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EFFECTS OF PERMETHRIN, A PYRETHROID INSECTICIDE, ON GLUCOSE AND LIPID METABOLISM

A Dissertation Presented

by

XIAO XIAO

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2017

The Department of Food Science

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Approved as to style and content by:

_____

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ABSTRACT

EFFECTS OF PERMETHRIN, A PYRETHROID INSECTICIDE, ON GLUCOSE AND LIPID METABOLISM

MAY 2017

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Over the last few decades, the decline of using organochlorine and organophosphorus insecticides has partially contributed to the rising utilization of newer synthetic insecticides, which are considered as less harmful and more environmental friendly than the older generation of insecticides. Pyrethroid insecticides are one of the newer insecticide classes reported with better biodegradability and low mammalian toxicity without sacrificing its insecticidal efficacy. Permethrin is one of the most widely used pyrethroid insecticides with structural similarity with natural pyrethrin insecticide from the flowers of *Chrysanthemum cinerariifolium*. Since its introduction in the 1970's, permethrin has been extensively used in medicine, military, household, industry and agriculture. Although a large body of evidence have supported that exposure to organochlorine and organophosphorus insecticides could increase the risk of developing obesity and diabetes, less attention has been drawn to pyrethroids. It has been reported recently that permethrin potentiated adipogenesis and insulin resistance *in vitro*. This study is designed to determine the effects of exposure to permethrin, along with the interaction with high-fat diet, on glucose and lipid metabolism *in vivo*. Our results demonstrated that chronic exposure to low level of permethrin could disturb glucose and lipid metabolisms in female and male mice in a diet-dependent manner. Exposure to

permethrin significantly increased insulin resistance in male and female mice fed high-fat diet as demonstrated by impaired insulin sensitivity, glucose tolerance and increased HOMA-IR. Permethrin treatment also significantly increased weight gain and adipose tissue weight in high-fat fed male mice but not female mice. Further mechanistic studies in mice showed that permethrin can target AMPK pathway, AKT pathway, and fatty acid oxidation to influence glucose and lipid metabolisms. *In vivo* studies in 3T3-L1 adipocytes showed that permethrin potentiated adipogenesis via calcium- and endoplasmic reticulum (ER) stress- mediated mechanisms. The current results suggest that exposure to permethrin can potentially disturb glucose and lipid metabolisms resulting in increased risk of developing obesity and type 2 diabetes.

Key words: Pyrethroids, permethrin, obesity, type 2 diabetes, adipogenesis, insulin resistance.

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CHAPTER 1

INTRODUCTION

Obesity is associated with energy imbalance with increased energy intake and reduced energy expenditure. Western diet, sedentary lifestyle, genetic defects and socioeconomic status have been widely accepted as the contributing factors linked with the pathogenesis of obesity. Type 2 diabetes are often associated with obesity, marked by elevated blood glucose level and insulin resistance [1]. More recently, a growing body of evidence has suggested that exposure to environmental pollutants, including insecticides, are linked with increased risk of obesity and type 2 diabetes [2-13]. Along with our continuing efforts to find bioactive food compounds to fight against obesity and diabetes, searching the contributing factors of these diseases can create new opportunities to protect the health and wellness of human beings.

Permethrin [(±)-3-Phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is one of the synthetic pyrethroid insecticides structurally based on the natural pyrethrins. First synthesized in 1973 and marketed in 1977, permethrin exhibits environmental stability and excellent potency against a wide spectrum of insect pests, while retaining a large margin of mammalian safety [14-16].

The insecticidal mechanism of action of permethrin relies on its ability to elicit a rapid functional disruption in the neuromuscular system by membrane depolarization. Permethrin is known to slow the inactivation of voltage sensitive sodium channel (VSSC), a pore forming transmembrane protein that consists of four homologous domains (I-IV) [14, 17, 18.]

In mammals, permethrin can be quickly biotransformed by ester cleavage and oxidation reactions and almost completely eliminated via urinary and fecal excretions within 12 days [16]. In the environment, permethrin can be degraded and disappeared rapidly (several hours to 58 days) by photolysis, microbial and plant biotranformations [16].

Based on these characteristics, permethrin became the first pyrethroid to be used to protect agricultural crops, public and animal health in the US [15, 18]. In the US alone, ~2.2 million lbs of permethrin have been sprayed annually to agricultural plots, residential areas and specific sites important to public health. Approximately 63% of this amount of permethrin is applied to residential areas [19]. Permethrin has been formulated in pet products and veterinary medications to control ectoparasitic arthropod pests, including over-the-counter formulations to control human head lice [20, 21]. Additionally, many biting arthropods show avoidance behaviors to permethrin, hence many permethrin treated materials (e.g., permethrin impregnated clothing including military uniforms, pet collars, and mosquito nets) have been developed and used to repel blood feeding arthropods [22]. The wide-spread use of those pyrethroids suggests that human exposure to permethrin is quite likely.

Previously, permethrin was reported to promote adipogenesis and induce insulin resistance in cell culture models similar to other types of insecticides [23-26]; however, there is a lack of *in vivo* study determining the effect of permethrin on glucose and lipid metabolisms. In addition, the molecular mechanisms regarding how permethrin potentiated adipogenesis and insulin resistance *in vitro* have not been fully explored. Thus, the purpose of this study was to investigate the effects of permethrin exposure on development of dietary-fat-induced obesity and type 2 diabetes in both female and male

mice. The molecular mechanisms of permethrin-potentiated adipogenesis in 3T3-L1 adipocytes were also studied.

CHAPTER 2

LITERATURE REVIEW

2.1 Insecticide introduction and classification

Insecticides, which are mostly neurotoxins, are a group of substances used to kill insect pests that are harmful to humans, crops, livestock, and pets [27]. The use of insecticides can be dated back to ancient civilization. The Sumerians used sulphur compounds against insects and mites around 4,500 years ago and the Chinese used arsenical compounds and mercury to control body lice about 3,200 years ago [28]. In the 20th century, the rise of modern synthetic chemistry and the demand for more food has contributed to the rising use and syntheses of numerous organic insecticides [29].

Insecticides can be classified based primarily on their chemical structures and mode of actions. Major classes of insecticides include organochlorines, organophosphorus, carbamates, pyrethroids, and neonicotinoids. Organochlorine insecticides were introduced in the 1940s, followed by organophosphorus insecticides (1950s), carbamates (1960s), pyrethroids (1970s), and neonicotinoids (1990s) [27]. A few examples of each type are shown in Table 2.1.

Table 2.1. Major classes of insecticides with examples

Organochlorines	Organophosphorus	Carbamates	Pyrethroids	Neonicotinoids
Aldrin	Chlorpyrifos	Aldicarb	Allethrin	Acetamiprid
DDT/DDE	Diazinon	Bendiocard	Bifenthrin	Clothanidin
Dieldrin	Dichlorvos	Carbaryl	Cismethrin	Dinotefuran
Heptachlor	Malathion	Dioxacarb	Cyhalothrin	Imidacloprid
Lindane	Parathion	Fenobucarb	Deltamethrin	Niternpyram
Methoxychlor	Profenofos	Isoprocarb	Permethrin	Thiacloprid
		Methomyl	Tefluthrin	Thiamethoxam

Most of the organochlorine insecticides are extremely lipophilic and chemically stable [30]. Organochlorine insecticides can be divided into dichlorodiphenyltrichloroethane (DDT)-type and chlorinated alicyclic-type (cyclodienes) based on their distinctive mechanisms of action. DDT-type insecticides (such as DDT and dicofol) are known to inhibit the closing of voltage-sensitive sodium channel (VSSC) in neurons, resulting in repetitive firing of action potentials, while chlorinated alicyclic-type insecticides (e.g. aldrin, dieldrin, heptachlor, and endosulfan) bind to γ-aminobutyric acid (GABA) chloride ionophore complex, which inhibits chloride influx into the nerve [30, 31]. DDT was once the most popular insecticide used in the 1940s to 1960s to reduce insect-born disease, such as malaria, yellow fever, and typhus, because of its broad-spectrum activity against insects and its relatively low mammal toxicity [32, 33]. DDE, also used as an insecticide, is the major biological metabolite and environmental breakdown product of DDT. Due to their toxicity on wild bird populations and high stability in the environment, most developed countries have banned the use of DDT and other chlorinated hydrocarbons, including the United States in 1972. Certain developing countries are still using organochlorines nowadays because of their effectiveness against certain disease-carrying insects and insect resistance to other insecticides [34]. Even though most countries have not used organochlorines for the last several decades, due to extremely stable chemical characteristics, DDE (as a major metabolite of DDT) can currently be found in human serum, adipose tissue, and many foods [30, 35].

Organophosphorus insecticides are irreversible inhibitors of cholinesterases, including acetyl cholinesterase, resulting in hyper-stimulation of cholinergic nerves (e.g. muscarinic and nicotinic acetylcholine receptor) [36]. As the largest insecticide class in the

world in 1980s, organophosphorus insecticides occupied 71% of world insecticides market in 1987; however, the use of organophosphorus insecticides dropped to around 52% in 1999 and to 13% in 2013 [37-39]. The use of organophosphorus insecticides was decreased due to their environmental persistence and mammalian toxicity, although some are considered as relatively less toxic to humans and less stable in the environment than organochlorines [27, 40].

Carbamates, which account for 6% of global insecticides [39], have the similar mechanism of action with organophosphorus insecticides, but their neurotoxic effects are relatively more moderate than organophosphorus because the inhibition of acetyl cholinesterase is reversible and carbamates are known to be rapidly metabolized by human and animals [30, 41].

Developed in the 1960s and 1970s, pyrethroids are structural analogs to naturally occurring insecticide, pyrethrin, found in *Chrysanthemum* flower heads. Pyrethroids can cause over excitation of the neuron by delaying the closing of VSSC, producing an effect similar to, but more pronounced than, DDT due to its better sodium channel binding capacity [18, 34]. Generally, pyrethroid can be divided into two types; Type I and Type II. Type I pyrethroid (non α-cyano moiety) mainly generates repetitive firing of action potential, which leads to the tremor syndrome (T-syndrome). Type II pyrethroids (α-cyano moiety) cause excessive membrane depolarization that leads to decreased action potential, which will eventually block nerve signal conduction, leading to choreoathetosis with salivation (CS syndrome) [42, 43]. By 2013, pyrethroids accounted for approximately 17% of the global insecticide market [39].

Neonicotinoids are a relatively new family of insecticides with structural

resemblance to nicotine [27]. Acting on nicotinic acetylcholine receptors, neonicotinoids can stimulate these receptors at low doses, while blocking these receptors at high doses, leading to paralysis and death [44]. Their high affinity for insect nicotinic acetylcholine receptors, but not to vertebrate nicotinic acetylcholine receptors, contributes to their selective toxicological properties [45, 46]. Ever since their first introduction in 1991, neonicotinoids have become the fastest growing class of insecticides used in protection of both agricultural crops and animal health, representing ~27% of the global insecticide market in 2013, which makes them the largest single insecticide class on the market [39, 47]. Due to the potential link between use of neonicotinoids and the reduction of bee population, the European Commission has banned use of three neonicotinoids (imidaclorprid, thiamethoxam, and clothianidin) in 2013 [48].

2.2 **Glucose and lipid metabolism overview**

Glucose, the most important carbohydrate as energy source, is utilized by various organs and tissues after being absorbed into the blood stream to form ATP through glycolysis and oxidative phosphorylation. On the other hand, dietary lipids such as triglycerides (TG) and free fatty acids (FFAs) also play critical roles in energy homeostasis. Fats are believed to provide even more energy per gram basis than glucose through β-oxidation and citric acid cycle [49]. The rise and fall of blood glucose and lipids level generate signals to induce the secretion of a variety of hormones to maintain energy homeostasis by controlling the storage of excessive energy in the fed state and breakdown of intracellular energy source in the fasting state. Through this hormone-

mediated mechanism, blood glucose and lipids (TG, FFAs, cholesterol etc.) are able to be maintained in a normal range in healthy individuals [50].

Insulin is the central hormone regulating glucose and lipid metabolism in the fed state. The rise of glucose and after meal generate signals to induce the secretion of insulin from pancreas. Insulin promotes glucose uptake and glycogen synthesis in muscle and fat while inhibiting glucose production from the liver by blocking glycogenolysis and gluconeogenesis. Insulin also stimulates lipogenesis and protein synthesis while inhibiting lipolysis and protein degradation. Skeletal muscle is the major target site for insulin-dependent glucose disposal (up to 75%), while adipose tissue accounts for only a small fraction [51, 52]. Insulin does not stimulate glucose uptake in liver, instead, it blocks glycogenolysis and gluconeogenesis while stimulating glycogen synthesis [52]. Other tissues not normally sensitive to insulin action such as brain and pancreatic β-cells are also important in regulating glucose homeostasis [52-54]. Thus, insulin resistance or deficiency can result in severe dysregulation of glucose and lipid homeostasis manifested by elevated fasting and postprandial glucose and lipids levels.

2.2.1 Insulin signaling pathways

Insulin initiates its action by binding to insulin receptors. Insulin receptors, including insulin like growth factor (IGF)-I receptor and insulin receptor-related receptor (IRR), are tyrosine kinases [55]. Insulin can increase the kinase activity of the insulin receptors, which subsequently phosphorylate insulin receptor substrates [56]. Among them, insulin receptor substrate (IRS)-1 is believed to play a major role in regulating insulin signaling [57-59]. Phosphorylated tyrosines in IRS serve as “docking sites” for proteins containing SH2 (Src-homology-2) domains, including the p85 regulatory subunit of

phosphoinositide 3-kinase (PI(3)K) and protein tyrosine phosphatase (SHP2). PI(3)K is critical in regulation of insulin action. The activation of PI(3)K can stimulate the phosphorylation of phosphoinositides to produce phosphatidylinositol-3-phosphates, especially phosphatidylinositol (3,4,5)-triphosphate (PIP3), which then bind to phosphoinositide-dependent kinase (PDK1). PDK1 belongs to the family of serine kinases that phosphorylates the serine/threonine kinase Akt (protein Kinase B). Akt also plays important role in transmission of insulin signal by controlling glucose transporter 4 (GLUT4) translocation and glycogen synthesis via phosphorylated inhibition of glycogen synthase kinase-3 (GSK-3) [60]. Atypical protein kinase Cs (PKCs), downstream of PI(3)K during insulin stimulation, are also suggested to play an important role in GLUT4 translocation [61].

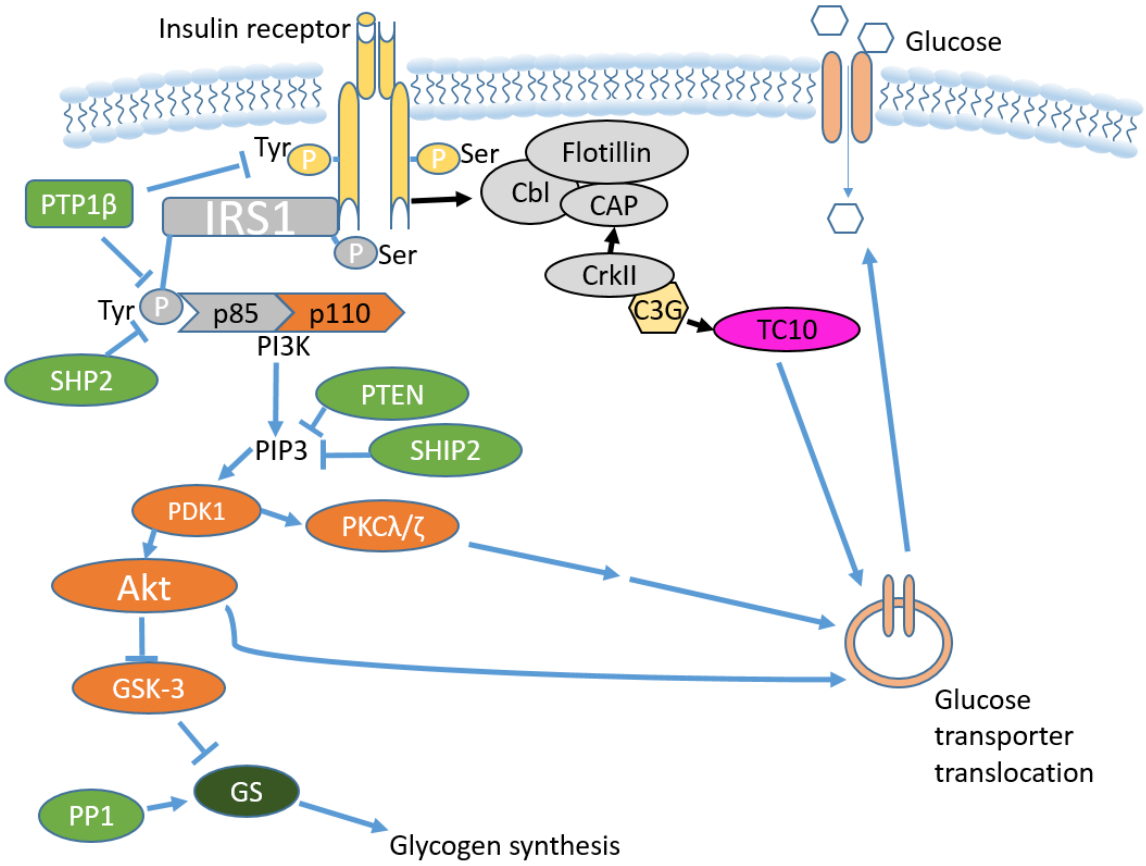


Figure 2.1. Insulin signaling pathways. Akt, protein kinase B; C3G, rap guanine nucleotide exchange factor 1; CAP, c-Cbl-associated protein; Cbl, casitas B-lineage lymphoma; CrkII, proto-oncogene c-Crk II; IRS-1, insulin receptor substrate-1; GS, glycogen synthase; GSK-3, glycogen synthase kinase-3; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKCλ/ζ, protein kinase C λ/ζ; PP1, protein phosphatase 1; PTEN, phosphatase and tensin homolog; PTP1β, protein tyrosine phosphtase 1β; Ser, serine; SHIP2, SH2 domain-containing inositol 5-phosphatase 2; SHP2, protein tyrosine phosphtase 2;Tyr, tyrosine.

On the other hand, other intracellular signals may attenuate or block insulin signaling. For example, in addition to tyrosine phosphorylation described above insulin receptor and IRS proteins may undergo serine phosphorylation, which decrease insulin signaling by attenuating insulin stimulated tyrosine phosphorylation [62]. Protein tyrosine phosphatases (PTPases) can also attenuate insulin signaling by rapid dephosphorylation

of insulin receptor and its substrates. Among them, protein tyrosine phosphatase 1B (PTP1B) are the most documented [63]. Overexpression of phosphatidylinositol-3-phosphates, such as phosphatase and tensin homologue (PTEN) and SH2 domain-containing inositol-5-phosphtase (SHIP2), can decrease the level of PIP3, thus reduce insulin sensitivity [64, 65].

In addition to PI(3)K activity, another pathway for insulin-stimulated glucose uptake via GLUT4 translocation have been identified [66]. Cbl (casitas B-lineage lymphoma) is a ubiquitin ligase that can be tyrosine phosphorylated by insulin receptor. Cbl is usually binding with c-Cbl-associated protein (CAP), an adapter protein, to form Cbl-CAP complex. Upon phosphorylation, the Cbl-CAP complex can be translocated to the lipid raft domains in the plasma membrane escorted by protein flotillin and adapter molecule Crk II (also known as proto-oncogene c-Crk or p38, a protein that in humans is encoded by the CRK gene) [67]. Crk II can also form a complex with rap guanyl nucleotide-exchange exchange factor 1 (C3G), which can subsequently activate G protein TC10 in the proximity of lipid rafts. This provides a second signal for GLUT4 translocation in parallel with PI(3)K pathway [52, 68].

2.2.2 Regulation of glucose metabolism in liver

Glycogen content is tightly regulated by insulin and glucagon. Insulin stimulates glycogen synthesis by activating glycogen synthase through the inhibition of kinases such as protein kinase A (PKA) or glycogen synthase kinase 3 (GSK-3) and activating protein phosphatase 1 (PP1) [52, 60, 69].

Gluconeogenesis and glycogenolysis is also tightly regulated by insulin. Insulin directly controls the activities of a series of enzymes by phosphorylation and

dephosphorylation cascades and mediate the gene expression encoding enzymes involved in gluconeogenesis and glycolysis [70]. Insulin inhibits the transcription of enzymes involved in gluconeogenesis, including phosphoenolpyruvate carboxylase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphatase, [71] while increases the transcription of glycolytic enzymes, including glucokinase and pyruvate kinase, and lipogenic enzymes, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). Forkhead family of transcription factors [72], peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) [73] and sterol regulatory element-binding protein 1 (SREBP-1) [74] are suggested to control gene expression of enzymes involved in insulin-regulated gluconeogenesis and glycogenolysis.

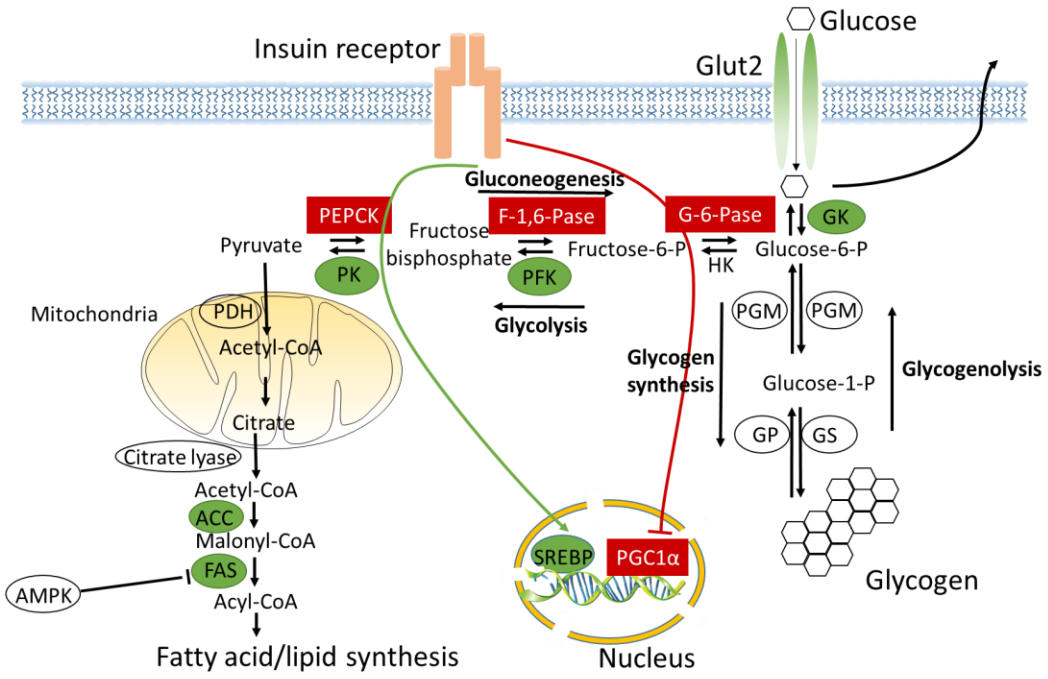


Figure 2.2. Regulation of gluconeogenesis, glycogenolysis, glycolysis, glycogen synthesis and lipid synthesis in the liver. ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; F-1,6-Pase, fructose-1,6-bisphosphatase; Fructose-6-P, fructose 6-phosphate; FAS, fatty acid synthase; HK, hexokinase; Glucose-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphtase; Glut2, glucose transporter 2; GK, glucokinase; GP, glycogen phosphorylase; GS, glycogen synthase; PDH, pyruvate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PGM, phosphoglucomutase; PK, pyruvate kinase; SREBP, sterol regulatory element-binding protein.

2.2.3 Insulin resistance

Insulin resistance is considered as a pathological state in which there is reduced responsiveness of circulating insulin [75]. Insulin resistance in obesity and type 2 diabetes is believed to be associated with many factors in insulin signaling, including decreased concentration of insulin receptor and IRS, decreased tyrosine kinase activities of insulin

receptor and IRS, activity of PI(3)K, glucose transporter translocation, and activities of various intracellular enzymes [66].

In addition, adipose tissue may also contribute to insulin resistance by elevating circulating FFAs, which may result in reduced glucose uptake, glycogen synthesis and glucose oxidation yet stimulated hepatic glucose output [76]. This process is believed to be associated with reduction in IRS-1 and PI(3)K activities and accumulation of triglycerides and fatty acid-derived metabolites (e.g. diacylglycerol, fatty acyl-CoA and ceramides) in muscle and liver [52]. A number of hormones secreted by fat cells, collectively called adipokines, including TNF-α, leptin, adiponectin and resistin, are also playing important roles in regulation of metabolism, energy expenditure and insulin resistance [52, 62]. Activation of PKC and/or inhibitor of nuclear factor κB (IκB) kinase and serine phosphorylation of the insulin receptors and its substrates might be another important mechanism [52].

2.2.4 Adipogenesis

When there is less energy expenditure than energy intake, most of the extra energy is stored in adipocytes in the form of triglycerides. As a result, adipose tissue mass arise by either increase in adipocyte cell size, cell number or both [50]. Adipocyte size is well correlated with the amount of stored triglycerides. It is suggested that mild obesity is generally associated with increases in cell size (hypertrophic obesity), while more severe obesity also involves increased fat cell number (hyperplastic obesity). Adipogenesis can be generally divided into several parts: preadipose proliferation, adipocytic differentiation, and lipogenesis. The molecular mechanism regulating

adipogenesis has been extensively studied in recent years. The key regulators have been identified as PPARɣ, the CCAAT-enhancer-binding proteins (C/EBP)s, and adipocyte determination and differentiation-dependent factor 1 (ADD1)/SREBP1 [50]. In addition to insulin, glucocorticoids, a steroid hormone secreted from adrenal gland, has also been suggested to play a central role in regulating adipogenesis. In fact, it has been demonstrated that the induction of PPARɣ gene by C/EBPβ,δ is largely dependent on the presence of glucocorticoid [50, 77].

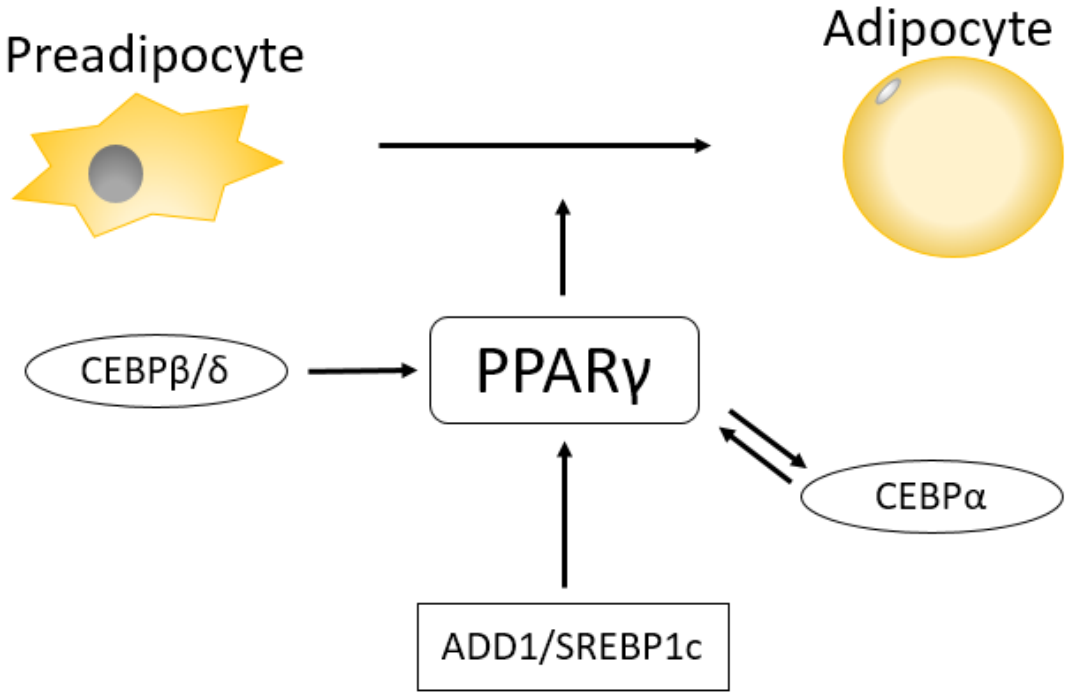


Figure 2.3. Regulation of adipogenesis. ADD1, adipocyte determination and differentiation-dependent factor 1; CEBPα, CCAAT/enhancer-binding protein alpha; CCAAT/enhancer-binding protein beta/delta CEBPβ/δ; PPARɣ, peroxisome proliferator-activated receptor gamma; SREBP1c, sterol regulatory element-binding transcription factor 1c.

2.2.5 Regulation of lipogenesis and lipolysis

The lipogenesis and lipolysis in adipocytes are well regulated by both hormone (e.g., insulin) and sympathetic stimulation (e.g., adrenergic) [70]. In the fed state, adipocytes can pick up glucose and store them primarily as lipid with the help of insulin and lipogenic enzymes, including lipoprotein lipase, pyruvate dehydrogenase, fatty acid synthase, and acetyl-CoA carboxylase. Insulin can inhibit lipolysis in adipocytes primarily through inhibition of hormone-sensitive lipase via reduction in cAMP concentration and PKA activation [78, 79].

In the fasting state or when energy expenditure is increased, adipocytes breakdown triglycerides into free fatty acids and glycerol and release them into blood stream for fatty acid oxidation in liver, muscle and brown adipose tissue (BAT). Unlike

white adipose tissue, which is designed for storage of excess energy, BAT is designed for energy expenditure by producing heat [50].

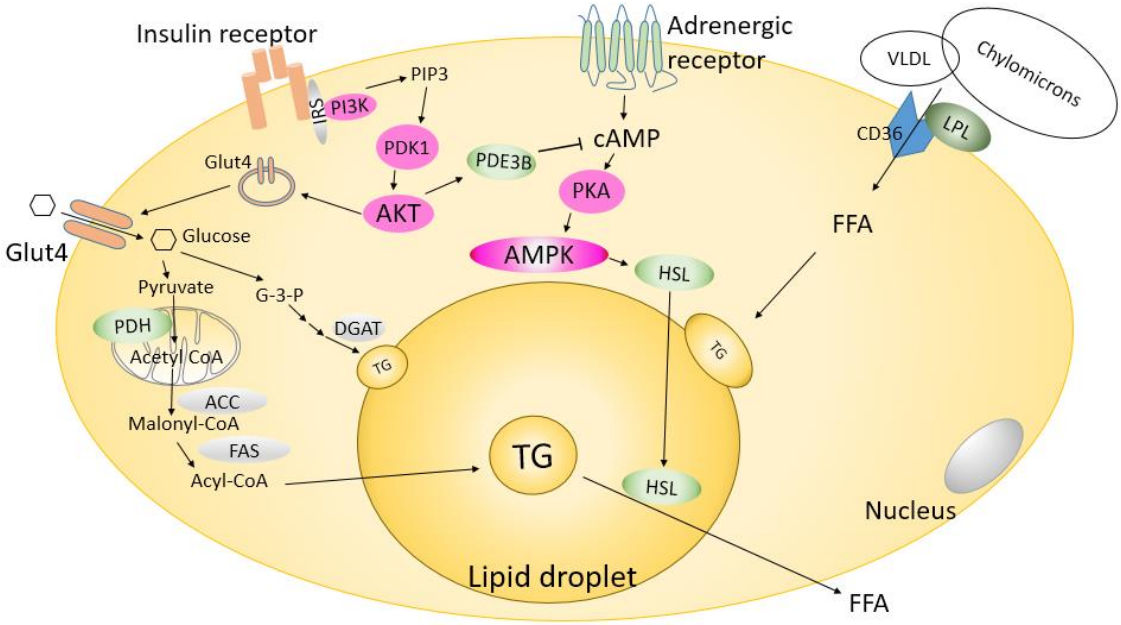


Figure 2.4. Regulation of lipogenesis and lipolysis in adipocyte. ACC, acetyl-CoA carboxylase; AKT, protein kinase B; AMPK, AMP-activated protein kinase; cAMP, cyclic adenosine monophosphate; CD36, cluster of differentiation 36;DGAT, diglyceride acyltransferase; FAS, fatty acid synthase; FFA, free fatty acid; Glucose-3-P, glycerol-3-phosphate; Glut4, glucose transporter 4; IRS, insulin receptor substrate; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; PDE3B, phosphodiesterase 3B; PDH, pyruvate dehydrogenase; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphatidylinositol-3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A;TG, triglycerides; VLDL, very-low-density lipoprotein.

2.3 Effects of insecticide exposure on glucose and lipid metabolisms

2.3.1 Effects of insecticides on risk of diabetes in human

Numerous epidemiological studies have found a potential association between insecticide exposure with increased risks of diabetes (summarized in Table 2.2). For this table, we have included elevated fasting blood glucose levels and insulin resistance as evidence of diabetes risk. Organochlorine and organophosphorus insecticides are the most studied, but few others reported on the role of pyrethroids, carbamates and

neonicotinoids on diabetes.

Most studies on organochlorines linked the blood levels of organochlorines or their metabolites with increased risk of diabetes in humans, as these are known to be persistent in human tissues, particularly fatty tissues. Overall, there is strong indication that exposure to these insecticides leads to increased risk of diabetes. In addition, Boada *et al.* [80] reported a negative correlation between blood levels of aldrin and p,p'-DDD and insulin-like growth factor-1, which are also known to have implications for development of insulin resistance [81].

Organophosphorus and carbamate insecticides have also been generally linked with increased risk of diabetes in humans, including pesticide applicators who were exposed to dichlorvos and trichlorfon [10]. Interestingly, Saldana et al. reported that wives of pesticide applicators of organophosphorus insecticides (diazinon and phorate) along with carbamate insecticide (carbofuran) have increased risks of gestational diabetes mellitus, while no association was found for other organophosphorus (malathion and terbufos) or carbamate insecticide (carbaryl) [82]. In addition, a study reported that patients with acute organophosphorus poisoning due to suicide attempts had hyperglycemia [83].

Reports on the effects of pyrethroids on diabetes from human studies are rather limited and no human study on neonicotinoids and diabetes is available currently. One study reported that pyrethroid mixture increases the risk of diabetes among pesticide factory workers in China [84]. Another study reported that exposure to pyrethroid insecticides (allethrin and prallethrin) increased plasma glucose levels in Indian men [85]. Since neonicotinoids have been used in the last few decades and they are designed to be relatively quickly degraded in biological systems, it is more challenging to investigate the

exposure to neonicotinoids on human health perspectives [86]. Overall, the majority of human studies have shown a positive association between exposure to certain type of insecticides and risk of diabetes.

Table 2.2. Effects of insecticide exposure on risk of diabetes in human

Insecticide[a]	Country	Subject	Risk of diabetes[c]	Other comments	Reference
Organochlorine					
Aldrin	USA	Pesticide applicators	↑		10
Chlordane	USA	General population	↑		87
	USA	Pesticides applicators	↑		10
p,p′-DDD	South Korea	Age ≥ 40	×		88
p,p′-DDE	Sweden	Aged 50-74 years old	×		89
	Sweden	Fishermen and their wives	↑		11
	USA	General population	↑		87
	USA	Native Americans	↑		2
	USA	Mexican Americans	↑		90
	Sweden	Fishermen's wives	↑		91
	USA	Non-diabetic	↑[c]		6
	Canada	First Nation Community members	↑		92
	Sweden	50-59 years old	↑		93
	USA	Great Lakes sport fish consumer	↑		94
	USA	General population	↑		95
	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Slovakia	Polluted area	↑		96
	Finland	57-70 years old	↑		97
	Belgium	Hospital patients, staff and volunteers	×		3
	USA	Non-diabetic	↑[d]		8
	Sweden	Age > 70	×		98
	Denmark	General population	↑		99
	Spain	General population	×		100
	Spain	Hospital patients	↑		101

	Canada	First Nations community members	×	↑ Blood DDE in diabetic individuals	102
	Benin	18-65 years old	↑		103
	Russia	8-9 years old boys	×[d]		104
	Belgium	General population	↑		105
	South Korea	Hospital patients	↑		106
	Slovakia	Polluted area	↑		107
	Thailand	General population	↑		108
	Saudi Arabia	30-50 years old	↑		109
	USA	Great Lake Sport fish consumer	↑		110
	Belgium	Aged 50-65 years old	↑	only in men	111
	France	Newborns	×	↓ Insulin & adiponectin level in female newborns	112
	Canada	Pregnant women	×[e]		113
o,p'-DDE	Denmark	General population	↑		99
p,p'-DDT	USA	Mexican Americans	↑		90
	USA	General population	↑		4
	USA	Non-diabetic	×[d]		5
	USA	General population	↑		95
	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Slovakia	Polluted area	↑		96
	USA	Non-diabetic	×[d]		8
	Denmark	General population	↑		99
	Spain	General population	×		100
	Benin	18-65 years old	↑		103
	South Korea	Hospital patients	↑		106
	Saudi Arabia	30-50 years old	↑		109
o,p′-DDT	South Korea	Age ≥ 40	↑		88

	Denmark	General population	↑		99
Dieldrin	USA	General population	×		95
HCB	Sweden	50-74 years old	↑		89
	USA	Native Americans	↑		2
	USA	Mexican Americans	×		90
	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Slovakia	Polluted area	×	↑ only prediabetes	96
	USA	Non-diabetic	×[d]		8
	Sweden	Age > 70	×		98
	Denmark	General population	↑		99
	Spain	General population	↑		100
	Spain	Hospital patients	×		101
	Canada	First Nations community members	×	↑ Blood HCB	102
	Russia	8-9 years old boys	↑[d]		104
	Slovakia	Polluted area	↑		107
	South Korea	> 40 years old	↑		114
	South Korea	Hospital patients	↑		106
	Belgium	50-65 years old	↑		111
cis-nonachlor	Canada	First Nations community members	↑		102
	South Korea	Hospital patients	↑		106
CB-153	Sweden	Fishermen and their wives	↑	only in men	11
	USA	General population	↑		87
β-HCH	Sweden	50-74 years old	×		89
	USA	Mexican Americans	↑		90
	USA	Non-diabetic	×[d]		5
	USA	Non-diabetic	×[c]		6
	USA	General population	↑		95

	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Slovakia	Polluted area	×	↑ only prediabetes	96
	Belgium	Hospital patients, staff and volunteers	↑		3
	USA	Non-diabetic	×[d]		8
	Denmark	General population	×		99
	Spain	General population	×		100
	Spain	Hospital patients	↑		101
	Canada	First Nations community members	×	↑ Blood β-HCH	102
	Benin	18-65 years old	↑		103
	Saudi Arabia	18-65 years old	↑		115
	Russia	8-9 years old boys	×[d]		104
	South Korea	Aged > 40 years	↑		114
	South Korea	Hospital patients	×		106
ɣ -HCH	USA	African and White Americans	×		7
	USA	Non-diabetic	×[d]		8
	Saudi Arabia	18-65 years old	↑		115
Heptachlor	USA	Pesticide applicators	↑		10
Heptachlor epoxide (A metabolite of heptachlor)	USA	General population	↑		95
	USA	General population	↑		116
	South Korea	Age ≥ 40	↑		88
	South Korea	Age > 40	↑		114
Mirex	USA	Native Americans	↓		2
	USA	General population	×		95
	USA	African and White Americans	↑		7
	South Korea	Age ≥ 40	↑		88
	USA	Non-diabetic	×[d]		8

	Canada	First Nations community members	×	↑ Blood mirex in diabetic individuals	102
Oxychlordane	Sweden	50-74 years old	×		89
	USA	General population	↑		87
	USA	Mexican Americans	↑		90
	USA	Non-diabetic	↑[d]		5
	USA	Non-diabetic	×[c]		6
	USA	General population	↑		95
	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Finland	57-70 years old	↑		97
	USA	Non-diabetic	×[d]		8
	Canada	First Nations community members	↑	↑ Blood oxychlordane in diabetic individuals	102
	South Korea	Aged > 40 years	↑		114
	Canada	Pregnant women	×[e]		113
TNC	Sweden	50-74 years old	×		89
	USA	General population	↑		87
	USA	Mexican Americans	↑		90
	USA	Non-diabetic	↑[d]		5
	USA	Non-diabetic	×[c]		6
	USA	General population	↑		95
	USA	African and White Americans	×		7
	South Korea	Age ≥ 40	↑		88
	Finland	57-70 years old	↑		97
	USA	Non-diabetic	×[d]		8
	Sweden	age > 70	↑		98
	Canada	First Nations community members	↑		102
	Benin	18-65 years old	↑		103

	Canada	Pregnant women	×[e]		113
Organophosphorus					
Dialkylpho-sphate (Metabolites)	France	Newborns	×	↑ Insulin level	112
Dimethylpho-sphate (Metabolites)	Canada	Pregnant women	↓[e]		113
Dimethylthio-phosphate (Metabolites)	Canada	Pregnant women	↓[e]		113
Mixture	Turkey	Overdose patients	↑[c]	Case report	83
	Iran	Farmers	↑		117
	India	A 15-year-old girl	↑[c]	Case report	118
Diazinon	Israel	Children	↑[c]		119
	USA	Wives of pesticide applicators	↑[e]		82
Dichlorvos	USA	Pesticides applicators	↑		10
Malathion	Canada	81-year-old mother and her 39-year old son	↑[c]	Case report	120
	USA	Wives of pesticide applicators	×[e]		82
	Egypt	Non-diabetic male famers	↑		121
Phorate	USA	Wives of pesticide applicators	↑[e]		82
Terbufos	USA	Wives of pesticide applicators	×		82
Trichlorfon	USA	Pesticides applicators	↑		10
Carbamates					
Carbaryl	USA	Wives of pesticide applicators	×[e]		82
Carbofuran	USA	Wives of pesticide applicators	↑[e]		82
Pyrethroids					
Allethrin	India	Mosquito repellent coils or mats users	↑[c]		85
Permethrin	USA	Pesticides applicators	×		10
Prallethrin	India	Mosquito repellent coils or mats users	↑[c]		85
Pyrethroid mixture	China	Pesticide factory workers	↑		84
Pyrethroid mixture	Bolivia	Male pesticide sprayers	↑		122

Other insecticide					
Amitraz	Turkey	Overdose patients	↑[c]	Case report	[123]
Mixture of chlopyrifos & cypermethrin	Morocco	A 30-year-old man	↑[c]	Case report	[124]

[a]Abbreviation for insecticides: 2,2′-bis (4-chlorophenyl)-1,1-dichlorodiethylene (p,p'- DDD), 2,2′-bis (4-chlorophenyl)-1,1-dichloroethylene (p,p'- DDE); 2,2′-bis (4-chlorophenyl)-1,1,1-trichloro-ethane (p,p'-DDT); Hexachlorobenzene (HCB); 2,2',4,4',5,5'-Hexachlorobiphenyl (CB-153); β-Hexachlorocyclohexane (β-HCH); ɣ-Hexachlorocyclohexane (ɣ-HCH); trans-nonachlor (TNC).
[b]Abbreviation: ↑, increase; ×, no association with; Gestational diabetes mellitus (GDM). Risk of diabetes including elevated blood glucose level[c], insulin resistance[d], and gestational diabetes mellitus[e].

