors and outcomes. Olive oil is the main source of MUFAs in the Mediterranean diet, followed by walnuts and chestnuts, which also provide PUFAs. It should be noted that this dietary pattern includes vegetables, fruits, and grains, which are also beneficial for cardiovascular health.[112]

The PREDIMED study followed for 5 years more than 5000 participants at high cardiovascular risk who were assigned to a Mediterranean diet supplemented with extra-virgin olive oil (50 g/day) or mixed nuts (30 g/day), both compared to control participants who consumed a diet with less fat content (30% of energy). The results showed that both intervention groups had fewer cardiovascular events (RR = 0.83).[17] Similar results were also observed with olive oil intake in the NHS study (1980-2010, n = 84 628, HR = 0.85), HPFS study (1986-2010, n = 42 908, HR = 0.85), Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (RR = 0.92), and European Prospective Investigation into Cancer and Nutrition (EPIC) study (HR = 0.87).[113] One of the arms of the EPIC study, conducted in the Dutch population, showed an increased risk of ischemic heart disease associated with MUFA intake (HR = 1.30).[106] However, it is worth noting that the authors of this study identified important confounding factors that could interfere with the final interpretation of the outcome, as they did not distinguish between cis and trans MUFAs.[106]

A review of studies published by the Cochrane Collaboration showed that the effectiveness of replacing SFAs with MUFAs in cardiovascular events is uncertain due to the small number of studies included.[96] The Dietary Guidelines for Americans,[7] however, state that the replacement of SFAs with MUFAs is associated with reduced cardiovascular risk, although the evidence is not so strong. A later cohort study showed that the replacement of 5% energy from SFAs with MUFAs reduced cardiovascular risk by 15%.[58]

5.4. Polyunsaturated Fatty Acids (Omega-6)

Regarding the effects ω6 series of UFAs on cardiovascular risk, randomized controlled trials and observational studies have provided evidence that the replacement of about 5 to 10% energy in the form of SFAs and refined carbohydrates (such as sugar, white bread, white rice) with ω6 reduces the risk of CVD without clinical evidence of adverse events.[114-117] The replacement of 1% energy from SFAs with ω6 has been associated with a reduction of 2 to 3% in the incidence of coronary heart disease.[94,118] This benefit may even be underestimated due to the large amount of SFAs in some foods that are also sources of ω6.

An important systematic review, which evaluated prospective cohort studies and randomized controlled trials involving individuals in primary and secondary prevention, showed that ω6 intake was not associated with a lower risk of CAD, in contrast to what was observed for fish or marine ω3 intake.[93] In fact, several studies have shown a lower reduction in cardiovascular outcomes with the replacement of SFAs with ω6 than with combined ω6 and ω3.[119]

The Cochrane Collaboration published a review of clinical trials evaluating the effect of ω6 intake on primary CVD prevention and concluded that the intake of ω6 fatty acids (linoleic, gamma-linolenic, dihomo-gamma-linolenic, and arachidonic acids) did not interfere with lipid or blood pressure markers; however, none of the studies assessed clinical outcomes.[65,120] In a more recent review, also conducted by the Cochrane Collaboration, which evaluated the effect of ω6 supplementation on risk factors (blood pressure, lipid profile, and adiposity) and cardiovascular outcomes (all-cause mortality, CVD mortality, and cardiovascular events), little or no benefit was observed from ω6 interventions on all-cause mortality (RR = 1.0; 95% CI: 0.88-1.12), CVD mortality (RR = 1.09; 95% CI: 0.76-1.55), and cardiovascular events (RR = 0.97; 95% CI: 0.81-1.15).[65] Likewise, ω6 intake was not associated with a lower risk of cardiac and cerebrovascular

events (RR = 0.84; 95% CI: 0.59-1.20) or stroke (RR = 1.36; 95% CI: 0.45-4.11). However, a slight reduction in the risk of acute myocardial infarction (AMI) was observed with increased ω6 intake (RR = 0.88; 95% CI: 0.76-1.02).[65]

Higher plasma concentration of ω6 was associated with lower risk of cardiovascular events, ischemic stroke, and CVD mortality, based on the results of a recent study analyzing data from 30 prospective studies, for a total of 68 659 participants enrolled.[121] In this publication, the authors reinforce the cardiovascular benefits of ω6 intake.

5.5. Polyunsaturated Fatty Acids (Marine Omega-3)

EPA and DHA have been investigated for their potential to reduce cardiovascular risk. The mechanisms proposed for cardiovascular benefits include reduced inflammatory markers and platelet aggregation, improved endothelial function, reduced blood pressure, and reduced triglyceridemia.[122-124] Marine ω3 fatty acids (DHA and EPA) exert numerous effects on different physiological and metabolic processes, which can influence the likelihood of developing CVD.

Although initial evidence suggests a protective effect of the intake of fish and marine ω3 fatty acids on cardiovascular events, especially in people with established CVD,[125-127] recent studies have not shown benefits of ω3 supplementation in people with previous manifestations of atherosclerotic disease.[128-130] A possible explanation is related to the characteristics of the population studied, especially regarding the more frequent use of well-known protective agents (e.g., statins, beta-blockers, angiotensin-converting enzyme inhibitors), the more aggressive control of traditional risk factors, and the larger number of revascularization procedures in more recent studies. Therefore, it is questioned whether ω3 fatty acids can bring real additional benefits when patients are treated according to current recommendations. Questions regarding formulation, dose, and duration of supplementation may also be raised. In the Alpha Omega[128] and SU.FOL.OM3 trials,[130] the dose of EPA+DHA (400 to 600 mg/day) may have been insufficient to produce a clinical benefit.

A recent meta-analysis of randomized controlled trials and prospective cohort studies evaluating the association between EPA+DHA intake and CAD risk showed a significant benefit only in populations at higher risk, including those with hypertriglyceridemia. The results of prospective cohort studies showed a significant reduction in the risk of any coronary event with higher intakes of EPA+DHA. Therefore, EPA+DHA intake appears to be associated with a reduced risk of coronary events, with greater benefit in populations at higher risk in randomized controlled studies.[131]

However, different formulations of ω3 and the populations studied seem to contribute to the results. Two recent controlled trials showed conflicting data, but there were differences in the dose and formulation of ω3 used. The ASCEND (A Study of Cardiovascular Events in Diabetes),[132] which evaluated 15 840 patients with diabetes mellitus but without evidence of CVD, showed no significant differences between patients who consumed 1.0 g of EPA+DHA and those who received placebo. A review conducted by the Cochrane Collaboration, which included 79 clinical trials, for a total of 1 120 059 participants enrolled with a 12- to 72-month follow-up, showed that EPA, docosapentaenoic acid (DPA), and DHA had little or no effect on all-cause mortality (RR = 0.98; 95% CI: 0.90-1.03), CVD mortality (RR = 0.95; 95% CI: 0.87-1.03), and cardiovascular events (RR = 0.99; 95% CI: 0.94-1.04).[133]

In the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT),[134] involving high-risk patients with elevated TG levels receiving statin therapy, the risk of ischemic events, including CVD death, was significantly lower in patients who received 2 g of icosapent-ethyl ester twice daily (total daily dose of 4 g) than in those who received placebo. In a total sample of 8179 patients (70.7% enrolled for secondary prevention) followed for a median of 4.9 years, there was a 25% reduction in the risk of the primary composite endpoint (HR = 0.75; 95% CI: 0.68-0.83; $P < 0.001$), key secondary endpoint events (HR = 0.74; 95% CI: 0.65-0.83; $P < 0.001$), and prespecified events, including the rate of CVD death (HR = 0.80; 95% CI: 0.66-0.98; $P = 0.03$). However, a higher rate of patients in the EPA group were hospitalized for atrial fibrillation or flutter, with no differences in the risk of bleeding. It is worth noting that icosapent ethyl is not a fatty acid found in food, and its indication, in pharmacological doses, is made at the physician's discretion.

Therefore, although there is a consensus that regular intake of fish rich in ω3 fatty acids should be part of a healthy diet, there is still no safe recommendation for supplementing fish-oil capsules. This occurs because the topic is still surrounded by controversy, fueled by conflicting results from clinical trials.

Using experimental models of atherosclerosis in mice, several studies have reported that fish oil and EPA can attenuate the atherosclerotic process, although the same has not been demonstrated in other experimental conditions.[135-140] Some population-based studies suggest an inverse association between fish or marine ω3 fatty acid intake and subclinical atherosclerosis markers, such as carotid intima-media thickness and coronary calcification, although this relationship seems to be subtle.[141-143] In a randomized trial of patients with CAD, supplementation with approximately 1.5 g/day of ω3 fatty acids for 2 years resulted in less progression and more regression of coronary atherosclerosis, as assessed by quantitative invasive angiography, compared to placebo, although the differences were small.[144] However, in another study, supplementation did not change the progression of carotid atherosclerosis, as assessed by ultrasound,[145] which disagrees with the results of the randomized trial conducted by Mita et al.,[146] who reported that highly purified EPA (1.8 g/day) attenuated the progression of carotid intima-media thickening in patients with diabetes.[146]

It is also possible that ω3 fatty acids play a protective role against cardiovascular events by modulating atherosclerotic plaque characteristics, making the plaque more stable. A randomized trial of patients awaiting carotid endarterectomy showed that atherosclerotic plaques readily incorporated ω3 fatty acids from fish-oil supplementation, making them

less vulnerable to rupture and instability phenomena,[147] an observation consistent with experimental findings.[139]

5.5.1. Effects on Peripheral Vascular Disease

Despite extensive research on the effects of ω3 fatty acids on improving vascular function, their effects on cardiovascular outcomes in individuals with peripheral arterial disease are less described. A meta-analysis of 5 studies with a total of 396 participants, published between 1990 and 2010, was conducted to evaluate this issue.[148-152] In patients with peripheral vascular disease, there is insufficient evidence to recommend ω3 fatty acids for the reduction of major cardiovascular events, need for revascularization or amputation, improvement in pain-free walking distance, or improvement in quality of life.[153]

5.5.2. Effects on Cardiac Arrhythmia and Sudden Cardiac Death

Experimental studies have shown antiarrhythmic effects of ω3 fatty acids, mainly attributable to a direct effect on ion channels.[154] Other mechanisms include modulation of the autonomic tone (improved heart rate variability), reduction in basal heart rate, and restriction of reperfusion-induced arrhythmias.[154] These effects may explain the beneficial results of ω3 fatty acids in the prevention of sudden cardiac death reported in some studies.

Several observational studies have suggested that ω3 fatty acids can provide particular protection against sudden cardiac death, especially in patients with AMI. This beneficial effect was also observed in a subanalysis of the GISSI-Prevenzione randomized trial,[155] but not in the most recent randomized trial, OMEGA.[129] This hypothesis was also confirmed in patients with implantable cardioverter defibrillators. The results were inconsistent, ranging from a slight beneficial effect of ω3 fatty acids on the reduction of severe ventricular arrhythmias in this subset of patients[156] to a proarrhythmic effect in some patients.[157]

Due to conflicting results, data from a meta-analysis were evaluated, for a total of 32 919 participants included in 9 studies. Of these, 16 465 patients received ω3 and 16 454 received placebo. There was a non-significant reduction in the risk of sudden cardiac death or ventricular arrhythmias with the use of ω3 fatty acids (OR = 0.82; 95% CI: 0.60-1.21; P = 0.21).[158]

Another review evaluated the results of studies using ω3 fatty acids in ventricular arrhythmias and sudden cardiac death, questioning whether these lipids produce antiarrhythmic, proarrhythmic, or neutral effects, which, in turn, would require randomized controlled trials with a specific design for these populations.[159]

5.5.3. Effects on Heart Failure

A large randomized controlled trial, the GISSI-HF trial, showed a slight reduction in mortality when ω3 (1 g/day) was supplemented in patients with New York Heart Association (NYHA) class II-IV heart failure (HF),[160] which is consistent with other epidemiological and observational studies that suggested an inverse relationship between fish or ω3 intake and HF-related events.[161,162]

Recommendations from national and international guidelines consider ω3 supplementation in HF a class IIb indication (level of evidence B) based on data from the GISSI-HF trial,[160] but not from other studies in which ω3 fatty acids have been supplemented.

In the GISSI-HF trial, which included 6975 patients with NYHA class II-IV HF or an ejection fraction <40% or who had been hospitalized in the preceding year for HF, 1 g of ω3 was added to standard therapy. This therapy included angiotensin-converting enzyme inhibitors/angiotensin receptor blockers in 94% of patients, beta blockers in 65%, and spironolactone in 39%. Patients were followed for a median of 3.9 years. Supplementation with ω3 fatty acids reduced by 8% the co-primary endpoint of CVD death or hospitalization: 10% in the relative risk of CVD death and 7% in cardiovascular hospitalizations.[160]

5.6. Polyunsaturated Fatty Acids (Vegetable Omega-3)

Although the real impact of vegetable-derived ω3 fatty acids on CVD is still under debate, most prospective observational studies suggest that ALA intake may protect against cardiovascular events.[163] In the HPFS study, the prospective analysis of more than 45 000 men showed that ω3 intake, both of marine and vegetable origin, was associated with a reduction in cardiovascular risk, with little influence of ω6 intake.[164] In the NHS study, which assessed cardiovascular outcomes in more than 76 000 women, ALA intake was inversely associated with the risk of sudden cardiac death, but not with other types of fatal coronary outcomes or non-fatal AMI.[165]

Meta-analyses and systematic reviews have produced conflicting results.[93,166,167] In the Alpha Omega randomized controlled trial, intake of a margarine supplemented with ALA for 40 months did not reduce the rate of cardiovascular events in patients who had had an AMI.[128] As for the effectiveness of ALA, there was a slight reduction in the risk of cardiovascular events (RR = 0.95; 95% CI: 0.83-1.07), CVD mortality (RR = 0.95; 95% CI: 0.72-1.26), and arrhythmias (RR = 0.79; 95% CI: 0.57-1.10).[133]

The role of the dietary ω6/ω3 ratio in the pathogenesis of cardiovascular, inflammatory, and autoimmune diseases has also been the subject of controversy in recent years. Humans have experienced dramatic changes in their diet regarding fatty acid intake in the last millennia. With the agricultural and industrial revolutions, there was an increase in the intake of cereals, oils, and grains rich in ω6, while the intake of ω3 decreased. The ω6/ω3 ratio, originally from 1:1 to 3:1, currently ranges from 15:1 to 40:1 in the Western diet.[168,169]

Most studies have concluded that, for general health promotion, the ω6/ω3 ratio should be lower than that currently observed in the general Western population.[170] Some experts advocate for a reduction in this ratio both by increasing ω3 intake and by reducing ω6 intake. Accordingly, in a prospective randomized secondary prevention trial of post-AMI patients, an experimental Mediterranean diet characterized, among other factors, by being richer in ALA (C18:3 – ω3) and oleic

acid (C18:1 – ω9) and poorer in linoleic acid (C18:2 – ω6) was associated with a reduction of up to 70% in overall mortality.[171] The diet included the replacement of corn oil with olive oil, with a consequent decrease in the ω6/ω3 ratio to up to 4:1.[171]

The evidence so far suggests that increased intake of ω3, particularly DHA and EPA, provides protection against CVD. In addition, several experts have questioned the validity of using the ω6/ω3 ratio alone in clinical practice and its relationship with cardiovascular risk.[172,173] Both fatty acids, ω6 and ω3, have been associated with beneficial effects on cardiovascular health. However, the importance of the ω6/ω3 ratio is based on the enzymatic competition between ω6 and ω3 due to the action of delta-6 desaturase, which converts both into different subspecies. On the one hand, high ω6 intake can decrease the metabolism of ω3 (ALA – C18:3) to EPA (C20:5) and DHA (C22:6),[174] thus limiting the benefits of ω3 fatty acids. On the other hand, the higher affinity of delta-6 desaturase for ω3 fatty acids may lead the essential metabolites derived from the bioconversion of ω6 not to be produced satisfactorily, which would support a recommendation for a small increase in its intake compared to ω3.[172]

In view of these issues and until further scientific evidence is available to support changes in current approaches, dietary recommendations should be based on the total intake of each fatty acid type (ω6 and ω3), and not only on the ω6/ω3 ratio.