2.3.2 Effects of insecticides on body weight change in human

Compared to the large number of epidemiological studies on insecticide and diabetes, a relatively small number of studies reported a link between insecticide exposure and obesity (summarized in Table 3). Three reported positive association between organochlorine exposure (DDE and β-hexachlorocyclohexane) and body mass index (BMI) [3, 8, 9], while others found no association between organochlorine insecticide exposure (including DDE and β-HCH) and BMI [3, 8, 9, 89].

For other markers of lipid metabolism, there were no effects of organochlorines on high-density lipoprotein cholesterol (HDL-cholesterol), except one study reported negative correlation between DDE and HDL [6]. Others reported that pyrethroid insecticides, allethrin and prallethrin, were linked with disturbed lipid metabolism by increasing triglycerides, phospholipids, very low-density lipoprotein cholesterol (VLDL-C), but no effects on HDL [85].

Table 2.3. Effects of insecticide exposure on body weight change in human

Insecticide[a]	Study information		Results[b]		Reference
	Country	**Description**	**BMI**	**Others**	
Organochlorine					
p,p'-DDE	Sweden	Women	=		89
	USA	Non-diabetic	N/A	= TG	6
	Belgium	Obese and lean men & women	=		3
	USA	African and White Americans	↑	↑ TG ↓ HDL-C	8
	Spain	Women in early pregnancy & their newborn children	↑	↑ Weight in the first 6 months; ↑ BMI at 14 months in infancy	9
	Denmark	General Population	N/A	↑ Lipid oxidation; ↑FFA	99
	Slovakia	Polluted area	↑	↑ TG & cholesterol; ↓ testosterone in males	107
	Belgium	Aged 50-65 years old	↑	Only in men	111
p,p'-DDT	USA	African and White Americans	=	= TG = HDL-C	8
HCB	Sweden	Women	=		89
	USA	African and White Americans	=	= TG = HDL-C	8
	Spain	Women in early pregnancy & their newborn children	=		9
	Denmark	General Population	N/A	↑ Lipid oxidation	99
	Saudi Adults	30-50 years old	N/A	↑ TG; ↓ HDL-cholesterol	115
	Slovakia	Polluted area	↑	↑ TG & cholesterol; ↓ testosterone in males	107
	Belgium	Aged 50-65 years old	↑	Only in women	111
β-HCH	Sweden	Women	=		89
	USA	Non-diabetic	N/A	= TG	6
	Belgium	Obese and lean men & women	↑	↑ Waist & subcutaneous abdominal fat mass	3
	USA	African and White Americans	=	= TG = HDL-C	8
	Spain	Women in early pregnancy & their newborn children	=		9

ɣ-HCH	USA	African and White Americans	=	= TG = HDL-C	8
Oxychlordane	Sweden	Women	=		89
	USA	Non-diabetic	N/A	↑ TG	6
	USA	African and White Americans	=	↑ TG = HDL-C	8
TNC	Sweden	Women	=		89
	USA	Non-diabetic	N/A	= TG	6
	USA	African and White Americans	=	= TG = HDL-C	8
Mirex	USA	African and White Americans	=	= TG = HDL-C	8
Organophosphorus					
Malathion	Egypt	Non-diabetic male famers	↑	↑ Waist circumference	121
Pyrethroids					
Allethrin	India	Men	N/A	↑ TG, phospholipids, lipid peroxides, & VLDL-C; ↓ Cholesterol &glycolipids; = HDL-C & LDL-C	85
Prallethrin	India	Men	N/A	↑ TG, phospholipids, lipid peroxides, & VLDL-C; ↓ Cholesterol & glycolipids; = HDL-C & LDL-C	85

[a]Abbreviation for insecticides: 2,2′-bis (4-chlorophenyl)-1,1-dichloroethylene (p,p'- DDE); 2,2′-bis (4-chlorophenyl)-1,1,1-trichloro-ethane (p,p'- DDT); Hexachlorobenzene (HCB); β-Hexachlorocyclohexane (β-HCH); ɣ-Hexachlorocyclohexane (ɣ-HCH); trans-nonachlor (TNC).
[b]Abbreviation for results: ↑, increase; ↓, decrease; =, no change; BMI, body mass index; GDM, gestional diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N/A, not available; TG, triglyceride; VLDL-C, very low-density lipoprotein cholesterol.

2.3.3 Effects of insecticides exposure on glucose and lipid metabolisms in animals

Although different classes of insecticides have slightly different mechanisms of insecticidal action, many share common characteristics by acting on the nerve system. Common symptoms after exposure to insecticides include spasm, muscular tremors, and convulsions [27, 37]. Overstimulation of the nervous system increases the energy demands and disturbs the functions of several organs, resulting in the disorder of energy

homeostasis that can lead to altered glucose and lipid metabolisms [125-128].

2.3.3.1. Effects of insecticides on blood glucose level in rodents

Studies have shown that exposure to all major types of insecticides induced hyperglycemia in experimental mice and/or rats (summarized in Table 2.4.). Among them, organochlorine and organophosphorus compounds are the most documented with relatively less reports on carbamates, pyrethroids, and neonicotinoids. Studies have demonstrated that exposure to certain organochlorines, organophosphorus, pyrethroids, and neonicotinoids elevated blood glucose levels, although inconsistent results have also been reported (Table 2.4.). This inconsistency may derive from the difference in dose and route of exposure, animal species, exposure duration, as well as methods to determine insulin resistance [129]. In fact, others suggested that insecticide-induced hyperglycemia is only temporary: blood glucose concentration increased initially and then decreased with the possibility of reaching hypoglycemia [129-131]. More importantly, our group recently reported that low doses of orally administrated permethrin or imidacloprid (levels lower than NOAEL) potentiate insulin resistance, only when high-fat diet was provided in mice [132-134]. These results suggest that low dose insecticide exposure should be evaluated along with other factors contributing to development of diabetes.

2.3.3.2. Effects of insecticides on body weight in rodents

Although it is known that overstimulation of the nervous system increases the energy demands that are potentially linked to reduced weight, a number of studies reported that exposure to insecticides can lead to increased body weight gain, while other studies found inconsistent results (summarized in Table 2.4.) [125-128]. Exposures to

organochlorine insecticides (DDE, HCB, and ɣ-HCH) have led to increased body weight gain in rodents (Table 2.4.) [135-138], including a study of parental trans-generational exposure to DDT linked to increased obesity rate in the offspring [139]. Similarly, others reported that perinatal exposure to DDT was linked to significant weight gain, but only in female offspring [140]. Another study found reduced weight after dieldrin exposure [141].

Organophosphorus insecticides have also been demonstrated to have moderate obesogenic effects. Chronic exposure to chlopyrifos under manifestation of cholinergic toxicity was reported to increase body weight in mice and rats [142, 143], including developmental exposure of chlorpyrifos on weight gain in male, but not female offspring [144]. Others reported reduced or no changes in weight after exposure to chlorpyrofos, dichlorvos, or dimethoate [145-147].

Limited data are available for effects of pyrethroids and neonicotinoids on weight gain. Exposure to cypermethrin (a pyrethroid) or imidacloprid (a neonicotinoid) significantly decreased body weight in mice or rats [148-150], while no effects of deltamethrin on weight in mice was reported [151]. Recently, our group reported that low doses of orally administrated permethrin or imidacloprid (levels of NOAEL and ADI) potentiate weight gain in male mice only when high-fat diet was provided [133, 134]. Taken collectively, the effects of insecticide exposure on body weight change are rather limited and inconsistent, likely depending on various factors, such as dose and route of administration, species, sex, and treatment duration. With new reports on low doses of insecticides and dietary fat interaction, it is possible that effects of insecticide exposure will be significant, particularly for relatively long-term low-dose exposure, when combined with other known contributing factors of obesity.

Table 2.4. Effects of insecticides-induced alteration of glucose and lipid metabolism in mice and rats

Insecticide	Treatment			Sex[d]	Results[e]						Reference
	Dose (mg/kg [a])	Route[b]	Duration[c]		BW	Glucose	Insulin	TG	Cholesterol	Other comments	
Mice											
Organochlorine											
DDE	0.4 & 2	Oral	Daily for 5 d, tested after 7, 14, 21 & 28d	M	↑ (2)	↑ (2; 7 & 21 d)	=	N/A	N/A	= Glucagon; = Insulin resistance; = pAkt Ser473/Akt (liver, muscle & fat); = Leptin & resistin	135
	2	Oral	Daily for 5d, 1 wk rest, +13 wk HFD or LFD w/o DDE	M	=	↑ in HFD (wk 4 & 8)	↓ in HFD	=	=	= Leptin & resistin; **Muscle**: ↑ Glut4 in HFD; **Fat**: = Glut4; **Liver**: ↓ Lipogenesis in HFD; ↑ FA oxidation in HFD; ↑ FA uptake in HFD; ↓ Gluconeogenesis in HFD	152
DDT	50	Oral	Single, tested after 0-18h & 7 d	M	N/A	↓ 5-7h, ↑ Baseline glucose in GTT after 7 d	= (18h)	N/A	N/A	↓ Glucose tolerance at 18h; ↓ Insulin secretion from isolated islet of pancreas at 18h, but not at 7d	153
	1.7	Oral	Daily for 16 d (GND11.5-PND5)	F & pups	↓ in ♂ (PND5); ↑ in adult ♀	↓ (PND5); = (6 m post-wean)	↑ in ♀ w/ HFD; = (6 m post-wean)	↑ in ♀ w/ HFD	↑ in ♀ w/ HFD;	↑ Insulin resistance in ♀ w/ HFD & ♂ w/ LFD; ↑ Fat mass in adult ♀; = Lipid level (during 6 m of post-weaning); = Food intake; = FFAs	140
Organophosphorus											
Chlorpyrifos	2	Oral (Diet)	8 wk	M[f]	↑	↑	↑	=	↑	↑ Insulin resistance; ↑ Food intake; ↑ Leptin in ApoE3[f]	142

Diazinon	6.5 (1/10 LD_{50})	i.p.	5 times weekly for 7 wk	M	N/A	↑	N/A	↑	↑	Tea and olive leaves extract prevented diazinon-disturbed glucose and lipid metabolism	154
Carbamates											
Furadan	0.125, 0.25, & 0.5	i.p.	Weekly for 2,4, & 6 wk	M	N/A	N/A	N/A	↑	↑	↑ Lipid content in serum, liver & kidney; ↑ Phospholipid in serum, liver, & kidney; ↓ Phospholipid in brain; = FFAs; ↓ Lipase activity in liver and serum	155
Pyrethroids											
Cypermethrin	10	Oral	Daily for 28 d	M	↓	↓	N/A	↑	↑; ↑ VLDL, ↓ HDL		149
Deltamethrin	1 & 3 (1/4 of NOAEL)	Oral (Diet)	Every 3 d in gestation & lactation in dams	F & ♂ pups	= in ♂ pups	N/A	N/A	N/A	N/A	Gene expression in fat of ♂ pups: ↓ Glucose transport: Glut4 (1) & Glut2; ↓ Lipogenic gene (1) : SREBP1, ACC-1, FABP4, CD36, LPL & SCD-1; ↓ Adipogenesis (1): PPARɣ & CEBPα	151
Neonicotinoids											
Imidacloprid	5,10, &15	Oral	Daily for 15 d	M	↓ (15)	N/A	N/A	N/A	N/A	↑ Liver & kidney weight (15)	148
	0.06, 0.6 & 6	Oral	Daily for 12 wk	M	↑	↑	↑	↑	=	↑ Leptin; = FFA; **Fat:** ↑ cell size; ↑ CD36, SREBP1, TNFα; ↓ CaMKKβ, pAMPKα/AMPKα, pACC/ACC; **Liver:**	134

									↑ PEPCK; ↓ CaMKKβ, PPARα, pAMPKα/AMPKα, pACC/ACC, Sirt1, PGC-1α; **Muscle:** ↓ GLUT4; ↑ PDK4; = CPT1	

Rat

Organochlorine

DDT	1	Oral (Diet)	180 d	M	N/A	N/A;	↓ in sedentary rats; Significant interaction DDT × Exercise	N/A	↓ in sedentary rats; ↑ in exercised rats; Significant interaction DDT × Exercise	= Food intake, carcass & liver lipids; Significant interaction DDT × Exercise (glucose tolerance)	156
	20	Oral	Daily for 189 d	F	=	N/A	N/A	N/A	N/A		138
	25 & 50	i.p.	Daily for 6 d (GND8-14)	F/M	↑ in F3	N/A	N/A	N/A	N/A	Gestational rats (F0) were treated and the third generation (F3) were observed	139
Dieldrin	30	Oral	Single, test after 24h	M	=	N/A	N/A	N/A	N/A	↑ Liver glycogen & TG; = Liver phospholipid & cholesterol	157
	2	Oral	6 m	M	↓	↑	N/A	N/A	=		141
HCB	*20 & 100; 40 & 200 w/ food deprivation;	Oral (Diet)	4 wk	F/M	↑ ♂ (40) w/ food deprivation, ↑ in ♂ (20 & 100) w/o food deprivation	N/A	N/A	N/A	N/A	= Food intake; ↑ Liver weight w/ food restriction (20); ↑ Liver hypertrophy w/ food deprivation (200)	137

ɣ-HCH (lindane)	5,10,20, & 40	Oral	Daily for 189 d; daily for 15 wk	F	↑ (20 & 40)	N/A	N/A	N/A	N/A	↑ Food intake & food efficiency (20 & 40); ↑ Liver weight; 40 caused death	138
Organophosphorus											
Acephate	2.5	s.c.	Daily for 8 wk	M	N/A	↑	N/A	N/A	N/A	↓ Liver glycogen; ↑ Pyruvic acid and lactic acid in liver, heart, kidney, brain & blood	158
	600	Oral	Daily for 8 wk	F/M	N/A	N/A	N/A	↓	=	↓ Total lipids; **Liver:** ↑ Total lipids (↓ microsome, = mitochondria, ↑ cytosol); ↑ Phospholipids & total cholesterol (↓ microsome, ↓ mitochondria, & ↑ cytosol); ↑ FFA & TG;	159
	140 (1/10 LD_{50})	Oral	Single, tested after 2-8h	M	N/A	↑ at 2h and return to normal	N/A	N/A	↓ in adrenal (2 & 6 h)	↑ Corticosterone (2 & 6 h); ↑ Liver glycogen (6 h) ↓ Gluconeogenesis (G6P & TAT) at 6 h; = Weights of adrenals & liver	160
Chlorpyrifos	1	s.c.	Daily for 4 d (PND1-4), test in adulthood	F/M	=	=	↑ in post-prandial ♂, = in fasting ♂; ↓ in ♀ ($p < 0.08$)	↑ in ♂	↑ in ♂	= FFAs	147
	5	s.c.	Daily for 4 m	F	↑ (starting at 2 m)	N/A	N/A	N/A	N/A	↑ Perinephric fat weight; = Weights of heart, liver & gastrocnemius muscle	143
	1, 2.5, & 4	Oral	Daily for 35 d, GND7-PND21 to dams	F& pups	↑ in ♂ pups starting at PND 51 (2.5); = in ♀ pups	N/A	N/A	N/A	N/A	↑ Body volume in ♂ pups at PND 100; ↓ Weight/volume ratio in ♂ pups at PND 100; ↓ Leptin	144

Diazinon	40 (1/3 LD_{50})	i.p.	Single, tested after 2 h	F	N/A	↑	N/A	N/A	N/A	↓ Brain glycogen; ↑ GP & PGM; = G6P; Atropine (cholinergic blocker), tolazoline (α-adrenergic blocker) and propranolol (β-adrenergic blocker) abolished or reduced diazinon-induced hyperglycemia & brain glycogenolysis	161
	40	i.p.	Single, tested after 2 h	F	N/A	↑; abolished by adrenal-ectomy	N/A	N/A	N/A	↑ Lactic acid; = Pyruvic acid; **Liver & brain:** ↓ Glycogen; ↑ Glycogenolysis (↑ GP & PGM, = G6P); ↑ Glycolysis (↑ HK & LDH, brain only; = G6PD); ↑ Gluconeogenesis (↑ F1,6D & PEPCK, liver only); Adrenalectomy abolished above changes	126
	128 (LD_{50}) -1 d; 64 – 2d; 16 – 8d; 8 – 32 d	Oral	1 (single), 2, 8 or 32 d	M	N/A	N/A	N/A	↑ at 128; = other doses	10, 15 d post-treatment: ↓ (64); ↓ (16); = (4); ↓ HDL; ↑ LDL (except ↓ at 16)	↓ Phospholipids	162
	15, 30, & 60	Oral	Single, tested after 2 h	M	N/A	↑	N/A	N/A	N/A	**Liver:** ↑ GP & PEPCK (30 & 60)	163
	15, 30, & 60 (1/20, 1/10, 1/5 LD_{50})	Oral	Single, tested after 2 h	M	N/A	↑	↓	N/A	N/A	↑ TNF-α; all effect abolished by cAMP & cGMP PDE inhibitor	164
	75 (1/4 LD_{50})	Oral	Single, tested after 28 d	M	N/A	↑	N/A	N/A	N/A	↑ Testosterone	165

	6.5 (1/10 LD_{50})	i.p.	Single, (tested after 2 wk)	M[g]	=	=	=	↓	↓ (Wistar); = HDL	↓ Glucose tolerance in GK; = Glut4 in fat	166
	15, 30, & 60	i.p.	Single, tested after 1 & 18 h	M	N/A	N/A	↓ (1h); = (18h)	N/A	N/A	↑ Langerhans islet GDH (1h, 30 & 60; 18h); ↑ Glutamate (1h; = 18 h) ↑ C-peptide; ↓ GDH gene expression (18h, 60)	167
Dichlorvos	4 (1/20 LD_{50}), 40 (1/2 LD_{50})	Oral	Single; 3, 7, 14 d	M	N/A	↑ (40)	N/A	N/A	N/A	↓ Glucose tolerance; **Liver:** ↑ GP & GS (40); ↓ uridine diphosphate glucose pyrophosphrylase	168
	6	s.c.	Daily for 8 wk	M	↓	↑	N/A	N/A	N/A	**Brain:** ↑ GP; ↓ Glycogen, HK, PFK, LDH & glucose uptake	146
	20 (1/4 LD_{50})	N/A	Single, tested after 1 or 3 d	M	N/A	N/A	N/A	N/A	N/A	**Liver:** ↓ Glycogen & GK activity; ↑ GK mRNA level; **Pancreas:** = GK activity & mRNA; = Insulin mRNA	169
Dimethoate	150	i.p.	15 & 30 d (every other days)	M	↑	= (15 d); ↑ (30 d)	↑	↑	= (15 d); ↑ (30 d)		170
	20 & 40 (1/20 & 1/10 LD_{50})	Oral	Daily for 30 d	M	↓ (40)	↑	N/A	N/A	N/A	↑ Lipase & amylase; ↓ Pancreas lipase & amylase; ↓ Glucose tolerance; ↑ Pancreas weight; = Weights of liver, kidney & adrenals	145
Isofenphos	20	Oral	Single, tested after 3-72h	M	N/A	N/A	N/A	N/A	N/A	↑ Muscle lipid; ↓ Sarcoplasmic esterase; ↓ Muscle lipase (13-18h)	171
Malathion	2000	s.c.	Single, tested after 0-6 h	F	N/A	↑	N/A	N/A	N/A		172
	500	i.p.	Single, tested after 0-48 h	F	N/A	↑ (first 6 h)	N/A	N/A	N/A	↑ Liver, kidney, heart, & spleen glycogen (all 6-12 h, except liver 6-24 h);	131

									= Brain glycogen	
	46 (1/25 LD_{50})	i.p.	15 d	M	N/A	=	N/A	N/A	N/A	↑ Liver glycogen, Adrenaline (8 d), noradrenaline (8 d) & dopamine (4 d).	173
	650	i.p.	Single, tested after 8 h	M	N/A	↑	N/A	N/A	N/A		174
	500	i.p.	Single, tested after 2 h	F	N/A	↑	N/A	N/A	N/A	↑ Lactate; = pyruvate; **Brain:** ↓ Glycogen; ↑ GP, PGM & HK; = G6P & G6PD	175
	125, 250, & 500	i.p.	Single, tested after 0.5,1, & 2 h	F	N/A	N/A	N/A	N/A	N/A	**Brain** (500 except glycogen): ↓ Glycogen (starting at 0.5 h); ↑ GP, PGM, HK & lactate; =G6P, G6PD, LDH & pyruvate; Adrenalectomy abolish these above changes; ↓ Succinic dehydrogenase; **Adrenal:** ↓ Ascorbic acid; ↓ Cholesterol	125
	5,10, & 20	Oral (Diet)	4 wk	M	N/A	↑	N/A	N/A	N/A	↑ Liver PEPCK & GP	176
	5,10, & 20	Oral (Diet)	4 wk	M	N/A	↑ (10 & 20)	↑ (10 & 20)	N/A	N/A	**Muscle:** ↑ PFK & GP (20); = HK	177
	100	Oral	Daily for 32 d	M	=	=	N/A	N/A	N/A	↓ Food intake; **Liver:** ↑ Weight, HK, & glycogen ; ↓ GP	129
	100	Oral	Daily for 32 d	M	N/A	=	N/A	N/A	N/A	↓ Liver lipids; ↑ glycogen; ↓ Muscle glycogen	130

	20	Oral	Daily for 28 d	M	N/A	↑	N/A	N/A	N/A	**Liver:** ↑ PEPCK; ↑ Mitochondrial GP; Administration of *Satureja khuzestanica* essential oil (225 mg/kg/day) abolished the malathion induced effect	178
	5,10, & 20	Oral (Diet)	28 d	M	N/A	↑ (10 & 20)	↑ (10 & 20)	N/A	N/A	↓ Pancreas insulin secretion (glucose stimulated); = (KCl stimulated)	128
	100 (1/21 LD_{50})	Oral	Single, tested after 24 h	M	N/A	N/A	N/A	N/A	N/A	↑ Liver glycogen & HK; ↓ Liver GP; Caffeic acid (100 mg/kg) abolished these effects;	127
	100 (1/21 LD_{50})	Oral	Daily for 32 d	M	N/A	N/A	N/A	↑	=; = HDL & LDL	↓ Hypothalamic CRH mRNA; ↑ iNOS; = nNOS	179
Parathion	0.1 & 0.2	s.c.	Daily for 4 d (PND1-4)	F & M	♂: ↑ (0.1); ♀: ↓ (0.1 & 0.2); ↓ in ♀ w/ HFD (0.2);	↑ (0.2) in only normal diet	=	↓ only ♀ in the fasted state	↑ in ♂ w/ HFD; ↓ in ♀	↓ Food intake in ♂ (0.2); ↑ Food intake in ♀ (0.1); ↓ HbA1c; ↓ FFA in ♀ w/ normal diet; ↓ β-hydroxybutyrate only in fasted ♂	180
Carbamates											
Carbendazim	0.48, 0.96, 2.4, & 4.8	s.c.	Single, tested after 12 & 24h	M	N/A	↓ after 12h (0.48 & 0.96); ↑ after 12h (4.8); ↑ after 24h	N/A	N/A	= after 12h; ↑ after 24h		181
Pyrethroids											
Cismethrin	4	i.v.	Single	M	N/A	↑	N/A	N/A	N/A	↑ Noradrenaline, adrenaline, & lactate	182
Cypermethrin	420 (1/10 LD_{50})	Oral (Diet)	6 m	M	N/A	=; ↑ in liver;	N/A	N/A	↓; = in liver	N/A	183

	0.06, 0.12, 0.3, & 0.6	s.c.	Single, tested after 12 & 24h	M	N/A	↑ after 12h (0.3 & 0.6); ↑ after 24h (0.06)	N/A	N/A	↑ after 12h (0.06 & 0.12); ↑ after 24 h (0.3 & 0.6)		181
Decamethrin	40	i.p.	Single	F	N/A	↑	N/A	N/A	N/A	↑ Lactate	184
Deltamethrin	1.28 (1/100 LD_{50})	Oral	Daily for 30 d	M	N/A	↑	N/A	↑	↑; ↑ LDL & VLDL; ↓ HDL	↑ Total lipid; Vitamin E attenuate adverse effect of deltamethrin	185
	1.5 & 2.6	i.v.	Single	M	N/A	↑	N/A	N/A	N/A	↑ Noradrenaline, adrenaline, & lactate	182
Neonicotinoids											
Imidacloprid	0, 5, 10, & 20	Oral	Daily for 90 d	F	↓ (20)	↑ (20)	N/A	=	=	↓ Food intake (20); ↑ Weight of liver, kidney, & adrenal (20)	150
	10, 30, & 90	Oral	Daily from GND 6-PND 21 to dams; Daily from PND21-PND 42 to pups	F& pups	=	N/A	N/A	N/A	N/A		186

[a] mg/kg BW/day unless otherwise specified (e.g. in diet), * doses are ppm in diet; NOAEL, no observed adverse effect level;
[b] s.c., subcutaneous injection; i.p., intraperitoneal injection; i.v., intravenous injection
[c] d, day(s); h, hour(s); m, month(s); wk, week(s); w/o, without; PND, postnatal days; GND, gestational day;
[d] F, female; M, male;
[e] Results are for all doses tested in each study, unless doses are indicated as numbers in parenthesis; all markers are from fasting blood samples unless otherwise specified; ↑, increase; ↓, decrease; = no change; w/, with; ♂, male; ♀, female; N/A, not available;
[f] apoE3 & C57BL/6N strain were used; [g]GK (Goto-Kakizaki) rats are a spontaneous animal model of non-insulin-dependent diabetes without obesity;
Acronyms used: ACC-1, Acetyl-CoA carboxylase 1; pACC, phosphorylated ACC; pAkt, phosphorylated Akt; AMPKα, AMP-activated protein kinase alpha; pAMPKα, phosphorylated AMPKα; BW, body weight; cAMP, cyclic adenosine monophosphate; CaMKKβ, calcium/calmodulin-dependent protein kinase kinase β; CD36, cluster of differentiation 36; CEBPα, CCAAT/enhancer-binding protein α; cGMP, cyclic guanosine monophosphate; CPT1, carnitine palmitoyltransferase I; CRH, corticotropin-releasing hormone; FA, fatty acid; FFAs, free fatty acids; FABP4, fatty acid binding protein 4; F1,6D, fructose 1,6-bisphosphatase; GDH, glutamate dehydrogenase; GK, glucokinase; GK rat, Goto-Kakizaki rat; Glut2, glucose transporter 2; Glut4, glucose transporter 4; G6P, glucose-6-phosphatase; G6PD, glucose-6-phosphatase dehydrogenase; GP, glycogen phosphorylase; GS, glycogen synthase; GTT, glucose tolerance test; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; HFD, high-fat diet; HK, hexokinase; HOMA-IR, homeostatic model assessment-insulin resistance; iNOS, inducible nitric oxide synthase; IL-6, interleukin 6; LDH, lactate dehydrogenase; LFD, low-fat diet; LPL, lipoprotein lipase; nNOS, neuronal nitric oxide synthase; PDE, phosphodiesterase inhibitor; PDK4, pyruvate dehydrogenase lipoamide kinase isozyme 4; PEPCK, phosphoenolpyruvate carboxykinase;

PFK, phosphofructokinase; PGC-1α, peroxisome proliferator-activated receptor gamma co-activator 1α; PGM, phosphoglucomutas; PK, pyruvate kinase; PPARɣ, peroxisome proliferator-activated receptor gamma; SCD-1, stearoyl-CoA desaturase-1; Sirt 1, NAD-dependent deacetylase sirtuin-1; SREBP1, sterol regulatory element-binding protein 1; TAT, tyrosine aminotransferase; TG, triglycerides; TNFα, tumor necrosis factor-α; VLDL, very low-density lipoprotein.

2.4 Mechanism of insecticides-induced change in glucose and lipid metabolism

Recent studies indicate that insecticides are reported to influence various organs and tissues, such as endocrine organs, liver, pancreas, muscle, and adipose tissue, which may lead to altered glucose and lipid metabolisms [30, 187-195]. A number of mechanisms were suggested to be influenced by insecticides; including oxidative stress and endoplasmic reticulum stress.

2.4.1 Liver

The liver, as one of the principal organs in regulation of glucose homeostasis, contributes to blood glucose level by maintaining a balance between storage of glucose via glycolysis and glycogenesis and release of glucose via glycogenolysis and gluconeogenesis [129, 176, 196-198]. It was suggested that since insecticides are designed to target the nervous system, exposure to insecticides may attribute to increased liver glucose production to meet the increased energy demand caused by overstimulation of the nervous system [163]. In fact, studies have demonstrated that exposure to certain insecticides can increase the activities of key enzymes involved in hepatic gluconeogenesis and glycogenolysis [126, 158, 163, 169, 176, 178, 199, 200]. Organophosphorus insecticides were shown to increase hepatic phosphoenolpyruvate carboxykinase (a key enzyme for gluconeogenesis) and glycogen phosphorylase (a key enzyme for glycogenolysis) in both *in vitro* and *in vivo* [163, 176, 178, 200]. Others, however, reported that insecticides increased hepatic glycogen levels by organochlorine (dieldrin) and organophosphorus (malathion) [131, 157]. The inconsistent results obtained from different

studies may be explained by initially increased blood glucose level caused by gluconeogenesis and glycogenolysis (the maximum increase was noted at 2 h after administration), followed by glycogenesis, and a return to normal blood glucose level 6–24 h after malathion treatment [129, 130].

2.4.2 Muscle

Muscle is tightly linked with the nervous system by neuromuscular junctions, thus susceptible to insecticide-induced neurotoxic effects. Previous reports have exhibited that exposure to malathion, an organophosphorus insecticide, decreased glycogen content in muscles [130, 177]. In particular, Pournourmohammadi et al. reported that this was due to increased activities of glycogen phosphorylase and phosphofructokinase (PFK), which are the key enzymes regulating glycogenolysis and glycolysis, respectively [177]. In addition, permethrin (a pyrethroid insecticide) and imidacloprid (a neonicotinoid insecticide) were previously shown to induce insulin resistance in C2C12 muscle cells via Akt signaling [24, 201].

2.4.3 Pancreas

Excessive stimulation of the cholinergic receptors by insecticides can result in disturbance of insulin and glucagon secretions, potentially due to pancreas tissue damage. Thus, insecticides that influence either acetylcholine level or its receptors by inhibiting acetylcholine esterase (organophosphorus and carbamates) or by acting as agonists to nicotinic acetylcholine receptors (neonicotinoids) can all potentially stimulate insulin release from the pancreas. Studies have found that exposure to malathion, an organophosphorus insecticide, resulted in elevated blood insulin levels in rats [128, 177].

Another study, however, found that exposure to malathion resulted in decreased glucose-stimulated insulin secretion accompanied with patchy degenerative changes in the islets of Langerhans [128]. Similarly, other human and animal studies also found that organophosphorus and carbamate insecticides can cause acute pancreatitis, which potentially influence insulin secretion [119, 202-212].

2.4.4 Adipose tissue

It is suggested that many lipophilic insecticides, such as DDE, malathion, or permethrin, can be easily trapped and stored in adipose tissues [128, 213, 214]. In addition, *in vitro* studies using 3T3-L1 adipocytes demonstrated that DDT [215, 216], DDE [216, 217], imidacloprid [23], and permethrin [201], potentiated adipogenesis. Another study, however, found that treatment of DDE to 3T3-L1 cells did not alter adipogenesis or lipolysis, but increased basal free fatty acid uptake and the release of leptin, resistin, and adiponectin, which may be potentially linked to increased risk of obesity and type 2 diabetes [218]. The same study also reported that oxychlordane and dieldrin (both organochlorines) increased basal free fatty acid uptake, but not insulin-stimulated glucose uptake in 3T3-L1 adipocytes [218].

2.4.5 Endocrine organs and brain

Currently more than 101 pesticides have been listed as proven or possible endocrine disruptors by the Pesticide Action Network UK [191]. Some of the insecticides disrupt the endocrine system by mimicking the action of estrogen. For example, certain organochlorines and carbamates were reported to inhibit androgen receptors; some organophosphorus insecticides were reported to increase the expression of estrogen

responsive genes; and certain pyrethroids potentiate the action of estrogen [195]. The endocrine disrupting activities of pesticides may potentiate the risk of obesity and type 2 diabetes and other related diseases [219, 220].

Along with altered glucose homeostasis, organophosphorus compounds have been demonstrated to elevate catecholamine levels [125, 173, 177, 221-225], which are abolished by adrenalectomy [125, 126]. This suggests a role of adrenal glands in organophosphorus-induced disturbance of glucose homeostasis.

Pyrethroids were reported to inhibit progesterone action, organophosphorus insecticides were shown to inhibit thyroid hormone receptor [195], and exposure to an organophosphorus, diazinon, was previously reported to increase the serum testosterone levels [165]. Organochlorine and carbamate insecticides showed anti-androgenic effects by inhibition of binding natural ligand to androgen binding receptors [191]. All these hormones are known to influence insulin sensitivity and glucose homeostasis [226-228]. It was suggested that insecticides were able to act as an agonist or an antagonist towards aryl hydrocarbon receptors and certain nuclear receptors, such as retinoic acid receptors, pregame X receptors, and peroxisome proliferator-activated receptors [229, 230].

Studies have reported decreased brain glycogen content with increased activity of glycogen phosphorylase by organophosphorus insecticide, malathion [126, 175] and dichlorvos [146]. It was suggested that malathion can interfere with oxygen uptake and promoted glycogenolysis and glycolysis in favor of anaerobic conditions to counteract the neurotoxic effects in rats [175]. Malathion was also shown to increase lactic acid concentration without altering pyruvate content in the brain [125]. Others reported that an organochlorine insecticide (dieldrin) can cause apoptosis and neural degeneration by

increasing acetylation of core histone H3 and H4 in neuronal cells [231]. Limited studies reported that exposure to insecticides could affect feeding behavior by acting on the neural circuits [191]. When low doses of imidacloprid or permethrin (at or lower than NOAEL) were administered in mice, no significant effects of either insecticide on food intake were observed [132-134].

2.4.6 Cellular Responses

2.4.6.1 Oxidative stress

Oxidative stress was often suggested to be associated with insecticide-induced toxicity *in vitro* and *in vivo* [163, 191, 194, 232-239]. It is also known that oxidative stress is linked with obesity and type 2 diabetes [240]. Thus, many mechanistic studies have suggested that insecticides disrupt glucose and lipid metabolisms via oxidative stress-mediated mechanisms, such as lipid peroxidation, mitochondrial dysfunction, inhibition of paraoxonase and glucose-6-phosphate dehydrogenase (G6PD), and nitrosative stress [30, 127, 187, 191, 194, 238, 241-244].

Glucose plays a critical role as an antioxidant against free radicals via the pentose phosphate pathway, in which nicotinamide adenine dinucleotide phosphate (NADP) is reduced to NADPH, which is subsequently used for the reduction of oxidized glutathione (GSH) from disulfides of GSH (GSSG) and cellular proteins [163]. The increased demand of glucose to counteract the increased reactive oxygen species induced by insecticides has been suggested to cause hyperglycemia, leading to stimulated hepatic glycogenolysis and gluconeogenesis pathways [163]. In fact, some studies found that antioxidants could attenuate insecticide-induced hyperglycemia [178, 185].

Abnormal mitochondrial respiratory chain functions can cause disturbance in intracellular energy homeostasis and has been involved in many diseases, including diabetes [245-248]. Exposure to insecticides can cause muscle fasciculation, which greatly increase the oxygen flow into corresponding tissues and organs [178, 187, 237, 238]. And this increased oxygen flow caused by insecticides may lead to elevated oxidative phosphorylation in mitochondria, which subsequently increase the production of reactive oxygen species as byproducts. In addition, some insecticides (rotenone or pyridaben) are known to disrupt mitochondrial respiratory chain reaction, mainly by inhibiting Complex I, II, III and V electron transport chain [249].

Studies have demonstrated that pancreatic beta cell failure induced by insecticide exposure could be the result of underlying mitochondrial dysfunction in the pancreas [191, 250, 251]. It is reported that the pancreas is more susceptible to reactive oxygen species than other tissues because of its relative low expression of defensive enzymes against reactive oxygen species [252-254].

Besides their inhibiting effect on acetylcholine esterase activity, organophosphorus compounds were reported to inhibit other esterases, such as the paraoxonase, a key enzyme involved in hydrolysis of oxons (active organophosphorus metabolites), which may potentially increase oxidative stress [255].

cAMP and cGMP signaling may also play an important role in insecticide-induced oxidative stress. Previous studies have found that increased cyclic nucleotides using phosphodiesterase inhibitors could protect against organophosphorus-induced lipid peroxidation in rat submandibular saliva [221] and liver cells [222]. Similarly, another report supported that increased intracellular levels of cAMP and cGMP by intraperitoneal

administration of phosphodiesterase inhibitors could exert protective effects against organophosphorus-induced hyperglycemia and oxidative/nitrosative stress in Langerhans islets cells in rats [164].

2.4.6.2 Endoplasmic reticulum stress

Recent studies have found endoplasmic reticulum (ER) stress play key roles in development of several chronic diseases, including obesity and type 2 diabetes [256-260]. There are a few studies reporting a correlation between several types of insecticides and ER stress. An organochlorine (endosulfan), carbamates (formetanate, methomyl, pyrimicarb), and a pyrethroid (bifenthrin) were reported to increase 78 kDa glucose-regulated protein GRP78, also known as binding immunoglobulin protein (BiP), which is one of the ER stress markers in human pulmonary A549 cells [261, 262]. Another pyrethroids insecticide, deltamethrin, was reported to induce ER stress in SK-N-AS neuroblastoma cells via elevation of intracellular calcium level and activation of eukaryotic translation initiation factor 2α (eIF2α) [42]. Currently, there is no study directly determining the role of ER stress caused by insecticides and development of obesity and type 2 diabetes.

2.4.6.3 Inflammatory responses

Inflammation plays essential role in protecting cells from harmful stimuli (e.g. pathogens, damaged cells, or irritants) by involving immune cells and inflammatory cytokines [e.g., tumor necrosis factor alpha (TNFα)] [263]. Recent evidence demonstrated that inflammation responses are also linked with the development of obesity and type 2 diabetes [263, 264]. Studies showed that exposure to insecticides disturbed glucose and lipid metabolism via increasing inflammatory responses *in vitro* and *in vivo* [134]. Imidacloprid,

a neonicotinoid insecticide, was reported to increase TNFα gene expression in fat tissue of male mice [134]. Acute oral exposure to diazinon, an organophosphorus insecticide, was reported to increase TNFα level in Langerhans islets of male rats [164]. Permethrin, a pyrethroid insecticide, increased the gene expression of TNFα in adipose tissue of male mice and 3T3-L1 adipocytes (Figure 6.5, Figure 4.5), which may be achieved by upregulation of Jun N terminal kinase (JNK) and CCAAT/enhancer binding protein homologous protein (CHOP) in the ER stress pathway (Section 6.4).

2.5 Conclusion and project rationale

The literature review has discussed that exposure to insecticides can disturb glucose and lipid homeostasis and potentially increase the risk of developing obesity and type 2 diabetes. Previous studies have largely focused on organochlorine and organophosphorus insecticides, with less studies on carbamates, pyrethroids and neonicotinoids. Furthermore, knowledge of how and when these metabolic perturbations may ultimately contribute to the development of obesity and related pathologies is also limited. Considering the currently wide use of pyrethroids and the declining use of organochlorines and organophosphorus, it is important to investigate the effects and mechanistic details of pyrethroids on alteration of glucose and lipid metabolism *in vitro* and *in vivo*. Permethrin is the single most widely used pyrethroid insecticide, accounting for ~60% of all pyrethroids [265]. It was previously shown that exposure to permethrin can potentiate adipogenesis in 3T3-L1 adipocytes and insulin resistance in C2C12 cells *in vitro* [201]; however, the effects of permethrin on glucose and lipid metabolisms *in vivo* and the mechanisms of how permethrin induce adipogenesis and insulin resistance *in vitro* have not been fully explored. The dissertation herein will evaluate the role of permethrin

on glucose and lipid metabolism *in vivo* and the molecular mechanisms of permethrin-potentiated adipogenesis in 3T3-L1 adipocytes.

CHAPTER 3

OBJECTIVES OF THE PROJECT

The long-term goal is to develop prevention and/or treatment strategies for obesity and type 2 diabetes. The objective of this project is to determine the potential contribution of permethrin (a pyrethroid insecticide) on development of obesity and diabetes. The central hypothesis is exposure to permethrin will alter lipid metabolism and glucose homeostasis along with dietary fat, resulting in potentiated weight gain and impaired insulin responses. The rationale of this proposed research is: by understanding biochemical mechanisms by which exposure to environmental contaminants (permethrin in this case) may result in development of obesity and type 2 diabetes, we will be able to direct more efficient prevention and/or treatment strategies for these and related pathologies now and in the future.

The project aims are as follows:

Specific Aim 1: **Determine the effects of permethrin and dietary fat interaction on development of obesity and type 2 diabetes *in vivo***. The hypothesis to be tested is permethrin will potentiate high-fat diet induced weight gain and development of insulin resistance in a dose-dependent manner.

Specific Aim 2: **Determine the mechanism of permethrin on impaired lipid metabolism in 3T3-L1 adipocytes.** The hypothesis to be tested is permethrin promotes adipogenesis by a calcium- and ER stress-dependent mechanisms.

CHAPTER 4

EXPOSURE TO PERMETHRIN PROMOTES HIGH-FAT-INDUCED WEIGHT GAIN AND INSULIN RESISTANCE IN MALE C57BL/6J MICE

4.1 Introduction

Permethrin [(±)-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] is one of the synthetic pyrethroid insecticides structurally based on the natural pyrethrins. First synthesized in 1973 and marketed in 1977, permethrin is a photo-stable ester composed of the dichloro analogue of chrysanthemic acid and the 3-phenoxybenzyl alcohol [266]. Four different stereoisomers can be found in the technical grade of permethrin products, and among these isomers, one with the [1R, cis] configuration is known to be the most potent form against insects (WHO 1990). In addition to the structural advancement for the enhanced environmental stability, permethrin exhibits excellent potency against a wide spectrum of insect pests, while retaining a large margin of mammalian safety [14-16].

The action of permethrin on its molecular targets has been extensively studied and well-reviewed previously [18, 267, 268]. Briefly, permethrin elicits a rapid functional disruption in the neuromuscular system by membrane depolarization. One of the important major target sites is located on the voltage sensitive sodium channel (VSSC) alpha-subunit, a pore forming transmembrane protein that consists of four homologous domains (I-IV) [17, 18]. Permethrin is known to slow the inactivation of VSSCs and produces the prolonged tail currents upon repolarization of the cell membrane under voltage-clamp electrophysiological recording conditions [267, 269, 270].

Permethrin in mammals can be quickly biotransformed by ester cleavage and oxidation reactions and almost completely eliminated via urinary and fecal excretions within 12 days [16]. Environmental fate of permethrin varies depending on the environmental conditions. A half-life of permethrin in soil under aerobic conditions has been estimated to be 28 days or less under laboratory study conditions [16]. In fact, permethrin can be degraded and disappeared from the environment rapidly (several hours to 58 days) by photolysis, microbial and plant bio-transformations, while very little movement in the soil [16]. Based on these characteristics, permethrin became the first pyrethroid to be used under a wide range of environmental conditions, approximately 17% of the global insecticide market was occupied by pyrethroid products by 2013 [15, 18, 39]. In the US alone, ~2.2 million lbs of permethrin have been sprayed annually to agricultural plots, residential areas and approximately 63% is applied to the residential area for public health [19]. Permethrin has been formulated in pet products and veterinary medications to control ectoparsitic arthropod pests, such as ticks and fleas. An over-the-counter 1% permethrin formulation for human head louse control has been available to treat infested school aged children [20, 21]. Additionally, many biting arthropods show avoidance behaviors to permethrin, hence many permethrin-treated materials (e.g., permethrin impregnated clothing including military uniforms, pet collars, and mosquito nets) have been developed and used to repel blood feeding arthropods [271]. The commercial availability of those pyrethroids suggests that human exposure to permethrin is unavoidable.

Previously, permethrin was reported to promote adipogenesis and induce insulin resistance in cell culture models similar to other types of membrane-depolarizing

insecticides [23-26]; however, there is a lack of *in vivo* study determining the effect of permethrin on glucose and lipid metabolisms. Thus, the purpose of this study is to investigate the effect of permethrin exposure on development of dietary-fat-induced obesity and type 2 diabetes using a mouse model.

4.2 Materials and methods

4.2.1 Materials

Permethrin (98%, mixture of *cis* and *trans* isomers) was from Sigma Aldrich Co. (St. Louis, MO). Insulin (human recombinant) was obtained from Novo Nordisk Inc. (Princeton, NJ). D-glucose solution (50%) was acquired from Hospira Inc. (Lake Forest, IL). Triglyceride (TG), cholesterol, glucose, and Pierce BCA protein assay kits were purchased from Thermo Scientific (Rockford, IL). Insulin ELISA kit was from ALPCO (Salem, NH). Leptin ELISA kit was purchased from R&D systems (Minneapolis, MN). Non-esterified fatty acid (NEFA) assay kit was from Wako Diagnostics (Richmond, VA). Rabbit antibodies of phosphorylated adenosine monophosphate-activated protein kinase α (pAMPKα), adenosine monophosphate-activated protein kinase α (AMPKα), phosphorylated acetyl-CoA carboxylase (pACC), and acetyl-CoA carboxylase (ACC) were purchased from Cell Signaling Technology (Danvers, MA). Mouse antibody of β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies were also from Cell Signaling Technology (Danvers, MA). High capacity cDNA reverse transcription kit, real time PCR primers and Taqman gene expression master mix were

obtained from Applied Biosystem (Carlsbad, CA). Other chemicals were either from Thermo Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO).

4.2.2 Animals and diet

All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst (Protocol # 2013-0014). Male C57BL/6J mice at 3 week of age from the Jackson's Laboratory (Bar Harbor, ME) were housed in pairs with a 12-h light-dark cycle in a temperature and humidity controlled room. Mice were adapted to new environment with low-fat semi-purified AIN-93-based diet in powdered form (TD94048, Harlan Laboratories, Madison, WI) for 3 weeks. All mice were given a baseline insulin tolerance test (ITT) in the 2nd week of adaptation and a baseline glucose tolerance test (GTT) in the 3rd week of the adaptation period. Then, animals were randomly divided into two dietary groups: low-fat (4 w/w % fat) and high-fat diet groups (20 w/w % fat, TD07518, Harlan Laboratories, Madison, WI). The diet composition is shown in Table 4.1. Permethrin was first dissolved in soybean oil and then mixed with other ingredients. Within each dietary fat group, control (without permethrin) and three difference doses of permethrin-containing diet were given to mice for 12 weeks. Body weight and food intake were measured weekly. Permethrin doses used in the current study were chosen based on acceptable daily intake (ADI) of permethrin of 50 µg/ kg body weight (BW)/day and the chronic no observed effect level (NOEL) of permethrin of 5000 µg/kg body weight/day [272, 273]. Permethrin concentration in low-fat diet were 0.43, 4.3 & 43 microgram per gram of diet to deliver 50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. For high-fat diet, actual permethrin concentration is 0.62, 6.2 & 62 microgram per gram of diet to deliver

50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. Estimated permethrin intake in low-fat diet fed animals were 58 ± 1 µg/kg BW/day, 594 ± 1 µg/kg BW/day and 6184 ± 151 µg/kg BW/day to deliver 50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. Estimated permethrin intake in high-fat diet fed animals were 72 ± 1 µg/kg BW/day, 610 ± 47 µg/kg BW/day and 6422 ± 133 µg/kg BW/day to deliver 50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. There were no significant differences on 3 permethrin doses delivered between high- vs. low-fat diets.

After 12 weeks of permethrin treatment, mice were sacrificed by CO_2 asphyxiation after 4 h of fasting. Blood was collected after cardiac puncture and serum were separated by centrifugation at 3,000 g for 20 mins at 4 °C. Internal organs [heart, liver, kidneys, pancreas, spleen and adipose tissue including epididymal, retroperitoneal, mesenteric and subcutaneous fat from abdominal area] were weighed, snap-frozen by liquid nitrogen and kept in -80°C for further analysis. A part of epididymal white adipose tissue was directly preserved in 10% neutralized formalin for histological analysis.

Table 4.1. Composition of experimental diet

Ingredient	Low-fat Diet	High-fat Diet
Casein	140	169.1
L-cystine	1.8	2.2
Corn starch	465.7	288.5
Maltodextrin	155	132
Sucrose	100	100
Soybean oil	40	200
Cellulose	50	50
Mineral Mix, AIN-93M-MX (TD 94049)	35	42.8
Vitamin Mix, AIN-93-VX (TD 94047)	10	12.4
Choline Bitartrate	2.5	3
tert-Butylhydroquinone (TBHQ)	0.008	0.04
Total	1000	1000

4.2.3 Determination of glucose homeostasis

Insulin tolerance test was carried out three times (2nd week of adaptation period, week 5 and week 9) according to the method described previously [274]. Mice were fasted for 4 hours before a bolus of insulin (0.75 U/kg) was injected intraperitoneally. Then, tail vein blood sample were obtained at 0, 15, 30, 60 and 120 minutes after insulin injection and tested for glucose level using a hand-held glucometer (Advocate, Pharma Supply Inc, Wellington, FL). The areas under the curve (AUC) were calculated using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA).

Intraperitoneal glucose tolerance test (IPGTT) were conducted at the 3rd week of adaptation, week 6 and week 11 according to a method described by Andrikopoulos and colleagues but with slight modification [275]. After 6 hours of fasting, a bolus of glucose solution (2 g/kg) was injected into the intraperitoneal cavity of each mouse. Blood glucose level was then measured at 0, 15, 30, 60, 120 min using a glucometer as described above. Blood samples at 0, 30, 60, 120 min were also obtained for insulin determination by lateral tail incision using a method described previously [276]. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated using HOMA2 calculator provided by University of Oxford [277].

4.2.4 Hematoxylin and eosin staining

Epididymal adipose tissues were fixed with 10% neutralized formalin solution before embedding into paraffin. 5-μm-thick sections were made for hematoxylin and eosin staining of nuclei and cytoplasma, respectively. Adipocyte size measurement was conducted by four individuals who were blinded to the treatment groups using ImageJ software (U.S. National Institutes of Health).

4.2.5 Western blot analysis

Immunoblot analysis was done based on a previous method [278]. Briefly, radioimmunoprecipitation assay buffer (Thermo scientific, Rockford, IL) containing protease & phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL) was used to homogenize and extract proteins from epididymal adipose tissue and liver samples. The sample lysates were then centrifuged and supernatant was used for protein quantification, and subject to electrophoresis using sodium dodecyl sulfate-polyacrylamide gel (SDS-

PAGE). After transferring to polyvinylidene fluoride membrane (Millipore, Bedford, MA), samples were incubated with primary and horseradish-conjugated secondary antibodies. Detection was performed using Image Station 4000MM instrument (Carestream Health, New Heaven, CT) by using Clarity ECL western blot substrate (Bio-Rad, Hercules, CA). Results were analyzed by ImageJ software (U.S. National Institutes of Health). Protein levels were normalized with β-actin protein concentration.

4.2.6 mRNA expression

The liver, gastrocnemius skeletal muscle and epididymal adipose tissue were homogenized using TRIzol reagent and total RNA was extracted according to manufacturer's protocol. Total RNA was subsequently reverse transcribed to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystem, Carlsbad, CA). TaqMan Gene Expression Assays for [Glucose transporter 4 (GLUT4, Mm00436615_m1), Sterol regulatory element-binding protein 1 (SREBP1, Mm00550338_m1), diacylglycerol O-acyltransferase 1 (DGAT1, Mm00515643_m1), diacylglycerol O-acyltransferase 2 (DGAT2, Mm00499536_m1), cluster of differentiation 36 (CD36, Mm00432403_m1), phosphoenolpyruvate carboxykinase 2 (PEPCK, Mm00551411_m1), pyruvate dehydrogenase kinase 4 (PDK4, Mm01166879_m1) were performed on StepOne Plus real time PCR instrument (Applied Biosystems, Carlsbad, CA). The oligonucleotide primers for TNFα (NM_013693.2) were purchased from Eurofins MWG Operon (Huntsville, AL). Threshold values were analyzed by comparative CT (ΔΔCT) method [279]. Relative quantities of gene expression with real-time quantitative PCR (RT-qPCR) were calculated relative to 18S ribosomal RNA.

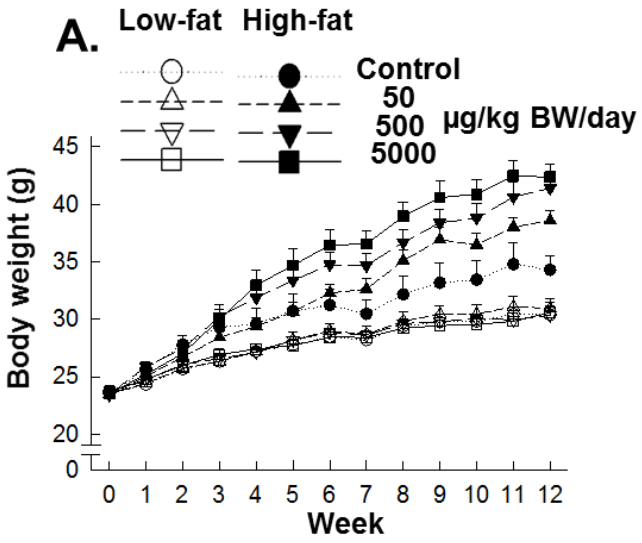
4.2.7 Statistical analysis

Data were analyzed by PROC MIXED using the SAS software (Version 9.3, SAS Institute Inc., Cary, NC, USA). Body weight (Figure 1A) data were analyzed by two-way repeated measure Analysis of Variance (ANOVA) and the slice option in the Least Square (LS) means statement. All the other results were analyzed by two-way ANOVA with LS means statement. The Tukey-Kramer's method was used for the multiple comparisons among the experimental groups. Letters (a, b, c, etc.) were used to present differences between each experimental group if there were significant interactions between diet and permethrin. *p*-values less than 0.05 were reported as statistically significant.

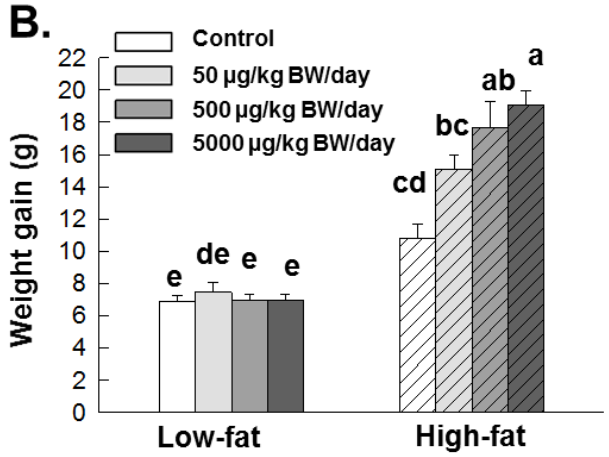
4.3 Results

4.3.1 Permethrin promoted weight gain without influencing energy intake in high-fat fed mice

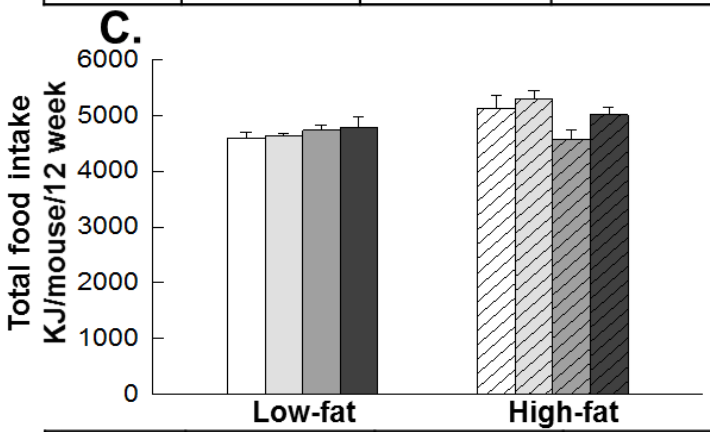
There was a significant effect of dietary fat ($p < 0.0001$), permethrin ($p = 0.0478$) and time ($p < 0.0001$) on body weight (Figure 4.1A). There was a significant three-way interaction (diet × permethrin × time) ($p = 0.0057$). Similarly, there was a significant effect of diet and permethrin treatment with significant interaction on body weight gain (Fig. 4.1B). These results suggest that permethrin promoted weight gain along with high-fat diet. There was significant effect of diet on energy intake (low-fat diet 4677.84 ± 56.26 kilojoules (KJ); high-fat diet 5007.39 ± 110.77 KJ), without permethrin effects or interaction (Fig. 4.1C).



Effect	Dietary fat (D)	Permethrin (P)	Time (T)	D × P × T
P-value	<.0001	0.0478	<.0001	0.0057



Effect	Dietary fat	Permethrin	Interaction
P-value	<.0001	<.0001	<.0001



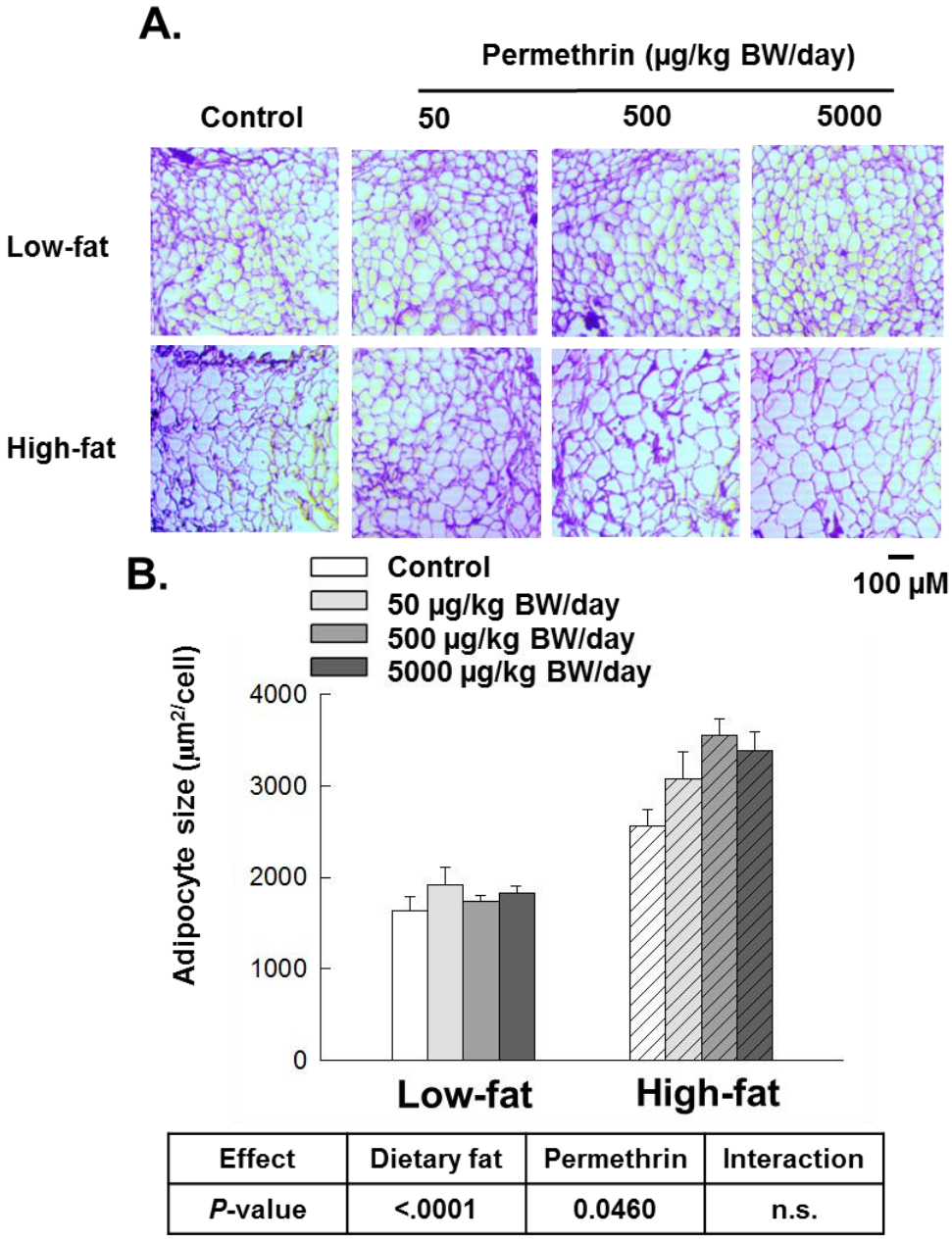
Effect	Dietary fat	Permethrin	Interaction
P-value	0.0139	n.s.	n.s.

Figure 4.1. Effects of permethrin treatment on body weight (A), weight gain (B) and energy intake (C) in male C57BL/6J mice. Mice were treated with either control or permethrin-containing diet [50, 500, 5000 µg/kg body weight (BW)/day] for 12 weeks in either low-fat or high-fat diet. (A) Blank symbols, low-fat fed mice; Filled symbols, high-fat fed mice. Circles, control; Up-triangles, 50 µg/kg BW/day; Down-triangles 500 µg/kg BW/day; Squares, 5000 µg/kg BW/day. Values represent means ± S.E. (n= 5-8). Means with different letters are significantly different ($p < 0.05$).

4.3.2 Effect of permethrin on organ weights and adipocyte size

Organ weights (liver, pancreas, heart, kidneys, and spleen) as well as adipose tissue weights (epididymal, subcutaneous, mesenteric, retroperitoneal, and total adipose tissue) are shown in Table 4.2. There were significant effects of diet on heart ($p = 0.0001$), kidney ($p = 0.0007$) and spleen ($p = 0.0001$) weights, but not liver and pancreas weights. Permethrin treatment had no significant effect on any of the organ weights (Table 4.2.). Significant diet and permethrin interactions were observed for heart and kidney weights.

There was a significant effect of diet on adipose tissue weights (Table 4.2., $p < 0.0001$ for all) as well as permethrin with an exception of mesenteric adipose tissue mass ($p < 0.05$ for all) with significant interactions ($p < 0.05$). Specifically, permethrin treatment in high-fat diet significantly increased total adipose tissue mass compared to high-fat control ($p < 0.0001$ with 500 µg/kg BW/day and $p = 0.0002$ with 5000 µg/kg BW/day), while no effects of permethrin was observed in low-fat diet groups. Consistently, adipocyte cell sizes were significantly increased by high-fat diet ($p < 0.0001$) and permethrin treatment ($p = 0.046$), while no diet and permethrin interaction was observed (Fig. 4.2B). Permethrin treatment at 500 and 5000 µg/kg BW/day significantly increased adipocyte size over control.



Effect	Dietary fat	Permethrin	Interaction
P-value	<.0001	0.0460	n.s.

Figure 4.2. Effects of permethrin treatment on epididymal adipocyte size in male C57BL/6J mice. Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg body weight/day) for 12 weeks in either low-fat or high-fat diet. Values represent means ± S.E. (n= 3).

Table 4.2. Effects of permethrin and dietary fat on organ weights (% of body weight) in male C57BL/6J mice

	Low-fat				High-fat				*p*-value		
		Permethrin doses			Permethrin doses						
	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Dietary fat	Perm	×
Liver	3.90±0.12	3.60±0.23	3.95±0.23	3.59±0.17	3.51±0.18	3.33±0.21	3.67±0.13	3.64±0.07	n.s.	n.s.	n.s.
Pancreas	0.43±0.04	0.50±0.05	0.46±0.01	0.44±0.02	0.54±0.05	0.45±0.04	0.36±0.03	0.38±0.03	n.s.	n.s.	n.s.
Heart	0.44±0.02^{ab}	0.49±0.03^{a}	0.48±0.02^{a}	0.52±0.01^{a}	0.47±0.03^{ab}	0.43±0.02^{ab}	0.35±0.04^{b}	0.35±0.02^{b}	0.0001	n.s.	0.0025
Kidneys	1.06±0.05^{abc}	1.12±0.04^{a}	1.07±0.04^{abc}	1.11±0.01^{ab}	1.12±0.07^{a}	0.99±0.02^{abc}	0.88±0.05^{bc}	0.87±0.04^{c}	0.0007	n.s.	0.0125
Spleen	0.30±0.02	0.28±0.04	0.30±0.03	0.30±0.04	0.26±0.03	0.21±0.01	0.17±0.02	0.18±0.02	0.0001	n.s.	n.s.
Adipose tissue											
Epididymal	2.19±0.20^{c}	2.19±0.17^{c}	1.74±0.17^{c}	1.79±0.14^{c}	3.57±0.42^{b}	4.57±0.27^{ab}	5.65±0.17^{a}	5.41±0.43^{a}	<.0001	0.0209	0.0001
Subcutaneous	1.36±0.24^{d}	1.47±0.15^{cd}	1.05±0.08^{d}	1.05±0.17^{d}	2.65±0.45^{c}	3.39±0.29^{b}	4.63±0.50^{ab}	4.95±0.21^{a}	<.0001	0.0093	0.0002
Mesenteric	1.32±0.09^{c}	1.52±0.13^{bc}	1.22±0.06^{c}	1.12±0.10^{c}	2.02±0.23^{ab}	2.17±0.14^{a}	2.74±0.22^{a}	2.52±0.19^{a}	<.0001	n.s.	0.0149
Retroperitoneal	0.59±0.08^{c}	0.63±0.09^{c}	0.39±0.05^{c}	0.42±0.07^{c}	1.09±0.12^{b}	1.56±0.11^{a}	2.00±0.09^{a}	1.77±0.09^{a}	<.0001	0.0016	<.0001

Total	5.45±0.57^{c}	5.81±0.46^{c}	4.40±0.28^{c}	4.38±0.46^{c}	9.34±1.13^{b}	11.70±0.69^{ab}	15.02±0.85^{a}	14.65±0.62^{a}	<.0001	0.0085	<.0001

Mice were treated with three doses of permethrin (50, 500, & 5000 µg/kg body weight/day). Values represent means ± SE (n=5-8).
Means with different superscripts within the same row are significantly different at $P<0.05$. Abbreviations: n.s., not significant; Perm, permethrin; ×, interaction

4.3.3 Effect of permethrin on glucose homeostasis

To determine the role of permethrin in diet-induced insulin resistance, ITT, GTT, the measurement of insulin during GTT, and HOMA-IR calculations were made. There were no significant differences on insulin responsiveness measured by ITT, GTT, insulin levels, or HOMA-IR during adaptation period (Fig. 4.3A, 4.3D, 4.3G, and Fig. 4.4A, respectively). In weeks 5 & 9, the high-fat diet group showed significantly increased insulin resistance versus the low-fat fed group ($p = 0.0006$ and <0.0001, respectively, Fig. 4.3B & 4.3C). Permethrin treatment only showed significant effect on insulin responsiveness in week 9 ($p = 0.0256$) with significant interaction ($p < 0.0001$) (Fig. 4.3B & 4.3C). In the high-fat dietary groups, animals with the middle and highest dose of permethrin (500 µg/kg BW/day and 5000 µg/kg BW/day, respectively) showed significantly increased insulin resistance when compared to high-fat control and the low dose of permethrin at week 9 (Fig. 4.3C). However, no significant effect of permethrin treatment was found between low-fat groups. At weeks 6 & 11, there were significant effects of dietary fat ($p < 0.0001$for GTT (Figure 4.3E & 4.3F), with a significant permethrin ($p = 0.0198$) and interaction effect ($p = 0.0061$) in week 11 only. In the high-fat dietary groups, animals with permethrin (500 µg/kg BW/day and 5000 µg/kg BW/day) groups showed significantly impaired glucose tolerance over high-fat control at week 11. When insulin levels were measured during glucose tolerance test as a marker of glucose homeostasis [280] (Figure 4.3G-4.3I), there was a significant effect of diet ($p < 0.0001$), permethrin ($p < 0.05$) and interaction effects ($p < 0.005$) on insulin levels in both weeks 6 and 11 (Fig. 4.3H and 4.3I). Results of HOMA-IR are shown in Figure 4.4. Both dietary fat and permethrin showed significant effects in HOMA-IR at week 6, 11 & 12.

Significant interactions were observed at week 6, but not week 11 & 12 (Fig. 4.4B-4.4D). Overall, these results suggest that permethrin may cause development of insulin resistance along with high-fat diet.

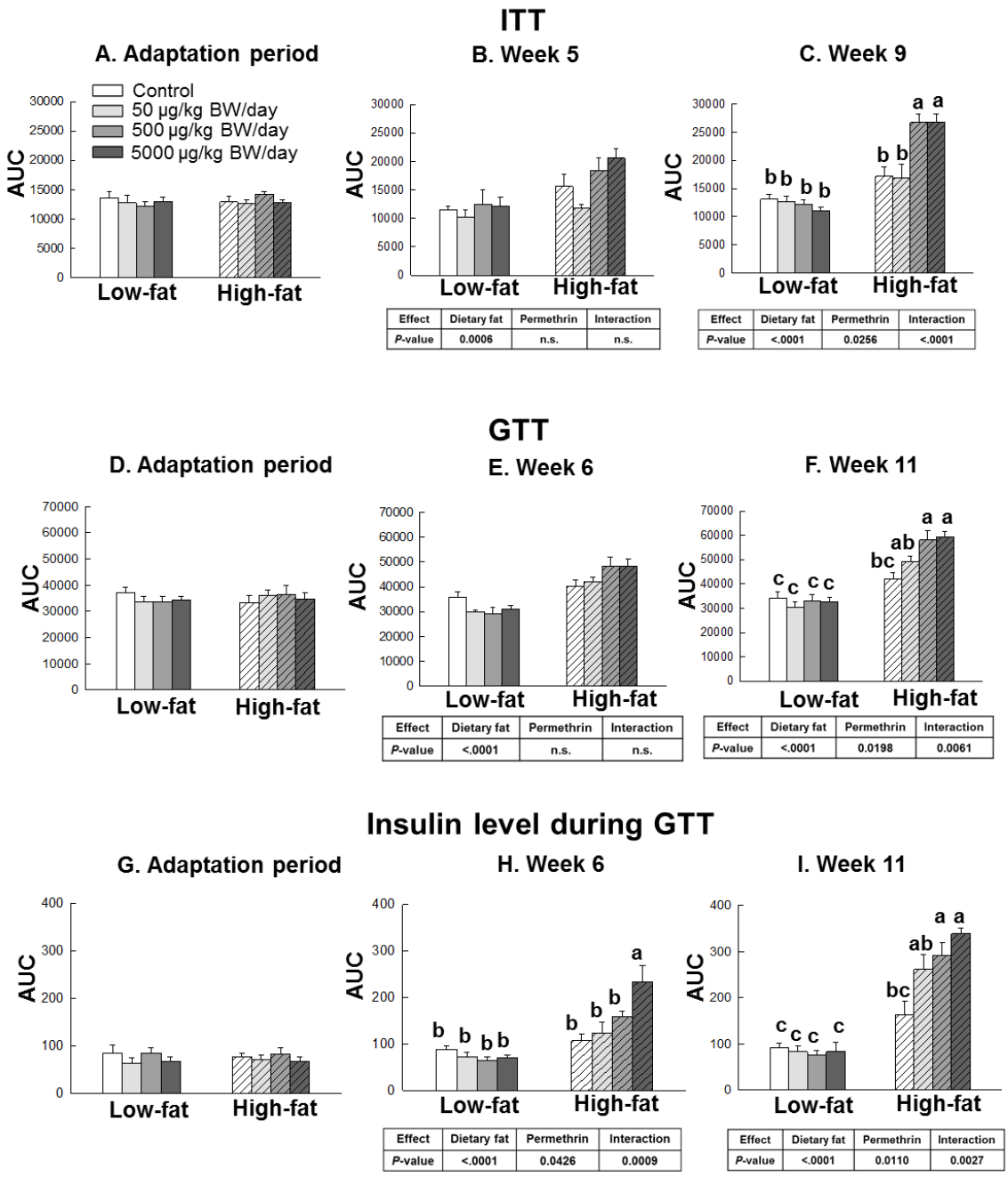


Figure 4.3. Effects of permethrin treatment on insulin responsiveness in male C57BL/6J mice. Insulin tolerance test (ITT, Figure 4.3A-4.3C), glucose tolerance test (GTT, Figure 4.3D-4.3F), insulin level during GTT (Figure 4.3G-4.3I). Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg body weight/day) for 12 weeks in each dietary group. Value represent means ± S.E. (n= 4-8). Means with different letters are significantly different ($p < 0.05$).

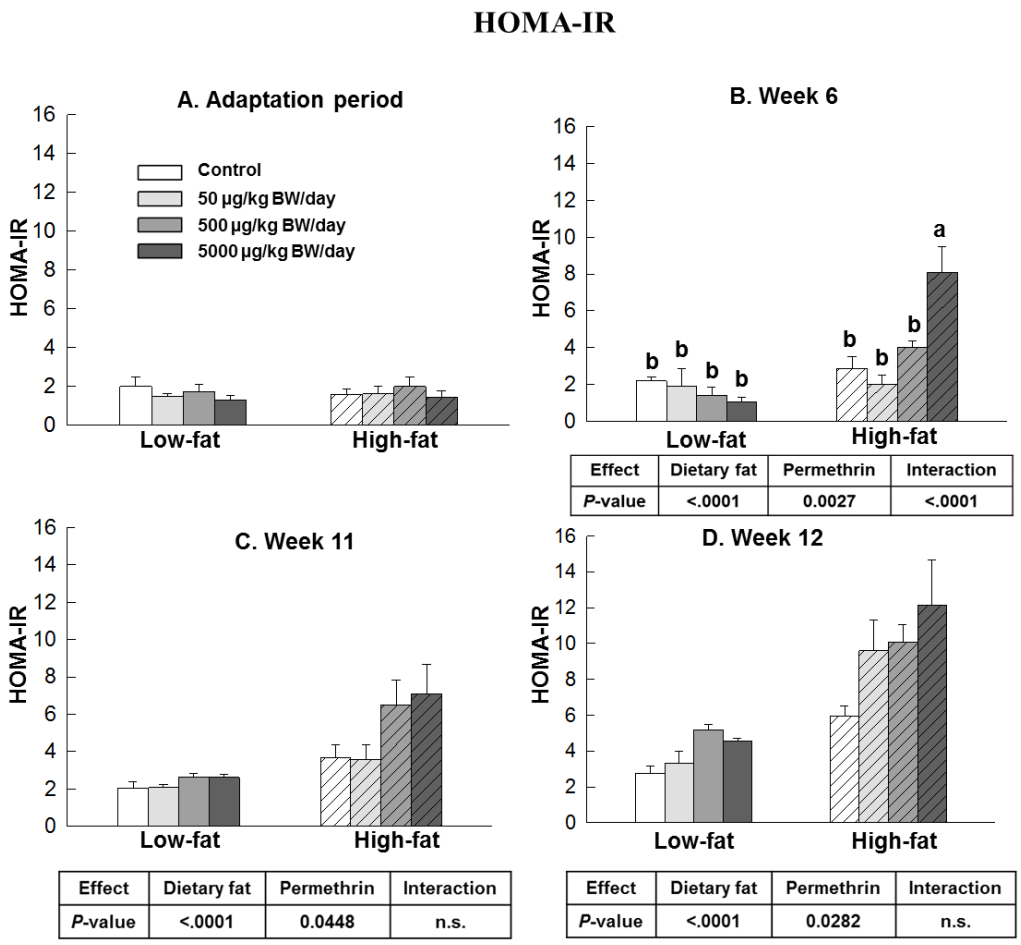


Figure 4.4. Effects of permethrin on HOMA-IR score in male C57BL/6J mice. HOMA-IR score was calculated during adaptation period, week 6, 11 and 12 with HOMA-IR calculator. Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg body weight/day) for 12 weeks in each dietary group. Value represent means ± S.E. (n= 4-8). Means with different letters are significantly different ($p < 0.05$).