5.7. Trans Fats

Several observational studies have associated the intake of trans fatty acids, or foods containing trans fats, with adverse cardiovascular outcomes.[76,175-180] An analysis of data from the NHS study showed that, for every 2% increase in trans fat intake, there was a 1.93-fold increase in the relative risk of coronary heart disease.[175] Likewise, the replacement of 2% energy from trans fats with UFAs reduced cardiovascular risk by 53%, as shown in the Seven Countries Study population.[181]

The Cardiovascular Health Study (CHS)[182] evaluated the plasma concentration of trans fatty acids (elaidic acid) in 2742 adults and showed that these fatty acids were associated with an increase in total mortality, mainly due to increased cardiovascular risk. A study evaluating the NHS and HPFS studies' databases also showed that trans fat intake increased total mortality to 13%, when comparing the highest to the lowest quintile of intake.[180]

This deleterious effect of trans fats on cardiovascular risk may be attributable to its action on increasing LDLc and decreasing ATP binding cassette transporters A1 (ABCA1) and G1 (ABCG1), responsible for cholesterol efflux from macrophages to ApoA-I and HDL, respectively.[183]

6. Endothelial Dysfunction

Endothelial dysfunction is one of the initial events in the genesis of CVD and results mainly from reduced production and/or availability of nitric oxide (NO) and from an imbalance between endothelium-derived vasodilator and vasoconstrictor factors.[184,185] Cardiovascular risk factors, such as oxidized LDL, dyslipidemia, hypertension, hyperglycemia, hyperinsulinemia, and smoking, can induce endothelial activation, which induces increased production of cytokines, chemokines, and reactive oxygen species (ROS), thus reducing the capacity for NO-dependent vasodilation. In addition, there is an increase in endothelial permeability, which facilitates the transport of LDL to the subendothelial layer, where LDL can undergo modifications (by oxidation or glycation) and trigger an inflammatory response. This can lead endothelial cells to express cell adhesion molecules and produce mediators that will promote chemotaxis of inflammatory cells, platelet activation, and smooth muscle cell (SMC) proliferation and migration, thus contributing to the genesis of atherosclerosis.[186,187] NO, on the other hand, is able to reduce the expression of inflammatory mediators and endothelial cell adhesion molecules and to decrease vascular reactivity, thus preventing vasoconstriction at the injury site.[188,189]

A high-fat diet has been shown to reduce the activation of the endothelial AMPK-PI3K-Akt-eNOS pathway, leading to endothelial dysfunction.[185,190,191] In experimental animals, consumption of a high-fat diet for 6 weeks increased the plasma concentration of pro-inflammatory cytokines and reduced adiponectin concentrations, while reducing NO production and promoting endothelial dysfunction.[192]

SFAs, especially palmitic acid, activate inflammatory responses and oxidative stress, which impair endothelial integrity and cause endothelial dysfunction. SFAs are able to activate the transcription nuclear factor kappa B (NF-κB), which controls inflammatory signaling and oxidative stress pathways,[193] and, consequently, induce endothelial dysfunction by increasing ROS and secreting pro-inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α.[194,195]

In a study on endothelial cells, palmitic acid inhibited insulin-dependent activation of endothelial NO synthase (eNOS), thereby reducing NO production, an effect mediated by the activation of PTEN (phosphatase and tensin homolog deleted on chromosome 10). Such phosphatase, when activated, reduces protein kinase B (Akt) phosphorylation.[196] In another study, treatment of endothelial cells with palmitic acid decreased NO production by reducing insulin-mediated phosphorylation of insulin receptor substrate-1 (IRS-1), Akt, and eNOS. This effect was dependent on increased palmitic acid-mediated IκB kinase (IKK)-β activation.[197]

SFAs can promote inflammation and endoplasmic reticulum (ER) stress in different cell types.[69,193,194,198,199] In cardiac fibroblasts, palmitic acid activated inflammatory pathways and induced mitochondrial dysfunction and ER stress, leading to increased ROS production and inflammasome activation, an effect that was mitigated by the presence of EPA.[198] In SMCs, palmitic acid is able to induce apoptosis through toll-like receptor 4 (TLR4) activation, increased ROS production, and increased caspase 3 and caspase 9 expression.[199] In macrophages, SFAs increase the content of oxidized LDL receptor-1 (LOX-1) with a subsequent increase in the uptake of oxidized LDL, leading to increased ROS production and ER stress, effects that were corrected by adding UFAs to the medium.[193] In endothelial cells, treatment with palmitic acid induced endothelial dysfunction and reduced eNOS and AMPK phosphorylation, with a subsequent reduction in NO production. Also, palmitic acid induced increases in ROS,

inducible nitric oxide synthase (iNOS), and apoptosis, actions that were attenuated by concomitant incubation with EPA.[194].

Habitual consumption of an SFA-rich diet was associated with changes in endothelial function in overweight young adults.[200] However, intervention studies assessing the effect of acute SFA intake on endothelial function have produced controversial results. The Dietary Intervention and VAScular function (DIVAS) study, involving adults with moderate cardiovascular risk, reported that 16-week isocaloric replacement of SFAs with MUFAs or linoleic acid had no effect on endothelial function, inflammatory markers, or insulin resistance. However, there was a reduction in the plasma concentrations of TC, LDLc, and E-selectin.[201] The DIVAS-2 study, which evaluated the acute effect of high-fat meals on endothelial function and cardiovascular risk markers in postmenopausal women, found no difference in the impact of different fatty acids on markers of endothelial function.[202]

SFAs, especially palmitic acid, activate inflammatory responses and oxidative stress, which impair endothelial integrity and cause endothelial dysfunction. Fish-oil supplementation significantly improved endothelial function in forearm resistance vessels.[123] Compared to placebo, systemic vascular compliance improved after 3 g/day of DHA or EPA for 7 weeks.[203] The proposed mechanisms include the incorporation of ω3 into membrane phospholipids, thus changing vascular compliance.[68] Attenuation of age-related vascular stiffness in patients with dyslipidemia and carotid artery distensibility is another proposed mechanism.[204] Endothelial dysfunction is closely associated with vascular wall inflammation. The effects of marine ω3 supplementation on in vivo endothelial function in humans are still controversial. An analysis of 33 intervention trials suggests that marine ω3 fatty acids may improve endothelial function in overweight dyslipidemic patients and in patients with diabetes, although the results are conflicting in patients with CVD and inconsistent in healthy individuals.[63]

A study of endothelial cells showed that elaidic acid can cause cell death by activating the caspase pathway,[205] as well as NF-κB activation by increasing ROS production, resulting in increased vascular cell and intercellular adhesion molecule (VCAM-1 and ICAM-1) expression and greater leukocyte adhesion.[206] Consistent with these results, a study in humans reported an increase in plasma concentrations of E-selectin and C-reactive protein (CRP) with trans fat intake.[207] Increased trans fat intake was also shown to increase the plasma concentrations of E-selectin, VCAM, and ICAM in 730 women who participated in the NHS study.[208] A study on endothelial cells investigating the effect of trans fatty acids on NF-κB activation showed that elaidic acid induced IκB phosphorylation, as assessed by an increase in IL-6 concentrations.[209] It also led to a decrease in both NO production and insulin signaling, and promoted pro-inflammatory signaling and cell death.[210]

6.1. Blood Pressure

In dietary intervention studies in overweight patients, those consuming a daily fish meal showed a decrease in systolic and diastolic blood pressure, and this reduction was even greater when combined with a weight loss program, even after adjustment for other covariates.[211] A meta-analysis conducted in the 1990s concluded that the effect of ω3 supplementation on blood pressure is dose-dependent, being effective from a dose of 3.0 g/day, with a reduction of 0.66 and 0.35 mm Hg in systolic and diastolic blood pressure, respectively, per gram of ω3 consumed.[212]

In another meta-analysis of 36 randomized trials, fish-oil supplementation (median dose of 3.7 g/day) reduced systolic blood pressure by 2.1 mm Hg and diastolic blood pressure by 1.6 mm Hg.[213] These modest results can be explained by the low degree of purity and low concentrations in the formulations used. Other studies using low doses (1.6 g of DHA and 0.6 g of EPA) have not shown benefits in blood pressure, possibly because of the low doses used. In high-risk patients, such as those on hemodialysis, 4-month supplementation with 2 g of ω3 was associated with lower systolic (−9 mm Hg) and diastolic (−11 mm Hg) blood pressure, compared to olive oil.[214]

In a meta-analysis involving patients with T2D, ω3 supplementation reduced diastolic pressure by 1.8 mm Hg.[215] Theobald et al.[216] also showed a reduction in blood pressure with the consumption of low doses of ω3.[216] However, when endothelial function or arterial stiffness rates are assessed, data are conflicting between studies.[216,217]

Schwingshackl et al.[218] conducted a systematic review and meta-analysis to investigate the impact of MUFAs on lipid metabolism, blood pressure, and cardiovascular events. The results showed that diets with MUFA content above 12% of energy had a beneficial effect only on diastolic and systolic blood pressure.

In addition to the benefits observed in the lipid profile,[219] the Mediterranean dietary pattern also improves blood pressure[220] and provides additional protection against oxidative stress,[221] inflammatory markers,[222] and endothelial dysfunction.[112] In this respect, it is noted that additional health benefits were conferred by other substances, independently of MUFAs. For such substances, there is currently no specific recommended intake.

Therefore, there is little evidence of the protective role of MUFAs against hypertension and endothelial dysfunction that could support specific recommendations.

6.2. Stroke

Elevated blood pressure is the main risk factor for stroke. Regarding SFA intake, some studies have observed little or no effect on stroke risk.[12,96,223,224] In the WHI study, which followed women for about 8 years, reduced SFA intake did not reduce the risk of stroke.[59] Conversely, other cohort studies, such as the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC), that followed 58 453 Japanese adults for 14 years,[225] and the PURE study,[102] reported an inverse association between SFA intake and stroke risk.

A meta-analysis found an inverse association between SFA intake and stroke risk only for Asian men with body mass index (BMI) <24 kg/m^2, indicating that factors such as ethnicity, sex, and body weight influence the association

between SFAs and stroke risk.[226] Thus, to date, there is no robust evidence to recommend the reduction of SFAs to prevent the risk of stroke.[96]

In randomized trials, the use of ω3 fatty acids, such as EPA, DHA and DPA (C20:5), reduced risk factors and mechanisms for cardiovascular events, including hypertension, hyperlipidemia, and endothelial dysfunction,[213,227,228] suggesting their protective role in CVD. However, the impact of these fatty acids on ischemic stroke is still controversial. Observational studies have shown inverse associations between self-reported dietary ω3 intake and ischemic stroke,[229] which were not confirmed in a meta-analysis of randomized trials using ω3 supplementation.[227] However, the meta-analysis data were derived from short-term supplementation studies of high-risk patients who, in general, had previous stroke, in which stroke was not a predetermined outcome. Therefore, it is not possible to generalize these results to populations in primary prevention.[230] In addition, ischemic stroke may be related to atherothrombotic or cardioembolic disease, whose pathophysiological mechanisms are different.[231] DHA can reduce the risk of atherothrombotic stroke by reducing endothelial dysfunction and atherosclerosis, whereas EPA and DPA can have a greater impact on cardioembolic stroke due to their effects on coagulation and atrial fibrillation.[232] Moreover, almost all studies of ω3 intake and stroke risk were based on self-reported dietary intake of these fatty acids, making it impossible to distinguish between the types of fatty acid consumed.

In a systematic review of 3 large US cohorts, the CHS, NHS and HPFS, the circulating levels of fatty acids were measured at baseline to assess their relationship with the incidence of ischemic stroke. Ischemic strokes were prospectively adjudicated and classified into atherothrombotic or cardioembolic, and the risk was calculated according to the circulating levels of fatty acids. Higher circulating levels of DHA were inversely associated with the incidence of atherothrombotic stroke, and DPA, with cardioembolic stroke. There was no association between EPA and stroke. These findings suggest differential benefits according to the ω3 fatty acid involved.[233]

7. Inflammation

SFAs are essential components of the lipid A present in the cell wall of Gram-negative bacteria – it is the endotoxic portion of lipopolysaccharide (LPS).[234] It is well documented that SFAs trigger inflammatory signaling, as they modulate both the NF-kB pathway, through the structure of TLR4 receptors,[235] and the TLR2 pathway.[236] Another mechanism that enhances the inflammatory process induced by SFA intake is intracellular NLRP3 inflammasome activation. The activated inflammasome then processes IL-1β and IL-18 into their mature forms, induced by NF-kB. Dietary SFAs have been shown to activate this mechanism via TLR4 receptors,[237] as have prostaglandins E2 (PGE2) derived from arachidonic acid,[238] with important implications for coronary heart disease[239] and comorbidities associated with T2D, such as diabetic retinopathy.[238]

In macrophages, lauric acid[240] showed greater inflammatory capacity, as assessed by the activation of the TLR4 pathway, compared to myristic, palmitic, and stearic acids, whereas MUFAs and PUFAs did not activate this pathway. The pretreatment of cells with different UFAs significantly reduced the pro-inflammatory effect induced by lauric acid.[241,242] Also, inhibition of TLR2 expression improved insulin action in muscle cells treated with palmitic acid as well as in skeletal muscle and adipose tissue in mice fed a high-SFA diet.[243] A study of 965 healthy young adults showed a positive association of plasma levels of myristic and palmitic acids with CRP levels, whereas stearic and linoleic acids were inversely associated.[244]

As precursors of eicosanoids and other anti-inflammatory mediators, ω3 fatty acids can exert anti-inflammatory effects, with benefits in several pathological conditions, including CVD. Many experimental studies have shown a wide range of ω3 anti-inflammatory effects, but in vivo investigations in humans have shown conflicting results.[154,245]

PUFAs of the ω3 series, such as EPA and DHA, are precursors of anti-inflammatory eicosanoids with cardiovascular benefits. Although experimental studies have demonstrated the anti-inflammatory effects of ω3, some studies in humans have shown conflicting results regarding cardiovascular outcomes.[133,154,245]

In cross-sectional and cohort studies, dietary intake of marine ω3 was associated with lower plasma levels of inflammatory markers, including adhesion molecules and CRP.[246,247] Concentrations of marine ω3 in plasma and in erythrocyte or granulocyte membranes were inversely associated with CRP concentrations in healthy individuals or patients with stable CAD.[248-250] An intervention study showed that food containing marine ω3 or supplementation with fish oil or DHA produced results compatible with attenuation of the inflammatory response in patients with T2D and hypertriglyceridemia.[251-253] In other trials, a diet supplemented with ω3 did not cause significant changes in inflammatory parameters in patients with cardiometabolic risk (1.24 g/day)[254] or in patients with previous AMI (5.2 g/day),[255,256] and the same was observed with PUFA supplementation in plasma CRP concentrations of healthy individuals (2.0 or 6.6 g/day).[256] Differences in the population profile, route of administration, supplementation dose, concomitant use of statins, and analyzed parameters may have contributed to the discrepant results. Therefore, the real clinical relevance of the anti-inflammatory effects of ω3 fatty acids of marine origin is still uncertain.