4.3.4 Effects of permethrin on serum markers

Results of serum analyses are shown in Table 4.3. There were significant effects of diet on insulin ($p < 0.0001$), glucose ($p = 0.0003$), leptin ($p < 0.0001$) and cholesterol ($p < 0.0001$), but not on free fatty acid or TG levels. Overall, there was a significant effect of permethrin treatment on insulin ($p = 0.0487$), glucose ($p = 0.0099$), leptin ($p < 0.0001$), TG ($p = 0.0357$) and cholesterol ($p = 0.0132$), but not on non-esterified fatty acids. There

was a significant interaction between dietary fat and permethrin treatment on glucose (p = 0.0011), leptin ($p < 0.0001$) and cholesterol ($p = 0.015$). In high-fat dietary groups, permethrin treatment at 5000 µg/kg BW/day significantly increased blood level of glucose ($p = 0.0033$) and leptin ($p < 0.0001$) than high-fat control. Permethrin treatment at 500 µg/kg BW/day significantly increased blood cholesterol levels than control in high-fat diet groups ($p = 0.0181$). However, permethrin had no significant effects on any serum parameters tested in low-fat dietary groups.

Table 4.3. Effects of permethrin and dietary fat on serum parameters in male C57BL/6J mice

	Low-fat				**High-fat**				***p*-value**		
		Permethrin doses				**Permethrin doses**					
	Control	**50 µg/kg**	**500 µg/kg**	**5000 µg/kg**	**Control**	**50 µg/kg**	**500 µg/kg**	**5000 µg/kg**	**Dietary fat**	**Perm**	**×**
Insulin (ng/mL)	0.77±0.11	0.86±0.29	1.43±0.48	1.31±0.08	2.05±0.34	3.76±0.47	2.89±0.35	3.01±0.64	<.0001	0.0487	n.s.
Glucose (mg/dL)	162.8±7.9^{b}	159.6±10.7^{b}	168.3±15.3^{b}	147.6±13.0^{b}	178.2±17.8^{b}	150.4±14.3^{b}	207.3±5.7^{ab}	260.1±13.3^{a}	0.0003	0.0099	0.0011
Leptin (ng/mL)	4.9±1.00^{c}	6.71±1.07^{c}	3.91±0.62^{c}	3.17±0.45^{c}	14.4±3.7^{b}	35.54±4.41^{b}	62.40±11.81^{a}	62.97±5.94^{a}	<.0001	<.0001	<.0001
NEFA (mEq/L)	1.20±0.08	1.21±0.14	1.46±0.11	1.10±0.02	1.06±0.09	1.20±0.14	1.13±0.08	1.28±0.04	n.s.	n.s.	n.s.
TG (mmol/L)	0.77±0.06	0.87±0.10	0.93±0.13	0.74±0.03	0.62±0.04	0.83±0.05	0.91±0.05	1.06±0.11	n.s.	0.0357	n.s.
Cholesterol (mg/dL)	162±9^{c}	156±14^{c}	169±10^{bc}	142±13^{c}	171±15^{bc}	167±18^{bc}	246±19^{a}	229±12^{ab}	<.0001	0.0132	0.015

Mice were treated with three doses of permethrin (50, 500, & 5000 µg/kg body weight/day). Values represent means ± SE (n=5-8). Means with different superscripts within the same row are significantly different at $p < 0.05$. Abbreviations: n.s., not significant; TG, triglyceride; NEFA, non-esterified fatty acid. Perm, permethrin; ×, interaction.

4.3.5 Effects of permethrin on markers of epididymal white adipose tissue

The AMP-activated protein kinase (AMPK) plays important role in regulating cellular energy metabolism as well as glucose and lipid metabolisms [281]. Based on previous report that permethrin potentiate adipogenesis via inhibiting the activation of AMPK [201], we have measured AMPKα activities in epididymal adipose tissue (Fig. 4.5). There was a significant effect of diet on phosphorylated AMPKα and AMPKα as well as ratio of pAMPKα/AMPKα ($p < 0.05$, Fig. 4.5A-4.5C), while significant permethrin effects were observed on pAMPKα and pAMPKα/AMPKα without any significant interactions for all.

As one of main down-stream targets of AMPK, ACC gets phosphorylated at Ser 79 (pACC) resulting in inactivation of ACC [282, 283] (Fig. 4.5D-4.5F). There was a significant effect of diet ($p = 0.0044$) and permethrin treatment ($p = 0.0215$) without interaction on pACC level (Figure 4.5D). For ACC level, there was a significant effect of diet ($p < 0.0001$) without any effects of permethrin or interaction (Fig. 4.5E). There was a significant effect of permethrin ($p = 0.0087$) without dietary and interaction effects on pACC/ACC ratio (Fig. 4.5F). Calcium/calmodulin-dependent protein kinase kinase-beta (CaMKKβ) is one of upstream regulator of AMPK [284]. The current results showed permethrin significantly decreased the protein level of CaMKKβ, while no effects of dietary fat and interaction were observed (Fig. 4.5H).

Glucose transporter-4 (GLUT4) is the major glucose transporter responsible for insulin-stimulated glucose uptake expressed in both adipose tissue and muscle [285]. There were significant effects of diet and permethrin without interaction on GLUT4 gene expression in adipose tissue (Fig. 4.5I). Next, we measured the expression of tumor

necrosis factor-α (TNFα), as it is an important inflammatory cytokine that plays key role in obesity induced insulin resistance in type 2 diabetes [286]. Significantly higher levels of TNFα expression were observed by both diet and permethrin with interaction in adipose tissue (Fig. 4.5J). Sterol regulatory element-binding protein 1 (SREBP1) is another important regulator of adipogenesis [287]. Permethrin significantly increased SREBP without effects of diet or interaction (Fig. 4.5K). We also tested CD36 (regulates fatty acid update) and acyl CoA: diacylglycerol acyltransferase 1 and 2 (DGAT, catalyze mammalian triacyglycerol synthesis and lipid droplet formation), however, there were no effects of diet or permethrin for these markers (data not shown) [288, 289].

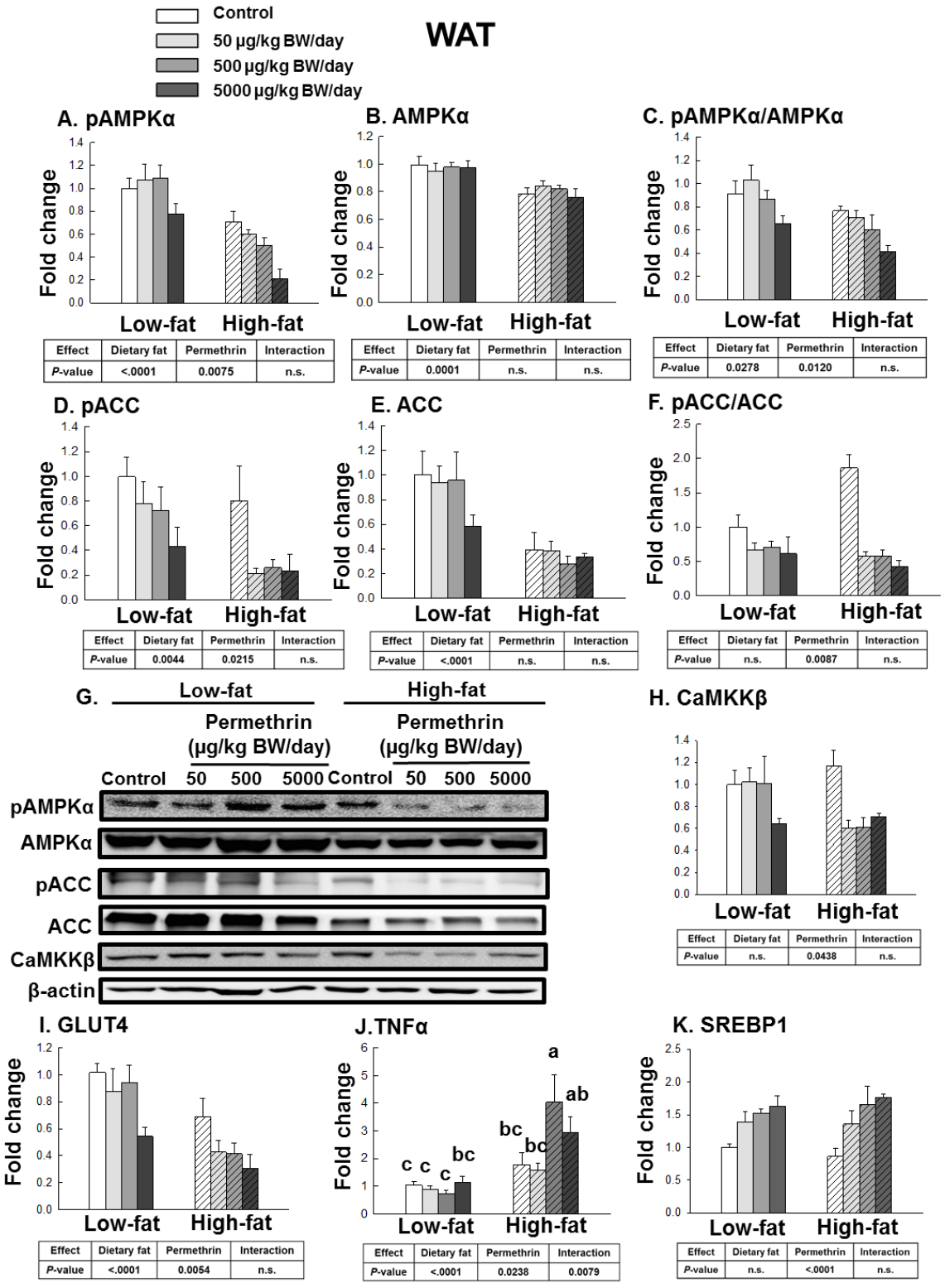
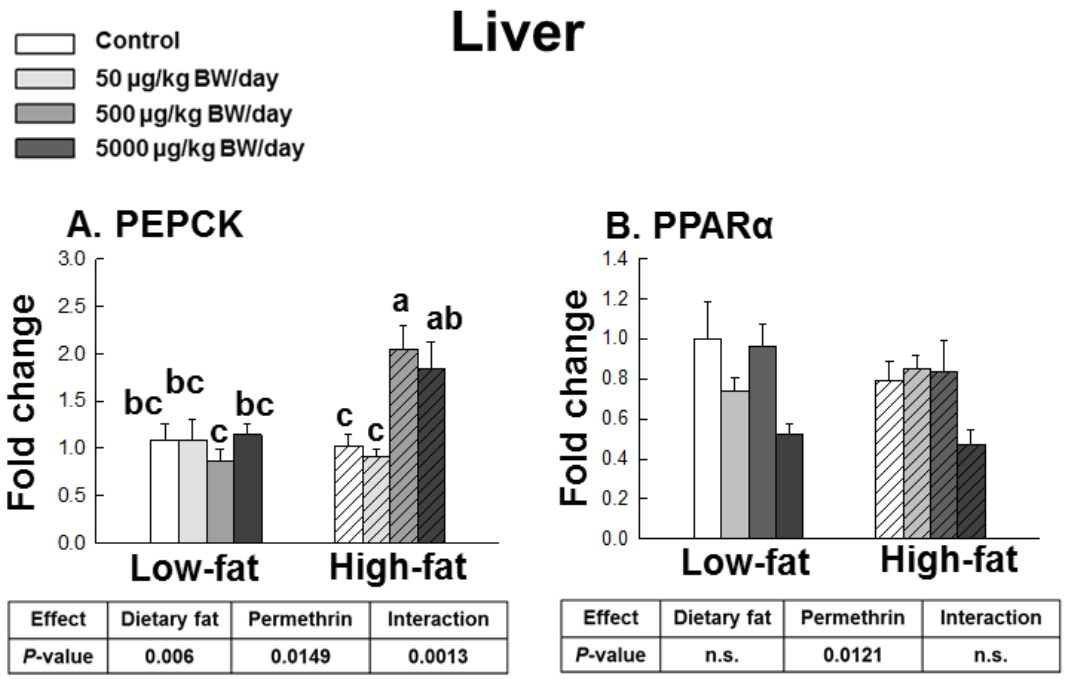

Control
50 μg/kg BW/day
500 μg/kg BW/day
5000 μg/kg BW/day
WAT
A. pAMPKα
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | <.0001 | 0.0075 | n.s.
B. AMPKα
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | 0.0001 | n.s. | n.s.
C. pAMPKα/AMPKα
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | 0.0278 | 0.0120 | n.s.
D. pACC
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | 0.0044 | 0.0215 | n.s.
E. ACC
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | <.0001 | n.s. | n.s.
F. pACC/ACC
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | n.s. | 0.0087 | n.s.
G.
Low-fat
High-fat
Permethrin (μg/kg BW/day)
Permethrin (μg/kg BW/day)
Control 50 500 5000 Control 50 500 5000
pAMPKα
AMPKα
pACC
ACC
CaMKKβ
β-actin
H. CaMKKβ
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | n.s. | 0.0438 | n.s.
I. GLUT4
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | <.0001 | 0.0054 | n.s.
J.TNFα
Fold change
c c c bc
bc bc a ab
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | <.0001 | 0.0238 | 0.0079
K. SREBP1
Fold change
Low-fat
High-fat
Effect | Dietary fat | Permethrin | Interaction
P-value | n.s. | <.0001 | n.s.

Figure 4.5. Effects of permethrin treatment on molecular targets involved in lipid metabolism and inflammation in epididymal white adipose tissue in male C57BL/6J mice. A. Protein levels of phosphorylated AMPKα (pAMPKα); B. AMPKα; C. pAMPKα to AMPKα ratio; D. Phosphorylated acetyl-CoA carboxylase (pACC); E. Acetyl-CoA carboxylase (ACC); F. pACC to ACC ratio; and G. Representative pictures. H. Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ); I, glucose transporter type 4 (GLUT4); J, tumor necrosis factor-α (TNFα); and K, sterol regulatory element-binding protein (SREBP1). Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg BW/day) for 12 weeks in each dietary group. Value represent means ± S.E. (n= 4-5). Means with different letters are significantly different ($p < 0.05$).

4.3.6 Effects of permethrin on the liver

The increase in hepatic gluconeogenesis is believed to play an important role in the elevation of fasting blood glucose level and pathogenesis of diabetes [290, 291]. Phosphoenolpyruvate carboxykinase (PEPCK) is the key enzyme regulating gluconeogenesis, as overexpression of hepatic PEPCK gene in mice lead to the development of non-insulin-dependent diabetes mellitus [290, 291]. There was a significant effect of diet ($p = 0.006$) and permethrin ($p = 0.0149$) with significant interaction on PEPCK level (Fig. 4.6A). Permethrin treatment at 500 ($p = 0.0056$) and 5000 µg/kg ($p = 0.0431$) significantly elevated PEPCK gene expression in the high-fat diet groups. However, no significant difference was found in the low-fat diet group.

PPARα is expressed principally in liver where it play important role in regulating fatty acid oxidation [292]. There was a significant effect of permethrin, but not diet or interaction, on suppressing PPARα gene expression in liver (Figure 4.6B).



Effect	Dietary fat	Permethrin	Interaction
P-value	0.006	0.0149	0.0013

Effect	Dietary fat	Permethrin	Interaction
P-value	n.s.	0.0121	n.s.

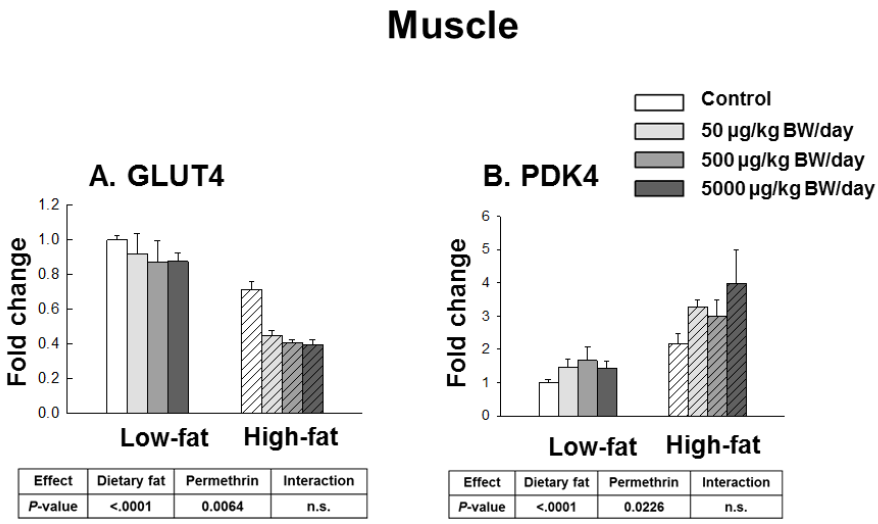
Figure 4.6. Effects of permethrin treatment on molecular targets involved in glucose and lipid metabolism in the liver of male C57BL/6J mice. A. Phosphoenolpyruvate carboxykinase (PEPCK); B. Peroxisome proliferator-activated receptor-α (PPARα). Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg BW/day) for 12 weeks in each dietary group. Value represent means ± S.E. (n= 3-5). Means with different letters are significantly different ($p < 0.05$).

4.3.7 Effects of permethrin on glucose metabolism in gastrocnemius skeletal muscle

In addition to adipose tissue, GLUT4 is expressed in the muscle, particularly GLUT4 level in slow muscle fibers play significant role in overall glucose metabolism [293, 294]. There was a significant effect of dietary fat ($p < 0.0001$) and permethrin ($p = 0.0064$) without interaction on GLUT4 gene expression in gastrocnemius muscle (Fig. 4.7A).

Pyruvate dehydrogenase kinase (PDK) plays key role in phosphorylation and inactivation of pyruvate dehydrogenase complex, which controls glucose oxidation [295].

Insulin suppresses the PDK4 gene expression in normal state. However, this effect is reduced in insulin resistance state resulting in increased PDK4 gene expression [296]. There was a significant effect of diet ($p < 0.0001$) and permethrin ($p = 0.0226$) without interaction on PDK4 gene expression in the muscle (Fig. 4.7B).



Effect	Dietary fat	Permethrin	Interaction
P-value	<.0001	0.0064	n.s.

Effect	Dietary fat	Permethrin	Interaction
P-value	<.0001	0.0226	n.s.

Figure 4.7. Effects of permethrin treatment on gene expression regulating glucose metabolism in gastrocnemius skeletal muscle of male C57BL/6J mice. A. Glucose transporter type 4 (GLUT4); B. pyruvate dehydrogenase kinase 4 (PDK4). Mice were treated with either control or permethrin-containing diet (50, 500, 5000 µg/kg BW/day) for 12 weeks in each dietary group. Value represent means ± S.E. (n= 4-5).

4.4 Discussion

The current study showed that daily administration of permethrin, at dose below NOEL, can potentiate high-fat diet induced weight and fat mass gains as well as altered insulin resistance in male mice, while no effects of permethrin were observed when it was supplemented in low-fat diet in these animals. To our knowledge, this is the first *in vivo* study reporting the potential role of permethrin in obesity and type 2 diabetes, along with dietary fat interaction.

AMPK serves as the intracellular energy gauge and is activated when intracellular ATP production decreases to provide energy. In addition, AMPK can also be activated by Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) and serine/threonine kinase 11 (STK11), also known as liver kinase B1 (LKB1) [297]. The current results showed that permethrin might target AMPK in adipocytes and are consistent with previous report that permethrin alters adipogenesis via AMPK-mediated mechanisms in 3T3-L1 adipocytes *in vitro* [201]. In addition, three other insecticides were reported to promote fat accumulation via AMPK-mediated mechanism [23, 216, 298].

Moreover, the current study shows that permethrin, along with high-fat diet, worsened insulin resistance. The link between exposures to organochlorine and organophosphorus insecticides and increased risk of development of type 2 diabetes, in both humans and animals, have been reported previously [6, 24, 83, 90, 91, 95, 96, 299]. In addition, two type I pyrethroids (allethrin and prallethrin) were previously reported to increase blood glucose level in male subjects [85]. Another study reported male pesticide sprayers exposed to pyrethroid mixture have higher prevalence of developing prediabetes [122]. Similarly, Wang et al. reported that increased the risk of diabetes among pyrethroid pesticide factory workers [300]. Animal studies have consistently reported the link between exposure to pyrethroids and the disturbance of glucose homeostasis [181, 182, 184, 185]. Exposures to cismethrin, decamehthrin, and deltamethrin were previously reported to increase blood glucose level in rats [182, 184, 185]. Elevated level of insulin was found in the serum of male rat exposed to α-cypermethrin [181].

In addition to animal studies, our group reported *in vitro* that permethrin reduced insulin-stimulated glucose uptake by decreasing the activation of AKT at Thr308 in

C2C12 muscle cells [201]. AKT plays significant role in inducing glucose uptake by stimulating GLUT4 translocation to the plasma membrane [301]. In this study, we did not measure the activity of AKT. However, we measured the gene expression of GLUT4 in adipose tissue and muscle. The current results indicated that permethrin treatment significantly decreased the gene expression of GLUT4 in both adipose tissue and muscle. Previously, developmental exposure to the pyrethroid insecticide deltamethrin was reported to decrease adipose tissue GLUT4 gene expression in male mice offspring [302]. Neonicotinoid insecticide imidacloprid was also reported to decrease muscle GLUT4 gene expression in mice fed with high-fat diet [134]. Thus, we suspect permethrin may cause insulin resistance via influencing AKT signaling and GLUT4 gene expression. In addition, we found increased TNFα gene expression in adipose tissue by permethrin treatment. TNFα is one of the major factors in obesity-induced insulin resistance [286]. It is known that TNFα can cause insulin resistance by increasing serine phosphorylation of insulin receptor substrate 1 (IRS-1) and inhibit insulin receptor activities [303]. Thus, the effect of permethrin on increased TNFα gene expression may have contributed to permethrin's effects on insulin resistance. Previously, it was reported that exposure to organophosphorus insecticide diazinon increased blood TNFα level [164]. Neonicotinoid insecticide imidacloprid was also reported to increase TNFα gene expression in adipose tissue of high-fat fed mice [134]. In this study, we did not measure the TNFα level in serum samples. Therefore, it is still not clear whether permethrin caused increased blood TNFα level in high-fat fed mice. In addition, increased PDK4 gene expression in muscle and increased PEPCK gene expression in liver by permethrin treatment would further contributed to altered glucose metabolism in these animals. Additional mechanistic

studies on insulin signaling pathway including AKT signaling and insulin-stimulated GLUT4 translocation will be needed to identify the exact mechanisms of permethrin on altered insulin responsiveness.

With significant usage of permethrin in a number of commercial products, it is not surprising that permethrin residues can be frequently found in agricultural products, household dusts, air, and diet [214, 304-306]. In fact, permethrin metabolites were frequently detected in adults and children urine samples [214, 304, 307]. Among various exposure routes, it is believed that dietary exposure is the main route of exposure to permethrin [214, 304]. Once absorbed, permethrin is rapidly metabolized by esterases and oxidases in the liver [214, 304, 308, 309]. Biological elimination half-life of permethrin ranged from 5 hours to 56 hours when administered orally 1 mg/kg in rat [310]. Due to highly lipophilic characteristic, however, permethrin is rather resistant to metabolism and may accumulate in adipose tissue [214].

It is also important to point out that permethrin doses used in the current study are relatively low (lower than NOEL); ADI (50 µg/kg BW/day) [272] and NOEL (5000 µg/kg BW/day) [273]. Thus, the results of the current study will be significant in that even at exposures that are considered 'safe', there may be significant effects on glucose and lipid metabolism in animals and humans when there are additional factors present, such as diet or other environmental contaminants.

Another potential mechanism is that permethrin along with its metabolites may serve as endocrine-disrupting agents, which contribute to lipogenesis [309, 311]. The major permethrin metabolites include, but not limited to, 3-phenoxybenzoic acid (free and glucuronide and glycine conjugates), the sulfate conjugate of 4'-hydroxy-3-

phenoxybenzoic acid, the sulfate conjugate of 2'hydroxy-3-phenoxybenzoic acid (from *cis*-permethrin only), the *trans*- and *cis*-dichlorovinyldimethylcyclopropane-carboxylic acids (free and glucuronide conjugates), and the 2-*trans*- and 2-*cis*-hydroxymethyl derivatives of each of the aforementioned *trans* and *cis* acids (free and glucuronide conjugates) [16, 308]. Cleavage and oxidation are believed to be the two major forms of metabolic reaction for permethrin. Most of the metabolic reaction begins at the terminal aromatic ring of the phenoxybenzyl moiety as well as the germinal dimethyl group of the cyclopropane ring and ends by conjugation [16]. It is generally accepted that *cis*-permethrin is harder to metabolize than *trans*-permethrin [312]. McCarthy *et al* found that two permethrin metabolites, 3-phenoxybenzyl alcohol 3-(4-hydroxy-3-phenoxy) benzyl alcohol, and 3-phenoxybenzaldehyde possess estrogenic activity, which is about 10^5 less than that of 17β-estradiol [309].

We have estimated doses of permethrin to be added in the diet based on average dietary intake and weight, although it was apparently that animals consumed higher doses of permethrin than originally intended (16-44% higher than intended doses, in Method section). However, no statistical differences were observed for doses of permethrin delivered to animals between two diets. Thus, any effects of permethrin observed only in high-fat fed animals still represent the role of dietary fat in permethrin's biological function. At this moment, it is not clear how dietary fat contributed to permethrin's effect on weight and fat mass gain and insulin resistance. We speculate that dietary fat caused metabolic changes in these animals, which may have been worsened with permethrin. Alternatively, since permethrin is highly lipophilic, it is likely that the absorption of permethrin with high-fat diet would be more efficient than that of low-fat diet. Thus, even

though there were no significant differences in permethrin doses administered, there may be dose-responses due to dietary fat content. Further investigation on the absorption efficacy of permethrin when delivered orally in the diet and metabolic consequence of permethrin are needed.

The current results also suggest significant increase of serum cholesterol levels by permethrin with high-fat diet. Previously, the link between exposures (including developmental exposure) to organochlorine, organophosphorus, and carbamate insecticides and increased cholesterol level in both humans and animals were reported [107, 140, 147, 154-156, 170, 180, 313]. In addition, pyrethroid insecticides, cypermethrin and deltamethrin, were reported to increase blood cholesterol level in mice and rat [149, 181, 185]. In particular, cypermethrin was reported to increase VLDL, while decreasing HDL in mice [149]. Similarly, deltamethrin was shown to increase LDL and VLDL, while decreasing HDL levels in rat [185]. One human study, however, reported negative correlation between chronic exposure to allethrin and prallethrin and blood cholesterol level in men [85]. Therefore, exposure of pyrethroid insecticides may increase the risk of hypercholesterolemia. Further human studies are needed to investigate the effects of pyrethroid insecticides on alteration of blood cholesterol levels.

In summary, the current study suggests the potential role of permethrin and dietary fat in development of excessive weight gain and insulin resistance in male mice. This is the first animal study determining the potential role of daily exposure to relatively low levels of permethrin in development of obesity and type 2 diabetes. Although it is not clear how permethrin elicit these effects, it is suggested that permethrin might target AMPK, fatty acid oxidation and insulin-sensitive glucose transporters. Based on the

current study, further studies are needed to identify the molecular targets of permethrin as well as effects of permethrin in female mice.

CHAPTER 5

PERMETHRIN ALTERS GLUCOSE METABOLISM WITH HIGH-FAT DIET AND DECREASES VOLUNTARY ACTIVITIES IN FEMALE C57BL/6J MICE

5.1 Introduction

Permethrin [(±)-3-phenoxybenzyl 3-(2,2-dichlorovinyl)-2,2 dimethylcyclopropanecarboxylate] is a synthetic insecticide that belongs to the pyrethroid family, which possesses structural resemblance to the natural pyrethrins. Developed in 1970s, synthetic pyrethroids demonstrate significantly improved photostability than its natural counterparts without sacrificing its potent insecticidal activities and low acute mammalian toxicity [18]. The use of pyrethroids has dropped slightly since 1997 [27], but they are still the second largest insecticide class current on the market, accounting for 16% of the global insecticides sales in 2015 [47].

In mammals, exposure to permethrin can produce syndromes of tremor and hyperactivities [34, 42, 43]. When orally exposed, permethrin can be quickly absorbed into the blood and subject to hydrolysis and degradation by esterases and cytochrome P450-dependent monooxygenases in the liver and other tissues with an elimination half-life of approximately 12h [18, 314, 315]. Permethrin is highly lipophilic, thus less likely to contaminate ground water. When exposed in the environment, permethrin can be rapidly broken down by microorganism or sunlight [316]. An experiment applying permethrin indoors near a window found that only 60% of permethrin remained after 20 days when it was exposed to daylight [316]. The half-life of permethrin in soil under aerobic laboratory conditions was reported to be less than 28 days [16].

Based on these characteristics, permethrin is one of the most widely-used synthetic pyrethroid insecticides in agricultural, veterinary, medical and household setting [310]. Products containing permethrin may be used on food and feed crops, on livestock, pets and/or on places where food is handled (e.g. restaurants) [33]. Other applications of permethrin include in public health mosquito control programs, human head lice control, and/or in clothing. Thus, human exposure to permethrin is quite likely.

Previously, some human and animal studies have found that exposure to pyrethroid insecticides can disturb glucose and lipid metabolisms and increase the risk of obesity and type 2 diabetes. [10, 84, 85, 122, 181-183, 185]. Our laboratory also demonstrated that exposure to permethrin can increase weight gain and insulin resistance in high-fat fed male mice as shown in the previous chapter. However, the effects of permethrin in females with high-fat diet induced obesity and insulin resistance have not been determined. In fact, some studies suggested exposure to insecticides may have sex-selectively influence body weights as well as glucose and lipid metabolisms [137, 140, 144, 180]. Thus, the purpose of this study was to investigate the effects of permethrin, along with dietary fat, on glucose and lipid metabolisms in female C57BL/6J mice.

5.2 Materials and methods

5.2.1 Materials

Permethrin (98%, mixture of *cis* and *trans* isomers) and HDL/LDL Quantitation kit were purchased from Sigma Aldrich Co. (St. Louis, MO). Insulin (human recombinant) was acquired from Novo Nordisk Inc. (Princeton, NJ). D-glucose solution

(50%) was obtained from Hospira Inc. (Lake Forest, IL). Glucose, cholesterol and TG kit were from Thermo Scientific (Rockford, IL)**.** Insulin ELISA kit was purchased from Mercodia (Winston Salem, NC). Leptin ELISA kit was from R&D systems (Minneapolis, MN). Free fatty acid assay kit was purchased from Cell Biolabs Inc. (San Diego, CA). Pierce BCA protein assay kit (Thermo scientific, Rockford, IL) was used for protein quantification. Rabbit antibodies of phosphorylated phosphoinositide-dependent kinase (pPDK), phosphorylated protein kinase B at threonine 308 (pAkt Thr308) and serine 473 (pAkt Ser473), Akt and Glucose transporter 4 (GLUT4) were purchased from Cell Signaling Technology (Danvers, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-rabbit secondary antibody was obtained from Cell Signaling Technology (Danvers, MA). Other chemicals were either purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA).

5.2.2 Animals and diet

All animal care and procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Amherst. Female C57BL/6J mice were purchased from the Jackson's Laboratory (Bar Harbor, ME) at 3 week of age and were housed two mice per cage with a 12h light-dark cycle in a temperature and humidity controlled room. Semi-purified AIN-93-based diet from Harlan Laboratories (TD94048 for low-fat and TD07518 for high-fat diets, Madison, WI) in powdered form were used. Permethrin was dissolved in soybean oil and mixed with other ingredients in diet. Diet and water were given to mice *ad libitum* throughout the experiment period except when

fasting was conducted prior to glucose measurement. After a week of adaptation with low-fat diet (4 w/w % fat), all mice were given a baseline test for insulin tolerance in the 2nd week of adaptation and glucose tolerance test in the 3rd week of adaptation. Then, animals were then randomly divided into two dietary groups: low-fat diet (4 w/w % fat) and high-fat diet group (20 w/w % fat). Within each dietary group, control diet (without permethrin) and three different doses of permethrin-containing diet were given to mice for 12 weeks. In the low-fat diet groups, permethrin concentration in diet is 0.26, 2.6, and 26 µg/g diet to deliver 50, 500 and 5000 µg/kg BW/day; in the high-fat diet group, permethrin concentration in diet is 0.36, 3.6 and 36 µg/g diet to deliver 50, 500 and 5000 µg/kg BW/day. Body weight and food intake were measured weekly. Food intake was measured as the total food intake per cage. Estimated permethrin intake in low-fat diet fed animals were 33 ± 1 µg/kg BW/day, 334 ± 2 µg/kg BW/day and 3387 ± 93 µg/kg BW/day for 50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. Estimated permethrin intake in high-fat diet fed animals were 31 ± 2 µg/kg BW/day, 374 ± 11 µg/kg BW/day and 3491 ± 100 µg/kg BW/day for 50 µg/kg BW/day, 500 µg/kg BW/day and 5000 µg/kg BW/day, respectively. There were no significant differences on 3 permethrin doses delivered between low- vs. high-fat diets. At the end of the study, mice were fasted for 4 hours and sacrificed by CO_2 asphyxiation. Blood was immediately collected by cardiac puncture and then sera were collected by centrifugation at 3,000 g for 20 mins at 4 °C. Internal organs (liver, heart, pancreas, kidneys, spleen, and white adipose tissues including epididymal, retroperitoneal and mesenteric fat pads) were weighed at sacrifice and kept in -80°C for further analyses.

5.2.3 Determination of glucose homeostasis

Insulin tolerance test (ITT) was conducted three times during the experiment (adaptation period, week 4, and week 10). Animals were fasted for 4 hours before test, tail-vein blood samples were obtained at 0, 15, 30, 60 and 120 minutes after intraperitoneal injection a bolus of insulin (0.75U/kg BW). Intraperitoneal glucose tolerance tests (GTT) were conducted in the adaptation period, at week 5 and week 11. Mice were fasted for 6 h prior to test. A bolus of glucose solution (2 g/kg BW) was injected into the intraperitoneal cavity, and blood was obtained from the tail end to measure glucose level at 0, 15, 30, 60, 120 min. Blood samples at 0, 30, 60, 120 min were also used for testing insulin level based on a method described previously [276]. All blood glucose levels were tested by using a glucometer with test strips (Advocate, Pharma Supply Inc, Wellington, FL). The areas under the curve (AUC) were calculated using SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA). HOMA-IR was calculated using HOMA2 calculator provided by University of Oxford [277].

5.2.4 Voluntary movement measurement (Non-exercise physical activity test)

Voluntary movement (non-exercise physical activity) was measured in week 1 and week 8 by using a method described previously [278, 317]. Briefly, individual mouse was put into a clear cage during the dark cycle(6:00 pm to 6:00 am). Diet and water (HydroGel, Portland, ME, USA) were provided *ad libitum* to mice during the measurement. Total travel distance (m) were recorded by an infrared camera with LoliTrack Quatro Video Tracking Software Version 1.0 (Loligo Systems, Tjele, Denmark). Data from early phase (6:00 pm to 8:00 pm) due to adaptation to new

environment and late phase (4:00 am to 6:00 am) due to sedentary behavior were excluded. Movement data from 8:00 pm to 4:00 am were used for analysis.

5.2.5 Western blot analysis

Immunoblot was conducted based on a method described previously [278]. Briefly, gastrocnemius skeletal muscle was frozen in liquid nitrogen and ground using a pestle and mortar. Sample was lysed using radioimmunoprecipitation assay buffer containing protease & phosphatase inhibitor cocktail (Thermo Scientific, Rockford, IL). Sample lysates were then centrifuged at 12,000g for 20 min at 4 °C. Protein concentration was determined using Pierce BCA protein assay kit (Thermo scientific, Rockford, IL). Samples were then separated by sodium dodecyl sulfate−polyacrylamide gel and transferred to polyvinylidene fluoride membrane (Millipore, Bedford, MA). Visualization was achieved with an Image Station 4000MM instrument (Carestream Health, New Heaven, CT) by using Clarity ECL Western blot substrate (Bio-Rad, Hercules, CA) after incubation with primary and horseradish peroxidase-conjugated secondary antibodies. The band densities were analyzed using ImageJ software (U.S. National Institutes of Health). Protein levels were normalized to GAPDH expression.

5.2.6 Statistical analysis

Data were analyzed by PROC MIXED using the SAS software (Version 9.3, SAS Institute Inc., Cary, NC, USA). Body weight (Figure 5.1A) data were analyzed by two-way repeated measure Analysis of Variance (ANOVA) with the slice option in the Least Square (LS) means statement. All the other results were analyzed by two-way ANOVA

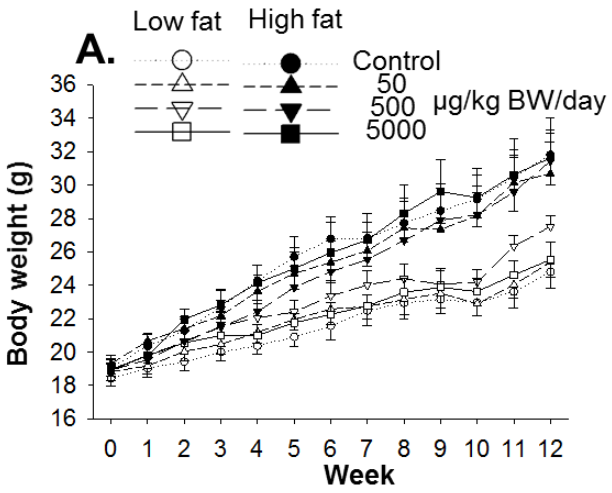
with LS means statement. The Tukey-Kramer's method was used for the multiple comparisons among the experimental groups. Letters (a, b, c, etc.) were used to present differences between each experimental group if there were significant interactions between dietary fat and permethrin. *p*-values less than 0.05 were reported as significant.

5.3 Results

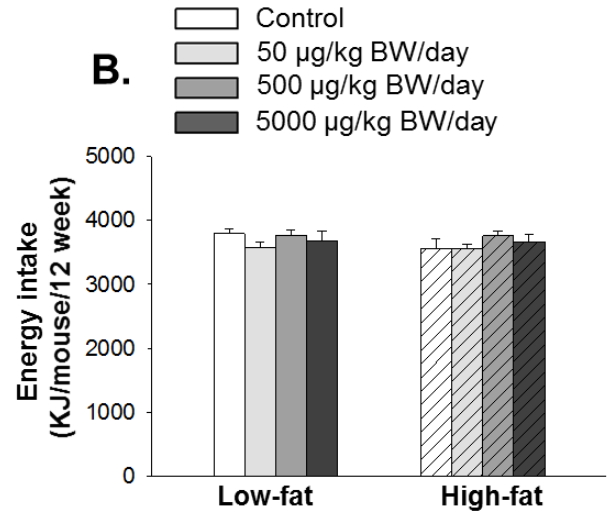
5.3.1 Effects of permethrin on body weight, energy intake and organ weights

Results of body weight and food intake are shown in Fig. 5.1A. Food intake was presented as the total energy intake over 12-week experimental period (Fig. 5.1B). There was a significant dietary fat effect on body weight; high-fat fed mice gained 2-fold more body weight than low-fat fed mice ($p < 0.0001$). However, no significant permethrin and interaction effect (dietary fat × permethrin × time) was observed. There was no significant difference in energy intake between high-fat and low-fat groups; 42.48-45.11 KJ/mouse/day in low-fat vs. 42.39-44.74 KJ/mouse/day in high-fat fed animals. There was no effect of permethrin treatment on energy intake.

Dietary fat significantly decreased the weight of liver, heart, spleen, and kidney, but not pancreas (Table 5.1). However, no significant effects of permethrin nor interaction effect were observed on any of the organ weights. Mice fed high-fat diet showed significantly higher adipose tissue weight than low-fat counterparts (1.8 fold, $p < 0.0001$ for all), while no permethrin and interaction effects were observed for all adipose tissue weights.



Effect	Dietary fat (D)	Permethrin (P)	Time (T)	D × P × T
p-value	<.0001	n.s.	<.0001	n.s.



Effect	Dietary fat	Permethrin	Interaction
p-value	n.s.	n.s.	n.s.

Figure 5.1. Effects of permethrin treatment on body weight (A) and energy intake (B) in female C57BL/6J mice. Low-fat or high-fat diet without or with permethrin [50, 500, 5000 µg/kg body weight (BW)/day] were given to mice *ad libitum* for 12 weeks. (A) Blank symbols, low-fat fed mice; Filled symbols, high-fat fed mice. Circles, control; Up-triangles, 50 µg/kg BW/day; Down-triangles 500 µg/kg BW/day; Squares, 5000 µg/kg BW/day. Values represent means ± S.E. (Figure 1A, n= 5-6; Figure 1B, n =3). Means with different letters are significantly different ($p < 0.05$).

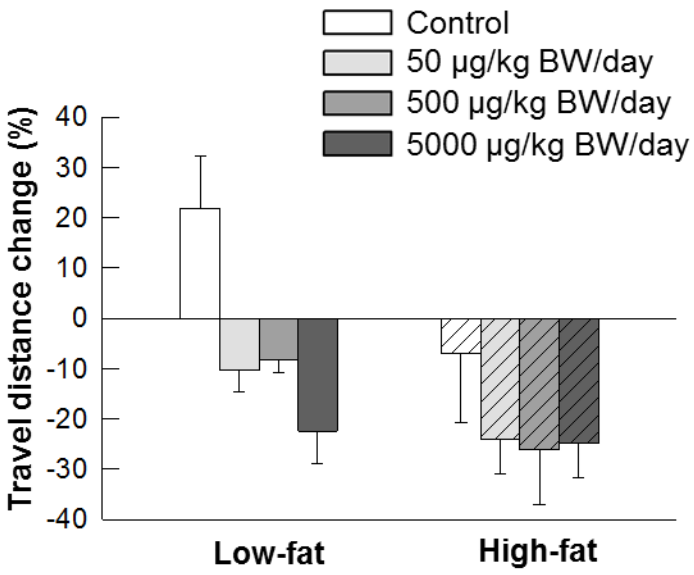
Table 5.1. Effects of permethrin and dietary fat on organ weights (% of body weight) in female C57BL/6J mice

	Low-fat				High-fat				*p*-value		
		Permethrin doses			Permethrin doses						
	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Dietary fat	Perm	×
Liver	4.15±0.19	4.18±0.08	3.84±0.16	4.05±0.10	3.42±0.23	3.44±0.22	3.63±0.13	3.62±0.07	< .0001	NS	NS
Heart	0.45±0.01	0.49±0.02	0.43±0.04	0.48±0.04	0.34±0.08	0.38±0.01	0.40±0.03	0.32±0.06	0.001	NS	NS
Spleen	0.38±0.02	0.34±0.02	0.34±0.02	0.35±0.01	0.31±0.01	0.39±0.06	0.32±0.02	0.32±0.01	0.0002	NS	NS
Kidney	1.07±0.01	1.10±0.05	0.99±0.04	1.12±0.08	0.92±0.05	0.97±0.04	0.95±0.02	0.91±0.03	0.0002	NS	NS
Pancreas	0.55±0.04	0.51±0.02	0.41±0.02	0.55±0.07	0.60±0.09	0.56±0.05	0.50±0.05	0.52±0.04	NS	NS	NS
Adipose tissue											
Omental	2.20±0.29	2.63±0.20	3.18±0.21	2.43±0.31	4.62±0.51	4.36±0.43	4.59±0.43	4.65±0.42	<.0001	NS	NS
Subcutaneous	2.00±0.25	2.49±0.1	3.34±0.17	2.64±0.41	5.55±0.72	4.87±0.58	5.37±0.66	5.31±0.45	<.0001	NS	NS
Retroperitoneal	0.46±0.07	0.57±0.07	0.67±0.08	0.55±0.11	1.09±0.15	1.10±0.06	1.21±0.11	1.16±0.12	<.0001	NS	NS
Mesenteric	1.09±0.10	1.43±0.13	1.49±0.17	1.13±0.12	1.99±0.31	1.94±0.25	2.09±0.09	2.06±0.26	<.0001	NS	NS
Total	5.75±0.65	7.12±0.53	8.68±0.52	6.75±0.94	13.25±1.65	12.26±1.26	13.25±1.17	13.18±1.17	<.0001	NS	NS

Values represent means ± SE (n=5-6). Abbreviations: NS, not significant;, Perm, permethrin; ×, interaction.

5.3.2 Permethrin treatment significantly decreased voluntary movement (non-exercise physical activity) along with high-fat diet

Results of voluntary movement as travel distance change between week 1 and week 8 (%) are shown in Fig. 5.2. There was a significant dietary fat effect on voluntary activities (2.3 fold, $p = 0.0186$). High-fat fed mice showed significantly decreased voluntary activities than low-fat fed mice. There was a significant effect of permethrin ($p = 0.0069$). Permethrin treatment at 50 and 5000 µg/kg BW/day significantly decreased voluntary activities over control (3.7 fold and 3.5 fold, respectively, $p < 0.05$). No interaction effect of permethrin and dietary fat was found.



Effect	Dietary fat	Permethrin	Interaction
p-value	0.0186	0.0069	n.s.

Figure 5.2. Effects of permethrin on voluntary movement (non-exercise physical activity test) in female C57BL/6J mice. Low-fat or high-fat diet without or with permethrin [50, 500, 5000 µg/kg body weight (BW)/day] were given to mice *ad libitum* for 12 weeks. Voluntary movement was measured in week 1 and week 8 from 6:00 pm to 6:00 am during dark circle. Results are presented as travel distance change between week 1 and week 8. Diet and water (HydroGel, Portland, ME, USA) were provided *ad libitum* to mice during the measurement. Total travel distance (m) were recorded by an infrared camera with LoliTrack Quatro Video Tracking Software Version 1.0 (Loligo Systems, Tjele, Denmark). Movement data from 8:00 pm to 4:00 am were used for analysis. Values represents means ±S.E. (n=5-6).

5.3.3 Effects of permethrin on serum markers of glucose and lipid metabolism

Results of serum analyses are shown in Table 5.2. Dietary fat showed significant effects on increasing insulin (1.6 fold), leptin (2.8 fold), cholesterol (1.1 fold) and high density lipoprotein cholesterol (1.2 fold) levels, but failed to show any significant effect on glucose, free fatty acids, triglycerides, and low-density lipoprotein cholesterol levels. Permethrin treatment significantly increased blood insulin (p = 0.0106) and glucose

levels (p = 0.0075) but failed to show any significant effect on leptin, free fatty acids, triglycerides, cholesterol, high-density lipoprotein and low-density lipoprotein cholesterol levels. There were no significant interactions between diet and permethrin on all serum markers tested except insulin. Permethrin treatment at 5000 µg/kg BW/day significantly increased insulin level than control in high-fat dietary groups (1.9 fold), but not in low-fat dietary groups.

Table 5.2. Effects of permethrin and dietary fat on serum parameters in female C57BL/6J mice

	Low-fat				High-fat				*p*-value		
		Permethrin doses				Permethrin doses					
	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Control	50 µg/kg	500 µg/kg	5000 µg/kg	Dietary fat	Perm	×
Insulin (ng/mL)	1.27±0.22^{b}	1.06±0.11^{b}	1.27±0.15^{b}	1.11±0.09^{b}	1.47±0.11^{b}	1.21±0.10^{b}	2.04±0.34^{ab}	2.80±0.47^{a}	0.0002	0.0106	0.01
Glucose (mg/dL)	167.0±9.4	197.0±12.1	225.6±14.5	176.6±16.2	170.8±14.4	151.4±12.5	198.0±7.3	196.1±13.7	NS	0.0075	NS
Leptin (ng/mL)	14.60±3.83	18.80±4.35	17.85±5.39	15.24±6.86	48.80±8.66	36.29±7.58	57.53±9.62	48.83±9.45	<0.0001	NS	NS
FFA (mEq/mL)	844±166	916±64	1051±170	904±160	935±146	853±335	973±115	590±188	NS	NS	NS
TG (mmol/L)	0.43±0.07	0.49±0.10	0.67±0.06	0.43±0.12	0.43±0.05	0.42±0.11	0.42±0.07	0.38±0.06	NS	NS	NS
Cholesterol (mg/dL)	126±5	155±11	155±10	149±19	151±8	162±8	163±8	173±6	0.0234	NS	NS
HDL-C (mg/dL)	109±4	117±10	113±8	116±3	115±12	129±7	132±9	145±8	0.0064	NS	NS
LDL-C (mg/dL)	26±2	26±4	27±3	29±7	28±4	26±2	28±4	19±2	NS	NS	NS

Values represent means ± SE (n=5-6). Means with different superscripts within the same row are significantly different at $p < 0.05$. Abbreviations: NS, not significant; FFA, free fatty acids; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Perm, permethrin; TG, triglycerides; ×, interaction.

5.3.4 Effect of permethrin on glucose homeostasis

To measure the effects of permethrin on glucose homeostasis, insulin tolerance test (ITT), glucose tolerance test (GTT) with insulin measurement, and HOMA-IR calculations were completed. There were no significant effects of dietary fat or permethrin on insulin responsiveness measured by ITT, GTT, insulin level, or HOMA-IR during adaptation period (Fig. 5.3A, 5.3D, 5.3G, and Fig. 5.4A). In week 4 & 10, high-fat fed animals showed significantly increased insulin resistance compared to low-fat fed animals (1.2 fold for both week 4 & 10, respectively, $p < 0.0001$ for both week, Fig. 5.3B & 5.3C). Permethrin treatment significantly decreased insulin responsiveness as demonstrated by ITT in week 10 (1.1 fold, $p = 0.0473$, Fig. 5.3C). However, no significant interaction between dietary fat and permethrin was found in week 4 & 10.

In week 5 & 11, there was a significant effect of dietary fat ($p = 0.0008$ for week 5 and $p < 0.0001$ for week 11) for GTT (Fig. 5.3E & 5.3F). Permethrin showed significant effect on increasing glucose tolerance in week 11 (1.2 fold, $p = 0.0369$). Permethrin treatments at 5000 µg/kg BW/day significantly increased glucose tolerance compared to control (1.2 fold, $p = 0.0383$). However, no significant interaction was found.

For insulin level during GTT, there was a significant effect of dietary fat on increasing blood insulin in week 5 & 11 (1.5 and 2.0 fold for week 5 and week 11, respectively, $p < 0.0001$ for both week 5 & 11, Fig. 5.3H & 5.3I). There was a significant effect of permethrin on increasing blood insulin level (1.5 fold, $p = 0.0271$) and interaction effect ($p = 0.0344$) in week 11 only (Fig. 5.3I). In high-fat diet, permethrin treatment at 5000 µg/kg BW/day significantly increased insulin level than control (2.0

fold, p =0.0056). However, no significant effect of permethrin was observed in low-fat dietary groups on insulin level during GTT.

To evaluate overall influence of permethrin on glucose homeostasis, we calculated HOMA-IR (Fig. 5.4). There was a significant effect of dietary fat on increasing HOMA-IR in week 5, 11 & 12 (1.3, 1.8, & 1.5 fold, p = 0.0096, 0.0003, & 0.0015, respectively). There was a significant effect of permethrin (p = 0.0116) on increasing HOMR-IR (1.2 fold, p = 0.0268) and interaction (p = 0.0268) in week 12 only. Permethrin treatment at 5000 µg/kg BW/day significantly increased insulin resistance in high-fat dietary group compared to control (p = 0.0138). However, no significant effect of permethrin was found in low-fat dietary groups.

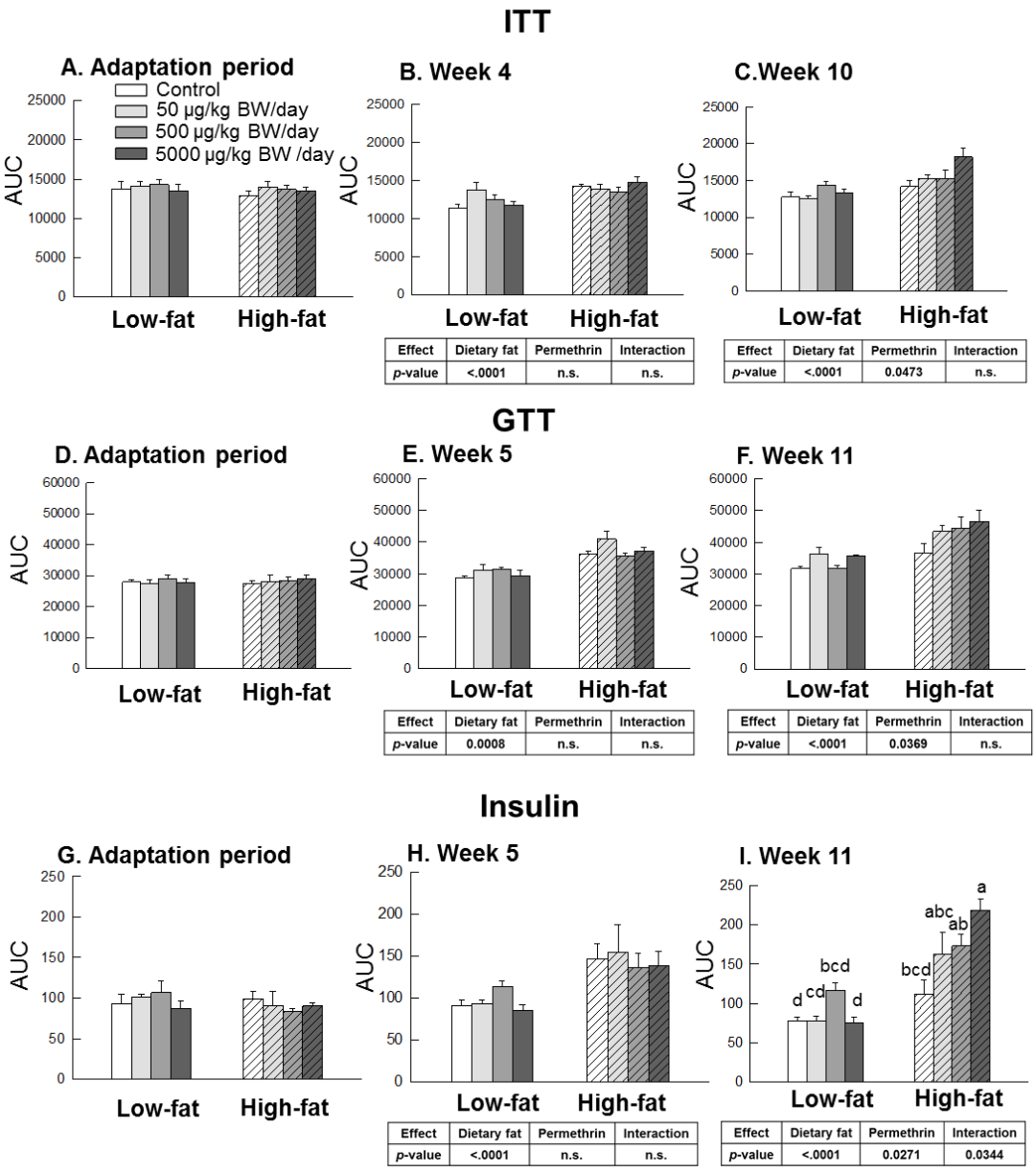
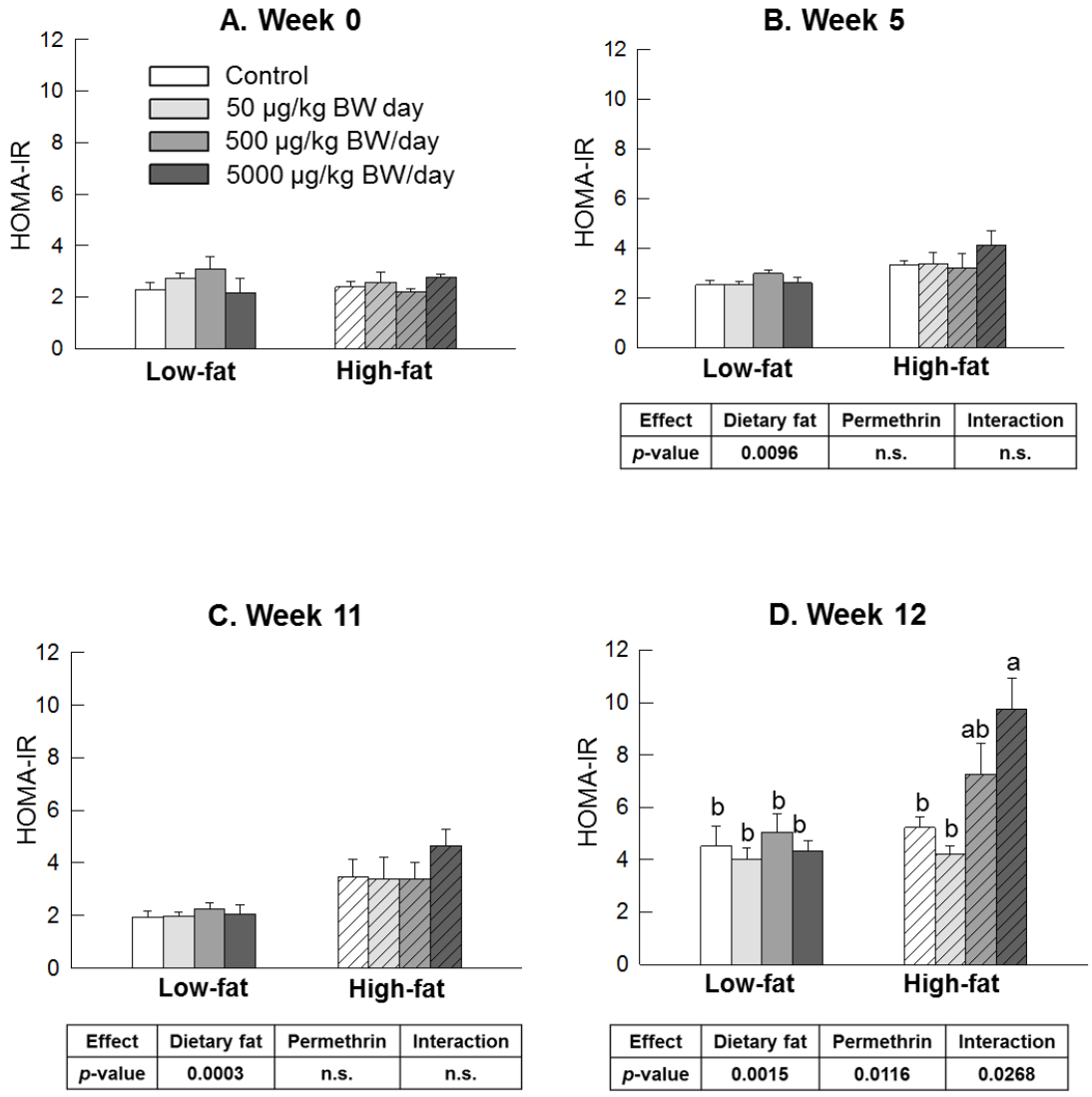


Figure 5.3. Effects of permethrin treatment on insulin responsiveness in female C57BL/6J mice. Low-fat or high-fat diet without or with permethrin [50, 500, 5000 µg/kg body weight (BW)/day] were given to mice *ad libitum* for 12 weeks. Insulin tolerance test (ITT, Figure 3A-C), glucose tolerance test (GTT, Figure 3D-F), insulin level during GTT (Figure 3G-I). Values represent means ± S.E. (n= 4-6). Means with different letters are significantly different ($p < 0.05$).



Effect	Dietary fat	Permethrin	Interaction
p-value	0.0096	n.s.	n.s.

Effect	Dietary fat	Permethrin	Interaction
p-value	0.0003	n.s.	n.s.

Effect	Dietary fat	Permethrin	Interaction
p-value	0.0015	0.0116	0.0268

Figure 5.4. Effects of permethrin on homeostasis model assessment - insulin resistance (HOMA-IR) score in female C57BL/6J mice. Low-fat or high-fat diet without or with permethrin [50, 500, 5000 µg/kg body weight (BW)/day] were given to mice *ad libitum* for 12 weeks. HOMA-IR score was calculated during adaptation period, week 6, 11 and 12 with HOMA-IR calculator. Values represent means ± S.E. (n= 4-6). Means with different letters are significantly different ($p < 0.05$).

5.3.5 Permethrin treatment significantly decreased the activation of AKT pathway in muscle

Skeletal muscle plays significant role in glucose uptake, which accounts for up to 75% of insulin-dependent glucose uptake [52]. In this study, we measured several key regulators in insulin-stimulated glucose uptake pathway, including Akt,

phosphoinositide-dependent kinase (PDK1), and GLUT4. Our results showed that permethrin and dietary treatment showed no significant difference in changing the protein level of Akt and pAkt Ser473. However, permethrin treatment significantly decreased pAkt Thr308 (1.1 fold, $p = 0.0386$, Fig 5.5A) and pAkt Thr308 to Akt ratio ($p = 0.0176$, Fig. 5.5D). Phosphorylated PDK1 (pPDK1) is one of the upstream markers of Akt. Dietary fat showed no significant effect on pPDK1 level. Permethrin treatment had a significant effect of decreasing pPDK1 ($p = 0.0245$). There was also a significant interaction of dietary fat and permethrin on pPDK1 level ($p = 0.0122$, Fig. 5.5F). Permethrin treatment at 5000 µg/kg significantly decreased pPDK1 in high-fat diet treatment group compared to high-fat control (2.1 fold, $p = 0.0242$). GLUT4 is the major glucose transporter in skeletal muscle, which plays significant role in mediating whole body glucose homeostasis [294]. Permethrin treatment significantly decreased GLUT4 protein level in muscle (1.3 fold, $p = 0.0078$, Fig. 5.5G). However, no dietary effect and interaction effect was observed. Taken together, these results showed that permethrin may target Akt signaling pathway to induce insulin resistance.

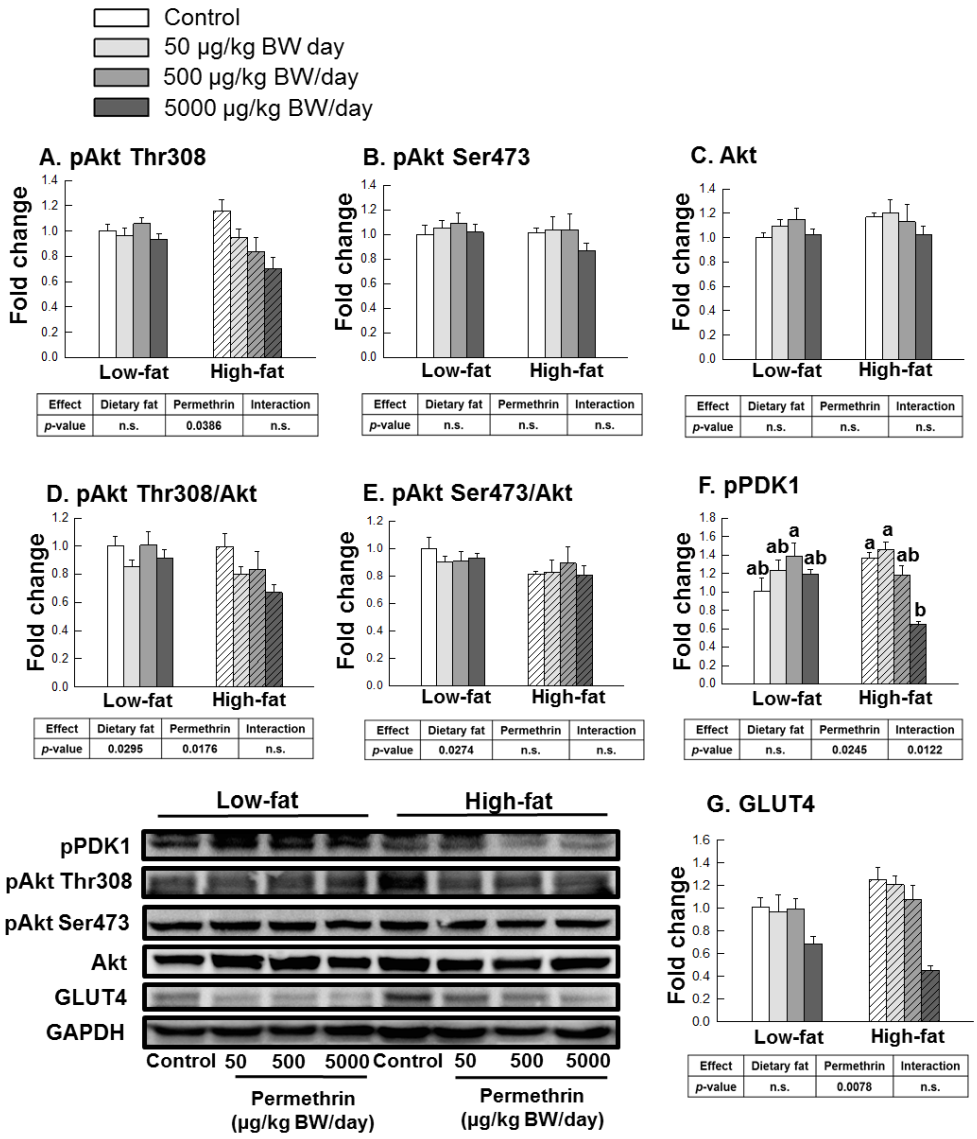


Figure 5.5. Effects of permethrin treatment on molecular targets involved in insulin signaling pathway in gastrocnemius skeletal muscle of female C57BL/6J mice. Low-fat or high-fat diet without or with permethrin [50, 500, 5000 µg/kg body weight (BW)/day] were given to mice *ad libitum* for 12 weeks. A. Protein levels of phosphorylated Akt (pAkt) Thr308; B. pAkt Ser473; C. Akt; D. pAkt Thr308 to Akt ratio; E. pAkt Ser473 to Akt ratio; F. phosphorylated phosphoinositide-dependent kinase-1 (PDK1); and G. Glucose transporter 4 (GLUT4). Values represent means ± S.E. (n= 4-6). Means with different letters are significantly different ($p < 0.05$).

5.4 Discussion

This is the first report to investigate the potential link between permethrin exposure and dietary fat on development of obesity and type 2 diabetes in female mice. The current results show that permethrin potentiated high-fat diet induced insulin resistance without significant induction of weight gain in C57BL/6J mice. This strain of mice is known to develop obesity and insulin resistance with high-fat diet [318]. High-fat diet caused significant weight gain and worsened insulin resistance compared to low-fat diet fed animals without any additional caloric intake in the current study. Permethrin did not have any effect on weight gain in this model. However, permethrin significantly worsened the insulin resistance caused by high-fat diet, while no effects of insulin resistance were observed with permethrin in low-fat diet. The actual doses of permethrin consumed were similar in both low- and high-fat diets, thus we do not think the different effects of permethrin on insulin resistance in low vs. high-fat diet were due to different doses of permethrin delivered. Currently, it is not clear how permethrin worsened high-fat diet induced insulin resistance in this model. It is possible, although, that due to highly lipophilic characteristics of permethrin, absorption may be more efficient with high dietary fat compared to that of low-fat diet. In fact, acute oral toxicity studies found that permethrin is much more toxic when dissolved in oil than other vehicles or its undiluted form [18].

The current results showed that permethrin significantly increased blood insulin and glucose levels, which indicated increased risk of type 2 diabetes. Generally, the maximal level of permethrin was reached within 4h after oral administration and then cleared from plasma with a elimination half-life of approximately 12h [314]. In addition, the

metabolic rate of permethrin in mammals also depends on different tissues and stereochemical configurations of permethrin. In this study, we used mixtures of *cis* and *trans* isomers of permethrin. Both the *cis* and *trans* isomers of permethrin were quickly metabolized in internal organs and tissues including the liver, kidney, muscle, spleen except fat, where the *cis* isomer was found to be much more difficult to eliminate with an estimated half-life of 12 days [315]. Major metabolites of permethrin mainly include 3-phenoxybenzoic acid (free and glucuronide and glycine conjugates) and the *trans*- and *cis*-dichlorovinyldimethylcyclopropane-carboxylic acids (free and glucuronide conjugates) [16]. The metabolites of permethrin were found to be of lower toxicity than the parent compound and are readily excreted [18, 315].

The current results showed that there was a significant effect of permethrin on decreasing voluntary activities of mice ($p = 0.0069$). It is known that pyrethroids can target neurons and influence physical activities in anthropods as well as mammals [319-322]. It was previously shown that daily intraperitoneal injection of deltamethrin, at 8.3-41.5 mg/kg body weights for 1-28 days, significantly decreased locomotive activity in mice [321]. A study reported that permethrin is more easily accumulated in the nervous system than plasma due to its lipophilic activity [314]. The maximum amounts of permethrin in nervous system (e.g. cerebellum, hippocampus, caudate putamen, frontal cortex, hypothalamus, and sciatic nerve) were about 1.5~7.5 times higher than in plasma after oral administration [314].

Our results indicated that there was a sex-dependent effect of permethrin on body weight. Permethrin treatment potentiated high-fat induced weight gain only in male mice as shown in previous chapter, but not in female mice. Previous studies on

organophosphorus insecticides and organochlorine insecticides also reported sex-selective metabolic disorder caused by insecticides [137, 144, 323]. Oral exposure to organochlorine insecticide, hexachlorobenzene, at 20 & 100 ppm in diet for four weeks, was reported to increase weight gain only in male rats [137]. Neonatal exposure to organophosphorus insecticide, parathion, increased body weight gain in males, but decreased body weight gain in female rats at 0.1 mg/kg BW/day [180]. Developmental exposure to organophosphorous insecticide, chlorpyrifos, initiated weight gain only in male offspring of rats [144]. Currently, we have limited knowledge about the sex-dependent effects of permethrin on weight gain. It is likely that the differences in male and female endocrine systems might contribute to this sex-dependent effect [324].

Development of insulin resistance is a major symptom for type 2 diabetes. Insulin acts via insulin receptor by auto-phosphorylation and then phosphorylates insulin receptor substrate (IRS) [52]. The phosphorylated tyrosines in IRS serve as "docking site" for other proteins that has SH2 (Ser-homology-2) domains (e.g. PI(3)K) and thus regulate their activities or subcellular locations [52]. Alternatively, IRS1 can undergo serine phosphorylation, which exerts inhibitory effect on insulin signaling [325]. Once activated, IRS can activate phosphoinositide 3-kinase (PI3K), which subsequently phosphorylate PDK1 and activate protein kinase B (Akt). Activated Akt increases translocation of glucose transporter 4 (GLUT4) from cytoplasm to plasma membrane to facilitate glucose uptake [52]. Thus, the role of Akt in insulin-stimulated glucose uptake is essential. Akt can be phosphorylated by PDK1 at Thr308 and by mTORC2 at Ser473 [201, 326-328]. Phosphorylation of both sites are required for Akt activation and the phosphorylation of Ser473 is not dependent upon phosphorylation of Thr308 or vice versa [327]. Our results

showed that permethrin impaired Akt activation by influencing Akt phosphorylation at Thr308, but not Ser473 in the gastrocnemius skeletal muscle. These results indicated that permethrin might target upstream of PDK1 to impair glucose uptake. Previously, it was suggested that permethrin can significantly reduce insulin-stimulated glucose uptake in C2C12 muscle cells by decreasing pAkt Thr308 via PDK1, but not Ser473 [201], which is consistent with current results. In addition, our results indicated that permethrin might influence glucose uptake by decreasing GLUT4 protein level in muscle, which is consistent with results from chapter 4.

Pyrethroid insecticides were previously shown to disturb glucose homeostasis and potentially increase the risk of diabetes in human and animals [181, 182, 184, 185, 300]. This study demonstrates that exposure to permethrin can induce high-fat diet induced insulin resistance without any significant influence on weight gain in female mice.

CHAPTER 6

PERMETHRIN POTENTIATES ADIPOGENESIS IN 3T3-L1 ADIPOCYTES VIA ALTERATION OF INTRACELLULAR CALCIUM AND ENDOPLASMIC RETICULUM STRESS

6.1 Introduction

Permethrin, a pyrethroid insecticide, was previously reported to potentiate obesity and insulin resistance *in vivo* as shown in previous chapters. In addition, our group also reported that permethrin can potentiate adipogenesis and insulin resistance *in vitro* [201]. However, the detailed molecular mechanisms underlying this permethrin-induced metabolic disorder have not been fully explored. Thus, the purpose of this chapter is to study the potential mechanism of permethrin-potentiated adipogenesis and insulin resistance in 3T3-L1 adipocytes.

The neurotoxicity of permethrin in insects as well as in mammals relies on its ability to bind and delay the closing of voltage sensitive sodium channels, allowing increased permeability of sodium ions [329]. At high oral doses, permethrin can produce syndromes, such as aggressive sparring, increased sensitivity to external stimuli, tremor, and prostration [330-332]. Low oral doses of permethrin can lead to dose-dependent decrease in locomotor activities and increase in the sensitivity of startle response to acoustic stimulus [322].

In addition to sodium channel, pyrethroid insecticides are also known to influence the function of voltage-sensitive calcium channels, resulting in increased calcium influx [18, 333, 334]. In fact, calcium channel α1 subunits belongs to a multigene family, which is evolutionarily related to the voltage-sensitive sodium channel gene family [18]. Previous *in*

vitro and *in vivo* studies in human and animals have shown that increased intracellular calcium is linked with augmented obesity, adipogenesis, as well as insulin resistance [335-338]. Thus, it is possible that permethrin may potentiate adipogenesis via increasing intracellular calcium level.

Endoplasmic reticulum (ER) is an important organelle in eukaryotic cells, which is responsible for protein synthesis, folding and dispatch [259]. In addition, ER also plays important roles for calcium storage, lipid metabolism, steroid hormone production, and detoxification of endogenous and exogenous compounds[339]. When there is an accumulation of unfolded or misfolded protein in the lumen of ER, the cell will activate a stress signaling pathway that leads to halting of protein translation, degrading unfolded and/or misfolded proteins, and increasing the manufacture of molecular chaperones involved in protein folding, which is called unfolded protein response (UPR) or ER stress [339]. Other factors that disrupt normal ER homeostasis, such as lipid accumulation, calcium depletion, changes in redox or energy status, etc., can also cause ER stress [339]. The aim of UPR is to restore normal ER function; however, prolonged UPR can often lead to inflammation and programmed cell death [340]. Recent study reported that pyrethroid insecticide deltamethrin can induce ER stress in nerve cells [42]. As ER stress is also linked with obesity, adipogenesis and insulin resistance [259, 339, 341, 342], we therefore propose that permethrin may potentiate adipogenesis via mediating intracellular calcium level and ER stress in 3T3-L1 adipocytes.

6.2 Materials and methods

6.2.1 Materials

Permethrin (98%, mixture of *cis* and *trans* isomers) were purchased from Sigma Aldrich Co. (St. Louis, MO). 3T3-L1 preadipocytes were purchased from American Type Culture Collection (Manassas, VA). Dubelco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), methylisobutylxanthin, dexamethasone, insulin, dimethyl sulfoxide, and protease inhibitor cocktail were purchased from Sigma-Aldrich (St. Louis, MO). Fura-2 in acetoxymethyl (AM) ester and BCA protein assay kit were purchased from Thermo Fisher Scientific (Agawam, MA).

Radioimmunoprecipitation assay buffer with ethylenediaminetetraacetic acid and ethylene glycol tetraacetic acid was purchased from Boston Bioproducts (Ashland, MA). Rabbit antibodies of inositol-requiring enzyme 1 alpha (IRE1α), phosphorylated protein kinase R-like endoplasmic reticulum kinase (p-PERK), PERK, X-box binding protein 1s (XBP1s), phosphorylated eukaryotic translation initiation factor 2 alpha (p-eIF2α), eIF2α, calmodulin (CaM) and goat antibodies of calcium/calmodulin-dependent protein kinase kinase 2 (CaMKKβ) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit antibodies of phosphorylated IRE1α (p-IRE1α) were obtained from Abcam (Cambridge, MA). Rabbit antibodies of phosphorylated insulin receptor substrate 1 Ser307 (p-IRS1 Ser307), binding immunoglobulin protein (BiP), endoplasmic reticulum oxidoreductase 1 alpha (Ero1-Lα) and mouse antibodies of C/EBP homologous protein (CHOP) were purchased from Cell Signaling Technology (Danvers, MA). Horseradish peroxidase-conjugated anti-rabbit secondary antibody was obtained from Cell Signaling Technology

(Danvers, MA). Other chemicals were either purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA). High capacity cDNA reverse transcription kit, real time PCR primers and Taqman gene expression master mix were obtained from Applied Biosystem (Carlsbad, CA). Other chemicals were either from Thermo Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO).

6.2.2 3T3-L1 cell culture

3T3-L1 preadipocytes were cultured according to a method described previously [23]. Briefly, 3T3-L1 preadipocytes were maintained in 5% CO_2 at 37˚C with DMEM containing 10% FBS and 1% penicillin-streptomycin until confluence (day -2). Two days after confluence, adipocyte differentiation was induced with a mixture of methylisobutylxanthin (0.5 mM), dexamethasone (1 µM), and insulin (1 µg/mL) in DMEM containing 10% FBS (Day 0). On day 2, this medium was replaced with DMEM containing 10% FBS and insulin (1 µg/mL) only. On day 4, cells were treated with DMEM containing 10% FBS only. On day 4 and thereafter, medium consisting of DMEM plus 10% FBS was subsequently replaced with fresh medium at 2 day intervals until harvest. Permethrin was dissolved in dimethyl sulfoxide (DMSO) prior to addition to culture medium to achieve various treatment concentrations (0.01, 0.1, 1, & 10 µM). These concentrations are based on the observation that serum and adipose tissue concentrations of cypermethrin administered at 2 mg/kg body weight for 10 weeks were in the range of 2-12 µM, while acceptable daily intake of permethrin is 0.05 mg/kg body weight (~60 nM) [18, 265]. Overall DMSO concentration in culture medium including control and permethrin treatment groups were 0.02%.