Studies involving ALA have shown an inverse relationship between ALA intake and inflammatory parameters, including serum CRP.[246,257,258] ALA supplementation reduced the concentration of inflammatory markers in patients with dyslipidemia, which occurred especially when the baseline diet was high in SFAs and low in MUFAs.[259]

Trans fat intake was positively associated with systemic inflammation, characterized by an increase in IL-1β, IL-6, TNF-α, and monocyte chemoattractant protein (MCP) levels in patients with CVD.[260] A case-control study of 111 patients with CAD showed that the incorporation of trans fatty acids into erythrocytes was associated with higher plasma levels of CRP and IL-6.[77]

8. Insulin Resistance and Diabetes

Inflammatory signaling induced by SFA intake can activate proteins with serine kinase activity, such as c-Jun N-terminal kinase (JNK) and IKK. These proteins negatively interfere with insulin signal transduction by reducing tyrosine phosphorylation of IRS-1.[261,262]

Intake of a high-SFA diet for 3 months reduced insulin sensitivity in individuals without diabetes.[263] In the LIPGENE cohort study, which evaluated 417 individuals with metabolic syndrome, reduced SFA intake had no impact on fasting plasma glucose and insulin concentrations, homeostasis model assessment of insulin resistance (HOMA-IR), insulin sensitivity, and inflammatory markers.[264] It is worth noting that, in the LIPGENE study, energy from SFAs was replaced with energy from UFAs or complex carbohydrates. In the Reading, Imperial, Surrey, Cambridge, and Kings (RISCK) trial, involving 548 overweight participants with high cardiometabolic risk, the isocaloric replacement of a SFA-rich diet (with high glycemic index) with a MUFA-rich diet (with high or low glycemic index) for 6 months caused no change in insulin sensitivity.[265] However, it was demonstrated that diets enriched with SFAs, especially palmitic acid, acutely induced insulin resistance in individuals with and without glucose intolerance.[266]

Prospective studies have found a positive association between SFA intake and glucose intolerance.[267,268] The HPFS study, which included 42 504 men, found an association of total fat and SFA intake with an increased risk of T2D, but the association was dependent of BMI.[269] In the Iowa Women's Health Study,[270] involving 35 988 women without a previous diagnosis of T2D, SFA intake was not associated with the risk of T2D; however, the risk of diabetes was inversely related to the replacement of SFAs with PUFAs. In addition, consumption of animal fat was associated with a 20% increase in T2D risk.[270] Another prospective study, the NHS study, which assessed the relationship between fat intake and T2D risk in 84 204 women, concluded that total fat and SFA intake was not associated with an increased risk of T2D.[271]

The WHI trial investigated the effects of dietary intervention in postmenopausal women followed for about 8 years and found that reduced intakes of total fat (9.1% of energy) and SFAs (3.2% of energy) did not change the risk of developing T2D. It is worth noting that the reduction in fat intake was offset by a 10% increase in carbohydrate intake.[272]

The development of T2D is known to result from the interaction of genetic factors and lifestyle, such as diet. The EPIC-InterAct study[273] evaluated potential interactions of genetic susceptibility and the effect of macronutrient intake on the risk of developing T2D and reported that SFA intake was not associated with T2D risk. Also, genetic susceptibility to T2D did not influence the relationship between macronutrient intake and T2D risk.[273] In another cohort of the EPIC-InterAct study, investigating the association between T2D risk and the concentration of different fatty acids in plasma phospholipids,[14] myristic, stearic, and palmitic acids were positively associated with T2D risk. It should be noted that a higher plasma concentration of these fatty acids was positively associated with the intakes of alcohol, margarine, and soft drinks and negatively with the intakes of fruit and vegetables, olive oil, and vegetable oil. Pentadecanoic acid (15:0) and heptadecanoic acid (17:0), however, were positively associated with the intakes of milk and dairy products, nuts, cakes, and fruit and vegetables and inversely associated with T2D risk.[14] Therefore, the observed deleterious effects cannot be attributed solely to the isolated activity of these SFAs, but rather to a context of inadequate diet.

A meta-analysis of observational studies found no association between SFA intake and T2D risk.[223] In a meta-analysis of cohort studies investigating the association between dietary patterns and T2D risk, a reduction in the risk of T2D was associated with healthy eating patterns, and not with a specific macronutrient.[274] In a meta-analysis of dietary intervention controlled studies evaluating the effect of isocaloric replacement of macronutrients on plasma glucose and insulin concentrations and on insulin resistance-related parameters, the replacement of SFAs with PUFAs reduced the glucose levels, glycated hemoglobin (HbA1c), C-peptide, and HOMA.[109]

To date, the evidence on the impact of SFAs on T2D risk is inconclusive. Results indicate that the influence of other dietary nutrients and components cannot be discarded, which is in line with international and Brazilian dietary guidelines. Therefore, the adoption of healthy eating patterns is recommended. Priority should be given to the consumption of fruit and vegetables, dairy products, lean meats, and complex carbohydrates, with low intake of simple carbohydrates, processed meats, and ultra-processed foods—such diet is considered more efficient in reducing the risk of cardiometabolic diseases.

Prospective cohort studies involving a large number of participants have suggested that a higher intake of ω3 fatty acids is associated with a higher incidence of T2D.[270,275] However, in a meta-analysis evaluating the relationship between marine ω3 PUFAs and T2D risk,[276] both the intake of fish and crustaceans (13 studies, RR per 100 g of fish/day = 1.12, 95% CI: 0.94-1.34) and supplementation with EPA+DHA (16 cohorts, RR per 250 mg/day = 1.04, 95% CI: 0.97-1.10) were not associated with the risk of diabetes. Plasma concentrations of EPA+DHA (5 cohorts, RR per 3% of total fatty acids = 0.94, 95% CI: 0.75-1.17) were also not associated with T2D risk.[276] Given the heterogeneity between studies and inconsistent effects related to follow-up duration, there is no evidence of beneficial or harmful effects of fish/seafood intake or EPA+DHA supplementation on the risk of developing diabetes.

However, there is evidence that higher plasma EPA/DHA levels may be associated with a lower risk of new-onset diabetes.[277] Despite the benefits described after ω3 intake in patients with T2D, a meta-analysis involving 23 randomized controlled trials showed no significant changes in HbA1c, fasting glucose, or fasting insulin when ω3 was supplemented at a mean dose of 3.5 g/day.[86]. Likewise, another meta-analysis of 26 controlled trials found that fish-oil supplementation, ranging from 2 to 22 g/day, did not change plasma HbA1c levels in patients with diabetes;[278] however, the high doses used in the studies should be taken into consideration. In addition, the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial showed that ω3 supplementation

did not reduce the rate of cardiovascular events in patients with glucose intolerance, impaired fasting glucose, and T2D.[279]

The effects of ALA on the glycemic profile have also been inconsistent.[217] However, it has been suggested that ALA intake may benefit glucose metabolism. Prospective data from the CHS study showed that higher plasma ALA levels were associated with a lower risk of new-onset T2D.[277] Similarly, in a large prospective analysis of more than 43 000 Chinese adults, ALA intake was inversely associated with the risk of incident T2D.[280] In a systematic review and meta-analysis of randomized controlled trials, ALA supplementation reduced blood glucose by 3.6 mg/dL.[67] Regarding flaxseed, a randomized controlled trial showed an improvement in insulin sensitivity.[281]

A systematic review identified 16 prospective studies, including cohort studies, that evaluated the relationship of ω3 intake and plasma levels with the incidence of T2D. Of a total of 540 184 individuals, 25 670 were cases of incident T2D.[276] Both ALA intake (n = 7 studies) and plasma ALA concentration (n = 6 studies) were not associated with T2D risk. Moderate heterogeneity (<55%) was observed for circulating ALA levels and diabetes, which may suggest a slightly lower risk of T2D.[276]

A review on ω3 fatty acids, cardiometabolic risk, and T2D concluded that there are no data demonstrating that ALA reduces the conversion of cardiometabolic risk to T2D or reduces mortality in people with T2D or cardiometabolic risk. ALA appears to reduce platelet aggregation in people with diabetes.[282]

Observational studies, using biological markers of fat intake or dietary surveys, suggest an inverse association between ω6 intake and T2D risk, although the data are not always consistent.[271,283] In the NHS study, involving 84 204 women aged 34 to 59 years without diabetes, CVD, or cancer who were prospectively followed for 6 years,ω6 intake assessed by validated food-frequency questionnaires was associated with a lower risk of T2D.[271] In men, a large prospective study, the HPFS study, also showed that the intake of ω6 as linoleic acid was associated with a lower risk of T2D in those aged <65 years and with BMI <25 kg/m^2.[269] Also, in the Singapore Chinese Health Study, in which more than 43 000 Chinese adults were prospectively assessed, neither ω6 intake nor the ω6/ω3 ratio was associated with new-onset T2D.[280]

Data from small intervention studies are also conflicting regarding the effect of ω6 on insulin sensitivity.[284] Further long-term, controlled studies are needed to identify the best dietary fatty acid composition to reduce the risk of T2D. Few data are available, and the effects of dietary fatty acid types (PUFAs and SFAs) on glycemic control in people with diabetes remain uncertain.[285]

Regarding trans fatty acids, experimental studies have shown adverse effects on glucose homeostasis and development of diabetes.[286-288] In addition, trans fatty acids increase plasma levels of TG, insulin, and postprandial glucose[289] and reduce glucose uptake by the skeletal muscle—changes that are accompanied by increased visceral and hepatic fat.[287] A study using data from the NHANES survey to investigate the association between trans fatty acids and metabolic syndrome found that plasma trans-fatty-acid concentration was positively associated with risk of metabolic syndrome and its individual components.[290] Even in small amounts, trans fatty acids have deleterious effects on glucose homeostasis, stimulating glycogenesis and increasing visceral fat.[286,289] Consumption of a trans fat-rich diet has been shown to induce greater weight gain, hepatic steatosis, and insulin resistance by suppressing the IRS-1 signaling pathway, with a consequent reduction in Akt and protein kinase C (PKC) phosphorylation.[286] In overweight patients with T2D, the intake of trans fatty acids has been consistently correlated with reduced insulin sensitivity and increased postprandial glucose and insulinemia.[291]

The CHS study, investigating the association of the incidence of T2D with both plasma phospholipid trans fat concentration and their consumption, found that plasma trans fatty acid concentrations were positively associated with the incidence of T2D after correction for de novo lipogenesis.[292] However, after adjusting for the intake of other foods, trans fatty acid intake was not associated with the incidence of T2D.[292] An important systematic review showed that trans fat intake was associated with a 28% increase in the risk of T2D, when studies with a low risk of bias were analyzed, in addition to being associated with increased all-cause mortality (34%), coronary heart disease mortality (28%), and cardiovascular risk (21%).[223]

9. Fatty Liver Disease

9.1. Hepatic Steatosis

The liver has a great metabolic capacity for the metabolism of all nutrients, especially fats. However, intracellular TG accumulation in more than 5% of hepatocytes characterizes nonalcoholic fatty liver disease (NAFLD),[293] a broad-spectrum clinical condition that initiates with hepatic steatosis and then progresses to nonalcoholic steatohepatitis (NASH), marked by the presence of fat and inflammatory infiltrate. This condition predisposes the person to the appearance of hepatic complications, such as fibrosis, cirrhosis, and hepatocellular carcinoma,[294,295] and extrahepatic complications, such as CVD and T2D.[296] The diagnosis should exclude secondary causes of hepatic steatosis, such as alcohol abuse, viral or autoimmune hepatitis, or steatosis due to use of steatogenic drugs.[296,297]

NAFLD is strongly associated with factors that compose the cardiometabolic risk profile, such as obesity, insulin resistance, T2D, and dyslipidemia.[296,297] About 90% of patients with NAFLD have at least one cardiometabolic risk factor, and 30% have all factors. The risk of NAFLD incidence has been shown to increase proportionally to the sum of factors related to cardiometabolic risk. For this reason, NAFLD is identified as the hepatic manifestation of cardiometabolic risk.[298] Individuals with T2D are at a 2-to-4-fold increased risk of progression to steatohepatitis together with the development of fatty liver disease complications.[294]

The development of NAFLD is related to an increased influx of free fatty acids (FFAs) to the liver, mainly due to increased lipolysis in adipose tissue, associated with insulin resistance and excess calories in the diet.[299] In patients with NAFLD, about 60% of hepatic TGs stem from adipose tissue lipolysis, 26% from de novo lipogenesis, and 14% from the

diet.[300] Additionally, there is an increase in hepatic lipogenesis together with a decrease in mitochondrial β-oxidation or VLDL secretion by the liver, contributing to hepatic lipid accumulation.[301,302] Hepatic lipid accumulation may then lead to inflammation, development of fibrosis, and loss of function. Fibrosis is the most important predictor of NAFLD-related mortality, and its presence increases the risk of death from CVD and liver diseases.[296]

Other factors may be related to the progression of the disease, such as: 1) increased ROS generation, promoting oxidative stress due to mitochondrial dysfunction or ER stress;[303] 2) lipid peroxidation; 3) activation of inflammatory pathways with a consequent increase in hepatic secretion of cytokines and inflammatory mediators such as TNF-α and IL-6, which may deteriorate the condition.[304] Moreover, lack of physical activity associated with a poor diet, i.e., rich in fats and excess calories, predisposes the development of NAFLD.[305]

Individuals with NAFLD have increased hepatic expression of genes related to fatty acid transport (fatty acid-binding proteins 4 and 5), TG hydrolysis (LPL), and recruitment of monocytes (MCP1) and PPAR-γ2.[306] PPAR-γ has been shown to induce SREBP-1c expression, with enhanced expression of genes that control proteins related to hepatic TG synthesis.[307]

Studies in animal models[308,309] or clinical trials using human participants[306,310] have strongly demonstrated the participation of a high-fat diet in the induction of hepatic steatosis. Insulin resistance plays a major role in hepatic lipid accumulation[311] and, within this context, the amount of fat (especially the type of fatty acid) influences hepatic lipogenesis and the action of insulin.[301-303]

9.2. Saturated Fatty Acids and Nonalcoholic Steatohepatitis

In hepatocytes, stearic acid and palmitic acid are able to induce apoptosis via excess JNK activation.[312] Another finding was that palmitate treatment can activate the IRE1α signaling pathway via TLR4. IRE1 is an ER transmembrane protein that governs the response to malformed proteins in the reticulum and induces apoptosis.[313]

A recent study demonstrated that palmitic acid promotes oxidative stress, ER stress, mitochondrial dysfunction, and inflammation in HepG2 cells. Animals that were given a high-fat diet rich in SFAs developed hepatic steatosis, NASH and fibrosis, conditions associated with ER stress, and insulin resistance. Conversely, the addition of oleic acid to the diet protected against SFA-induced hepatic lipotoxicity.[314] SFA or sucrose intake by experimental animals induced SFA accumulation in the liver, ER stress, and apoptosis compared to a PUFA-rich diet.[315]

A study in humans showed that total fat intake and SFA intake were positively associated with hepatic lipid content.[316] A 7-week randomized double-blind study in healthy individuals revealed that diets rich in palmitic or linoleic acid promoted similar weight gain. However, excess calories from SFAs increased the deposition of liver fat, visceral adipose tissue, and total fat as well as reduced the percentage of lean tissue when compared to a PUFA-rich diet. Additionally, increased body and liver fat correlated positively with elevated plasma concentrations of palmitic acid and inversely with linoleic acid.[317]

A recent study showed that an additional consumption of 1000 kcal in the form of SFAs for 3 weeks led to a greater increase in intrahepatic lipid content (55%) when compared to the same extra intake of UFAs or sugars, which elevated hepatic lipid content by 15% and 33%, respectively. SFA intake also induced insulin resistance and increased plasma ceramide concentrations by 49%.[318]

9.3. Unsaturated Fatty Acids and Nonalcoholic Steatohepatitis

In the liver, SCD1 is the enzyme primarily responsible for inserting double bonds in saturated chains of fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0), converting them to palmitoleic acid (C16:1) and oleic acid (C18:1), respectively. The aim is to control excess SFA content in the body, either from food or from excess endogenous conversion of palmitic acid derived from de novo lipogenesis. In NAFLD, lipogenic pathways are activated, and desaturation (SCD1) and oxidation pathways are reduced. This is partly due to insulin resistance and mainly due to a local inflammatory process.[319] Errazuriz et al.[320] found that patients with NAFLD had reduced liver fat (assessed by spectroscopic magnetic resonance imaging [MRI]) when they consumed a MUFA-rich diet for 12 weeks (22% of energy) compared to the control group (8% of energy). Such changes occurred even though the diets were isocaloric and the participants had no weight loss at the end of the study.[320]

In a randomized study conducted by Bozzetto et al.,[321] patients with T2D were assigned to one of the following interventions: (1) high-MUFA diet; (2) high-carbohydrate/high-fiber/low-glycemic index diet; (3) high-carbohydrate/high-fiber/low-glycemic index diet plus exercise; or (4) high-MUFA diet plus exercise. There was a reduction of up to 30% in hepatic lipid content in patients assigned to the high-MUFA diet, regardless of exercise.[321] The same group of researchers demonstrated, in a subsequent study, that liver fat reduction was due to the activation of hepatic oxidative pathways, based on measurement of β-hydroxybutyrate. Despite having identified an increase in β-oxidation, they found no increase in the ratio of palmitoleic to palmitic acid, which implies that there was no difference in SCD1 activity.[322]

Together with the lipolytic action promoted by MUFAs, the anti-inflammatory action coordinated by oleic acid may be involved in the potency of the restoration of liver function, as demonstrated by Morari et al.[323] In their study, HepG2 cells treated with oleic acid showed increased gene expression and protein content of IL-10, a protein with a potent anti-inflammatory action. Oleic acid activates the protein PGC-1α, which binds to another protein, cMAF. In the form of a dimer, PGC-1α and cMAF migrate to the nucleus and induce exclusive transcription of the IL-10 gene.[323]

Similarly, PUFAs have different hepatic metabolic responses. Omega-6 fatty acids (linoleic and arachidonic acids) and ω3 fatty acids (ALA, EPA, and DHA) participate in hepatic metabolism but are primarily intended for constitution of cell membranes, intracellular signaling as second messengers, and other functions, thus being diverted from their use as an energy substrate.[324] In 2007,

Yamaguchi et al.[325] experimentally inhibited hepatic TG synthesis. Despite an improvement in steatosis, liver damage intensified and then progressed to fibrosis and cirrhosis. The study demonstrated that, with an increase in FFAs in the cytoplasm, there was greater ROS oxidation, inducing important inflammation.[325]

Although some studies suggest an improvement in NAFLD with ω3 fatty acid supplementation,[326] there are still inconsistencies in the literature regarding its benefits.[327,328] In a randomized study of children with NAFLD, daily intake of 1300 mg of ω3 fatty acids for 6 months reduced aspartate aminotransferase and gamma-glutamyl transpeptidase, in addition to increasing serum adiponectin, but these changes were not sufficient to reduce the degree of steatosis on ultrasound.[329] Some of the inconsistencies found in the studies are usually related to the experimental design, the certification of the content of the chosen capsule, and the choice of placebo, among other factors. Tobin et al.[330] conducted a randomized, double-blind study in which they treated 291 patients with a concentrated ω3 fatty acid capsule (460 mg EPA + 380 mg DHA) or placebo (olive oil) for 24 weeks. MRI-proton density fat fraction assessment showed a significant reduction in hepatic lipid content, similar in both groups, which was attributed to adherence to a healthy dietary pattern.[330]

Although ω3 fatty acids reduce TG synthesis by blocking SREBP,[83,331,332] results of clinical trials[329,330] do not support the recommendation of ω3 fatty acid supplementation in the treatment of NAFLD and NASH, as discussed in a position statement by the American Association for the Study of Liver Diseases.[297]

9.4. Trans Fatty Acids and Nonalcoholic Steatohepatitis

A high-fat diet enriched with trans fatty acids induced an increase in the expression of transcription factors involved in hepatic lipogenesis (SREBP-1c and PPAR-γ) and reduced MTP, suggesting less ability to export TGs, which led to the development of NASH.[308] A study that evaluated 4242 participants in the NHANES cohort showed a positive association between plasma concentration of trans fatty acids and NAFLD, which was estimated by plasma biomarkers of liver function such as alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase.[333]

Diet composition may influence the development of NAFLD,[334] and, within this context, excess SFAs may contribute to intrahepatic lipid accumulation.[318] Conversely, healthy dietary patterns rich in UFAs, such as the Mediterranean diet, seem to have beneficial effects, including improved steatosis even if there is no weight loss.[335,336] However, further prospective studies comparing the effect of macronutrients on NAFLD and evaluating pre- and post-treatment histological components are needed.

The treatment of NAFLD consists primarily of weight loss, which is achieved by reducing energy intake by approximately 30%. Losing 3% to 5% of body weight reduces steatosis, and losing 7% to 10% of baseline weight contributes to the improvement of histological components of steatohepatitis and fibrosis.[337] Physical activity combined with caloric restriction aids weight loss and maintenance.[297]

Thus, individuals with NAFLD should be instructed to follow a calorie-restricted diet and practice physical activity to lose weight. The adoption of healthy dietary patterns should be encouraged, including a large amount and a varied range of fruits and vegetables, in addition to favoring complex carbohydrates over simple carbohydrates, with increased UFA intake and adequate SFA intake.[297]

10. Lipid Metabolism in Adipose Tissue

Adipose tissue is composed of adipocytes, preadipocytes, immune cells, fibroblasts, lymph nodes, and nervous tissue. The adipocyte is the only cell that can store fat without compromising its function, which primarily is to promote lipogenesis and lipolysis.[338] Additionally, adipose tissue is able to secrete several bioactive substances such as leptin, cytokines (TNF, IL-6, MCP1, IL-1β), and other adipokines, performing autocrine, paracrine, and endocrine functions.[339] Such actions can be modulated by different fatty acids from the diet.

In response to excess energy and in an attempt to restore tissue homeostasis, the adipose tissue undergoes a remodeling process consisting of adipocyte hypertrophy and hyperplasia and high cytokine secretion, which characterizes them as proinflammatory cytokines.[340] However, in the long term, secretion of TNF-α, IL-6, iNOS, and MCP1, together with recruitment of inflammatory cells such as neutrophils, T cells, and macrophages, promote inflammation, fibrosis,[339-341] and insulin resistance in adipose tissue,[342] which plays a key role in the metabolic derangements observed in obese patients.[343]

Cell signaling mediated by TNF-α receptors culminates in NF-kB activation, which increases cytokine secretion and characterizes local inflammation. In this condition, the adipocyte shows increased lipolysis with increased FFA release. SFAs derived from adipocyte lipolysis activate TLR4s in tissue-resident macrophages, intensifying the local inflammatory response and establishing a vicious circle.[344] Concomitantly with these actions, there is a gradual polarization of macrophages from the M2 subpopulation (anti-inflammatory action linked to resolution of injury) to the M1 subpopulation (classic activation pathway associated with Th1 response). Thus, there is an intensification of the inflammatory state and induction of insulin resistance in adipose tissue.[339] In obese patients, other factors such as adipose tissue hypoxia, ER stress, and endotoxemia also contribute to the maintenance of inflammation in adipose tissue.

Insulin has an important effect on adipose tissue, as it inhibits lipolysis and stimulates lipogenesis and glucose and FFA uptake. The activation of inflammatory pathways antagonizes the action of insulin by inducing resistance to the hormone and favors the appearance of diseases associated with cardiometabolic risk.[343]

TGs from the diet are packaged into chylomicrons and hydrolyzed by the action of LPL,[343] releasing FFAs, which are then directed to adipose tissue and to a lesser extent to the muscle.[345] Thus, the type of fatty acid in adipose tissue has a strong correlation with the fatty acid in the diet.

10.1. Saturated Fatty Acids and Adipose Tissue Metabolism

An in vitro study showed that preincubation of adipocytes with palmitic acid induced cell hypertrophy with a consequent increase in MCP1 secretion and hydroperoxide concentration, a marker of oxidative stress.[345] These effects were not observed with oleic acid.[345,346] In another study, palmitic acid activated NF-kB and increased the expression of proinflammatory cytokines in 3T3-L1 adipocytes.[347] In experimental animals, a high-fat diet rich in lauric acid induced the activation of proinflammatory cytokines (TNF-α, IL-6, MCP1, IL-1β, IFNγ) and activated serine kinases such as IKKβ and JNK in adipose tissue, with a reduction in AMPK phosphorylation.[348] Conversely, it increased the production of cytokines with anti-inflammatory action in an attempt to rescue tissue homeostasis.[348]

In animal models, consumption of a high-fat diet rich in palmitic acid led to increased dendritic cell infiltrate in adipose tissue, together with the development of insulin resistance. In dendritic cells, palmitic acid induced increased expression of maturation markers such as CD40, CD80, MHCII, and TLR4. An increased expression of caspase-1 and IL-1β genes suggests parallel activation of the inflammasome pathway, another intracellular structure involved in the control of inflammatory tone.[237]

A subsequent study conducted by the same research group showed that a SFA-rich diet induced insulin resistance, reduced glucose uptake, and increased plasma insulin concentrations. Moreover, there was a reduction in the expression of IRS1 and glucose transporter type 4 in adipose tissue, as well as tyrosine phosphorylation of IRS1 and AKT. These effects were not observed in the groups undergoing the MUFA-rich diet.[349]

Kolak et al.[350] found that an increase in macrophage infiltrate, MCP1 and PAI1 expression, and ceramide accumulation occurred in subcutaneous adipose tissue regardless of BMI. In addition, these changes positively correlated with hepatic lipid accumulation.

A cross-sectional study that included 484 participants in Japan showed that SFA consumption (assessed by fatty acid concentration in plasma phospholipids) correlated with a reduction in adiponectin and an increase in resistin and visfatin, which are adipokines related to insulin resistance and adipogenesis.[351]

A study of overweight individuals that evaluated the additional consumption of 1000 kcal/day in SFAs (coconut oil and butter), UFAs (olive oil and nuts), or sugars showed that SFAs induced insulin resistance and increased the expression of genes related to inflammatory pathways in adipose tissue.[318]

10.2. Unsaturated Fatty Acids and Adipose Tissue Metabolism

Adipose tissue stores SFAs more efficiently; however, if there is a high proportion of UFAs in the diet, lipid deposition on adipose tissue may follow the same dietary profile.[352] Because of the difficulty in investigating tissue dispersion profile of fatty acids obtained from food in humans, most of the studies are conducted in animals.[353] Providing a high-fat diet to mice for only 3 days was shown to increase the amounts of palmitic and oleic acid in adipose tissue, with oleic acid being deposited preferably in the mesenteric adipose tissue.

The study also showed that only oleic acid was able to change the inflammatory profile of M1 macrophages to the anti-inflammatory M2 profile, both in animal tissue and in adipocyte culture.[353] In the LIPIGENE study, 39 patients with cardiometabolic risk assigned to a high-oleic acid diet showed increased expression of genes that control autophagy (Beclin-1 and ATG7) and apoptosis (CASP3 and CASP7) compared to both the low-fat, high-complex carbohydrate group and the high-complex carbohydrate, high-ω3 fatty acid group.[354]

Several studies have demonstrated the correlation between arachidonic acid content in adipose tissue and AMI.[355-358] A case-cohort study showed a strong correlation (39% of participants) between arachidonic acid content in adipose tissue and AMI.[359] This is explained by the rapid release of arachidonic acid by the adipocyte, which is a substrate for the synthesis of proinflammatory and prothrombotic eicosanoids, favoring inflammation and destabilization of the atherosclerotic plaque. In addition, this fatty acid has been associated with insulin resistance and may increase cardiovascular risk.[359]

The known anti-inflammatory potential of ω3 fatty acids seems to positively interfere with the control of tissue inflammation in patients, but more robust evidence is still needed. Spencer et al.[360] treated insulin-resistant but nondiabetic patients with 4 g of ω3 fatty acid (ethyl ester) for 12 weeks and observed a significant reduction in MCP1, and thus macrophages, in adipose tissue but not in the muscle. These phenomena were not followed by a reduction in plasma cytokine concentration, insulin sensitivity, or adiponectin. In a coculture experiment of adipocytes and macrophages from the same participants, the adipocytes of patients who consumed ω3 fatty acids had reduced MCP1 content even in the presence of macrophages.[360] In a randomized, double-blind controlled study, overweight and obese pregnant women were supplemented with 2 g of ω3 fatty acid (EPA + DHA) twice a day, from gestational week 10 to birth. Plasma concentration of CRP decreased significantly, followed by reduced TLR4 in adipose tissue and decreased gene expression of TNF, IL-6, and IL-8 in placental tissue.[361]

Difficulties in the development of general recommendations for fatty acid intake in patients with diseases are due to a wide variation in experimental protocols, including different types of food, duration of diets, conflicts of interest of the study authors, and the quality of scientific information, among others.

In a double-blind, placebo-controlled study, insulin-resistant patients were given a daily supplementation of 3.9 g of ω3 fatty acids (EPA + DHA) for 6 months and underwent adipose tissue biopsy before and after the intervention. No benefit associated with tissue metabolism was observed.[362] However, in a study of human adipocytes, EPA induced an increase in the expression of genes involved in adipocyte "beiging". Proteins involved in mitochondrial biogenesis, such as uncoupling protein 1 and carnitine palmitoyltransferase 1, were stimulated. The same study showed, however, that arachidonic acid reduced mitochondrial respiration and then

energy expenditure.[363] Finally, considering the evidence found to consolidate the decision-making process regarding ω3 fatty acids and their relationship with adipose tissue function, Iturari et al.[364,363] treated 55 obese, nondiabetic patients eligible for bariatric surgery with 3.3 g of ω3 fatty acids (EPA + DHA) for 8 weeks. There was a significant reduction in subcutaneous adipose tissue, content of chemokines CCL2 and CCL3, IL-6, hypoxia-inducible factor 1-alpha and transforming growth factor-beta, and CD40, as well as an increase in adiponectin. No changes induced by ω3 fatty acid consumption in visceral adipose tissue were observed in the experimental group compared to the placebo group.