6.2.3 Measurement of intracellular calcium

Intracellular calcium was determined using Fura-2 acetoxymethyl (AM) ester, a ratiometric fluorescence Ca^{2+} indicator, as described previously with slight modifications [343, 344]. Briefly, 3T3-L1 cells were seeded in 96-well plate and induced to differentiation (day 0). Permethrin treatment was started on day 0 of differentiation until harvest. On day 6, cells were treated with Fura-2-AM- containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-ringer buffer for 1 h at 37 °C. Then, the HEPES-ringer buffer was removed and the cells were washed with phosphate-buffered saline (PBS) twice. The cells were then incubated in PBS for a further 30 minutes to allow complete de-esterification of intracellular acetoxymethyl esters. The intracellular Ca^{2+} level were calculated by using excitation at 340 nm and 380 nm and emission at 510 nm [345].

6.2.4 Western blot analysis

Cell lysates were prepared in radioimmunoprecipitation assay (RIPA) buffer with ethylenediaminetetraacetic acid (EDTA) and ethylene glycol tetraacetic acid (EGTA) supplemented with protease inhibitor cocktail and phosphatase inhibitors. Protein concentrations were determined using BCA protein assay kit. Aliquots from the cell lysates were separated using 10% SDS-polyacrylamide gel and transferred to an Immobilin P membrane (Millipore, Bedford, MA). Primary antibodies were purchased from either Cell Signaling Technologies (Danvers, MA) or Santa Cruz Biotechnologies (Dallas, TX). Detection were performed using an enhanced chemiluminescence solution

(GE Healthcare, Piscataway, NJ) with an Image Station 4000MM (Carestream Health, New Haven, CT). Blot image and results were quantified using Image J software.

6.2.5 Real time PCR analysis

Cells were harvested with Trizol reagent to extract total RNA under RNase free condition. Then total RNA was reverse transcribed to cDNA using high-capacity reverse transcription kit (Applied Biosystems, Carlsbad, CA). mRNA level of tumor necrosis factor alpha (TNFα, Mm00443258_m1), calmodulin (Mm01336281_g1), calcium/calmodulin dependent protein kinase kinase 2 (CaMKKβ, Mm00520236_m1) were analyzed by performing Taqman probe-based gene expression analysis (Applied Biosystems, Carlsbad, CA). 18s rRNA (Mm03928990_g1) was used as an internal standard.

6.2.6 Statistical analysis

Data were expressed as mean ± S.E. and analyzed with SAS program by one-way ANOVA with Tukey's range test (SAS 9.3, Cary, NC). Significance of differences was defined at the $P < 0.05$ level.

6.3 Results

6.3.1 Permethrin treatment dose-dependently increased intracellular calcium level

Intracellular calcium, as a ubiquitous second messenger, plays important role in regulating cellular activities, such as cell growth, proliferation, and apoptosis [346, 347] .

Recent evidence demonstrated that intracellular calcium signaling is linked with obesity, adipogenesis and insulin resistance [336-338, 348]. Fig 6.1. shows effect of permethrin on intracellular calcium level in 3T3-L1 adipocytes. Permethrin treatment (0.01, 0.1, 1, & 10 μM) dose-dependently increased intracellular calcium level compared to control in 3T3-L1 adipocytes ($p < 0.05$).

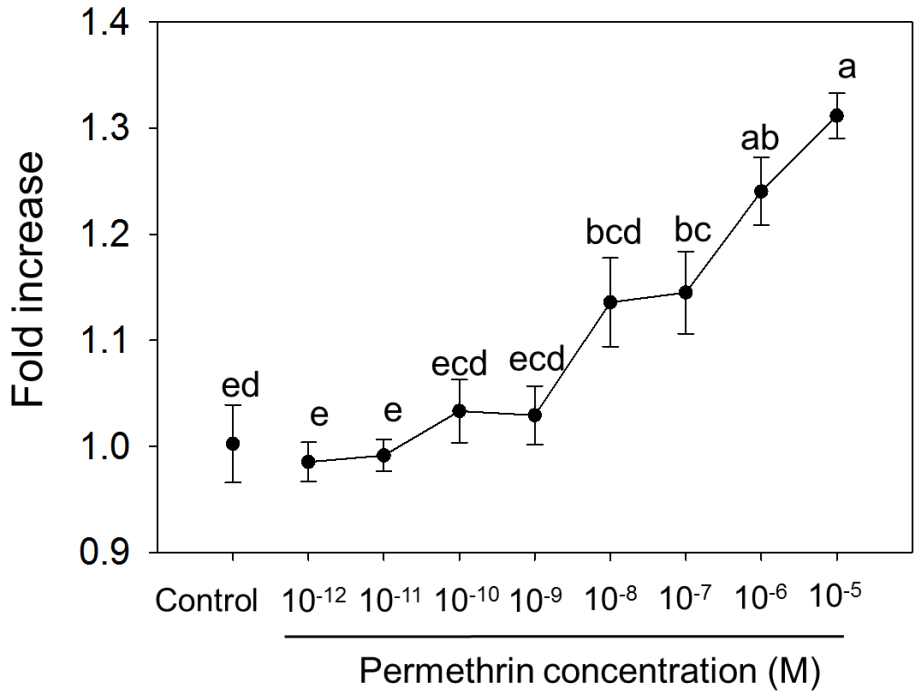


Figure 6.1. Permethrin dose-dependently increased intracellular calcium level in 3T3-L1 adipocytes. 3T3-L1 cells were treated with permethrin (10^{-12} ~ 10^{-5}M) for 6 days of differentiation. Intracellular calcium level was measured on day 6 with a fluorescent calcium indicator fura-2 in acetyoxymethyl form. Numbers are mean ±SE (n=6). Means with different letters are significantly different ($p < 0.05$).

6.3.2 Permethrin treatment dose-dependently increased calmodulin (CaM) and calcium/calmodulin dependent protein kinase kinase 2 (CaMKKβ) in 3T3-L1 adipocytes

Calmodulin is a ubiquitous calcium-binding protein present in all eukaryotic cells [349]. Permethrin treatment (10 μM) significantly increased calmodulin gene expression (*p*

= 0.0123) and protein level ($p < 0.05$) compared to control in 3T3-L1 adipocytes. CaMKKβ plays a role in calcium/calmodulin-dependent (CaM) kinase cascade and acts as one of the upstream activators of AMP-activated protein kinase (AMPK) [350]. Our results showed that permethrin (10 µM) increased both gene expression and protein level of CaMKKβ compared to control in 3T3-L1 adipocytes ($p < 0.05$).

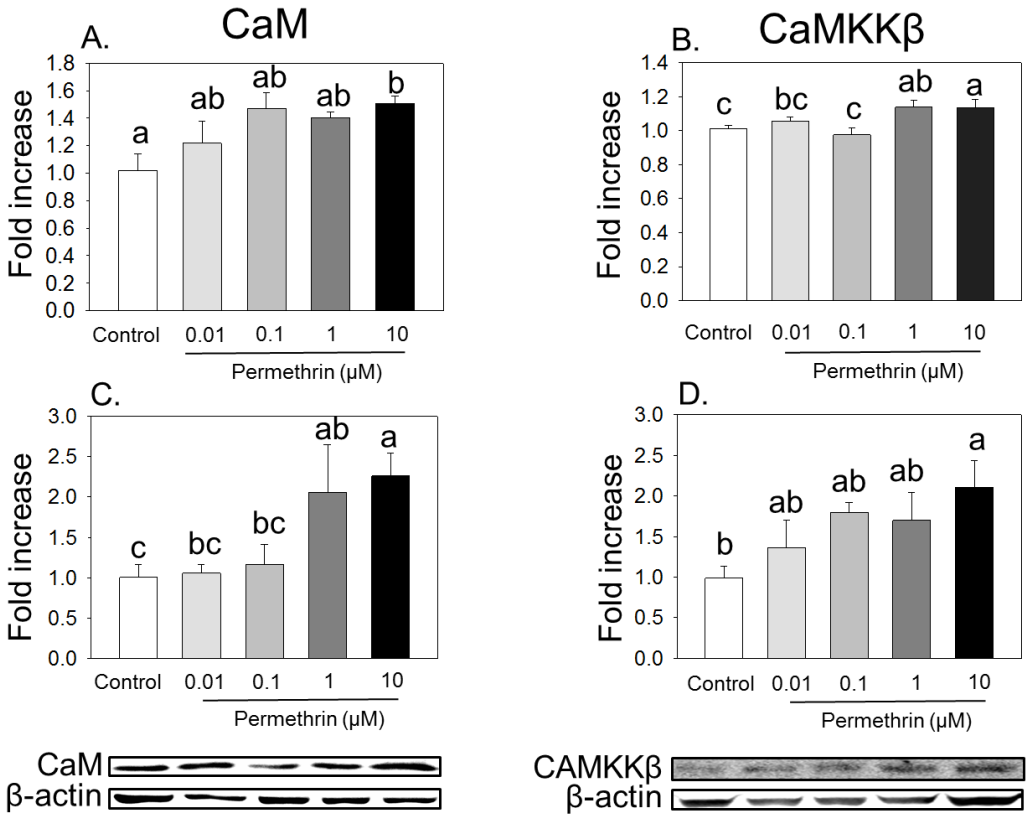


Figure 6.2. Permethrin dose-dependently increased calmodulin (CaM) and calcium/calmodulin dependent protein kinase kinase 2 (CaMKKβ) gene expression and protein level in 3T3-L1 adipocytes. 3T3-L1 cells were treated with permethrin (0.01, 0.1, 1, & 10 µM) for 6 days of differentiation. Cells were harvested on day 6. Gene expression and protein level of CaM (Fig. 2A & 2C, respectively) and CaMKKβ (Fig. 2B & 2D, respectively) were shown. Numbers are mean ± SE (n=3-4). Means with different letters are significantly different ($p < 0.05$).

6.3.3 Permethrin treatment significantly increased ER stress

BiP, a molecular chaperone localized in the lumen of ER, binds to misfolded and newly synthesized proteins and prepares them for subsequent folding, oligomerization or degradation [339]. In addition, Ero1-Lα, an ER membrane-associated N-glycoprotein, is also involved in protein folding [351]. Both BiP and Ero1-Lα are upregulated under ER stress [339, 351]. Our results showed that treatment of permethrin (10 μM) significantly increased level of BiP and Ero1-Lα ($p < 0.05$) than control. PERK is an ER transmembrane protein kinase that phosphorylates the α subunit of translation initiation factor 2 (eIF2α) at Ser 51 in response to ER stress [259]. The phosphorylation status of PERK and eIF2α is therefore an important marker of the presence of ER stress [259]. Phosphorylation of eIF2α subsequently induces expression of CHOP, which is another downstream marker of PERK and eIF2α pathway under ER stress [352]. Permethrin treatment (1 & 10 μM) significantly increased phosphorylation of PERK and eIF2α ($p < 0.05$) as well as protein level of CHOP than control ($p < 0.05$). Another important ER stress sensor is inositol-requiring enzyme 1 (IRE1), an ER transmembrane protein that can undergo phosphorylation to activate the UPR [353, 354]. The phosphorylation of IRE1 will lead to splicing of X-box binding protein (XBP1) mRNA into transcription factor XBP1s, which subsequently increase the synthesis of ER chaperones and phospholipids [339]. Our results showed that permethrin treatment (10 μM) significantly increased phosphorylation and protein level of IRE1α than control ($p < 0.05$). In addition, the level of XBP1s is also significantly increased by permethrin (0.1, 1, & 10 μM) than control ($p < 0.05$). Taken together, these results indicated that permethrin treatment significantly increased ER stress in 3T3-L1 adipocytes.

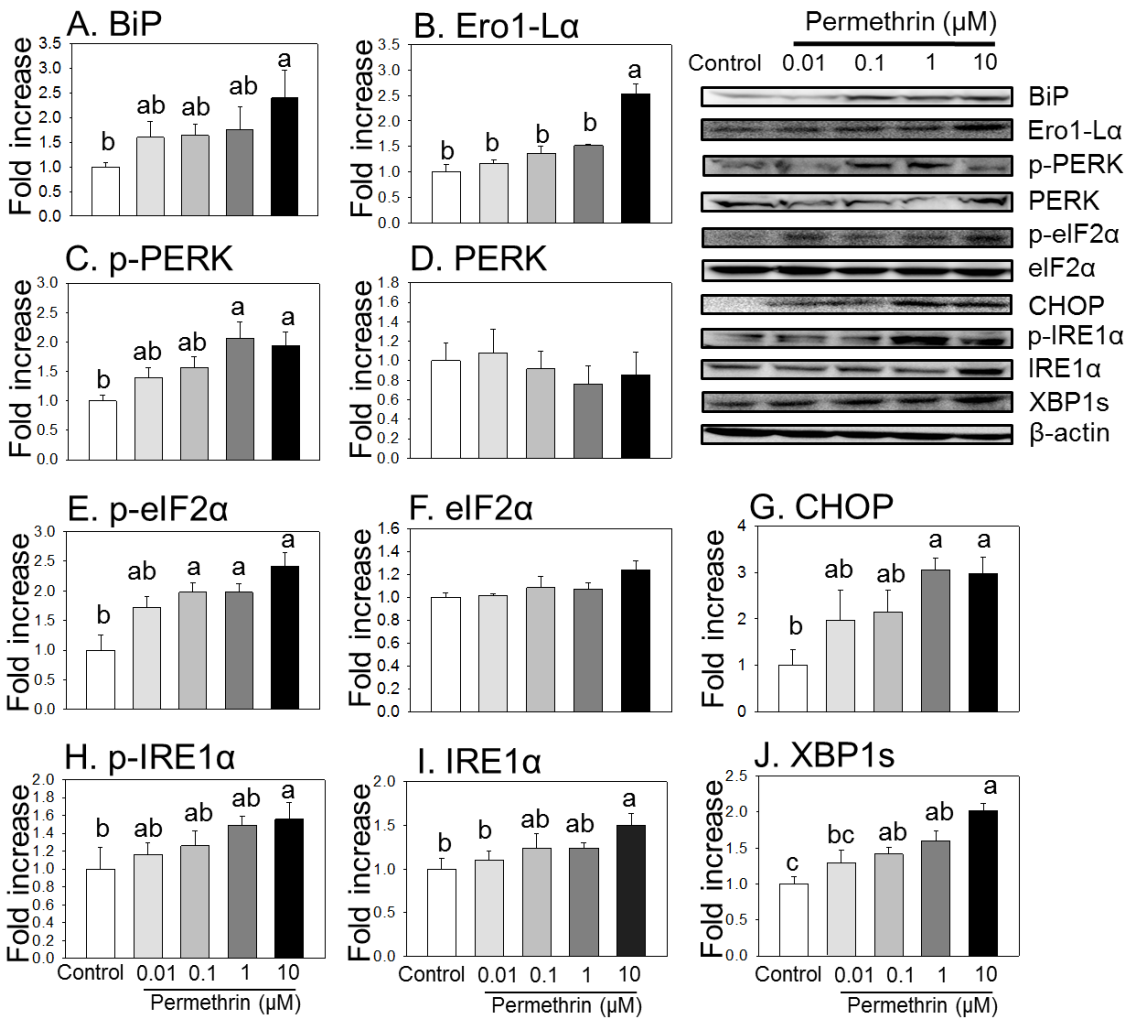
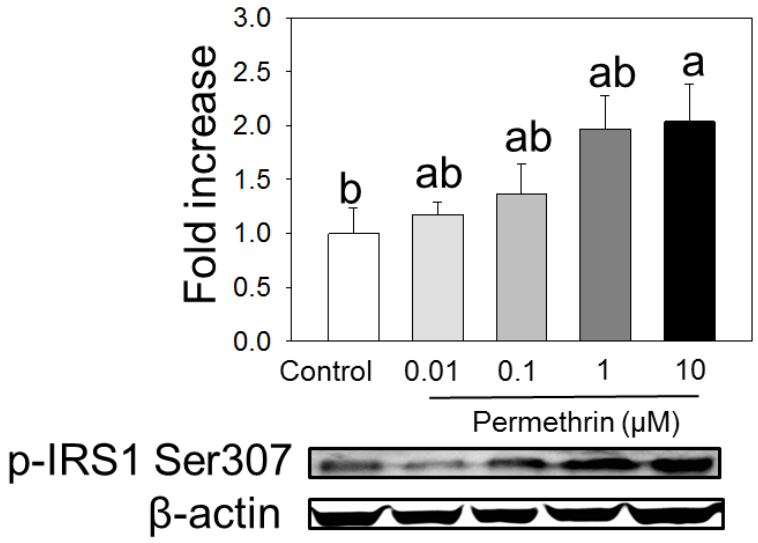


Figure 6.3. Permethrin induced ER stress in 3T3-L1 adipocytes. 3T3-L1 cells were treated with permethrin (0.01, 0.1, 1, & 10 μM) for 6 days of differentiation. Cells were harvested on day 6 for immunoblotting. Protein expression of binding immunoglobulin protein (BiP, Fig. 3A); endoplasmic reticulum oxidoreductase 1 alpha (Ero1-Lα, Fig. 3B); phosphorylated RNA-like endoplasmic reticulum kinase (p-PERK, Fig. 3C); PERK (Fig. 3D); phosphorylated eukaryotic initiation factor 2 alpha (p-eIF2α, Fig. 3E); eIF2α (Fig. 3F); C/EBP homologous protein (CHOP, Fig. 3G); phosphorylated inositol-requiring enzyme 1 alpha (p-IRE1α, Fig. 3H); IRE1α (Fig. 3I); X-box binding protein 1s (XBP1s, Fig. 3J) were shown. Numbers are mean ±SE (n=3-4). Means with different letters are significantly different ($p < 0.05$).

6.3.4 Permethrin treatment significantly increased serine phosphorylation of insulin receptor substrate 1



Phosphorylation of IRS1 at Serine 307 is known to cause insulin resistance [325].

The results showed that permethrin treatment (10 μM) significantly increased p-IRS1 Ser307 ($p < 0.05$).

Figure 6.4. Permethrin increased serine phosphorylation of insulin receptor substrate 1 (IRS1) in 3T3-L1 adipocytes. 3T3-L1 cells were treated with permethrin (0.01, 0.1, 1, & 10 μM) for 6 days of differentiation. Cells were harvested on day 6 for immunoblotting. Protein expression of phosphorylated insulin receptor substrate 1 at Serine 307 (p-IRS1 Ser307) were shown. Numbers are mean ±SE (n=3-4). Means with different letters are significantly different ($p < 0.05$).

6.3.5 Permethrin treatment significantly increased gene expression of inflammatory markers

We further investigated the influence of permethrin treatment on gene expression of TNFα, one of the key regulators in inflammatory response. All permethrin treatment (0.01, 0,1, 1, & 10 μM) significantly increased TNFα gene expression level than control.

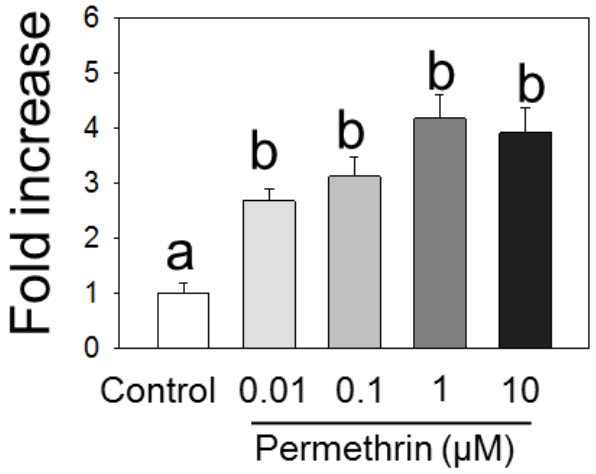


Figure 6.5. Permethrin increased mRNA expression of tumor neocrosis factor α (TNFα) in 3T3-L1 adipocytes. 3T3-L1 cells were treated with permethrin (0.01, 0.1, 1, &10 μM) for 6 days of differentiation and then RNA were extracted using Trizol solution. Extracted RNA were reverse transcribed to cDNA and then real time PCR (RT-PCR) were performed. Relative quantities of gene expression with RT-PCR were calculated relative to 18S ribosomal RNA. Numbers are mean ±SE (n=3). Means with different letters are significantly different ($p < 0.05$).

6.4 Discussion

Results from current study suggest that permethrin increased intracellular calcium and ER stress in 3T3-L1 adipocytes. To our knowledge, this is the first report linking alteration of calcium and ER stress in 3T3-L1 adipocytes by permethrin treatment.

The current results showed that permethrin dose-dependently increased intracellular calcium level in 3T3-L1 adipocytes. In fact, pyrethroid insecticides were known to influence the function of voltage-sensitive calcium channels, resulting in

increased calcium influx [18, 333, 334]. Permethrin and other pyrethroids (e.g., cyfluthrin, deltamethrin) were potent enhancers of both calcium uptake and neurotransmitter release via influencing N-type voltage sensitive calcium channels [329, 333]. Cismethrin and deltamethrin were shown to increase intracellular calcium in rat synaptosomes [333]. Deltamethrin were also shown to increase intracellular calcium and ER stress in neuroblastoma cells [42]. As calcium channel α1 subunits is genetically related to the voltage-sensitive sodium channel [18], it is likely that permethrin may act directly on the VSSC to increase calcium influx.

Another important intracellular compartment that might contribute to elevated intracellular calcium level is the ER. ER serves as an important intracellular calcium store with calcium concentration approximately 1000 times higher than in the cytosol [346]. The calcium concentration in the ER is mainly regulated by three types of proteins: (1) Ca^{2+} pumps [e.g. sacro/endoplasmic-reticulum Ca^{2+} -ATPase (SERCA)] for uphill transport of Ca^{2+} from the cytosol to the ER lumen; (2) Ca^{2+} binding proteins [e.g. calsequestrin, calreticulin, calnexin, 78-kDa glucose-regulated protein/immunoglobulin heavy chain binding protein (GRP78/BiP), GRP94, and various protein disulfide isomerases (PDI)] for storage of calcium in the ER and; (3) Ca^{2+} channels regulating ER calcium release to cytosol along its electrochemical gradient [(e.g. ryanodine-receptors (RyR) or the inositol 1,4,5,-trisphosphate (IP_3)-receptors (IP_3R)] [355].

In normal conditions, the $[Ca^{2+}]_{ER}$ is maintained at a relative stable level to prevent ER Ca^{2+} depletion or overload [355]. The release of Ca^{2+} from ER will trigger a series of cellular responses that lead to an increase of Ca^{2+} entry into the cytosol of the cell, a phenomenon known as capacitative calcium entry or "store-operated" Ca^{2+} entry

[356]. Two key players involved in this process are STIM1 and STIM2 proteins, which are ubiquitously expressed transmembrane proteins with a luminal Ca^{2+} sensor [357, 358]. The depletion of ER Ca^{2+} can lead to the oligomerization of STIM1 or STIM2 and interact with Orai proteins [358]. These tetrameric Ca^{2+} channels in the plasma membrane are then responsible for an increased Ca^{2+} entry [359-361]. Recent evidence shows that ER calcium depletion is also linked with ER stress [355].

In this study, permethrin increased the protein level of Ero1-Lα, an oxidoreductase enzyme involved in protein folding by catalyzing the formation and isomerization of protein disulfide bonds in the ER of eukaryotes [362]. In addition to its role in protein folding and oxidation, Ero1-Lα is also involved in regulating ER calcium release [351]. Upregulation of Ero1-Lα leads to loss of ER calcium and hyper-oxidation of ER lumen, which in turn triggers ER stress [351]. In this study, we did not measure calcium level in the ER. It is likely that permethrin caused ER calcium depletion, which subsequently lead to store-operated calcium entry from outside of the cell into the cytosol of the cell.

A large amount of experimental evidence support that ER stress is associated with obesity, adipogenesis, and insulin resistance [257-259, 341, 342, 363, 364]. Three canonical ER stress pathways have been discovered (Fig. 6.6), which are initiated with three different ER stress transducers: PERK, IRE1, and activating transcription factor 6 (ATF6). These transducers are present in the ER membrane and under normal conditions bind to ER chaperones BiP. Increased binding of BiP to luminal misfolded proteins and its dissociation from PERK, IRE1, and ATF6, will activate these transducers. PERK activation will further phosphorylate eIF2α, which slows protein translation and relieve

ER workload. eIF2α phosphorylation induces expression of CHOP, which is involved in apoptosis [339]. The current results showed that permethrin dose-dependently increased the phosphorylation of PERK, eIF2α and the protein level of CHOP. These results indicate that permethrin can cause ER stress by activating PERK pathway. As CHOP is linked with increased gene expression in inflammatory responses [339]. This is consistent with our results that permethrin treatment significantly increased TNFα level than control.

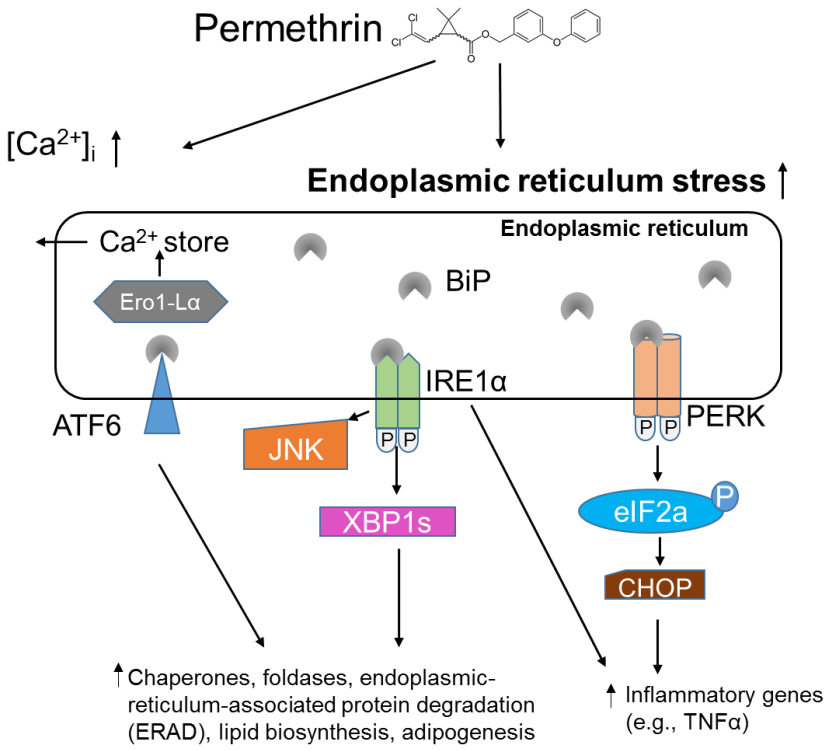


Figure 6.6. Potential mechanism of permethrin-potentiated adipogenesis and insulin resistance in 3T3-L1 adipocytes. Permethrin increases intracellular calcium level and ER stress pathway evidenced by elevated protein levels of binding immunoglobulin protein (BIP) and endoplasmic reticulum oxidoreductase 1 alpha (Ero1-Lα). Permethrin also activates ER stress transducer inositol-requiring enzyme 1 alpha (IRE1α) and protein kinase R-like endoplasmic reticulum kinase (PERK). IRE1 activation causes activation of Jun N terminal kinase (JNK) and splicing of X-box binding protein (XBP1) mRNA. The splicing of XBP1 leads to the translation of transcription factor XBP1s, which upregulate ER chaperones, endoplasmic reticulum-associated protein degradation (ERAD), lipid biosynthesis and adipogenesis. Activated PERK will further phosphorylate eukaryotic translation initiation factor 2 (eIF2α), induces expression of C/EBP homologous protein (CHOP), which increases inflammatory responses. Permethrin may activate ATF6, leading to similar results as IRE1α-XBP1s pathway.

IRE1 activation causes splicing of XBP1 mRNA and translation into the transcription factor XBP1s [339]. XBP1s upregulates ER chaperones, components of the endoplasmic-reticulum-associated protein degradation (ERAD) machinery and phospholipid biosynthesis [339]. The current results indicated that permethrin can activate the IRE1-XBP1s pathway as indicated by increased phosphorylation of IRE1 and protein

level of XBP1s. In addition, IRE1 can also activates Jun N terminal kinase (JNK) by recruiting the scaffold protein tumor necrosis factor receptor-associated factor 2 (TRAF2) [339]. Activated JNK is known to cause insulin resistance via phosphorylation of IRS1 Ser307 [365, 366]. This is consistent with our finding that permethrin treatment significantly increased phosphorylation of IRS1 at Ser 307. In addition, recent evidence also demonstrated that the IRE1α-XBP1s pathway is indispensable for adipogenesis [341]. Currently, we do not have direct evidence that permethrin treatment potentiated adipogenesis via IRE1α-XBP1s pathway. Future studies using IRE1α- or XBP1- deficient 3T3-L1 adipocytes by permethrin treatment are needed to confirm our hypothesis.

ATF6 is another ER stress transducer present in the ER membrane. ATF6 can also regulate genes involved in ERAD, lipid biosynthesis, and ER expansion in addition to IRE1 pathway [339, 367]. In this study, we did not measure the markers involved in ATF6 pathway. As it is also an important pathway for lipid biosynthesis, further studies are needed to investigate whether permethrin influence adipogenesis via ATF6.

In conclusion, our results showed that permethrin may potentiate adipogenesis via increasing of intracellular calcium level and ER stress. Future studies are needed to investigate the potential role of permethrin on ER stress *in vivo.*

CHAPTER 7

FUTURE DIRECTIONS

Currently, limited knowledge is known about how dietary fat contributed to permethrin's effect on weight gain and insulin resistance. The current results showed that permethrin potentiated weight gain and insulin resistance only in high-fat fed mice. Considering permethrin is highly lipophilic, it is likely that the absorption and accumulation of permethrin with high-fat diet would be more efficient than with low-fat diet. Further investigation on the absorption and accumulation efficacy of permethrin when delivered orally in low-fat and high-fat diet are needed.

Meanwhile, there is a growing evidence showing that early-life exposure to insecticides is associated with metabolic disorder later in life [144, 147, 151, 180, 368-370]. Children are more susceptible to chemical exposure because of higher food and water contamination per kilogram body weight, and higher surface-to-volume ratio [371]. In addition, the metabolic detoxification mechanisms are not fully developed in youth, which potentially increase the susceptibility to insecticides [372]. The current studies are limited to that adulthood exposure to permethrin can potentiate high-fat diet induced obesity and type 2 diabetes. Thus, future studies are needed to investigate whether early-life exposures (e.g. prenatal, neonatal, etc.) can induce metabolic change later in life, especially when other factors are present, such as diet, as well as sex dependent responses.

Calcium ions play important role in cellular signaling as well as in adipogenesis [335]. The current results showed that permethrin dose-dependently increased intracellular

calcium level in 3T3-L1 adipocytes; however, the mechanism how permethrin induces calcium increase remains unclear. Permethrin could directly act on the voltage sensitive calcium channel to cause extracellular calcium entry into 3T3-L1 adipocytes followed by calcium-induced calcium release from ER [373]; permethrin could directly cause calcium release from intracellular calcium storage sites (e.g. ER and/or mitochondria) followed by store-operated calcium entry from extracellular space [356]. Thus, future studies are needed to determine the route of permethrin-induced calcium mobilization in adipocytes. In addition, the current results demonstrate that permethrin induced ER stress in 3T3-L1 adipocytes. As elevated intracellular calcium and ER stress are linked with obesity and type 2 diabetes *in vivo* [259, 336], we speculate that permethrin, along with high-fat diet, could promote obesity and type 2 diabetes via calcium- and ER stress- mediated mechanism *in vivo*. Future studies are needed to verify our hypothesis.

BIBLIOGRAPHY

[1] Rosen, E. D.; Walkey, C. J.; Puigserver, P.; Spiegelman, B. M., Transcriptional regulation of adipogenesis. *Genes & development* 2000, *14*, 1293-307.

[2] Codru, N.; Schymura, M. J.; Negoita, S.; Akwesasne Task Force on, E.; Rej, R.; Carpenter, D. O., Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. *Environ Health Perspect* 2007, *115*, 1442-7.

[3] Dirinck, E.; Jorens, P. G.; Covaci, A.; Geens, T.; Roosens, L.; Neels, H.; Mertens, I.; Van Gaal, L., Obesity and persistent organic pollutants: possible obesogenic effect of organochlorine pesticides and polychlorinated biphenyls. *Obesity* 2011, *19*, 709-14.

[4] Everett, C. J.; Frithsen, I. L.; Diaz, V. A.; Koopman, R. J.; Simpson, W. M.; Mainous, A. G., Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and DDT with diabetes in the 1999-2002 National Health and Nutrition Examination Survey. *Environ Res* 2007, *103*, 413-418.

[5] Lee, D. H.; Lee, I. K.; Jin, S. H.; Steffes, M.; Jacobs, D. R., Association between serum concentrations of persistent organic pollutants and insulin resistance among nondiabetic adults. *Diabetes care* 2007, *30*, 622-628.

[6] Lee, D. H.; Lee, I. K.; Porta, M.; Steffes, M.; Jacobs, D. R., Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. *Diabetologia* 2007, *50*, 1841-1851.

[7] Lee, D. H.; Steffes, M. W.; Sjodin, A.; Jones, R. S.; Needham, L. L.; Jacobs, D. R., Low dose of some persistent organic pollutants predicts type 2 diabetes: a nested case-control study. *Environ Health Persp* 2010, *118*, 1235-1242.

[8] Lee, D. H.; Steffes, M. W.; Sjodin, A.; Jones, R. S.; Needham, L. L.; Jacobs, D. R., Jr., Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. *PloS one* 2011, *6*, e15977.

[9] Mendez, M. A.; Garcia-Esteban, R.; Guxens, M.; Vrijheid, M.; Kogevinas, M.; Goni, F.; Fochs, S.; Sunyer, J., Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. *Environ Health Perspect* 2011, *119*, 272-8.

[10] Montgomery, M. P.; Kamel, F.; Saldana, T. M.; Alavanja, C. R.; Sandler, D. P., Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993-2003. *Am J Epidemiol* 2008, *167*, 1235-1246.

[11] Rylander L, R.-H. A., and Hagmar L, A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. *Environ Health* 2005, *4*.

[12] Taylor, K. W.; Novak, R. F.; Anderson, H. A.; Birnbaum, L. S.; Blystone, C.; DeVito, M.; Jacobs, D.; Kohrle, J.; Lee, D. H.; Rylander, L.; Rignell-Hydbom, A.; Tornero-Velez, R.; Turyk, M. E.; Boyles, A. L.; Thayer, K. A.; Lind, L., Evaluation of the Association between Persistent Organic Pollutants (POPs) and Diabetes in Epidemiological Studies: A National Toxicology Program Workshop Review. *Environ Health Persp* 2013, *121*, 774-783.

[13] Thayer, K. A.; Heindel, J. J.; Bucher, J. R.; Gallo, M. A., Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environ Health Perspect* 2012, *120*, 779-89.

[14] Clark, J. M.; Symington, S. B., Advances in the mode of action of pyrethroids. *Top Curr Chem* 2012, *314*, 49-72.

[15] Soderlund, D. M., Resmethrin, the first modern pyrethroid insecticide. *Pest Manag Sci* 2015, *71*, 801-7.

[16] WHO, Environmental health criteria for permethrin, vol.94. WHO, Geneva. 1990.

[17] Vijverberg, H. P.; van den Bercken, J., Annotation. Action of pyrethroid insecticides on the vertebrate nervous system. *Neuropathol Appl Neurobiol* 1982, *8*, 421-40.

[18] Soderlund, D. M.; Clark, J. M.; Sheets, L. P.; Mullin, L. S.; Piccirillo, V. J.; Sargent, D.; Stevens, J. T.; Weiner, M. L., Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. *Toxicology* 2002, *171*, 3-59.

[19] Feo, M. L.; Eljarrat, E.; Barcelo, D., Determination of pyrethroid insecticides in environmental samples. *Trac-Trend Anal Chem* 2010, *29*, 692-705.

[20] Durand, R.; Bouvresse, S.; Berdjane, Z.; Izri, A.; Chosidow, O.; Clark, J. M., Insecticide resistance in head lice: clinical, parasitological and genetic aspects. *Clin Microbiol Infect* 2012, *18*, 338-44.

[21] Clark, J. M.; Yoon, K. S.; Lee, S. H.; Pittendrigh, B. R., Human lice: Past, present and future control. *Pestic Biochem Physiol* 2013, *106*, 162-171.

[22] National Research Council, Health Effects of Permethrin-Impregnated Army Battle-Dress Uniforms. 1994.

[23] Park, Y.; Kim, Y.; Kim, J.; Yoon, K. S.; Clark, J.; Lee, J.; Park, Y., Imidacloprid, a neonicotinoid insecticide, potentiates adipogenesis in 3T3-L1 adipocytes. *J Agric Food Chem* 2013, *61*, 255-9.

[24] Kim, J.; Park, Y.; Yoon, K. S.; Clark, J. M.; Park, Y., Imidacloprid, a neonicotinoid insecticide, induces insulin resistance. *The Journal of toxicological sciences* 2013, *38*, 655-60.

[25] Moreno-Aliaga, M. J.; Matsumura, F., Effects of 1,1,1-trichloro-2,2-bis (p-chlorophenyl)-ethane (p,p '-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. *Biochemical pharmacology* 2002, *63*, 997-1007.

[26] Howell, G. I. I. I.; Mangum, L., Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicology in vitro : an international journal published in association with BIBRA* 2011, *25*, 394-402.

[27] Casida, J. E.; Durkin, K. A., Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu Rev Entomol* 2013, *58*, 99-117.

[28] Unsworth, J., History of pesticide use, international union of pure and applied chemistry; http://agrochemicals.iupac.org/index.php?option=com_sobi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31. 2010.

[29] van Emden, H. F.; Peakall, D. B., Beyond silent spring. Springer. ISBN 978-0-412-72800-6. 1996.

[30] Karami-Mohajeri, S.; Abdollahi, M., Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review. *Hum Exp Toxicol* 2011, *30*, 1119-40.

[31] Coats, J. R., Mechanisms of toxic action and structure-activity relationships for organochlorine and synthetic pyrethroid insecticides. *Environ Health Perspect* 1990, *87*, 255-62.

[32] Casida, J. E.; Quistad, G. B., Golden age of insecticide research: past, present, or future? *Annu Rev Entomol* 1998, *43*, 1-16.

[33] NPIC, DDT General Fact Sheet. National Pesticide and Information Center. 1999.

[34] Davies, T. G.; Field, L. M.; Usherwood, P. N.; Williamson, M. S., DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB life* 2007, *59*, 151-62.

[35] USDA, Pesticide Data Program Annual Summary. United States Department of Agriculture. 2014.

[36] Abou-Donia, M. B., Organophosphorus ester-induced chronic neurotoxicity. *Arch Environ Health* 2003, *58*, 484-97.

[37] Nauen, R.; Bretschneider, T., New modes of action of insecticides. *Pestic Outlook* 2002, *13*.