Despite the potential metabolic benefits from ω3 fatty acid consumption, there is no consensus on its relevance for the treatment of dysmetabolism regarding adipose tissue function. Conversely, there is a greater body of evidence supporting incremental metabolic benefits of MUFAs for conditions associated with dysmetabolism.

11. Food

11.1. Coconut Oil

Coconut oil is composed almost entirely (92%) of SFAs, of which lauric acid accounts for approximately 50%, followed by myristic acid (16%), palmitic acid (8%), and finally caprylic, capric, and stearic acids. Regarding essential fatty acid concentrations, coconut oil has a low concentration of linoleic acid (18:2) and no linolenic acid (18:3).[43,365]

The largest coconut oil producers are the Philippines, Indonesia, and India, extracting two different types of oil: one is refined, bleached, and deodorized, and the other is virgin, cold-pressed, with no refining processes.[366] Coconut oil consumption has grown significantly in recent years, and this is partly due to the fact that its properties have been erroneously associated with those of medium-chain triglycerides, formed mainly by caprylic acid (8:0) and capric acid (10:0),[367] which are absorbed bound to albumin and reach the liver via portal system, with no consequent increase in TGs. Lauric acid, the main fatty acid in coconut oil, is largely transported by the lymphatic system after being absorbed,[38] and its presence in chylomicrons is dose-dependent.[38]

Beneficial associations regarding coconut oil consumption possibly stem from a study conducted on people from Pukapuka and Tokelau, two Polynesian islands that exhibit low incidence of CVD. The typical diet of this population is rich in saturated fat, and coconut is the main source of fat and energy; protein is obtained mainly from fish, and carbohydrate is obtained from native fruits such as breadfruit. In addition, the diet is high in fiber and low in sucrose and processed foods, because of the limited access to these foods.[368] This situation has changed in recent decades, possibly because of the migration to Western dietary habits, even though coconut oil consumption was maintained. In 2010, about 40% of the Polynesian population was diagnosed with chronic diseases (CVD, T2D, and hypertension), which were responsible for three-quarters of deaths in the archipelago.[369]

Coconut oil is able to increase plasma concentrations of TC and LDLc compared to other fats such as olive oil[370] and safflower oil.[371] A study in humans showed that lauric acid elevates TC and LDLc, compared to a MUFA-rich diet, but less markedly than palmitic acid.[372,373] Mendis et al.[373] found that the isocaloric replacement of coconut oil, typically found in the diet of Sri Lankan people, with soybean oil rich in PUFAs reduced the plasma concentrations of TC, LDLc, and TG in normolipidemic individuals. The same result was obtained with corn oil in dyslipidemic individuals.[219]

Furthermore, studies showing increased HDLc concentrations with coconut oil intake have shown a concomitant increase in LDLc, which is known to elevate cardiovascular risk.[374]

SFAs are known to activate inflammatory signaling pathways, as well as ER stress, autophagy, and apoptosis, via activation of TLRs linked to the innate immune response.[375] TLRs recognize pathogen-associated molecular patterns such as LPS, found in the cell wall of gram-negative bacteria, and then alert the immune system. When activated, TLRs trigger signaling that culminates in the transcription and secretion of proinflammatory cytokines.[375]

Lauric acid, among all SFAs, has the greatest inflammatory potential.[241] An in vitro study in macrophages showed that lauric acid induced NF-κB activation, leading to increased expression of cyclooxygenase-2 via activation of TLRs 2 and 4.[376] The ability of lauric acid to activate inflammatory pathways by activating TLR4, leading to inflammatory cytokine secretion and T-cell activation, has already been described in different cell types.[241, 377]

A study that compared the effect of consuming coconut, palm, or olive oil for 5 weeks on inflammatory parameters of normocholesterolemic individuals found no difference in plasma concentrations of homocysteine and inflammatory markers such as TNF-α, IL-1β, IL-6, INF-γ, and IL-8. However, in that study, the standard deviation was excessively high and may have masked differences in inflammatory profile.[370]

Valente et al.[378] evaluated the acute effect of a diet rich in coconut oil compared to olive oil in 15 overweight women and found no difference regarding energy metabolism and lipid oxidation.

Regarding the antioxidant properties attributed to polyphenols found in virgin coconut oil, studies are still preliminary and were conducted mostly in experimental animals, thus their findings cannot be translated into humans.

To date, there are no randomized controlled studies and epidemiological studies evaluating the effect of coconut oil on lipid profile, inflammatory profile, and cardiovascular outcome. Thus, there is no evidence to indicate coconut oil as a substitute for UFA-rich vegetable oils.

11.2. Palm Oil

Palm oil, together with interesterified fats, has been widely used by the industry as a substitute for trans fat in food. Despite being a vegetable oil, palm oil is composed of SFAs (45% palmitic acid and 5% stearic acid) and UFAs (40% oleic acid and 10% linoleic acid). Thus, an increase in direct consumption of palm oil, or indirect consumption via processed foods, will contribute to a greater SFA intake, which elevates cardiovascular risk.

In humans, a palm oil-rich diet increased plasma concentrations of TC and LDLc compared to consumption of high-UFA vegetable oil.[379] A meta-analysis of intervention studies found that, compared to vegetable oils with low SFA concentrations such as canola, soybean, and olive oil, palm oil increased the concentrations of TC, LDLc, and, to a modest extent, HDLc, which is consistent with the effect of SFAs on lipoprotein profile. Compared to trans fat, the increase in HDLc was more pronounced, as trans fat intake reduces its concentrations.[380] Conversely, palm oil seems to have similar effects to animal fat on plasma lipids.[380,381]

Palm oil consumption should be kept within the recommended SFA intake range. Despite being a vegetable oil, palm oil is very rich in palmitic acid and thus seems to act similarly to animal fats.

11.3. Chocolate

Chocolate is obtained from the cocoa bean, which comes mainly from countries in South America and the west coast of Africa. In addition to cocoa, cocoa butter, sugar, milk, and lecithin, other ingredients such as nuts, cereals, and fruits may be incorporated into the manufacture of chocolate, characterizing it as a high-energy density product rich in carbohydrates and fats. Chocolate also has polyphenols and minerals such as potassium, magnesium, iron, and zinc. Approximately 63% of cocoa fat is composed of stearic (34%) and palmitic (27%) acids. The remaining 37% are in the form of MUFAs (33.5%) and PUFAs (3.5%).[382]

Because it is rich in stearic acid, cocoa fat has a neutral effect on cholesterolemia. Studies that investigated food consumption in humans show that, compared to palmitic acid, stearic acid reduced plasma concentrations of TC and LDLc in a similar way to oleic acid. In addition, stearic acid increased oleic acid concentrations in plasma CE and TG,[383] which is explained by the fact that stearic acid is rapidly converted to oleic acid in the liver by the action of SCD1.[48] More recent data from the EPIC study showed a positive association between stearic acid concentrations in plasma phospholipids and risk of both coronary heart disease[108] and T2D.[14] However, it is important to note that stearic acid is also endogenously produced by de novo lipogenesis.

Stearic acid intake appears to have a neutral effect on cholesterolemia; however, it must be taken into account that chocolate is also a source of calories and simple sugars, which may contribute to weight gain and increased cardiovascular risk.

11.4. Butter

Butter derives from the cream obtained from milk that was skimmed; therefore, its fat comes exclusively from dairy fat. In a portion of butter, about 51.5% of fatty acids are SFAs, including palmitic (24%), stearic (10%), myristic (8%), and lauric (2%) acids, while the rest is composed of MUFAs (22%) and PUFAs (1.5%).[25]

A randomized study evaluating the impact of butter SFAs compared to isocaloric diets rich in UFAs on cardiometabolic risk showed that butter consumption increased the concentrations of TC, LDLc, and ApoB.[384] In a prospective cohort study of more than 26 000 individuals, consumption of butter, together with milk and milk products, was inversely associated with incidence of T2D.[385] In two other cohorts followed up for 10 and 20 years, no association was found between butter consumption and CVD.[386,387] However, it should be noted that in the MESA study cohort,[387] even in the highest quintile, the median consumption of butter was less than 5 g/day per person.

A systematic review of cohort studies with a high degree of evidence found no association between butter consumption and risk of CVD, CAD, and stroke. Conversely, there was an inverse association with risk of T2D.[388]

The results of the studies should be interpreted with caution, as the actions of SFAs in plasma lipids and cardiovascular health have been well consolidated. The use of butter should be part of a healthy, individualized dietary pattern that considers the added energy value and promotes weight management when required.

11.5. Dairy

Milk and milk products are an important source of calcium and high biological value protein. Conversely, whole-fat dairy consumption may increase the intake of SFAs, especially myristic acid, which has a strong correlation with increased cardiovascular risk. Skim dairy consumption is part of the DASH diet recommendations, a dietary pattern that was originally developed for the treatment of hypertension and, because of its cardiometabolic benefits,[389] is recommended as a healthy dietary pattern for all adults.[390]

More recently, studies have shown that dairy consumption is inversely associated with risk of T2D[14,391] stroke,[392] and CVD.[110] In those studies, plasma concentrations of pentadecanoic acid (15:0) and heptadecanoic acid (17:0) were used as markers of dairy consumption, as, because they are not endogenously synthesized, they must be obtained from the diet, and dairy is their main source.

It is important to note that the food matrix is a determining factor in cardiovascular risk, as, in addition to macronutrients, food provides micronutrients and fibers that contribute to a favorable cardiovascular outcome within the context of healthy dietary patterns. In contrast, the inclusion of processed foods rich in simple, refined sugars and additives such as food coloring agents, preservatives, and thickeners, may negatively impact cardiovascular risk. Additionally, the use of lipid-lowering drugs such as statins may mitigate or even mask the effects of SFA consumption on cardiovascular risk.[106]

11.6. Meat

The most consumed types of meat are beef, chicken, and pork, which are important nutritional sources of high biological value proteins, providing all essential amino acids, vitamins, and minerals. The amount of fat and the distribution of fatty acids will vary according to the source and the type of meat cut. Overall, meats contain mostly MUFAs and SFAs (especially palmitic and stearic acids) and a small amount of PUFAs.[25, 28]

A positive association between meat consumption and cardiovascular risk has been observed in some studies[110] but

not in others.[393] A study of more than 780 individuals found that consumption of red and processed meats correlated with a less healthy dietary pattern but not with CVD and T2D risk markers.[394] A prospective cohort study of more than 74 thousand individuals showed an association between greater consumption of (processed and unprocessed) meat and increased risk of CVD mortality (such association was found even in individuals with greater consumption of fruits and vegetables).[395]

An increased risk of all-cause and CVD mortality was associated with greater consumption of red and processed meats but not with consumption of unprocessed meats alone in two meta-analyses.[396,397] Processed meats are also rich in sodium and nitrogen compounds such as nitrates, which may contribute to a deleterious effect on cardiovascular risk because of their effects on blood pressure and endothelial function.

It is well established that high consumption of red and processed meats is associated with an increased cardiovascular risk, which is why their intake should be moderate and consistent with the total SFA intake recommended in the diet.

12. Gut Microbiota

High-fat diets, especially those rich in SFAs, are able to change the composition of gut microbiota,[398-400] induce decreased bacterial diversity, increased intestinal permeability, metabolic endotoxemia, and low-grade systemic inflammation,[401-407] and influence the development of several chronic diseases such as obesity, diabetes, and atherosclerosis.[408] Loss of intestinal epithelium integrity allows LPS from the cell membrane of gram-negative bacteria to translocate into plasma, culminating in metabolic endotoxemia.[401,403]

A greater consumption of high-SFA diets has been shown to increase intestinal paracellular permeability by interfering in tight-junction proteins, and thereby plasma concentrations of LPS are elevated.[409,410] Changes in intestinal permeability are related to the regulation of tight-junction proteins, a protein complex that maintains cell-cell junctions in the intestinal epithelium, forming a barrier against the passage of macromolecules.[411]

A study in mice found that a high-SFA diet induced greater formation of taurocholic acid, which allowed the expansion of sulfate-reducing bacteria such as *Bilophila wadsworthia*, an effect that was not observed in a high-PUFA diet. That study shows that changes in the composition of bile acids due to the type of dietary fat may cause dysbiosis, compromising host homeostasis.[400]

An increase in intestinal permeability induced by a high-fat diet, consisting mainly of SFAs, leads to changes in gut microbiota and increased inflammatory response, triggered by TLR4 activation by LPS.[412] Another mechanism may be associated with decreased secretion of the enzyme intestinal alkaline phosphatase by the duodenal brush border, which is responsible for detoxifying LPS, thus protecting against endotoxemia.[413]

An experimental study showed that a high-fat diet, especially when combined with a high-sugar diet, induces dysbiosis and inflammation in the intestinal epithelium and changes the activation of the vagal afferent pathway, actions that may impair the regulation of food intake, contributing to hyperphagia and development of obesity.[414]

12.1. Dietary Patterns and Gut Microbiota

Diet components have an important impact on the profile of gut microbiota. Therefore, different dietary patterns can modulate gut microbiota in distinct ways.

A study that investigated the association of dietary variables with gut microbiota identified 97 nutrients associated with relative abundance data or with presence/absence of microbiomes. The nutrients were divided into four groups: amino acids and choline; carbohydrates; fats; and fibers and vegetables. The study showed that the fat versus fiber groups were antagonistically associated with bacterial abundance,[415] i.e., bacteria that were positively associated with fat tended to be negatively associated with fibers. The same pattern of association was seen for the amino acid and protein versus carbohydrate groups and the fat versus carbohydrate groups. In addition, microbial rates that correlated with BMI also correlated with higher consumption of fats and SFAs.[415]

A recent randomized study of 217 healthy individuals compared the effect of isocaloric diets containing increasing concentrations of fat (20%, 30%, and 40%) and the same amount of fiber (14 g/day).[416] The high-fat diet increased fecal concentrations of palmitic, stearic, and arachidonic acids. The latter was positively associated with increased plasma concentrations of inflammatory mediators such as CRP as well as PGE2 and thromboxane B2, both derived from arachidonic acid. An important result of that study was that, even with adequate amounts of fiber in the diet, a high fat consumption prevented the formation of short-chain fatty acids (SCFAs) by bacteria.[416] Additionally, increased fat consumption reduced bacterial diversity.

12.2. Importance of Dietary Pattern in Short-chain Fatty Acid Synthesis

The production of glycoside hydrolases, which are responsible for the breakdown of some saccharides, is very limited in the human body. Conversely, some intestinal bacteria encode enzymes capable of digesting a wide range of polysaccharides, such as fibers.[417] The fermentation of soluble fibers promotes the formation of SCFAs, especially propionate (C3), acetate (C4), and butyrate (C5), which, in addition to serving as an energy substrate for colonocytes, perform systemic actions such as favoring glucose homeostasis.[418,419]

The presence of SCFAs induces secretion of intestinal incretins, such as GLP-1 and PYY, which act on the central nervous system by promoting satiety and reducing food consumption, decreasing gastric emptying time, increasing intestinal transit, in addition to stimulating insulin synthesis and secretion by the pancreas.[418]

A reduction in fiber consumption may impact the composition of the gut microbiota and the production of SCFAs. A prospective study of 17 obese individuals evaluated the impact of two high-protein/high-fat/low-fiber diets. The results show that both diets decreased fecal production of SCFAs and increased the concentration of branched-chain

fatty acids, phenylacetic acid, and nitrogenous compounds, which are detrimental to colonic health.[420]

13. Dietary Cholesterol

13.1. Plasma Concentration of Lipids and Lipoproteins

The relationship between dietary cholesterol and plasma TC has been shown to be linear in observational cohort studies.[421,422] However, observational studies have limitations such as the presence of confounding variables, which may increase the magnitude of correlations, both positive and negative, and selection biases.[423] Furthermore, dietary cholesterol consumption is generally associated with increased consumption of SFAs, which are known to increase LDLc and cardiovascular risk.[424]

In recent years, there has been an intense discussion about the role of dietary cholesterol in the incidence of atherosclerotic complications. In response to that, the AHA no longer limits egg consumption as a way of protecting against CVDs. Thus, the Dietary Guidelines for Americans withdrew a recommendation for restricting cholesterol intake to no more than 300 mg per day.[7] However, the guidelines suggest that dietary cholesterol remains important and should be considered for developing healthy dietary patterns. They also highlight that dietary cholesterol consumption should be as low as possible, as recommended by the Institute of Medicine.[425] As noted, food sources containing high amounts of cholesterol are usually also rich in SFAs, such as fatty meats and high-fat dairy products. Therefore, the recommendation focuses on restricting SFAs to less than 10% per day, which should be sufficient to control dietary cholesterol.[7]

It is worth mentioning that not all people respond the same way to dietary cholesterol consumption, as the response is highly variable depending on genetic and metabolic factors[426,427] Lipid profile responses to dietary cholesterol were examined in 19 intervention studies. Cholesterol intake, mainly from eggs, led to an increase in both LDLc and HDLc, resulting in a slight increase in the LDLc/HDLc ratio. However, the analysis of this ratio can be very simplistic, as, while LDLc is an excellent marker of cardiovascular risk and changes in its value show a marked relationship with cardiovascular risk, changes in HDLc do not express possible changes in the functionality of HDL particles, which extends far beyond reverse cholesterol transport.[428]

Cholesterol consumption up to 400 mg/day from eggs is not associated with increased plasma TG concentrations in overweight individuals with diabetes or prediabetes.[429]

13.2. Risk of Developing Type 2 Diabetes

Observational and randomized studies have shown conflicting results regarding the association between dietary cholesterol consumption and risk of T2D. A case-control study demonstrated a 2-fold increase in the risk of T2D in individuals who consumed 3 to 4.9 eggs per week and a 3-fold increase in those who consumed more than 5 eggs per week, after adjusting for confounding factors such as BMI, family history of diabetes, smoking, physical activity, and plasma TG concentration.[430] An investigation that used data from two prospective randomized studies, Physicians' Health Study I (1982-2007) and Women's Health Study (1992-2007), demonstrated that during follow-up (20 years in men and 11.7 years in women) the development of diabetes was higher in those who consumed more than 5 eggs per week in men and more than 7 eggs per week in women, after multivariate adjustments.[431] However, other studies of populations from different regions have not shown the same association. A prospective study of the Japanese population (Japan Public Health Center-based Prospective Study) with a 5-year follow-up concluded that high intake of dietary cholesterol or eggs was not associated with a higher risk of T2D.[432] Opposite results were observed in the male population of the KuopioI Schaemic Heart Disease Risk Factor Study, which found that a higher egg consumption was associated with a lower risk of T2D in a 19.3-year follow-up.[433]

In the Jackson Heart Study, in an African American population, a higher prevalence of T2D was observed in those who consumed more eggs (> 5 eggs/week vs < 1 egg/month); however, a prospective analysis showed no association between egg consumption and incidence of T2D.[434]

In systematic reviews and meta-analyses with healthy individuals, there was also no consensus on the association between egg consumption and increased risk of CVD and T2D.[435,436] The results can be explained in part by confounding factors such as SFA intake and dietary energy intake, which favor weight gain and development of metabolic syndrome.[437]

13.3. Risk of Cardiovascular Diseases in Type 2 Diabetes

Another issue under discussion is the role of dietary cholesterol in cardiovascular risk in individuals with T2D or metabolic syndrome.

Observational and prospective studies associate egg consumption with a higher risk of CVD in the general population, while others only found association in individuals with T2D.[438] A meta-analysis concluded that the consumption of > 1 egg per day increased by 1.69 times the risk of developing CVD compared to the consumption of no eggs or < 1 egg per week. However, egg consumption was not associated with mortality.[439]

A randomized study of individuals with prediabetes or T2D (DIADEGG Study) who were assigned a diet with high (2 eggs/day for 6 days/week) or low (< 2 eggs/week) egg consumption for 3 months concluded that greater consumption of dietary cholesterol did not change plasma concentrations of HDLc, LDLc, and TC. The study also showed that there was no increase in risk factors for CVD in patients with T2D.[429]

In the NHS population, lower consumption of dietary cholesterol (assessed by the intake of eggs and meat) in patients with T2D was associated with healthier quality of life and thus lower risk of CVD. When quality-of-life factors were controlled for, the association between cholesterol consumption and risk of CVD was attenuated, suggesting that the improvement in quality of life is also associated with cardiovascular risk, and not only with dietary cholesterol.[440]

Results based on the Framingham Offspring Study population, which was followed up for 20 years, demonstrated

no association of dietary cholesterol consumption with fasting lipid profile or risk of CVD in individuals with altered fasting blood glucose or T2D.[441]

An analysis of the prospective PREDIMED study population, which included participants with no previous cardiovascular events who were followed up for an average of 5.8 years, concluded that low or moderate egg consumption did not increase the risk of CVD in individuals either with or without T2D.[442]

Results of prospective randomized and observational studies, as well as systematic reviews and meta-analyses, are inconclusive regarding the association between greater consumption of dietary cholesterol and greater risk of CVD in individuals with T2D because of the high heterogeneity of the populations evaluated and methods used.

13.4. Impact on Cardiovascular Diseases

The available scientific evidence is conflicting regarding the impact of cholesterol intake on cardiovascular risk. Several studies suggest lack of association between dietary cholesterol and CAD or stroke, although there are limitations to be considered in the results.[427,443,444] In Asians, the highest quartile of dietary cholesterol consumption did not correlate with increased subclinical atherosclerosis assessed by calcium scoring.[445] In Finns, consumption of more than 400 mg of cholesterol per day was not associated with increased intima-media thickness or incidence of CAD.[446] However, in Americans, adding 300 mg of cholesterol to a baseline diet containing an average of 300 mg of cholesterol per day was associated with a 17% increase in CVD risk.[447]

Because high cholesterol consumption may be associated with an increased risk of developing CVD, and such risk may be dose-dependent, monitoring cholesterol intake is recommended.[447]

14. EGG

Egg is a low-SFA source of dietary cholesterol with high nutrient density and low cost. A chicken egg (50 g) contains high biological value protein (7.5 g), SFAs (1.6 g), MUFAs (1.8 g), PUFAs (0.9 g), and cholesterol (approximately 200 mg). Egg yolk is also rich in choline (147 mg), an essential nutrient for liver and muscle functions.[25,448]

The impact of egg consumption on lipid profile is quite variable.[449] In healthy adolescents, the consumption of more than 3 eggs per week is not associated with changes in lipid profile.[450] Similarly, in normolipidemic and physically active adults, the consumption of 2 eggs per day did not change plasma concentrations of lipoproteins after 12 weeks of study.[451] Conversely, a meta-analysis of 28 studies evaluating the consumption of from 5 eggs per week to 3 eggs per day showed that egg consumption in hyper-responsive individuals increases the concentration of TC by 5.60 mg/dL (95% CI: 3.11-8.09; $P < 0.0001$), LDLc by 5.55 mg/dL (95% CI: 3.14-7.69; $P < 0.0001$), and HDLc by 2.13 mg/dL (95% CI: 1.10-3.16; $P < 0.0001$), having a neutral effect on TG concentration compared to no egg consumption.[452] Nonetheless, there is evidence that egg consumption is associated with larger LDLc particles, which are less susceptible to oxidation and penetration into the endothelium.[449]

Findings on the impact of egg consumption on CVD risk, remain conflicting. A meta-analysis assessing the impact of consuming 1 egg per day versus < 2 eggs per week on the risk of CAD and stroke found no association between egg consumption and coronary risk in 7 studies of low heterogeneity.[453] Conversely, there was a 12% reduction in the risk of stroke with increased egg consumption and no dose-response relationship in the risk trend for stroke with increased egg consumption.[453]

In a cohort study of the Chinese population, high egg consumption (7 or more eggs per week) compared to low egg consumption (< 1 egg per week) was not associated with cardiovascular mortality, CAD, or stroke.[454] A study evaluating American population cohorts, considering an average consumption of 0.5 eggs per day (3 to 4 eggs per week), concluded that each additional 0.5 eggs consumed per day is associated with a 6% increase in risk of CVD (95% CI: 1.03-1.10) and an 8% increase in all-cause mortality (95% CI: 1.04-1.11). However, after statistical adjustment for cholesterol consumption, both associations were no longer significant, with an adjusted hazard ratio of 0.99 (95% CI: 0.93-1.05) for CVD incidence and an adjusted hazard ratio of 1.03 (95% CI: 0.97-1.09) for all-cause mortality.[447] A recent analysis of the results of 3 prospective cohort studies that included 177 000 individuals showed that moderate egg consumption (1 egg/day) was not associated with an increased risk of mortality or CVD.[455]

In high cardiovascular risk individuals, the degree of atherosclerosis (assessed by coronary angiography) was lower among those who consumed > 1 egg per week compared to those who consumed < 1 egg per week.[456] Similarly, the consumption of 2 eggs per day for 6 weeks did not affect endothelial function in individuals with CAD.[457]

A systematic review of cohort studies evaluating patients with T2D concluded that the consumption of at least 1 egg per day increased the risk of developing CVD by 69% (AMI, CAD, stroke, and ischemic heart disease) when compared to the consumption of < 1 egg per week, with no association with increased mortality.[439]

With regard to HF, a Swiss study assessing the results of two prospective cohorts concluded that daily consumption of 1 egg did not increase the risk of HF among men and women, but the consumption of > 1 egg per day increased the risk of HF by 30% in men, and the causal effect remains unclear.[444]

A review of current evidence is not able to establish a causal relationship between egg consumption and CVD. However, divergent results of observational studies suggest caution in egg consumption, especially among patients with T2D and those who are hyper-responsive to dietary cholesterol. Because eggs have high nutrient and protein density, they may be included in the diet as long as being part of a healthy dietary pattern.

14.1. Trimethylamine N-oxide in Cardiovascular Diseases

Studies have shown that the gut microbiota is involved in the development of CAD,[458] and trimethylamine N-oxide (TMAO) is an emerging research focus on the study of atherosclerosis progression. TMAO is an amine oxide that can be naturally found in the diet but also be metabolized from choline (abundant in eggs), carnitine (red meat), betaine,

and phosphatidylcholine. These precursors are converted to trimethylamine (TMA) in the small intestine by specific bacteria such as Firmicutes, proteobacteria, and actinobacteria found in the gut microbiota.[459,460] TMA is absorbed and oxidized to TMAO through a reversible reaction in the liver, then catalyzed by the enzyme flavin-containing monooxygenase 3.[461]

Fish seems to be the largest food source of TMAO. Studies assessing fish intake show an increase in plasma TMAO concentrations (50 times higher) when compared to other food sources of carnitine or choline. Nevertheless, urinary excretion of TMAO and dimethylamine (derived from TMA) following fish consumption is higher compared to that of meat, dairy, fruits, vegetables, or grains.[462-464]

Elevated plasma TMAO concentrations correlated with increased risk of major cardiovascular events, prevalence of CVD, poorer prognosis, and increased risk of death.[461] This is because TMAO can exacerbate the inflammatory response in the vascular wall and induce the production of ROS. More recently, the role of TMAO in modulating cholesterol and bile acid metabolism and promoting atherosclerosis progression has been demonstrated.[463]

A mechanism by which TMAO may contribute to the progression of CVD is through an increased expression of scavenger receptors, which are responsible for the uptake of oxidized LDL, including class A scavenger receptors and surface protein CD36 in macrophages, both involved in cholesterol absorption. Some studies also suggest that TMAO prevents reverse cholesterol transport, which may contribute to the pathogenesis of CVD, promoting cholesterol accumulation in macrophages.[464]

Vascular events such as AMI and stroke in individuals with high plasma TMAO concentrations may be related to increased platelet activity due to cytoplasmic release of calcium, which may predispose the person to hypercoagulation and increased thrombotic events.[465,466]

A meta-analysis of studies recruiting over 26 000 participants followed up for about 4 years showed an increased relative risk (7.6%) of all-cause mortality for each increment of TMAO.[467]

A recent study evaluated the relationship of consumption of different protein sources (red meat, white meat, or vegetable protein) in TMAO metabolism. Long-term red meat consumption increased plasma TMAO concentrations by more than 3 times, as well as urinary excretion, compared to the other groups.[468] Studies on egg consumption have not found an association between egg consumption and increased TMAO. A study of 50 healthy participants showed that the consumption of 2 eggs (400 mg choline) per day did not change plasma TMAO concentrations.[460,468]

14.2. Hepatic Steatosis

Animal experiments suggest that high-cholesterol diets induce the progression of NASH, especially if combined with high-fat and high-energy diets.[469-472] However, there are no human studies showing the effect of dietary cholesterol on the development of hepatic steatosis. The current guideline for the treatment of NAFLD makes no reference to cholesterol consumption and etiology or treatment of this disease.[297]

15. Interesterified Fats

Interesterified fats have been used as a substitute for trans fatty acids, which are prepared using partial hydrogenation of vegetable oils. Interesterified fats are prepared using a fully hydrogenated solid base that is blended with a vegetable oil. Blended solid fractions such as palm stearin or lauric acid (found in coconut oil) and palm olein are used to prepare this solid base.[26]

The main characteristic of interesterified fats is the lack of trans fatty acids; however, they have a high concentration of SFAs. Interesterification is carried out through a chemical process that uses sodium methoxide as a catalyst, which promotes rearrangement of fatty acids in the glycerol molecule.[26] This forms TGs with new physical, organoleptic, and chemical properties, with enriched SFAs in the sn-2 position of glycerol, which is normally occupied by PUFAs in vegetable oils.[473] In this process, a large amount of TGs consisting of 3 SFAs are formed. Palmitic acid (more frequently) and stearic acid are the fatty acids most used in the food industry to replace trans fat.[473]

15.1. Studies in Animals

The consumption of a normolipidic diet containing interesterified fat produced from soybean oil, compared to a diet with soybean oil, by Wistar rats for 8 weeks resulted in higher expression of ATF3, an ER stress marker, and a higher concentration of the inflammatory cytokine TNF-α, with no difference in weight gain and glucose tolerance. However, greater weight gain was observed after 16 weeks of treatment, together with increased retroperitoneal adipose tissue mass and impaired glucose tolerance in the group that consumed interesterified fat.[474]

The effect of coconut oil, rice bran oil or sesame oil blended or subjected to enzymatic interesterification, with SFA/MUFA/PUFA ratio of 1:1:1 and PUFA/SFA ratio of 0.8:1, consumed for 60 days, was also evaluated in Wistar rats.[475] In animals fed interesterified oils, concentrations of TC, LDLc, and TG were reduced compared to animals fed blended oils. This was due to an increased expression of hepatic LDL receptor and the protein SREBP2, which induces cholesterol synthesis, compared to the same fat that had not undergone interesterification.[476]

Long-term consumption of a high-fat diet enriched with interesterified fat containing palmitic acid by LDL receptor knockout mice did not increase plasma cholesterol concentrations. However, there an increased concentration of cholesterol in LDL particles, a condition that resulted in higher atherosclerotic lesion, together with greater arterial macrophage infiltration.[477] Another study by the same research group demonstrated that long-term consumption of those diets in the same animal model led to greater weight gain, expanded adipose tissue, and adipocyte hypertrophy with greater inflammation, evidenced by increased pIKK and TNF-α levels.[478]

Other studies have evaluated the effect of a normolipidic diet containing interesterified fat rich in palmitic acid by female animals during pregnancy and lactation on the offspring. The results show that interesterified fat consumption predisposes

the offspring to the development of obesity in adulthood,[479,480] suggesting a negative epigenetic influence. In addition, a study conducted by Misan et al. (2015)[480] found that, after 90 days of life, the offspring showed greater weight gain as well as lower EPA concentration and greater leukocyte circulation in the brain, with no increase in TLR4.