[38] Casida, J. E.; Quistad, G. B., Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets. *Chem Res Toxicol* 2004, *17*, 983-98.

[39] Sparks, T. C., Insecticide discovery: An evaluation and analysis. *Pestic Biochem Physiol* 2013, *107*, 8-17.

[40] Chambers, J. E., Toxicity of pesticides. In: Basic Environmental Toxicology, pp. 185-198. Cockerham, L. G. and Shane, B. S., Eds. CRC Press, Boca Raton. 1994.

[41] Risher, J. F.; Mink, F. L.; Stara, J. F., The toxicologic effects of the carbamate insecticide aldicarb in mammals: a review. *Environ Health Perspect* 1987, *72*, 267-81.

[42] Hossain, M. M.; Richardson, J. R., Mechanism of pyrethroid pesticide-induced apoptosis: role of calpain and the ER stress pathway. *Toxicological sciences : an official journal of the Society of Toxicology* 2011, *122*, 512-25.

[43] Narahashi, T., Neurophysiological effects of insecticides. In: Handbook of Pesticide Toxicology, pp. 335-351. Kreger, Eds. Academic Press, San Diego. 2001.

[44] Gervais, J. A.; Luukinen, B.; Buhl, K.; Stone, D., "Imidacloprid technical fact sheet" (PDF). National Pesticide Information Center. 2012.

[45] Chao, S. L.; Dennehy, T. J.; Casida, J. E., Whitefly (Hemiptera: Aleyrodidae) binding site for imidacloprid and related insecticides: a putative nicotinic acetylcholine receptor. *J Econ Entomol* 1997, *90*, 879-82.

[46] Matsuda, K.; Buckingham, S. D.; Kleier, D.; Rauh, J. J.; Grauso, M.; Sattelle, D. B., Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol Sci* 2001, *22*, 573-80.

[47] Sparks, T. C.; Nauen, R., IRAC: Mode of action classification and insecticide resistance management. *Pestic Biochem Physiol* 2015, *121*, 122-8.

[48] The European Commision, Decision by the European Commission to restrict the use of imidacloprid, thiamethoxam and clothianidin (Regulation EU No. 485/2013), 2016. http://eur-lex.europa.eu/eli/reg_impl/2013/485/oj.

[49] Stryer, L., Biochemistry. (Fourth Ed). New York: W.H. Freeman and Company. pp. 510–515, 581–613, 775–778. ISBN 0 7167 2009 1995.

[50] Spiegelman, B. M.; Flier, J. S., Adipogenesis and obesity: Rounding out the big picture. *Cell* 1996, *87*, 377-389.

[51] Klip, A.; Paquet, M. R., Glucose-Transport and Glucose Transporters in Muscle and Their Metabolic-Regulation. *Diabetes care* 1990, *13*, 228-243.

[52] Saltiel, A. R.; Kahn, C. R., Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001, *414*, 799-806.

[53] Kulkarni, R. N.; Bruning, J. C.; Winnay, J. N.; Postic, C.; Magnuson, M. A.; Kahn, C. R., Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 1999, *96*, 329-339.

[54] Bruning, J. C.; Michael, M. D.; Winnay, J. N.; Hayashi, T.; Horsch, D.; Accili, D.; Goodyear, L. J.; Kahn, C. R., A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Molecular cell* 1998, *2*, 559-569.

[55] Patti, M. E.; Kahn, C. R., The insulin receptor--a critical link in glucose homeostasis and insulin action. *J Basic Clin Physiol Pharmacol* 1998, *9*, 89-109.

[56] White, M. F., The IRS-signalling system: A network of docking proteins that mediate insulin action. *Molecular and cellular biochemistry* 1998, *182*, 3-11.

[57] Tamemoto, H.; Kadowaki, T.; Tobe, K.; Yagi, T.; Sakura, H.; Hayakawa, T.; Terauchi, Y.; Ueki, K.; Kaburagi, Y.; Satoh, S.; et al., Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 1994, *372*, 182-6.

[58] Araki, E.; Lipes, M. A.; Patti, M. E.; Bruning, J. C.; Haag, B., 3rd; Johnson, R. S.; Kahn, C. R., Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 1994, *372*, 186-90.

[59] Fasshauer, M.; Klein, J.; Kriauciunas, K. M.; Ueki, K.; Benito, M.; Kahn, C. R., Essential role of insulin receptor substrate 1 in differentiation of brown adipocytes. *Molecular and cellular biology* 2001, *21*, 319-29.

[60] Cross, D. A. E.; Alessi, D. R.; Vandenheede, J. R.; Mcdowell, H. E.; Hundal, H. S.; Cohen, P., The Inhibition of Glycogen-Synthase Kinase-3 by Insulin or Insulin-Like Growth-Factor-1 in the Rat Skeletal-Muscle Cell-Line-L6 Is Blocked by Wortmannin, but Not by Rapamycin - Evidence That Wortmannin Blocks Activation of the Mitogen-Activated Protein-Kinase Pathway in L6-Cells between Ras and Raf. *Biochemical Journal* 1994, *303*, 21-26.

[61] Standaert, M. L.; Galloway, L.; Karnam, P.; Bandyopadhyay, G.; Moscat, J.; Farese, R. V., Protein kinase C-zeta as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes - Potential role in glucose transport. *The Journal of biological chemistry* 1997, *272*, 30075-30082.

[62] Hotamisligil, G. S.; Peraldi, P.; Budavari, A.; Ellis, R.; White, M. F.; Spiegelman, B. M., IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996, *271*, 665-8.

[63] Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P., Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 1999, *283*, 1544-8.

[64] Ogg, S.; Ruvkun, G., The C-elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. *Molecular cell* 1998, *2*, 887-893.

[65] Clement, S.; Krause, U.; Desmedt, F.; Tanti, J. F.; Behrends, J.; Pesesse, X.; Sasaki, T.; Penninger, J.; Doherty, M.; Malaisse, W.; Dumont, J. E.; Le Marchand-Brustel, Y.; Erneux, C.; Hue, L.; Schurmans, S., The lipid phosphatase SHIP2 controls insulin sensitivity. *Nature* 2001, *409*, 92-97.

[66] Pessin, J. E.; Saltiel, A. R., Signaling pathways in insulin action: molecular targets of insulin resistance. *J Clin Invest* 2000, *106*, 165-9.

[67] Baumann, C. A.; Ribon, V.; Kanzaki, M.; Thurmond, D. C.; Mora, S.; Shigematsu, S.; Bickel, P. E.; Pessin, J. E.; Saltiel, A. R., CAP defines a second signalling pathway required for insulin-stimulated glucose transport. *Nature* 2000, *407*, 202-7.

[68] Chiang, S. H.; Baumann, C. A.; Kanzaki, M.; Thurmond, D. C.; Watson, R. T.; Neudauer, C. L.; Macara, I. G.; Pessin, J. E.; Saltiel, A. R., Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature* 2001, *410*, 944-8.

[69] Brady, M. J.; Nairn, A. C.; Saltiel, A. R., The regulation of glycogen synthase by protein phosphatase 1 in 3T3-L1 adipocytes. Evidence for a potential role for DARPP-32 in insulin action. *The Journal of biological chemistry* 1997, *272*, 29698-703.

[70] Pilkis, S. J.; Granner, D. K., Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annual review of physiology* 1992, *54*, 885-909.

[71] Sutherland, C.; O'Brien, R. M.; Granner, D. K., New connections in the regulation of PEPCK gene expression by insulin. *Philos Trans R Soc Lond B Biol Sci* 1996, *351*, 191-9.

[72] Nakae, J.; Park, B. C.; Accili, D., Insulin stimulates phosphorylation of the forkhead transcription factor FKHR on serine 253 through a Wortmannin-sensitive pathway. *The Journal of biological chemistry* 1999, *274*, 15982-5.

[73] Yoon, J. C.; Puigserver, P.; Chen, G. X.; Donovan, J.; Wu, Z. D.; Rhee, J.; Adelmant, G.; Stafford, J.; Kahn, C. R.; Granner, D. K.; Newgard, C. B.; Spiegelman, B. M., Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001, *413*, 131-138.

[74] Foretz, M.; Pacot, C.; Dugail, I.; Lemarchand, P.; Guichard, C.; Le Liepvre, X.; Berthelier-Lubrano, C.; Spiegelman, B.; Kim, J. B.; Ferre, P.; Foufelle, F., ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Molecular and cellular biology* 1999, *19*, 3760-3768.

[75] Savage, D. B.; Petersen, K. F.; Shulman, G. I., Mechanisms of insulin resistance in humans and possible links with inflammation. *Hypertension* 2005, *45*, 828-33.

[76] Bergman, R. N.; Ader, M., Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab* 2000, *11*, 351-6.

[77] Wu, Z. D.; Bucher, N. L. R.; Farmer, S. R., Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBP beta, C/EBP delta, and glucocorticoids. *Molecular and cellular biology* 1996, *16*, 4128-4136.

[78] Anthonsen, M. W.; Ronnstrand, L.; Wernstedt, C.; Degerman, E.; Holm, C., Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. *The Journal of biological chemistry* 1998, *273*, 215-221.

[79] Kitamura, T.; Kitamura, Y.; Kuroda, S.; Hino, Y.; Ando, M.; Kotani, K.; Konishi, H.; Matsuzaki, H.; Kikkawa, U.; Ogawa, W.; Kasuga, M., Insulin-induced phosphorylation and activation of cyclic nucleotide phosphodiesterase 3B by the serine-threonine kinase Akt. *Molecular and cellular biology* 1999, *19*, 6286-96.

[80] Boada, L. D.; Lara, P. C.; Alvarez-Len, E. E.; Losada, A.; Zurnbado, M. L.; Limihana-Canhal, J. M.; Apolinario, R.; Serra-Majem, L.; Luzardo, O. P., Serum levels of insulin-like growth factor-I in relation to organochlorine pesticides exposure. *Growth Horm Igf Res* 2007, *17*, 506-511.

[81] Aguirre, G. A.; De Ita, J. R.; de la Garza, R. G.; Castilla-Cortazar, I., Insulin-like growth factor-1 deficiency and metabolic syndrome. *J Transl Med* 2016, *14*, 3.

[82] Saldana, T. M.; Basso, O.; Hoppin, J. A.; Baird, D. D.; Knott, C.; Blair, A.; Alavanja, M. C. R.; Sandler, D. P., Pesticide exposure and self-reported gestational diabetes mellitus in the agricultural health study. *Diabetes care* 2007, *30*, 529-534.

[83] Amanvermez, R.; Baydin, A.; Yardan, T.; Basol, N.; Gunay, M., Emergency laboratory abnormalities in suicidal patients with acute organophosphate poisoning. *Turk J Biochem* 2010, *35*, 29-34.

[84] Wang, J.; Zhu, Y.; Cai, X.; Yu, J.; Yang, X.; Cheng, J., Abnormal glucose regulation in pyrethroid pesticide factory workers. *Chemosphere* 2011, *82*, 1080-2.

[85] Narendra, M.; Kavitha, G.; Helah Kiranmai, A.; Raghava Rao, N.; Varadacharyulu, N. C., Chronic exposure to pyrethroid-based allethrin and prallethrin mosquito repellents alters plasma biochemical profile. *Chemosphere* 2008, *73*, 360-4.

[86] Casida, J. E., Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. *J Agric Food Chem* 2011, *59*, 2923-31.

[87] Lee, D. H.; Lee, I. K.; Song, K.; Steffes, M.; Toscano, W.; Baker, B. A.; Jacobs, D. R., A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes - Results from the National Health and Examination Survey 1999-2002. *Diabetes care* 2006, *29*, 1638-1644.

[88] Son, H. K.; Kim, S. A.; Kang, J. H.; Chang, Y. S.; Park, S. K.; Lee, S. K.; Jacobs, D. R.; Lee, D. H., Strong associations between low-dose organochlorine pesticides and type 2 diabetes in Korea. *Environ Int* 2010, *36*, 410-414.

[89] Glynn, A. W.; Granath, F.; Aune, M.; Atuma, S.; Darnerud, P. O.; Bjerselius, R.; Vainio, H.; Weiderpass, E., Organochlorines in Swedish women: determinants of serum concentrations. *Environ Health Perspect* 2003, *111*, 349-55.

[90] Cox, S.; Niskar, A. S.; Narayan, K. M. V.; Marcus, M., Prevalence of self-reported diabetes and exposure to organochlorine pesticides among Mexican Americans: Hispanic Health and Nutrition Examination Survey, 1982-1984. *Environ Health Perspect* 2007, *115*, 1747-1752.

[91] Rignell-Hydbom, A.; Rylander, L.; Hagmar, L., Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. *Hum Exp Toxicol* 2007, *26*, 447-52.

[92] Philibert, A.; Schwartz, H.; Mergler, D., An exploratory study of diabetes in a First Nation community with respect to serum concentrations of p,p'-DDE and PCBs and fish consumption. *Int J Environ Res Public Health* 2009, *6*, 3179-89.

[93] Rignell-Hydbom, A.; Lidfeldt, J.; Kiviranta, H.; Rantakokko, P.; Samsioe, G.; Agardh, C. D.; Rylander, L., Exposure to p,p'-DDE: a risk factor for type 2 diabetes. *PloS one* 2009, *4*, e7503.

[94] Turyk, M.; Anderson, H.; Knobeloch, L.; Imm, P.; Persky, V., Organochlorine exposure and incidence of diabetes in a cohort of great lakes sport fish consumers. *Environ Health Persp* 2009, *117*, 1076-1082.

[95] Everett, C. J.; Matheson, E. M., Biomarkers of pesticide exposure and diabetes in the 1999-2004 national health and nutrition examination survey. *Environ Int* 2010, *36*, 398-401.

[96] Ukropec, J.; Radikova, Z.; Huckova, M.; Koska, J.; Kocan, A.; Sebokova, E.; Drobna, B.; Trnovec, T.; Susienkova, K.; Labudova, V.; Gasperikova, D.; Langer, P.; Klimes, I., High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. *Diabetologia* 2010, *53*, 899-906.

[97] Airaksinen, R.; Rantakokko, P.; Eriksson, J. G.; Blomstedt, P.; Kajantie, E.; Kiviranta, H., Association between type 2 diabetes and exposure to persistent organic pollutants. *Diabetes care* 2011, *34*, 1972-1979.

[98] Lee, D. H.; Lind, P. M.; Jacobs, D. R., Jr.; Salihovic, S.; van Bavel, B.; Lind, L., Polychlorinated biphenyls and organochlorine pesticides in plasma predict development of type 2 diabetes in the elderly: the prospective investigation of the vasculature in Uppsala Seniors (PIVUS) study. *Diabetes care* 2011, *34*, 1778-84.

[99] Faerch, K.; Hojlund, K.; Vind, B. F.; Vaag, A.; Dalgard, C.; Nielsen, F.; Grandjean, P., Increased serum concentrations of persistent organic pollutants among prediabetic individuals: potential role of altered substrate oxidation patterns. *J Clin Endocr Metab* 2012, *97*, E1705-E1713.

[100] Gasull, M.; Pumarega, J.; Tellez-Plaza, M.; Castell, C.; Tresserras, R.; Lee, D. H.; Porta, M., Blood concentrations of persistent organic pollutants and prediabetes and diabetes in the general population of Catalonia. *Environ Sci Technol* 2012, *46*, 7799-810.

[101] Arrebola, J. P.; Pumarega, J.; Gasull, M.; Fernandez, M. F.; Martin-Olmedo, P.; Molina-Molina, J. M.; Fernandez-Rodriguez, M.; Porta, M.; Olea, N., Adipose tissue concentrations of persistent organic pollutants and prevalence of type 2 diabetes in adults from Southern Spain. *Environ Res* 2013, *122*, 31-7.

[102] Pal, S.; Blais, J. M.; Robidoux, M. A.; Haman, F.; Krummel, E.; Seabert, T. A.; Imbeault, P., The association of type 2 diabetes and insulin resistance/secretion with persistent organic pollutants in two First Nations communities in northern Ontario. *Diabetes Metab* 2013, *39*, 497-504.

[103] Azandjeme, C. S.; Delisle, H.; Fayomi, B.; Ayotte, P.; Djrolo, F.; Houinato, D.; Bouchard, M., High serum organochlorine pesticide concentrations in diabetics of a cotton producing area of the Benin Republic (West Africa). *Environ Int* 2014, *69*, 1-8.

[104] Burns, J. S.; Williams, P. L.; Korrick, S. A.; Hauser, R.; Sergeyev, O.; Revich, B.; Lam, T.; Lee, M. M., Association between chlorinated pesticides in the serum of prepubertal Russian boys and longitudinal biomarkers of metabolic function. *Am J Epidemiol* 2014, *180*, 909-919.

[105] Dirinck, E. L.; Dirtu, A. C.; Govindan, M.; Covaci, A.; Van Gaal, L. F.; Jorens, P. G., Exposure to persistent organic pollutants: relationship with abnormal glucose metabolism and visceral adiposity. *Diabetes care* 2014, *37*, 1951-8.

[106] Kim, K. S.; Lee, Y. M.; Kim, S. G.; Lee, I. K.; Lee, H. J.; Kim, J. H.; Kim, J.; Moon, H. B.; Jacobs, D. R., Jr.; Lee, D. H., Associations of organochlorine pesticides and polychlorinated biphenyls in visceral vs. subcutaneous adipose tissue with type 2 diabetes and insulin resistance. *Chemosphere* 2014, *94*, 151-7.

[107] Langer, P.; Ukropec, J.; Kocan, A.; Drobna, B.; Radikova, Z.; Huckova, M.; Imrich, R.; Gasperikova, D.; Klimes, I.; Trnovec, T., Obesogenic and diabetogenic impact of high organochlorine levels (HCB, p,p'-DDE, PCBs) on inhabitants in the highly polluted Eastern Slovakia. *Endocr Regul* 2014, *48*, 17-24.

[108] Teeyapant, P.; Ramchiun, S.; Polputpisatkul, D.; Uttawichai, C.; Parnmen, S., Serum concentrations of organochlorine pesticides p,p'-DDE in adult Thai residents with background levels of exposure. *The Journal of toxicological sciences* 2014, *39*, 121-7.

[109] Al-Othman, A. A.; Abd-Alrahman, S. H.; Al-Daghri, N. M., DDT and its metabolites are linked to increased risk of type 2 diabetes among Saudi adults: a cross-sectional study. *Environ Sci Pollut R* 2015, *22*, 379-386.

[110] Turyk, M.; Fantuzzi, G.; Persky, V.; Freels, S.; Lambertino, A.; Pini, M.; Rhodes, D. H.; Anderson, H. A., Persistent organic pollutants and biomarkers of diabetes risk in a cohort of Great Lakes sport caught fish consumers. *Environ Res* 2015, *140*, 335-44.

[111] Van Larebeke, N.; Sioen, I.; Hond, E. D.; Nelen, V.; Van de Mieroop, E.; Nawrot, T.; Bruckers, L.; Schoeters, G.; Baeyens, W., Internal exposure to organochlorine pollutants and cadmium and self-reported health status: a prospective study. *Int J Hyg Environ Health* 2015, *218*, 232-45.

[112] Debost-Legrand, A.; Warembourg, C.; Massart, C.; Chevrier, C.; Bonvallot, N.; Monfort, C.; Rouget, F.; Bonnet, F.; Cordier, S., Prenatal exposure to persistent organic pollutants and organophosphate pesticides, and markers of glucose metabolism at birth. *Environ Res* 2016, *146*, 207-217.

[113] Shapiro, G. D.; Dodds, L.; Arbuckle, T. E.; Ashley-Martin, J.; Ettinger, A. S.; Fisher, M.; Taback, S.; Bouchard, M. F.; Monnier, P.; Dallaire, R.; Morisset, A. S.; Fraser, W., Exposure to organophosphorus and organochlorine pesticides, perfluoroalkyl substances, and polychlorinated biphenyls in pregnancy and the association with impaired glucose tolerance and gestational diabetes mellitus: The MIREC Study. *Environ Res* 2016, *147*, 71-81.

[114] Lee, Y. M.; Kim, K. S.; Kim, S. A.; Hong, N. S.; Lee, S. J.; Lee, D. H., Prospective associations between persistent organic pollutants and metabolic syndrome: A nested case-control study. *Sci Total Environ* 2014, *496*, 219-225.

[115] Al-Othman, A.; Yakout, S.; Abd-Alrahman, S. H.; Al-Daghri, N. M., Strong associations between the pesticide hexachlorocyclohexane and type 2 diabetes in Saudi Adults. *Int J Env Res Pub He* 2014, *11*, 8984-8995.

[116] Patel, C. J.; Bhattacharya, J.; Butte, A. J., An environment-wide association Study (EWAS) on type 2 diabetes mellitus. *PloS one* 2010, *5*, e10746.

[117] Malekirad, A. A.; Faghih, M.; Mirabdollahi, M.; Kiani, M.; Fathi, A.; Abdollahi, M., Neurocognitive, mental health, and glucose disorders in farmers exposed to organophosphorus pesticides. *Arh Hig Rada Toksikol* 2013, *64*, 1-8.

[118] Swaminathan, K.; Sundaram, M.; Prakash, P.; Subbiah, S., Diabetic ketoacidosis: an uncommon manifestation of pesticide poisoning. *Diabetes care* 2013, *36*, e4.

[119] Weizman, Z.; Sofer, S., Acute pancreatitis in children with anticholinesterase insecticide intoxication. *Pediatrics* 1992, *90*, 204-206.

[120] Meller, D.; Fraser, I.; Kryger, M., Hyperglycemia in anticholinesterase poisoning. *Can Med Assoc J* 1981, *124*, 745-8.

[121] Raafat, N.; Abass, M. A.; Salem, H. M., Malathion exposure and insulin resistance among a group of farmers in Al-Sharkia governorate. *Clinical biochemistry* 2012, *45*, 1591-5.

[122] Hansen, M. R.; Jors, E.; Lander, F.; Condarco, G.; Schlunssen, V., Is cumulated pyrethroid exposure associated with prediabetes? A cross-sectional study. *J Agromedicine* 2014, *19*, 417-26.

[123] Avsarogullari, L.; Ikizceli, I.; Sungur, M.; Sozuer, E.; Akdur, O.; Yucei, M., Acute amitraz poisoning in adults: clinical features, laboratory findings, and management. *Clin Toxicol (Phila)* 2006, *44*, 19-23.

[124] Badrane, N.; Askour, M.; Berechid, K.; Abidi, K.; Dendane, T.; Zeggwagh, A. A., Severe oral and intravenous insecticide mixture poisoning with diabetic ketoacidosis: a case report. *BMC Res Notes* 2014, *7*, 485.

[125] Matin, M. A.; Sattar, S.; Husain, K., Modification of malathion induced neurochemical changes by adrenalectomy in rats. *Mol Chem Neuropathol* 1990, *13*, 119-28.

[126] Matin, M. A.; Husain, K.; Khan, S. N., Modification of diazinon-induced changes in carbohydrate-metabolism by adrenalectomy in rats. *Biochemical pharmacology* 1990, *39*, 1781-1786.

[127] Rezg, R.; Mornagui, B.; El-Fazaa, S.; Gharbi, N., Caffeic acid attenuates malathion induced metabolic disruption in rat liver, involvement of acetylcholinesterase activity. *Toxicology* 2008, *250*, 27-31.

[128] Pournourmohammadi, S.; Ostad, S. N.; Azizi, E.; Ghahremani, M. H.; Farzami, B.; Minaie, B.; Larijani, B.; Abdollahi, M., Induction of insulin resistance by malathion: Evidence for disrupted islets cells metabolism and mitochondrial dysfunction. *Pestic Biochem Physiol* 2007, *88*, 346-352.

[129] Rezg, R.; Mornagui, B.; El-Arbi, M.; Kamoun, A.; El-Fazaa, S.; Gharbi, N., Effect of subchronic exposure to malathion on glycogen phosphorylase and hexokinase activities in rat liver using native PAGE. *Toxicology* 2006, *223*, 9-14.

[130] Rezg, R.; Mornagui, B.; Kamoun, A.; El-Fazaa, S.; Gharbi, N., Effect of subchronic exposure to malathion on metabolic parameters in the rat. *C R Biol* 2007, *330*, 143-7.

[131] Gupta, P. K., Malathion induced biochemical changes in rat. *Acta pharmacologica et toxicologica* 1974, *35*, 191-4.

[132] Xiao, X.; Kim, Y.; Kim, D.; Yoon, K. S.; Clark, J. M.; Park, Y., Exposure to permethrin alters glucose metabolism in response to high fat diet in female C57BL/6J mice. *In: The Toxicologist: Supplement to Toxicological Sciences* 2015, *144*, Society of Toxicology, 2015, Abstract no. 2154.

[133] Xiao, X.; Sun, Q.; Kim, Y.; Kim, D.; Yoon, K. S.; Clark, J. M.; Park, Y., Exposure to permethrin increases body fat mass and alters glucose metabolism in response to high fat diet in male C57BL/6J mice *In: The Toxicologist: Supplement to Toxicological Sciences* 2016, *150*, Society of Toxicology, 2016, Abstract no. 2889.

[134] Sun, Q.; Xiao, X.; Kim, Y.; Kim, D.; Yoon, K. S.; Clark, J. M.; Park, Y., Imidacloprid promotes high fat diet-induced adiposity and insulin resistance in male C57BL/6J mice. *J Agric Food Chem* 2016, *64*, 9293-9306.

[135] Howell, G. E.; Meek, E.; Kilic, J.; Mohns, M.; Mulligan, C.; Chambers, J. E., Exposure to p,p '-dichlorodiphenyldichloroethylene (DDE) induces fasting hyperglycemia without insulin resistance in male C57BL/6H mice. *Toxicology* 2014, *320*, 6-14.

[136] Howell, G. E.; Mulligan, C.; Meek, E.; Chambers, J. E., Effect of chronic p,p '-dichlorodiphenyldichloroethylene (DDE) exposure on high fat diet-induced alterations in glucose and lipid metabolism in male C57BL/6H mice. *Toxicology* 2015, *328*, 112-122.

[137] Villeneuve, D. C.; Van Logten, M. J.; Den Tonkelaar, E. M.; Greve, P. A.; Vos, J. G.; Speijers, G. J. A.; Van Esch, G. J., Effect of food deprivation on low level hexa chloro benzene exposure in rats. *Sci Total Environ* 1977, *8*, 179-186.

[138] Chadwick, R. W.; Cooper, R. L.; Chang, J.; Rehnberg, G. L.; McElroy, W. K., Possible antiestrogenic activity of lindane in female rats. *J Biochem Toxicol* 1988, *3*, 147-58.

[139] Skinner, M. K.; Manikkam, M.; Tracey, R.; Guerrero-Bosagna, C.; Haque, M.; Nilsson, E. E., Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. *Bmc Medicine* 2013, *11*.

[140] La Merrill, M.; Karey, E.; Moshier, E.; Lindtner, C.; La Frano, M. R.; Newman, J. W.; Buettner, C., Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. *PloS one* 2014, *9*, e103337.

[141] Shakoori, A. R.; Rasul, Y. G.; Ali, S. S., The effect of long term administration of dieldrin on biochemical components in blood serum of albino rats. *Folia Biol (Krakow)* 1984, *32*, 213-22.

[142] Peris-Sampedro, F.; Cabre, M.; Basaure, P.; Reverte, I.; Domingo, J. L.; Teresa Colomina, M., Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice. *Environ Res* 2015, *142*, 169-176.

[143] Meggs, W. J.; Brewer, K. L., Weight gain associated with chronic exposure to chlorpyrifos in rats. *J Med Toxicol* 2007, *3*, 89-93.

[144] Lassiter, T. L.; Brimijoin, S., Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. *Neurotoxicology and teratology* 2008, *30*, 125-30.

[145] Kamath, V.; Rajini, P. S., Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. *Toxicology* 2007, *231*, 137-46.

[146] Sarin, S.; Gill, K. D., Dichlorvos induced alterations in glucose homeostasis: possible implications on the state of neuronal function in rats. *Molecular and cellular biochemistry* 1999, *199*, 87-92.

[147] Slotkin, T. A.; Brown, K. K.; Seidler, F. J., Developmental exposure of rats to chlorpyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. *Environ Health Perspect* 2005, *113*, 1291-4.

[148] Arfat;, Y.; Mahmood;, N.; Tahir;, M. U.; Rashid;, M.; Anjum;, S.; Zhao;, F.; Li;, D.-J.; Sun;, Y.-L.; Hu;, L.; Zhihao;, C.; Yin;, C.; Shang;, P.; Qian, A.-R., Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice. *Toxicol Rep* 2014, *1*, 554-561.

[149] Ince, S.; Kucukkurt, I.; Demirel, H. H.; Turkmen, R.; Sever, E., Thymoquinone attenuates cypermethrin induced oxidative stress in Swiss albino mice. *Pestic Biochem Physiol* 2012, *104*, 229-235.

[150] Bhardwaj, S.; Srivastava, M. K.; Kapoor, U.; Srivastava, L. P., A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2010, *48*, 1185-90.

[151] Armstrong, L. E.; Driscoll, M. V.; Donepudi, A. C.; Xu, J.; Baker, A.; Aleksunes, L. M.; Richardson, J. R.; Slitt, A. L., Effects of developmental deltamethrin exposure on white adipose tissue gene expression. *Journal of biochemical and molecular toxicology* 2013, *27*, 165-171.

[152] Howell, G. E., 3rd; Mulligan, C.; Meek, E.; Chambers, J. E., Effect of chronic p,p'-dichlorodiphenyldichloroethylene (DDE) exposure on high fat diet-induced alterations in glucose and lipid metabolism in male C57BL/6H mice. *Toxicology* 2015, *328*, 112-22.

[153] Yau, D. T.; Mennear, J. H., The inhibitory effect of DDT on insulin secretion in mice. *Toxicol Appl Pharmacol* 1977, *39*, 81-8.

[154] Al-Attar, A. M.; Abu Zeid, I. M., Effect of tea (Camellia sinensis) and olive (Olea europaea L.) leaves extracts on male mice exposed to diazinon. *BioMed research international* 2013.

[155] Gupta, M.; Mukherjee, S.; Gupta, S. D.; Dolui, A. K.; Dey, S. N.; Roy, D. K., Changes of lipid spectrum in different tissues of Furadan-treated mice. *Toxicology* 1986, *38*, 69-79.

[156] Berdanier, C. D.; de Dennis, S. K., Effect of exercise on the responses of rats to DDT. *J Toxicol Environ Health* 1977, *2*, 651-6.

[157] Bhatia, S. C.; Venkitasubramanian, T. A., Mechanism of dieldrin-induced fat accumulation in rat liver. *J Agric Food Chem* 1972, *20*, 993-6.

[158] Deotare, S. T.; Chakrabarti, C. H., Effect of acephate (orthene) on tissue levels of thiamine, pyruvic acid, lactic acid, glycogen and blood sugar. *Indian J Physiol Pharmacol* 1981, *25*, 259-64.

[159] Choudhari, P. D.; Chakrabarti, C. H., Effect of acephate (orthene), an organophosphorus insecticide, on lipid metabolism in albino rats. *Indian J Exp Biol* 1984, *22*, 45-9.

[160] Joshi, A. K. R.; Rajini, P. S., Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: Role of gluconeogenesis. *Toxicology* 2009, *257*, 40-45.

[161] Husain, K.; Ansari, R. A., Influence of cholinergic and adrenergic blocking drugs on hyperglycemia and brain glycogenolysis in diazinon-treated animals. *Canadian journal of physiology and pharmacology* 1988, *66*, 1144-7.

[162] Ibrahim, N. A.; El-Gamal, B. A., Effect of diazinon, an organophosphate insecticide, on plasma lipid constituents in experimental animals. *J Biochem Mol Biol* 2003, *36*, 499-504.

[163] Teimouri, F.; Amirkabirian, N.; Esmaily, H.; Mohammadirad, A.; Aliahmadi, A.; Abdollahi, M., Alteration of hepatic cells glucose metabolism as a non-cholinergic detoxication mechanism in counteracting diazinon-induced oxidative stress. *Hum Exp Toxicol* 2006, *25*, 697-703.

[164] Ghafour-Rashidi, Z.; Dermenaki-Farahani, E.; Aliahmadi, A.; Esmaily, H.; Mohammadirad, A.; Ostad, S. N.; Abdollahi, M., Protection by cAMP and cGMP phosphodiesterase inhibitors of diazinon-induced hyperglycemia and oxidative/nitrosative stress in rat Langerhans islets cells: Molecular evidence for involvement of non-cholinergic mechanisms. *Pestic Biochem Physiol* 2007, *87*, 261-270.

[165] Alahyary, P.; Poor, M. I.; Azarbaijani, F. F.; Nejati, V., The potential toxicity of diazinon on physiological factors in male rat. *Pak J Biol Sci* 2008, *11*, 127-30.

[166] Ueyama, J.; Kamijima, M.; Asai, K.; Mochizuki, A.; Wang, D.; Kondo, T.; Suzuki, T.; Takagi, K.; Takagi, K.; Kanazawa, H.; Miyamoto, K.; Wakusawa, S.; Hasegawa, T., Effect of the organophosphorus pesticide diazinon on glucose tolerance in type 2 diabetic rats. *Toxicol Lett* 2008, *182*, 42-7.

[167] Jamshidi, H. R.; Ghahremani, M. H.; Ostad, S. N.; Sharifzadeh, M.; Dehpour, A. R.; Abdollahi, M., Effects of diazinon on the activity and gene expression of mitochondrial glutamate dehydrogenase from rat pancreatic Langerhans islets. *Pestic Biochem Physiol* 2009, *93*, 23-27.

[168] Teichert-Kuliszewska, K.; Szymczyk, T., Changes in rat carbohydrate metabolism after acute and chronic treatment with dichlorvos. *Toxicol Appl Pharmacol* 1979, *47*, 323-30.

[169] Romero-Navarro, G.; Lopez-Aceves, T.; Rojas-Ochoa, A.; Fernandez Mejia, C., Effect of dichlorvos on hepatic and pancreatic glucokinase activity and gene expression, and on insulin mRNA levels. *Life Sci* 2006, *78*, 1015-20.

[170] Reena, K.; Ajay, K.; Sharma, C. B., Haematological changes induced by dimethoate in rat. *Arh Hig Rada Toksikol* 1989, *40*, 23-7.

[171] Calore, E. E.; Sesso, A.; Puga, F. R.; Cavaliere, M. J.; Calore, N. M.; Weg, R., Sarcoplasmic lipase and non-specific esterase inhibition in myofibers of rats intoxicated with the organophosphate isofenphos. *Exp Toxicol Pathol* 1999, *51*, 27-33.

[172] Ramu, A.; Drexler, H., Hyperglycemia in acute malathion intoxication in rats. *Isr J Med Sci* 1973, *9*, 635-9.

[173] Gowda, H.; Uppal, R. P.; Garg, B. D., Effect of malathion on adrenal activity, liver glycogen & blood glucose in rats. *Indian J Med Res* 1983, *78*, 847-51.

[174] Rodrigues, M. A.; Puga, F. R.; Chenker, E.; Mazanti, M. T., Short-term effect of malathion on rats' blood glucose and on glucose utilization by mammalian cells in vitro. *Ecotoxicol Environ Saf* 1986, *12*, 110-3.

[175] Matin, M. A.; Husain, K., Cerebral glycogenolysis and glycolysis in malathion-treated hyperglycaemic animals. *Biochemical pharmacology* 1987, *36*, 1815-7.

[176] Abdollahi, M.; Donyavi, M.; Pournourmohammadi, S.; Saadat, M., Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following subchronic exposure to malathion. *Comp Biochem Physiol C Toxicol Pharmacol* 2004, *137*, 343-7.

[177] Pournourmohammadi, S.; Farzami, B.; Ostad, S. N.; Azizi, E.; Abdollahi, M., Effects of malathion subchronic exposure on rat skeletal muscle glucose metabolism. *Environ Toxicol Pharmacol* 2005, *19*, 191-6.

[178] Basiri, S.; Esmaily, H.; Vosough-Ghanbari, S.; Mohammadirad, A.; Yasa, N.; Abdollahi, M., Improvement by Satureja khuzestanica essential oil of malathion-induced red blood cells acetylcholinesterase inhibition and altered hepatic mitochondrial glycogen phosphorylase and phosphoenolpyruvate carboxykinase activities. *Pestic Biochem Physiol* 2007, *89*, 124-129.

[179] Rezg, R.; Mornagui, B.; Benahmed, M.; Chouchane, S. G.; Belhajhmida, N.; Abdeladhim, M.; Kamoun, A.; El-fazaa, S.; Gharbi, N., Malathion exposure modulates hypothalamic gene expression and induces dyslipedemia in Wistar rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2010, *48*, 1473-7.

[180] Lassiter, T. L.; Ryde, I. T.; Mackillop, E. A.; Brown, K. K.; Levin, E. D.; Seidler, F. J.; Slotkin, T. A., Exposure of neonatal rats to parathion elicits sex-selective reprogramming of metabolism and alters the response to a high-fat diet in adulthood. *Environ Health Perspect* 2008, *116*, 1456-62.

[181] Veerappan, M.; Hwang, I.; Pandurangan, M., Effect of cypermethrin, carbendazim and their combination on male albino rat serum. *Int J Clin Exp Pathol* 2012, *93*, 361-369.

[182] Cremer, J. E.; Seville, M. P., Comparative effects of two pyrethroids, deltamethrin and cismethrin, on plasma catecholamines and on blood glucose and lactate. *Toxicol Appl Pharmacol* 1982, *66*, 124-33.

[183] Shakoori, A. R.; Ali, S. S.; Saleem, M. A., Effects of six months' feeding of cypermethrin on the blood and liver of albino rats. *J Biochem Toxicol* 1988, *3*, 59-71.

[184] Ray, D. E.; Cremer, J. E., Action of decamethrin (a synthetic pyrethroid) on the rat. *Pestic Biochem Physiol* 1979, *10*, 333-340.

[185] Yousef, M. I.; Awad, T. I.; Mohamed, E. H., Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology* 2006, *227*, 240-247.

[186] Gawade, L.; Dadarkar, S. S.; Husain, R.; Gatne, M., A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2013, *51*, 61-70.

[187] Abdollahi, M.; Ranjbar, A.; Shadnia, S.; Nikfar, S.; Rezaie, A., Pesticides and oxidative stress: a review. *Med Sci Monit* 2004, *10*, RA141-7.

[188] Hectors, T. L. M.; Vanparys, C.; van der Ven, K.; Martens, G. A.; Jorens, P. G.; Van Gaal, L. F.; Covaci, A.; De Coen, W.; Blust, R., Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia* 2011, *54*, 1273-1290.

[189] Hernandez, A. F.; Parron, T.; Tsatsakis, A. M.; Requena, M.; Alarcon, R.; Lopez-Guarnido, O., Toxic effects of pesticide mixtures at a molecular level: Their relevance to human health. *Toxicology* 2013, *307*, 136-145.

[190] Kuo, C. C.; Moon, K.; Thayer, K. A.; Navas-Acien, A., Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. *Current diabetes reports* 2013, *13*, 831-49.

[191] Mostafalou, S.; Abdollahi, M., Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. *Toxicol Appl Pharmacol* 2013, *268*, 157-77.

[192] Rahimi, R.; Abdollahi, M., A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. *Pestic Biochem Physiol* 2007, *88*, 115-121.