15.2. Studies in Humans

In humans, both partially hydrogenated and interesterified soybean oil provided an increase in the LDLc/HDLc ratio when compared to palm oil. In addition to the change in plasma lipid concentrations, interesterified fat had an adverse effect on glucose metabolism, reducing plasma insulin concentration and increasing fasting glucose.[481] However, a more recent study showed no changes in fasting glucose and insulin following interesterified fat consumption.[482] However, when compared to margarine containing high levels of linoleic acid and moderate levels of trans fat, the consumption of margarines containing palm oil (lauric, myristic, palmitic, oleic, and linoleic acids) or interesterified palm oil favored an increase in LDLc concentrations in hypercholesterolemic men.[483] A likely explanation to those different results is that Sundram et al.[481] used interesterified fat composed of stearic acid, while Filippou et al.[482] used palmitic acid. Both studies compared interesterified fat with palm oil.

Additionally, interesterification has been shown to transfer significant amounts of palmitic acid to the sn-2 position and UFAs to the sn-1 and sn-3 positions, which had an effect on plasma chylomicrons.[484]

Studies also showed that interesterified fat induced a lower postprandial plasma TG concentration in healthy menopausal women,[485] in healthy young adults,[486] and in hypertriglyceridemic adults[487] compared to palm oil.

Regarding the influence of nutritional status and the intake of interesterified fat consumption on lipoprotein profile,[488] interesterification was found to increase postprandial TG concentration (85%) in obese individuals. This was not observed in healthy individuals, suggesting that interesterification may affect them differently from those who are at risk of developing CVD and T2D.

In healthy individuals, interesterification did not change plasma lipid concentrations but favored a lower concentration of D-dimer, a fibrin degradation product associated with risk of CVD.[489]

To date, there is no scientific evidence for reaching a conclusion on the effect of the interesterification process on metabolic parameters, development of atherosclerosis, and cardiovascular outcome. However, it is important to note the high content of SFAs in interesterified fat that is currently used by the food industry.

16. Medium-chain Triglycerides

Medium-chain TGs are defined as structured lipids composed of a mixture of saturated-chain fatty acids, containing from 6 to 12 carbons, formed by caproic acid (C6: 1 to 2%), caprylic acid (C8: 65 to 75%), capric acid (C10: 25 to 35%), and lauric acid (C12: 1 to 2%).[367,490] The fatty acids of medium-chain TGs are obtained by fractionation of coconut or palm oils.[491] Except for lauric acid, the other fatty acids are absorbed via the portal system and, because they are not incorporated into chylomicrons, they do not induce an increase in plasma TG levels.[491,492] Lauric acid is preferably transported via the lymphatic system by chylomicrons.[38,493] For this reason, for the management of familial hyperchylomicronemia, when LPL is absent, the use of medium-chain TGs composed mostly of caproic, caprylic, and capric acids is indicated.[491]

17. Familial Chylomicronemia Syndrome

Familial chylomicronemia syndrome (FCS) is a rare autosomal recessive disease that affects 1 to 2 people per million.[494,495] It is characterized by severe hypertriglyceridemia, even when fasting, due to a deficiency in the enzyme LPL or in other proteins required for normal lipase activity. The most common homozygous mutations in FCS are found in the genes LPL, APOA5, GPBIHBP1 (glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1), APOC2, and LMF1 (lipase maturation factor 1), but compound heterozgous mutations may also appear in different genes that cause FCS.[496-498] TG concentrations are often 10 to 100 times higher than those found in normal individuals (< 150 mg/dL), ranging from 1500 to 15 000 mg/dL or higher.[499,500] Hypertriglyceridemia in FCS stems from the inability to metabolize TGs and other fats. TGs are normally metabolized via an LPL-dependent pathway.[500] Although an LPL-independent pathway exists, it is not sufficient to compensate for the loss of LPL function. In FCS, accumulation of chylomicrons and their remnants cannot be metabolized, and they build up in the plasma. In the pancreas, there is impairment of blood flow and activation of the inflammatory process, resulting in pancreatitis,[501-503] and this condition accounts for 10% of all causes of pancreatitis[501]. Patients with elevated TG-induced pancreatitis have more severe conditions, longer hospitalizations, required stay in the intensive care unit, high rates of progression to pancreatic necrosis, and a higher frequency of organ failure and mortality.[504] Pancreatitis may also progress to a chronic condition, with exocrine and endocrine pancreatic insufficiency, including pancreatic diabetes (type 3c), which can be fatal. Recurrent abdominal pain, lipemia retinalis, hepatosplenomegaly, lipemic plasma, eruptive xanthoma, and poor quality of life are other common findings.[505-510] Because those patients are not able to metabolize TGs, the current nutritional guidance consists of a very-low-fat diet (< 10-15% of total energy, or about 15-20 g of fat per day), restriction of refined carbohydrates, and alcohol withdrawal.[511] Additionally, individuals with FCS of all ages should be regularly monitored for the consumption of micronutrients, particularly fat-soluble vitamins.[511] Depending on individual tolerability, medium-chain TGs may be indicated for energy intake in the diet.[491] Medications that are known to elevate TGs should also be used with caution, such as diuretics, beta-blockers, systemic corticosteroids, retinoids, bile acid sequestrants, protease inhibitors, and antidepressants (sertraline). Supplementation with ω3 fatty acids and other drugs used to treat hypertriglyceridemia has been inconsistent in reducing TGs.[512-514]

18. Practical Aspects of Nutritional Intervention

The nutritional composition of the diet must be adjusted to the objectives proposed for each individual, considering the individual's energy needs and cultural preferences. Several nutritional strategies can contribute to cardiovascular prevention provided they are based on the exclusion of trans fats, adequate SFA intake, and proportionally greater UFA intake, in addition to encouraging the consumption of fruits, vegetables, and whole grains.[9,515]

Foods of animal origin – such as meat, milk, and dairy products – naturally have a higher SFA content, while vegetable oils have a higher UFA content, except for coconut and palm oils, which are rich in SFAs. Among vegetable oils (Table 1), soybean, canola and corn oils are most used, which have a good distribution of fatty acids. Soybean and canola oils have an additional advantage over corn oil: they have lower SFA content and higher ALA (ω3) content, which is essential for humans and is a precursor to EPA and DHA, also found in fish (Figures 1 and 2).

The amount of fat from meat varies according to the type of cut. Therefore, lean meat cuts, such as pork loin and pork tenderloin, have a SFA content similar to that of commonly recommended beef cuts, such as knuckle and rump steak (Figure 3), making it possible to expand the options of protein-source foods with a cardioprotective focus.

Whole-milk dairy products have higher amounts of SFA than those produced with skimmed or semi-skimmed milk. Regarding cheese, those with lower water content and harder, such as parmesan cheese, proportionally have a higher SFA concentration than Brazilian cream cheese, Minas cheese, and ricotta cheese (Figure 4). The choice between product types should consider the serving size, since even dairy products with less fat content may be important sources of SFAs if consumed in large amounts.

Nutritional guidance should enable consumers to understand the composition of foods, especially processed foods, since the amount and type of nutrients, especially fats, may vary within the same product type depending on the manufacturer (Table S1, Supplementary Material). In this context, adequate food labeling becomes essential for the processes of nutritional education and consumer choice. Another important aspect to be considered is food preparation. Deep frying, for example, can add a large amount of fat to food items, thus considerably increasing the energy intake. It is important to note that vegetable oils, which are sources of ω3 and ω6, should not be substituted for tropical oils (palm and coconut oils) or animal fats (lard and butter), as they are rich in SFAs and do not provide adequate amounts of essential dietary fatty acids. This guidance is in line with the latest AHA recommendation for cardiovascular risk prevention[8,9] and with the ESC/EAS guidelines, which recommend occasional use of tropical oils in small amounts.[10]

Finally, care should be taken in recommending the use of dietary supplements that have not been scientifically proven to provide health benefits. Therefore, non-pharmacological strategies to reduce cardiovascular risk should consider the available evidence that points to benefits, safety, costs, and tolerability, in addition to possible effects of drug-nutrient interactions. Another important aspect is that the misuse of supplements may compromise adherence to both pharmacological and nutritional treatment.[516]

19. Labeling and Trans Fatty Acids

The use of trans fats brings a number of advantages to the food industry, such as cost reduction, longer shelf life, high melting point, and wide possibilities of use. However, their association with increased cardiovascular risk is clearly established, so that several international and national guidelines recommend their exclusion from the diet. Reducing NCDs is one of the goals of the WHO Global Strategy on Diet, Physical Activity and Health,[517] which, in line with international guidelines,[9,10,518] recommends eliminating trans fats from the diet.[517]

In Brazil, the National Health Surveillance Agency (ANVISA), which is responsible for food labeling regulation, established in 2003 that food labels must state the amount per serving of trans fats present in the product.[519] However, despite the mandatory requirement, ANVISA resolution allows foods that contain an amount less than or equal to 0.2 g per serving to be declared as trans fat-free (labeled as "zero trans fat" or "does not contain trans fats"). It is important to note that this tolerance may lead to increased trans fat intake through the high intake of foods declared as trans fat-free, but which contain values close to 0.2 g per serving.[520] In addition, the serving declared on the label and considered trans fat-free is often smaller than the average amount of the product consumed.[520] Therefore, it is important that consumers receive guidance on how to identify the presence \of trans fats in the list of ingredients in order to avoid the intake of foods containing trans fats.

20. Final Considerations

This position statement shows that recent findings regarding the effects of fatty acids on intracellular signaling pathways and the results of clinical and epidemiological studies support the current nutritional guidelines for the prevention and treatment of cardiometabolic diseases. The grade of recommendation and level of evidence in regard of the effect of fatty acids on cardiovascular diseases are shown in table 2 and 3. International guidelines recommend eliminating trans fatty acids from the diet, reducing SFA intake, and including, in appropriate amounts, foods that are sources of UFAs. Epidemiological studies show that both excessive SFA intake and insufficient PUFA intake are associated with increased cardiovascular risk. In addition, the effects of fatty acid intake still depend on the dietary pattern in which they are consumed, since the replacement of SFAs with refined carbohydrates can increase cardiovascular risk. However, when isocalorically replaced with UFAs or even with complex carbohydrates, cardiovascular outcomes tend to be favorable. The benefits attributed to an adequate fatty acid profile are only observed in the presence of healthy eating patterns.

21. Nutritional Amounts of Fatty Acids and Cholesterol in Foods

Table 1 – Nutritional table with amounts of fatty acids and cholesterol in oils and fats. Food composition per 100 g of edible portion: fatty acids and cholesterol

Food	Total	Saturated fatty acids (g/100 g)					Monounsaturated fatty acids (g/100 g)		Polyunsaturated fatty acids (g/100 g)					Trans fats (g/100 g)	Cholesterol (mg)
		Total	Lauric acid 12:0	Myristic acid 14:0	Palmitic acid 16:0	Stearic acid 18:0	Total	Oleic acid 18:1	Total	ALA 18:3	EPA 20:5	DHA 22:6	Linoleic acid 18:2	Elaidic acid 18:1t	
Palm oil	100	43.1	0.28	0.79	36.77	4.61	40.1	39.86	16.1	0.83	0	0	15.69	0	NA
Extra-virgin olive oil	100	14.9	0	0	11.30	2.96	75.5	74.01	9.5	0.75	0	0	8.74	0	NA
Lard	100	39.2	0.2	1.3	23.8	13.5	45.1	41.2	11.2		0	0	10.2	0	95
Spray whipped cream with vegetable fat	27.3	25.9	10.70	3.64	2.63	7.46	0.1	0.05	0.1		0	0	0.08	0	tr.
Commercial mayonnaise made with eggs	30.5	4.1	0	0.02	2.84	0.37	6.4	6.24	15.4	1.43	0	0	13.86	0	42
Cocoa butter	100	59.7	0	0.1	25.5	33.2	32.9	32.6	3	0.1	0	0	2.8	0	0
Unsalted butter	86	51.5	2.11	7.96	23.87	9.64	21.9	19.80	1.5	0.27	0	0	1.22	2.31	214
Unsalted margarine with interesterified oil (65% lipids)	67.1	20.9	2.35	0.94	12.41	4.15	14.4	14.07	26.5	2.58	0	0	23.79	0.12	NA
Avocado oil	100	11.5	0	0	10.9	0.66	70.5	67.88	13.48	0.95	0	0	12.53	0	0
Cottonseed oil	100	25.9	0	0.8	22.7	2.3	17.8	17.0	51.9	0.2	0	0	51.5	0	0
Canola oil	100	7.9	0	0.06	4.59	2.21	62.6	61.14	28.4	6.78	0	0	20.87	0	NA
Coconut oil	99	82.4	41.8	16.6	8.63	2.5	6.3	6.25	1.7	0.019	0	0	1.67	0.02	0
Sesame oil	100	14.2	0		8.9	4.8	39.7	39.3	41.7	0.3	0	0	41.3	0	0
Sunflower oil	100	10.8	0	0.07	6.10	3.42	25.4	25.15	62.6	0.39	0	0	62.22	0	NA
Corn oil	100	15.2	0		12.12	2.18	33.4	33.04	50.9	0.96	0	0	49.44	0	NA
Soybean oil	100	15.2	0	0.08	10.83	3.36	23.3	22.98	60.0	5.72	0	0	53.85	0	NA

Source: Núcleo de Estudos e Pesquisas em Alimentação – NEPA/Universidade Estadual de Campinas (UNICAMP). Tabela brasileira de composição de alimentos/NEPA-UNICAMP. Versão II. 2. ed. Campinas, SP: NEPA-UNICAMP, 2006. Available at: www.unicamp.br/nepa.25 USDA Food Composition Databases. United States Department of Agriculture. Agricultural Research Service USDA National Nutrient Database for Standard Reference Legacy Release, April 2018. USDA Branded Food Products Database. Available at: https://ndb.nal.usda.gov/ndb/search/list?home=true.520 ALA: alpha-linolenic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; NA: not applicable; tr.: trace.

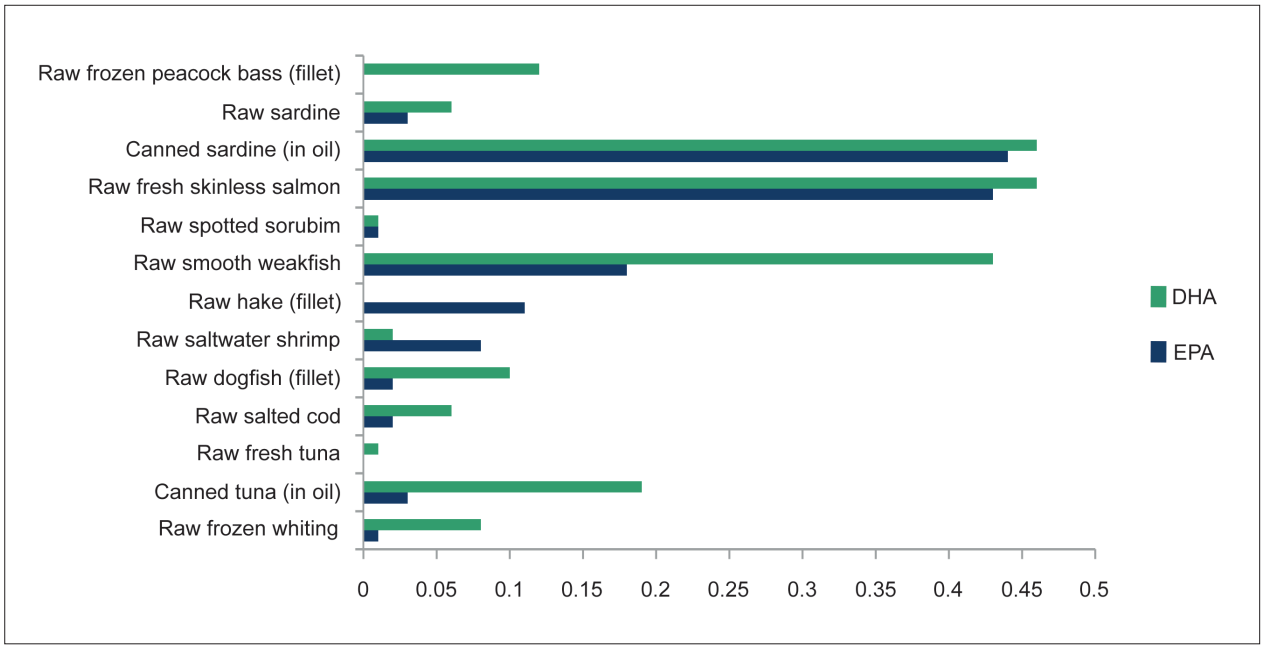

Figure 1 – *Content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish (g/100 g)*

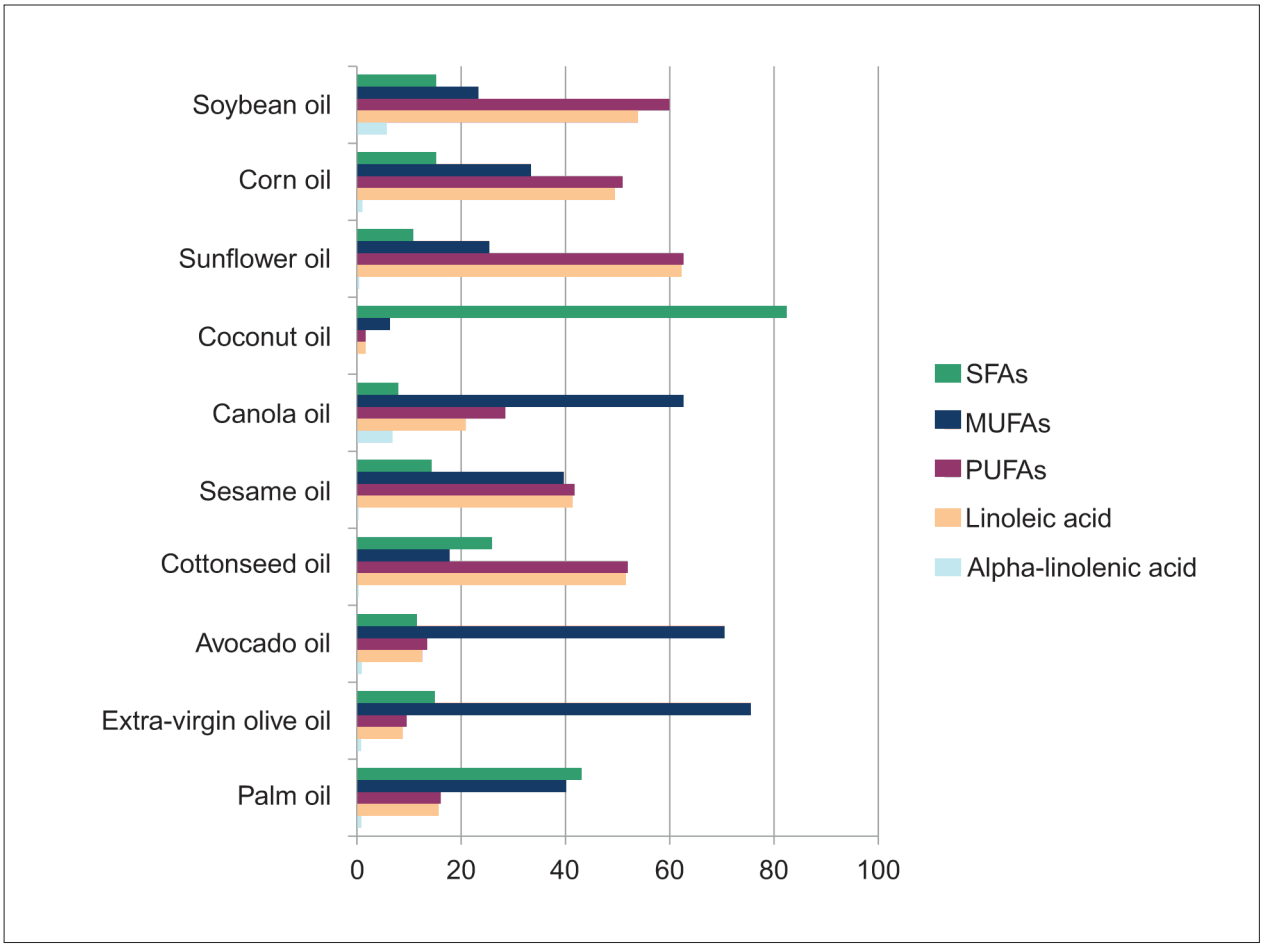

Figure 2 – *Content of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs) in vegetable oils (g/100 g)*

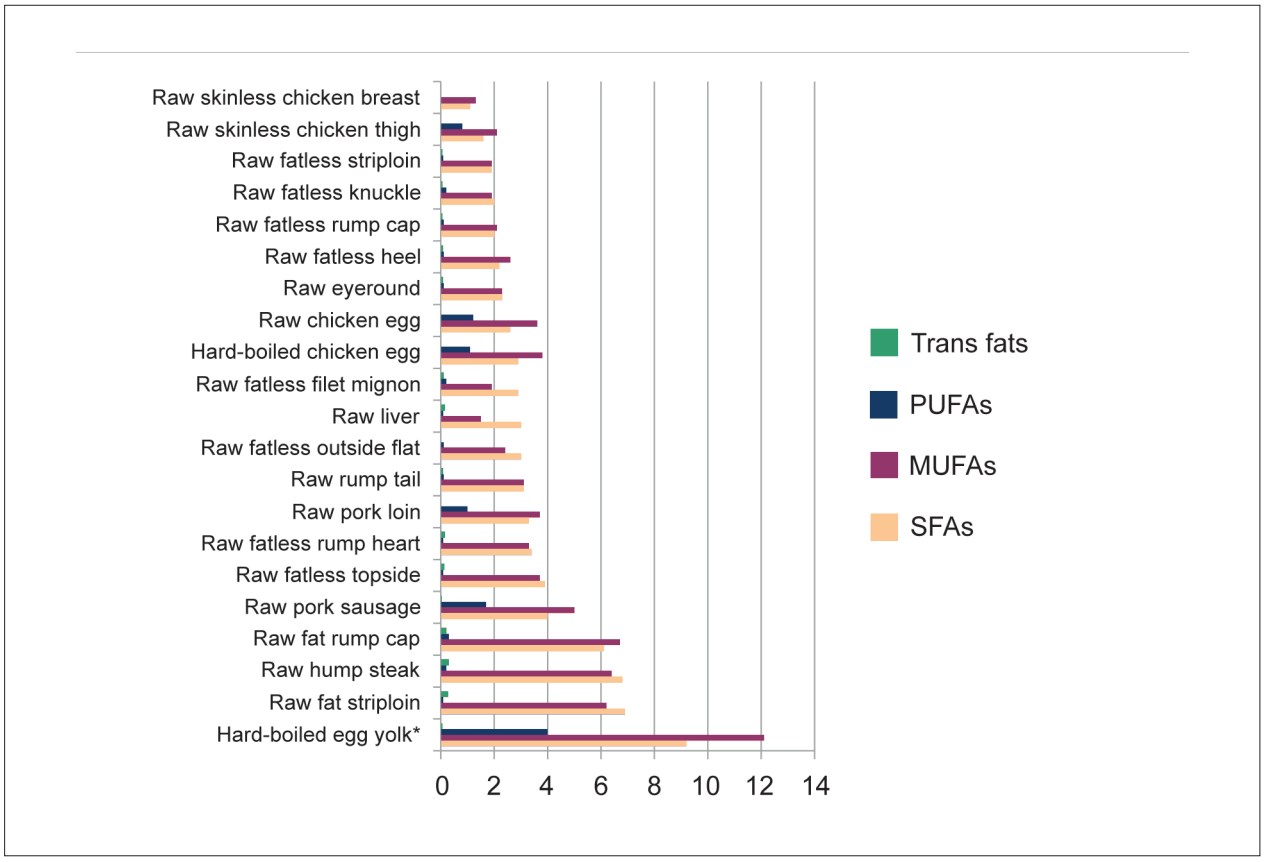

Figure 3 – *Content of monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFAs), and trans fatty acids in meats and eggs (g/100 g)*

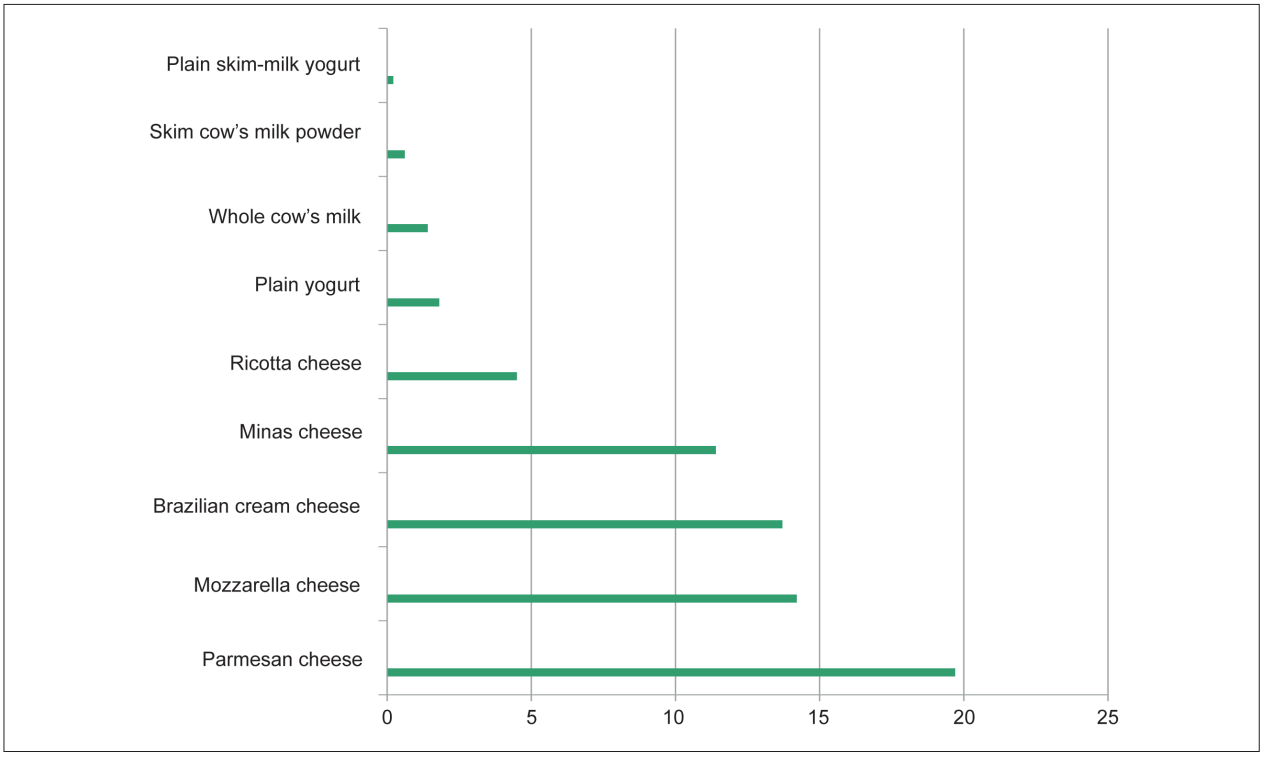

Figure 4 – *Total content of saturated fatty acids in dairy products (g/100 g)*

22. Grade of Recommendations and Level of Evidence: Fatty Acids and Cardiovascular Disease

Table 2 – Dietary Fatty Acids and Cardiovascular Risk

Recommendation	Grade of recommendation	Level of evidence
Trans fatty acids must be excluded from the diet	III	A
Limit SFA consumption to < 7% of total energy Intake for individuals with high cardiovascular risk, such as people living with Diabetes Mellitus and familial hypercholesterolemia	I	A
Partially replacement of SFA with PUFA, should be recommended to intensify the reduction of plasma LDLc concentrations	I	A
Partially replacement of SFA with omega-6 PUFA can be recommended to improve insulin sensitivity	IIa	B
Replacement of SFA with PUFA can be recommended to reduce cardiovascular risk	IIa	A
Dietary recommendations should be based on total PUFA consumption and not on Omega-6/Omega-3 ratio	IIa	C
Stimulating the consumption of Omega-3 PUFA from vegetal sources, as part of a healthy diet, can be recommended to reduce cardiovascular risk	IIb	B
Stimulating the consumption of fish, as part of a healthy diet, should be recommended to reduce cardiovascular risk	I	B
Tropical oils (palm and coconut) should be used occasionally in limited amounts, because of their high SFA content	III	B

Table 3 – Supplementation of omega-3 and cardiovascular risk

Supplementation of marine Omega-3 (2-4 g/dia) can be recommended in severe hypertriglyceridemia (> 500 mg/dL), as part of the treatment at the physician's discretion	I	B
Purified Omega-3: Supplementation with formulation containing EPA (icosapent ethyl, 4 g/day) in patients with high cardiovascular risk and high levels of plasma triglycerides, on statin treatment, can be recommended since it seems to reduce the risk of major adverse cardiovascular events, including cardiovascular mortality, as part of the treatment at the physician's discretion. This product is not locally accessible	I	A

Erratum

In the "Position Statement on Fat Consumption and Cardiovascular Health – 2020", with DOI: https://doi.org/10.36660/abc.20201340, published in the journal Arquivos Brasileiros de Cardiologia, 116(1):160-212, on page 160, correct author name Lis Mie Misuzawa Beda to: Lis Mie Masuzawa Beda.

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*Supplemental Materials

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Statement