[193] Rezg, R.; Mornagui, B.; El-Fazaa, S.; Gharbi, N., Organophosphorus pesticides as food chain contaminants and type 2 diabetes: a review. *Trends Food Sci Tech* 2010, *21*, 345-357.

[194] Soltaninejad, K.; Abdollahi, M., Current opinion on the science of organophosphate pesticides and toxic stress: A systematic review. *Med Sci Monitor* 2009, *15*, Ra75-Ra90.

[195] McKinlay, R.; Plant, J. A.; Bell, J. N. B.; Voulvoulis, N., Endocrine disrupting pesticides: implications for risk assessment. *Environ Int* 2008, *34*, 168-183.

[196] Yang, M. C.; McLean, A. J.; Rivory, L. P.; Le Couteur, D. G., Hepatic disposition of neurotoxins and pesticides. *Pharmacol Toxicol* 2000, *87*, 286-91.

[197] Hers, H. G., Mechanisms of blood glucose homeostasis. *J Inherit Metab Dis* 1990, *13*, 395-410.

[198] Nordlie, R. C.; Foster, J. D.; Lange, A. J., Regulation of glucose production by the liver. *Annu Rev Nutr* 1999, *19*, 379-406.

[199] Bergman, R. N., New concepts in extracellular signaling for insulin action: the single gateway hypothesis. *Recent progress in hormone research* 1997, *52*, 359-85; discussion 385-7.

[200] Kauffman, F. C.; Davis, L. H.; Whittaker, M., Activation of glycogen phosphorylase in rat pheochromocytoma PC12 cells and isolated hepatocytes by organophosphates. *Biochemical pharmacology* 1990, *39*, 347-54.

[201] Kim, J.; Park, Y.; Yoon, K. S.; Clark, J. M.; Park, Y., Permethrin alters adipogenesis in 3T3-L1 adipocytes and causes insulin resistance in C2C12 myotubes. *Journal of biochemical and molecular toxicology* 2014, *28*, 418-24.

[202] Dressel, T. D.; Goodale, R. L.; Arneson, M. A.; Borner, J. W., Pancreatitis as a complication of anticholinesterase insecticide intoxication. *Ann Surg* 1978, *189*, 199-204.

[203] Hsiao, C. T.; Yang, C. C.; Deng, J. F.; Bullard, M. J.; Liaw, S. J., Acute pancreatitis following organophosphate intoxication. *J Toxicol Clin Toxicol* 1996, *34*, 343-7.

[204] Moore, P. G.; James, O. F., Acute pancreatitis induced by acute organophosphate poisoning. *Postgrad Med J* 1981, *57*, 660-2.

[205] Marsh, W. H.; Vukov, G. A.; Conradi, E. C., Acute pancreatitis after cutaneous exposure to an organophosphate insecticide. *Am J Gastroenterol* 1988, *83*, 1158-60.

[206] Lankisch, P. G.; Muller, C. H.; Niederstadt, H.; Brand, A., Painless acute pancreatitis subsequent to anticholinesterase insecticide (parathion) intoxication. *Am J Gastroenterol* 1990, *85*, 872-5.

[207] Harputluoglu, M. M.; Kantarceken, B.; Karincaoglu, M.; Aladag, M.; Yildiz, R.; Ates, M.; Yildirim, B.; Hilmioglu, F., Acute pancreatitis: an obscure complication of organophosphate intoxication. *Hum Exp Toxicol* 2003, *22*, 341-3.

[208] Gokel, Y.; Gulalp, B.; Acikalin, A., Parotitis due to organophosphate intoxication. *J Toxicol Clin Toxicol* 2002, *40*, 563-5.

[209] Panieri, E.; Krige, J. E.; Bornman, P. C.; Linton, D. M., Severe necrotizing pancreatitis caused by organophosphate poisoning. *J Clin Gastroenterol* 1997, *25*, 463-5.

[210] Kandalaft, K.; Liu, S.; Manivel, C.; Borner, J. W.; Dressel, T. D.; Sutherland, D. E.; Goodale, R. L., Organophosphate increases the sensitivity of human exocrine pancreas to acetylcholine. *Pancreas* 1991, *6*, 398-403.

[211] Goodale, R. L.; Manivel, J. C.; Borner, J. W.; Liu, S.; Judge, J.; Li, C.; Tanaka, T., Organophosphate sensitizes the human pancreas to acinar cell injury - an ultrastructural-study. *Pancreas* 1993, *8*, 171-175.

[212] Moritz, F.; Droy, J. M.; Dutheil, G.; Melki, J.; Bonmarchand, G.; Leroy, J., Acute-pancreatitis after carbamate insecticide intoxication. *Intens Care Med* 1994, *20*, 49-50.

[213] Karmaus, W.; Osuch, J. R.; Eneli, I.; Mudd, L. M.; Zhang, J.; Mikucki, D.; Haan, P.; Davis, S., Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. *Occup Environ Med* 2009, *66*, 143-149.

[214] Morgan, M. K.; Sheldon, L. S.; Croghan, C. W.; Jones, P. A.; Chuang, J. C.; Wilson, N. K., An observational study of 127 preschool children at their homes and daycare centers in Ohio: Environmental pathways to cis- and trans-permethrin exposure. *Environ Res* 2007, *104*, 266-274.

[215] Moreno-Aliaga, M. J.; Matsumura, F., Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (p,p'-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. *Biochemical pharmacology* 2002, *63*, 997-1007.

[216] Kim, J.; Sun, Q.; Yue, Y.; Yoon, K. S.; Whang, K. Y.; Marshall Clark, J.; Park, Y., 4,4'-Dichlorodiphenyltrichloroethane (DDT) and 4,4'-dichlorodiphenyldichloroethylene (DDE) promote adipogenesis in 3T3-L1 adipocyte cell culture. *Pestic Biochem Physiol* 2016, *131*, 40-5.

[217] Mangum, L. H.; Howell, G. E., III; Chambers, J. E., Exposure to p,p '-DDE enhances differentiation of 3T3-L1 preadipocytes in a model of sub-optimal differentiation. *Toxicol Lett* 2015, *238*, 65-71.

[218] Howell, G., 3rd; Mangum, L., Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicology in vitro : an international journal published in association with BIBRA* 2011, *25*, 394-402.

[219] Alonso-Magdalena, P.; Quesada, I.; Nadal, A., Endocrine disruptors in the etiology of type 2 diabetes mellitus. *Nature reviews. Endocrinology* 2011, *7*, 346-53.

[220] Gore, A. C.; Chappell, V. A.; Fenton, S. E.; Flaws, J. A.; Nadal, A.; Prins, G. S.; Toppari, J.; Zoeller, R. T., Executive summary to EDC-2: The endocrine society's second scientific statement on endocrine-disrupting chemicals. *Endocrine reviews* 2015, *36*, 593-602.

[221] Abdollahi, M.; Bahreini-Moghadam, A.; Emami, B.; Fooladian, F.; Zafari, K., Increasing intracellular cAMP and cGMP inhibits cadmium-induced oxidative stress in rat submandibular saliva. *Comp Biochem Physiol C Toxicol Pharmacol* 2003, *135C*, 331-6.

[222] Abdollahi, M.; Chan, T. S.; Subrahmanyam, V.; O'Brien, P. J., Effects of phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and susceptibility to a mitochondrial toxin. *Molecular and cellular biochemistry* 2003, *252*, 205-11.

[223] Ramu, A.; Slonim, A. E.; London, M.; Eyal, F., Hyperglycemia in acute malathion poisoning. *Isr J Med Sci* 1973, *9*, 631-4.

[224] Fukuyama, G. S.; Adie, P. A., Blood levels of adrenaline and noradrenaline during anticholinesterase poisoning. *Arch Int Pharmacodyn Ther* 1963, *146*, 56-64.

[225] Sungur, M.; Guven, M., Intensive care management of organophosphate insecticide poisoning. *Crit Care* 2001, *5*, 211-5.

[226] Bruns, C. M.; Kemnitz, J. W., Sex hormones, insulin sensitivity, and diabetes mellitus. *ILAR J* 2004, *45*, 160-9.

[227] Fernandez-Real, J. M.; Lopez-Bermejo, A.; Castro, A.; Casamitjana, R.; Ricart, W., Thyroid function is intrinsically linked to insulin sensitivity and endothelium-dependent vasodilation in healthy euthyroid subjects. *J Clin Endocr Metab* 2006, *91*, 3337-3343.

[228] Polderman, K. H.; Gooren, L. J.; Asscheman, H.; Bakker, A.; Heine, R. J., Induction of insulin resistance by androgens and estrogens. *The Journal of clinical endocrinology and metabolism* 1994, *79*, 265-71.

[229] Kojima, H.; Takeuchi, S.; Nagai, T., Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor. *J Health Sci* 2010, *56*, 374-386.

[230] Lemaire, G.; Balaguer, P.; Michel, S.; Rahmani, R., Activation of retinoic acid receptor-dependent transnocription by orgachlorine pesticides. *Toxicol Appl Pharm* 2005, *202*, 38-49.

[231] Song, C.; Kanthasamy, A.; Anantharam, V.; Sun, F.; Kanthasamy, A. G., Environmental neurotoxic pesticide increases histone acetylation to promote apoptosis in dopaminergic neuronal cells: relevance to epigenetic mechanisms of neurodegeneration. *Molecular pharmacology* 2010, *77*, 621-32.

[232] Kalender, S.; Ogutcu, A.; Uzunhisarcili, M.; Acikgoz, F.; Durak, D.; Ulusoy, Y.; Kalender, Y., Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicology* 2005, *211*, 197-206.

[233] Bagchi, D.; Bagchi, M.; Hassoun, E. A.; Stohs, S. J., In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology* 1995, *104*, 129-40.

[234] Yang, Z. P.; Dettbarn, W. D., Diisopropylphosphorofluoridate-induced cholinergic hyperactivity and lipid peroxidation. *Toxicol Appl Pharmacol* 1996, *138*, 48-53.

[235] Begum, K.; Rajini, P. S., Augmentation of hepatic and renal oxidative stress and disrupted glucose homeostasis by monocrotophos in streptozotocin-induced diabetic rats. *Chem-Biol Interact* 2011, *193*, 240-245.

[236] Mostafalou, S.; Eghbal, M. A.; Nili-Ahmadabadi, A.; Baeeri, M.; Abdollahi, M., Biochemical evidence on the potential role of organophosphates in hepatic glucose metabolism toward insulin resistance through inflammatory signaling and free radical pathways. *Toxicol Ind Health* 2012, *28*, 840-851.

[237] Mostafalou, S.; Abdollahi, M., The role of environmental pollution of pesticides in human diabetes. *Int J Pharmacol* 2012, *8*, 139-140.

[238] Abdollahi, M.; Mostafalou, S.; Pournourmohammadi, S.; Shadnia, S., Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. *Comp Biochem Physiol C Toxicol Pharmacol* 2004, *137*, 29-34.

[239] Slaninova, A.; Smutna, M.; Modra, H.; Svobodova, Z., A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinol Lett* 2009, *30 Suppl 1*, 2-12.

[240] Furukawa, S.; Fujita, T.; Shimabukuro, M.; Iwaki, M.; Yamada, Y.; Nakajima, Y.; Nakayama, O.; Makishima, M.; Matsuda, M.; Shimomura, I., Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004, *114*, 1752-61.

[241] Akhgari, M.; Abdollahi, M.; Kebryaeezadeh, A.; Hosseini, R.; Sabzevari, O., Biochemical evidence for free radical-induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. *Hum Exp Toxicol* 2003, *22*, 205-211.

[242] Ranjbar, A.; Pasalar, P.; Abdollahi, M., Induction of oxidative stress and acetylcholinesterase inhibition in organophosphorous pesticide manufacturing workers. *Hum Exp Toxicol* 2002, *21*, 179-182.

[243] Shadnia, S.; Azizi, E.; Hosseini, R.; Khoei, S.; Fouladdel, S.; Pajoumand, A.; Jalali, N.; Abdollahi, M., Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. *Hum Exp Toxicol* 2005, *24*, 439-45.

[244] Matsuoka, T.; Kajimoto, Y.; Watada, H.; Kaneto, H.; Kishimoto, M.; Umayahara, Y.; Fujitani, Y.; Kamada, T.; Kawamori, R.; Yamasaki, Y., Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. *J Clin Invest* 1997, *99*, 144-50.

[245] Abdul-Ghani, M. A.; DeFronzo, R. A., Mitochondrial dysfunction, insulin resistance, and type 2 diabetes mellitus. *Current diabetes reports* 2008, *8*, 173-8.

[246] Kim, J. A.; Wei, Y.; Sowers, J. R., Role of mitochondrial dysfunction in insulin resistance. *Circulation research* 2008, *102*, 401-14.

[247] Lowell, B. B.; Shulman, G. I., Mitochondrial dysfunction and type 2 diabetes. *Science* 2005, *307*, 384-7.

[248] Ma, Z. A.; Zhao, Z.; Turk, J., Mitochondrial dysfunction and beta-cell failure in type 2 diabetes mellitus. *Exp Diabetes Res* 2012, *2012*, 703538.

[249] Gomez, C.; Bandez, M. J.; Navarro, A., Pesticides and impairment of mitochondrial function in relation with the parkinsonian syndrome. *Frontiers in bioscience : a journal and virtual library* 2007, *12*, 1079-93.

[250] Lim, S.; Ahn, S. Y.; Song, I. C.; Chung, M. H.; Jang, H. C.; Park, K. S.; Lee, K. U.; Pak, Y. K.; Lee, H. K., Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. *PloS one* 2009, *4*, e5186.

[251] Lee, H. K., Mitochondrial dysfunction and insulin resistance: the contribution of dioxin-like substances. *Diabetes Metab J* 2011, *35*, 207-15.

[252] Grankvist, K.; Marklund, S. L.; Taljedal, I. B., CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. *The Biochemical journal* 1981, *199*, 393-8.

[253] Kakkar, R.; Mantha, S. V.; Radhi, J.; Prasad, K.; Kalra, J., Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. *Clin Sci (Lond)* 1998, *94*, 623-32.

[254] Ho, E.; Bray, T. M., Antioxidants, NFkappaB activation, and diabetogenesis. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine* 1999, *222*, 205-13.

[255] Mackness, M. I.; Harty, D.; Bhatnagar, D.; Winocour, P. H.; Arrol, S.; Ishola, M.; Durrington, P. N., Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 1991, *86*, 193-9.

[256] Cao, S. S.; Kaufman, R. J., Targeting endoplasmic reticulum stress in metabolic disease. *Expert opinion on therapeutic targets* 2013, *17*, 437-448.

[257] Hotamisligil, G. S., Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 2010, *140*, 900-17.

[258] Hummasti, S.; Hotamisligil, G. S., Endoplasmic reticulum stress and inflammation in obesity and diabetes. *Circulation research* 2010, *107*, 579-91.

[259] Ozcan, U.; Cao, Q.; Yilmaz, E.; Lee, A. H.; Iwakoshi, N. N.; Ozdelen, E.; Tuncman, G.; Gorgun, C.; Glimcher, L. H.; Hotamisligil, G. S., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 2004, *306*, 457-461.

[260] van der Kallen, C. J.; van Greevenbroek, M. M.; Stehouwer, C. D.; Schalkwijk, C. G., Endoplasmic reticulum stress-induced apoptosis in the development of diabetes: is there a role for adipose tissue and liver? *Apoptosis : an international journal on programmed cell death* 2009, *14*, 1424-34.

[261] Skandrani, D.; Gaubin, Y.; Beau, B.; Murat, J. C.; Vincent, C.; Croute, F., Effect of selected insecticides on growth rate and stress protein expression in cultured human A549 and SH-SY5Y cells. *Toxicology in vitro : an international journal published in association with BIBRA* 2006, *20*, 1378-86.

[262] Skandrani, D.; Gaubin, Y.; Vincent, C.; Beau, B.; Claude Murat, J.; Soleilhavoup, J. P.; Croute, F., Relationship between toxicity of selected insecticides and expression of stress proteins (HSP, GRP) in cultured human cells: effects of commercial formulations versus pure active molecules. *Biochimica et biophysica acta* 2006, *1760*, 95-103.

[263] Monteiro, R.; Azevedo, I., Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm* 2010, *2010*.

[264] Lumeng, C. N.; Saltiel, A. R., Inflammatory links between obesity and metabolic disease. *J Clin Invest* 2011, *121*, 2111-7.

[265] California Environmental Protection Agency, Summary of toxicology data: Pyrethrins. . 1987.

[266] Elliott, M.; Farnham, A. W.; Janes, N. F.; Needham, P. H.; Pulman, D. A.; Stevenson, J. H., A photostable pyrethroid. *Nature* 1973, *246*, 169-70.

[267] Choi, J. S.; Soderlund, D. M., Structure-activity relationships for the action of 11 pyrethroid insecticides on rat Na(v)1.8 sodium channels expressed in Xenopus oocytes. *Toxicol. Appl. Pharmacol.* 2006, *211*, 233-244.

[268] Ray, D. E.; Fry, J. R., A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacol Ther* 2006, *111*, 174-93.

[269] Tan, J.; Liu, Z.; Wang, R.; Huang, Z. Y.; Chen, A. C.; Gurevitz, M.; Dong, K., Identification of amino acid residues in the insect sodium channel critical for pyrethroid binding. *Molecular pharmacology* 2005, *67*, 513-22.

[270] Yoon, K. S.; Symington, S. B.; Lee, S. H.; Soderlund, D. M.; Clark, J. M., Three mutations identified in the voltage-sensitive sodium channel alpha-subunit gene of permethrin-resistant human head lice reduce the permethrin sensitivity of house fly Vssc1 sodium channels expressed in Xenopus oocytes. *Insect Biochem Mol Biol* 2008, *38*, 296-306.

[271] National Research Council, Health effects of permethrin-impregnated army battle-dress uniforms. National Academy of Sciences, Washington DC. 1994.

[272] WHO, Permethrin (40:60 cis:trans isomer ratio) world health organization WHO specifications and evaluations for public health pesticides 1990.

[273] CEPADP, California enviromental protection agency department of pesticide regulation medical toxicology branch summary of toxicology data pyrethrins. 1987.

[274] Di Gregorio, G. B.; Hensley, L.; Lu, T.; Ranganathan, G.; Kern, P. A., Lipid and carbohydrate metabolism in mice with a targeted mutation in the IL-6 gene: absence of development of age-related obesity. *American journal of physiology. Endocrinology and metabolism* 2004, *287*, E182-7.

[275] Andrikopoulos, S.; Blair, A. R.; Deluca, N.; Fam, B. C.; Proietto, J., Evaluating the glucose tolerance test in mice. *American journal of physiology. Endocrinology and metabolism* 2008, *295*, E1323-32.

[276] Christensen, S. D.; Mikkelsen, L. F.; Fels, J. J.; Bodvarsdottir, T. B.; Hansen, A. K., Quality of plasma sampled by different methods for multiple blood sampling in mice. *Laboratory animals* 2009, *43*, 65-71.

[277] Wallace, T. M.; Levy, J. C.; Matthews, D. R., Use and abuse of HOMA modeling. *Diabetes care* 2004, *27*, 1487-1495.

[278] Kim, Y.; Kim, D.; Good, D. J.; Park, Y., Effects of postweaning administration of conjugated linoleic acid on development of obesity in nescient basic helix-loop-helix 2 knockout mice. *J Agric Food Chem* 2015, *63*, 5212-23.

[279] Schmittgen, T. D.; Livak, K. J., Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008, *3*, 1101-8.

[280] Ayala, J. E.; Samuel, V. T.; Morton, G. J.; Obici, S.; Croniger, C. M.; Shulman, G. I.; Wasserman, D. H.; McGuinness, O. P.; Consortium, N. I. H. M. M. P. C., Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. *Disease models & mechanisms* 2010, *3*, 525-34.

[281] Kahn, B. B.; Alquier, T.; Carling, D.; Hardie, D. G., AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. *Cell metabolism* 2005, *1*, 15-25.

[282] Ha, J.; Daniel, S.; Broyles, S. S.; Kim, K. H., Critical phosphorylation sites for acetyl-CoA carboxylase activity. *The Journal of biological chemistry* 1994, *269*, 22162-8.

[283] Canto, C.; Auwerx, J., AMP-activated protein kinase and its downstream transcriptional pathways. *Cellular and molecular life sciences : CMLS* 2010, *67*, 3407-23.

[284] Hawley, S. A.; Pan, D. A.; Mustard, K. J.; Ross, L.; Bain, J.; Edelman, A. M.; Frenguelli, B. G.; Hardie, D. G., Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. *Cell metabolism* 2005, *2*, 9-19.

[285] Bell, G. I.; Kayano, T.; Buse, J. B.; Burant, C. F.; Takeda, J.; Lin, D.; Fukumoto, H.; Seino, S., Molecular biology of mammalian glucose transporters. *Diabetes care* 1990, *13*, 198-208.

[286] Hotamisligil, G. S., The role of TNFalpha and TNF receptors in obesity and insulin resistance. *Journal of internal medicine* 1999, *245*, 621-5.

[287] Kim, J. B.; Spiegelman, B. M., ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes & development* 1996, *10*, 1096-107.

[288] Coburn, C. T.; Knapp, F. F., Jr.; Febbraio, M.; Beets, A. L.; Silverstein, R. L.; Abumrad, N. A., Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *The Journal of biological chemistry* 2000, *275*, 32523-9.

[289] Harris, C. A.; Haas, J. T.; Streeper, R. S.; Stone, S. J.; Kumari, M.; Yang, K.; Han, X.; Brownell, N.; Gross, R. W.; Zechner, R.; Farese, R. V., Jr., DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. *Journal of lipid research* 2011, *52*, 657-67.

[290] Valera, A.; Pujol, A.; Pelegrin, M.; Bosch, F., Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Sciences of the United States of America* 1994, *91*, 9151-4.

[291] Beale, E. G.; Harvey, B. J.; Forest, C., PCK1 and PCK2 as candidate diabetes and obesity genes. *Cell Biochem. Biophys.* 2007, *48*, 89-95.

[292] Reddy, J. K.; Hashimoto, T., Peroxisomal beta-oxidation and peroxisome proliferator-activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr* 2001, *21*, 193-230.

[293] Gaster, M.; Staehr, P.; Beck-Nielsen, H.; Schroder, H. D.; Handberg, A., GLUT4 is reduced in slow muscle fibers of type 2 diabetic patients - Is insulin resistance in type 2 diabetes a slow, type 1 fiber disease? *Diabetes* 2001, *50*, 1324-1329.

[294] Huang, S.; Czech, M. P., The GLUT4 glucose transporter. *Cell metabolism* 2007, *5*, 237-52.

[295] Sugden, M. C.; Holness, M. J., Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. *Am J Physiol-Endoc M* 2003, *284*, E855-E862.

[296] Kim, Y. I.; Lee, F. N.; Choi, W. S.; Lee, S.; Youn, J. H., Insulin regulation of skeletal muscle PDK4 mRNA expression is impaired in acute insulin-resistant states 10.107/db05-1606. *Diabetes* 2006, *55*, 2311-2317.

[297] Mihaylova, M. M.; Shaw, R. J., The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol* 2011, *13*, 1016-23.

[298] Sun, Q.; Qi, W.; Yang, J.; Yoon, K. S.; Clark, J. M.; Park, Y., Fipronil promotes adipogenesis via AMPK alpha-mediated pathway in 3T3-L1 adipocytes. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 2016, *92*, 217-223.

[299] Lee, D. H.; Jacobs, D. R., A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: Results from the National Health and Nutrition Examination Survey 1999-2002 - Response to Porta. *Diabetes care* 2006, *29*, 2568-2568.

[300] Wang, J. S.; Zhu, Y. Q.; Cai, X. A.; Yu, J. M.; Yang, X. P.; Cheng, J. X., Abnormal glucose regulation in pyrethroid pesticide factory workers. *Chemosphere* 2011, *82*, 1080-1082.

[301] Kohn, A. D.; Summers, S. A.; Birnbaum, M. J.; Roth, R. A., Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *The Journal of biological chemistry* 1996, *271*, 31372-8.

[302] Armstrong, L. E.; Driscoll, M. V.; Donepudi, A. C.; Xu, J.; Baker, A.; Aleksunes, L. M.; Richardson, J. R.; Slitt, A. L., Effects of developmental deltamethrin exposure on white adipose tissue gene expression. *Journal of biochemical and molecular toxicology* 2013, *27*, 165-71.

[303] Hotamisligil, G. S., Mechanisms of TNF-alpha-induced insulin resistance. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association* 1999, *107*, 119-25.

[304] Heudorf, U.; Angerer, J., Metabolites of pyrethroid insecticides in urine specimens: current exposure in an urban population in Germany. *Environ Health Perspect* 2001, *109*, 213-7.

[305] Lu, C.; Barr, D. B.; Pearson, M. A.; Walker, L. A.; Bravo, R., The attribution of urban and suburban children's exposure to synthetic pyrethroid insecticides: a longitudinal assessment. *J Expo Sci Environ Epidemiol* 2009, *19*, 69-78.

[306] Whyatt, R. M.; Camann, D. E.; Kinney, P. L.; Reyes, A.; Ramirez, J.; Dietrich, J.; Diaz, D.; Holmes, D.; Perera, F. P., Residential pesticide use during pregnancy among a cohort of urban minority women. *Environ Health Perspect* 2002, *110*, 507-514.

[307] Baker, S. E.; Olsson, A. O.; Barr, D. B., Isotope dilution high-performance liquid chromatography-tandem mass spectrometry method for quantifying urinary metabolites of synthetic pyrethroid insecticides. *Arch Environ Contam Toxicol* 2004, *46*, 281-8.

[308] Gaughan, L. C.; Unai, T.; Casida, J. E., Permethrin metabolism in rats. *J Agric Food Chem* 1976, *25*, 9-17.

[309] McCarthy, A. R.; Thomson, B. M.; Shaw, I. C.; Abell, A. D., Estrogenicity of pyrethroid insecticide metabolites. *J Environ Monit* 2006, *8*, 197-202.

[310] Tornero-Velez, R.; Davis, J.; Scollon, E. J.; Starr, J. M.; Setzer, R. W.; Goldsmith, M. R.; Chang, D. T.; Xue, J.; Zartarian, V.; DeVito, M. J.; Hughes, M. F., A pharmacokinetic model of cis- and trans-permethrin disposition in rats and humans with aggregate exposure application. *Toxicological sciences : an official journal of the Society of Toxicology* 2012, *130*, 33-47.

[311] Tyler, C. R.; Beresford, N.; van der Woning, M.; Sumpter, J. P.; Thorpe, K., Metabolism and environmental degradation of pyrethroid insecticides produce compounds with endocrine activities. *Environ Toxicol Chem* 2000, *19*, 801-809.

[312] Nishi, K.; Huang, H.; Kamita, S. G.; Kim, I. H.; Morisseau, C.; Hammock, B. D., Characterization of pyrethroid hydrolysis by the human liver carboxylesterases hCE-1 and hCE-2. *Archives of biochemistry and biophysics* 2006, *445*, 115-23.

[313] Peris-Sampedro, F.; Cabre, M.; Basaure, P.; Reverte, I.; Domingo, J. L.; Teresa Colomina, M., Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice. *Environ Res* 2015, *142*, 169-76.

[314] Anadon, A.; Martinez-Larranaga, M. R.; Diaz, M. J.; Bringas, P., Toxicokinetics of permethrin in the rat. *Toxicol Appl Pharmacol* 1991, *110*, 1-8.

[315] Gaughan, L. C.; Unai, T.; Casida, J. E., Permethrin Metabolism in Rats. *J Agr Food Chem* 1977, *25*, 9-17.

[316] Toynton, K.; Luukinen, B.; Buhl, K.; Stone, D., Permethirn Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/archive/Permtech.html. 2009.

[317] Kim, J. H.; Park, Y.; Kim, D.; Good, D. J.; Park, Y., Dietary conjugated nonadecadienoic acid prevents adult-onset obesity in nescient basic helix-loop-helix 2 knockout mice. *The Journal of nutritional biochemistry* 2013, *24*, 556-66.

[318] Surwit, R. S.; Kuhn, C. M.; Cochrane, C.; McCubbin, J. A.; Feinglos, M. N., Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 1988, *37*, 1163-7.

[319] Baatrup, E.; Bayley, M., Effects of the pyrethroid insecticide Cypermethrin on the locomotor activity of the wolf spider Pardosa amentata: quantitative analysis employing computer-automated video tracking. *Ecotoxicol Environ Saf* 1993, *26*, 138-52.

[320] Frank, J. P.; Kellner, T. P., Deltamethrin Risk Characterization Document. 2000, *1*.

[321] Nieradko-Iwanicka, B.; Borzecki, A., Subacute poisoning of mice with deltamethrin produces memory impairment, reduced locomotor activity, liver damage and changes in blood morphology in the mechanism of oxidative stress. *Pharmacological reports : PR* 2015, *67*, 535-41.

[322] Crofton, K. M.; Reiter, L. W., The effects of type I and II pyrethroids on motor activity and the acoustic startle response in the rat. *Fundam Appl Toxicol* 1988, *10*, 624-34.

[323] Lassiter, T. L.; Ryde, I. T.; MacKillop, E. A.; Brown, K. K.; Levin, E. D.; Seidler, F. J.; Slotkin, T. A., Exposure of neonatal rats to parathion elicits sex-selective reprogramming of metabolism and alters the response to a high-fat diet in adulthood. *Environ Health Persp* 2008, *116*, 1456-1462.

[324] Mugford, C. A.; Kedderis, G. L., Sex-dependent metabolism of xenobiotics. *Drug Metab Rev* 1998, *30*, 441-98.

[325] Aguirre, V.; Werner, E. D.; Giraud, J.; Lee, Y. H.; Shoelson, S. E.; White, M. F., Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *The Journal of biological chemistry* 2002, *277*, 1531-7.

[326] Sarbassov, D. D.; Guertin, D. A.; Ali, S. M.; Sabatini, D. M., Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005, *307*, 1098-101.

[327] Alessi, D. R.; Andjelkovic, M.; Caudwell, B.; Cron, P.; Morrice, N.; Cohen, P.; Hemmings, B. A., Mechanism of activation of protein kinase B by insulin and IGF-1. *Embo J* 1996, *15*, 6541-51.

[328] Jacinto, E.; Facchinetti, V.; Liu, D.; Soto, N.; Wei, S.; Jung, S. Y.; Huang, Q.; Qin, J.; Su, B., SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. *Cell* 2006, *127*, 125-37.

[329] Soderlund, D. M., Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. *Archives of toxicology* 2012, *86*, 165-81.

[330] Verschoyle, R. D.; Barnes, J. M., Pestic Biochem Phys *Pestic Biochem Phys* 1972, *2*, 308.

[331] Verschoyle, R. D.; Aldridge, W. N., Structure-activity relationships of some pyrethroids in rats. *Archives of toxicology* 1980, *45*, 325-329.

[332] Bradbury, S. P.; Coats, J. R., Comparative toxicology of the pyrethroid insecticides. *Rev Environ Contam Toxicol* 1989, *108*, 133-77.

[333] Clark, J. M.; Symington, S. B., Pyrethroid action on calcium channels: neurotoxicological implications. *Invert Neurosci* 2007, *7*, 3-16.

[334] Cao, Z.; Shafer, T. J.; Murray, T. F., Mechanisms of pyrethroid insecticide-induced stimulation of calcium influx in neocortical neurons. *The Journal of pharmacology and experimental therapeutics* 2011, *336*, 197-205.

[335] Jones, B. H.; Kim, J. H.; Zemel, M. B.; Woychik, R. P.; Michaud, E. J.; Wilkison, W. O.; Moustaid, N., Upregulation of adipocyte metabolism by agouti protein: possible paracrine actions in yellow mouse obesity. *The American journal of physiology* 1996, *270*, E192-6.

[336] Zemel, M. B.; Kim, J. H.; Woychik, R. P.; Michaud, E. J.; Kadwell, S. H.; Patel, I. R.; Wilkison, W. O., Agouti regulation of intracellular calcium: role in the insulin resistance of viable yellow mice. *Proceedings of the National Academy of Sciences of the United States of America* 1995, *92*, 4733-7.

[337] Zemel, M. B.; Shi, H.; Greer, B.; Dirienzo, D.; Zemel, P. C., Regulation of adiposity by dietary calcium. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2000, *14*, 1132-8.

[338] Draznin, B.; Sussman, K. E.; Eckel, R. H.; Kao, M.; Yost, T.; Sherman, N. A., Possible role of cytosolic free calcium concentrations in mediating insulin resistance of obesity and hyperinsulinemia. *J Clin Invest* 1988, *82*, 1848-52.

[339] Cnop, M.; Foufelle, F.; Velloso, L. A., Endoplasmic reticulum stress, obesity and diabetes. *Trends Mol Med* 2012, *18*, 59-68.

[340] Xu, C.; Bailly-Maitre, B.; Reed, J. C., Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 2005, *115*, 2656-64.

[341] Sha, H. B.; He, Y.; Chen, H.; Wang, C.; Zenno, A.; Shi, H.; Yang, X. Y.; Zhang, X. M.; Qi, L., The IRE1 alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell metabolism* 2009, *9*, 556-564.

[342] Basseri, S.; Lhotak, S.; Sharma, A. M.; Austin, R. C., The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. *Journal of lipid research* 2009, *50*, 2486-501.

[343] Takahashi, A.; Camacho, P.; Lechleiter, J. D.; Herman, B., Measurement of intracellular calcium. *Physiological reviews* 1999, *79*, 1089-125.

[344] Grynkiewicz, G.; Poenie, M.; Tsien, R. Y., A new generation of Ca2+ indicators with greatly improved fluorescence properties. *The Journal of biological chemistry* 1985, *260*, 3440-50.

[345] Molecular Probes, Fura and Indo Ratiometric Calcium Indicators Product Information; https://tools.thermofisher.com/content/sfs/manuals/mp01200.pdf. 2011.

[346] Berridge, M. J.; Lipp, P.; Bootman, M. D., The versatility and universality of calcium signalling. *Nature reviews. Molecular cell biology* 2000, *1*, 11-21.

[347] Berridge, M. J.; Bootman, M. D.; Lipp, P., Calcium--a life and death signal. *Nature* 1998, *395*, 645-8.

[348] Kim, J. H.; Kiefer, L. L.; Woychik, R. P.; Wilkison, W. O.; Truesdale, A.; Ittoop, O.; Willard, D.; Nichols, J.; Zemel, M. B., Agouti regulation of intracellular calcium: role of melanocortin receptors. *The American journal of physiology* 1997, *272*, E379-84.

[349] Stevens, F. C., Calmodulin: an introduction. *Can J Biochem Cell Biol* 1983, *61*, 906-10.

[350] Woods, A.; Dickerson, K.; Heath, R.; Hong, S. P.; Momcilovic, M.; Johnstone, S. R.; Carlson, M.; Carling, D., Ca2+/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell metabolism* 2005, *2*, 21-33.

[351] Seervi, M.; Sobhan, P. K.; Joseph, J.; Ann Mathew, K.; Santhoshkumar, T. R., ERO1alpha-dependent endoplasmic reticulum-mitochondrial calcium flux contributes to ER stress and mitochondrial permeabilization by procaspase-activating compound-1 (PAC-1). *Cell Death Dis* 2013, *4*, e968.

[352] Oyadomari, S.; Mori, M., Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell death and differentiation* 2004, *11*, 381-9.

[353] Urano, F.; Wang, X.; Bertolotti, A.; Zhang, Y.; Chung, P.; Harding, H. P.; Ron, D., Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000, *287*, 664-6.

[354] Shamu, C. E.; Walter, P., Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus. *Embo J* 1996, *15*, 3028-39.

[355] Mekahli, D.; Bultynck, G.; Parys, J. B.; De Smedt, H.; Missiaen, L., Endoplasmic-reticulum calcium depletion and disease. *Cold Spring Harbor perspectives in biology* 2011, *3*.

[356] Berridge, M. J., Capacitative calcium entry. *The Biochemical journal* 1995, *312 (Pt 1)*, 1-11.

[357] Stathopulos, P. B.; Zheng, L.; Li, G. Y.; Plevin, M. J.; Ikura, M., Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry. *Cell* 2008, *135*, 110-122.

[358] Brandman, O.; Liou, J.; Park, W. S.; Meyer, T., STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell* 2007, *131*, 1327-1339.

[359] Schindl, R.; Muik, M.; Fahrner, M.; Derler, I.; Fritsch, R.; Bergsmann, J.; Romanin, C., Recent progress on STIM1 domains controlling Orai activation. *Cell calcium* 2009, *46*, 227-232.

[360] Deng, X. X.; Wang, Y. J.; Zhou, Y. D.; Soboloff, J.; Gill, D. L., STIM and Orai: dynamic intermembrane coupling to control cellular calcium signals. *Journal of Biological Chemistry* 2009, *284*, 22501-22505.

[361] Cahalan, M. D., STIMulating store-operated Ca2+ entry. *Nature Cell Biology* 2009, *11*, 669-677.

[362] Frand, A. R.; Cuozzo, J. W.; Kaiser, C. A., Pathways for protein disulphide bond formation. *Trends Cell Biol* 2000, *10*, 203-10.

[363] Hotamisligil, G. S., Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes* 2005, *54 Suppl 2*, S73-8.

[364] Park, S. W.; Zhou, Y. J.; Lee, J.; Lee, J.; Ozcan, U., Sarco(endo) plasmic reticulum Ca2+-ATPase 2b is a major regulator of endoplasmic reticulum stress and glucose homeostasis in obesity. *Proceedings of the National Academy of Sciences of the United States of America* 2010, *107*, 19320-19325.

[365] Gual, P.; Le Marchand-Brustel, Y.; Tanti, J. F., Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie* 2005, *87*, 99-109.

[366] Hirosumi, J.; Tuncman, G.; Chang, L.; Gorgun, C. Z.; Uysal, K. T.; Maeda, K.; Karin, M.; Hotamisligil, G. S., A central role for JNK in obesity and insulin resistance. *Nature* 2002, *420*, 333-6.

[367] Bommiasamy, H.; Back, S. H.; Fagone, P.; Lee, K.; Meshinchi, S.; Vink, E.; Sriburi, R.; Frank, M.; Jackowski, S.; Kaufman, R. J.; Brewer, J. W., ATF6alpha induces XBP1-independent expansion of the endoplasmic reticulum. *J Cell Sci* 2009, *122*, 1626-36.

[368] Slotkin, T. A., Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity? *Reproductive Toxicology* 2011, *31*, 297-301.

[369] Adigun, A. A.; Wrench, N.; Seidler, F. J.; Slotkin, T. A., Neonatal organophosphorus pesticide exposure alters the developmental trajectory of cell-signaling cascades controlling metabolism: differential effects of diazinon and parathion. *Environ Health Perspect* 2010, *118*, 210-5.

[370] Lassiter, T. L.; Ryde, I. T.; Levin, E. D.; Seidler, F. J.; Slotkin, T. A., Neonatal exposure to parathion alters lipid metabolism in adulthood: Interactions with dietary fat intake and implications for neurodevelopmental deficits. *Brain research bulletin* 2010, *81*, 85-91.

[371] Garry, V. F., Pesticides and children. *Toxicol Appl Pharmacol* 2004, *198*, 152-63.

[372] Sheets, L. P., A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroid insecticides. *Neurotoxicology* 2000, *21*, 57-63.

[373] Roderick, H. L.; Berridge, M. J.; Bootman, M. D., Calcium-induced calcium release. *Current biology : CB* 2003, *13*, R425.