# Role of Adipose-to-Muscle Communication in PCB126-induced Metabolic Defects

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**Abstract**

Despite the importance of muscle in the development of type 2 diabetes, few studies have investigated the effect of polychlorinated biphenyls (PCBs) on muscle energy metabolism. Previous results from our lab suggested that PCB126 exposure induced an indirect negative effect on muscle mitochondrial function. Since PCBs are stored in adipose tissue, we hypothesized that PCB126 alters adipokine secretion which in turn affects muscle metabolism. **Objectives.** Study the adipose-to-muscle communication in PCB126-induced metabolic defects. **Methods.** Communication between adipocytes and myotubes was reproduced by exposing C2C12 or mouse primary myotubes to the conditioned medium (CM) of 3T3L1 adipocytes exposed to environmentally relevant PCB126 levels. **Results.** PCB126 significantly increased adipokine secretion and decreased mitochondrial function, glucose uptake and glycolysis in insulin resistant (IR) but not in insulin sensitive 3T3L1. However, exposure of myotubes to CM of IR 3T3L1 only decreased glucose uptake and insulin sensitivity, without altering myotubes glycolysis or mitochondrial function. **Conclusion.** Our results suggest that the increased adipokine secretion by adipocytes could explain the decreased muscle glucose uptake and insulin sensitivity when exposed to PCB126.

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**Abbreviation List**

ΔΔCT – comparative CT

AA – Antibiotic Antimycotic

ACC – Acetyl-CoA carboxylase

ADH – Aldehyde dehydrogenase

AdipoR1 – Adiponectin receptor 1

AdipoR2 – Adiponectin receptor 2

AhR – Aryl hydrocarbon receptor

AMEM – Alpha modification of eagle's medium 1X

AMPK – AMP-activated protein kinase

ARNT – Aryl hydrocarbon receptor nuclear translocator

ATP – Adenosine triphosphate

bHLH-PAS – Basic helix-loop-helix-PER-ARNT-SIM

BMI – Body mass index

BSA – Bovine serum albumin

cDNA – Complimentary DNA

CM – Conditioned media

CYP1A1 – Cytochrome C 1A1

CYP1A2 – Cytochrome C 1A2

CYP1B2 – Cytochrome C 1B2

DAG - Diacylglycerol

DDE – 1,1-Dichloro-2,2-bis(p-chlorophenyl) ethylene

DMEM – Dulbecco's modification of eagle's medium 1X

DNA – Deoxyribonucleic acid

ECAR – Extracellular acidification rate

EDCs – Endocrine disrupting chemicals

eNOS – Endothelial nitric oxide synthase

ETC – Electron transport chain

FA – Fatty acids

FBS – Fetal bovine serum

FGF-b – Basic fibroblast growth factor

FFA – Free fatty acids

GLUT4 – Glucose transporter 4

HBS – Hepes Buffered Saline

hrs and hr – hour(s)

IL-6 – Interleukin-6

IL-8 – Interleukin-8

IR – Insulin resistance

IRS-1 – Insulin receptor substrate-1

IS – Insulin sensitive

JNK – c-Jun- N-terminal kinase

LPL – Lipoprotein lipase

MAPK – Mitogen-activated protein kinase

M.C – Maximal capacity

MCP-1 – Monocyte chemoattractant protein 1

M.G.C – Maximal glycolytic capacity

mRNA – Messenger RNA

NHANES – National Health and Nutrition Examination Survey

NF-κB – Nuclear factor kappa-B

NO – Nitric oxide

OCR – Oxygen consumption rate

p38MAPK – p38 mitogen-activated protein kinase

PAI-1 – Plasminogen activator inhibitor-1

PCBs – Polychlorinated biphenyls

PKB – Protein kinase B

PDK1 – Phosphoinositide-dependent protein kinase-1

PI3K – Phosphoinositide 3-kinase

PIP3 – Phosphatidylinositol (3,4,5)-triphosphate

PLIN5 – Perilipin-5

PLSD – Fisher's protected least significant difference

POPs – Persistent organic pollutants

PPARα – Peroxisome proliferator-activated receptor alpha

PPARγ – Peroxisome proliferator-activated receptor gamma

PtdIns 4,5 P2 – Phosphatidylinositol 4,5-biphosphate

RNA – Ribonucleic acid

ROS – Reactive oxygen species

RT-qPCR – Real time quantitative polymerase chain reaction

SA-PE – Streptavidin-Phycoerythrin conjugate

SEM – Standard error of the mean

Ser – Serine

SOC3 – Suppressor of cytokine signaling 3

T1D – Type 1 diabetes

T2D – Type 2 diabetes

TCDD – 2,3,7,8-Tetrachlorodibenzodioxin

TEF – Toxicity equivalency factor

TEQ – Toxic equivalence

TG – Triglycerides

Thr – Threonine

TS – Transport solution

XREs – Xenobiotic response elements

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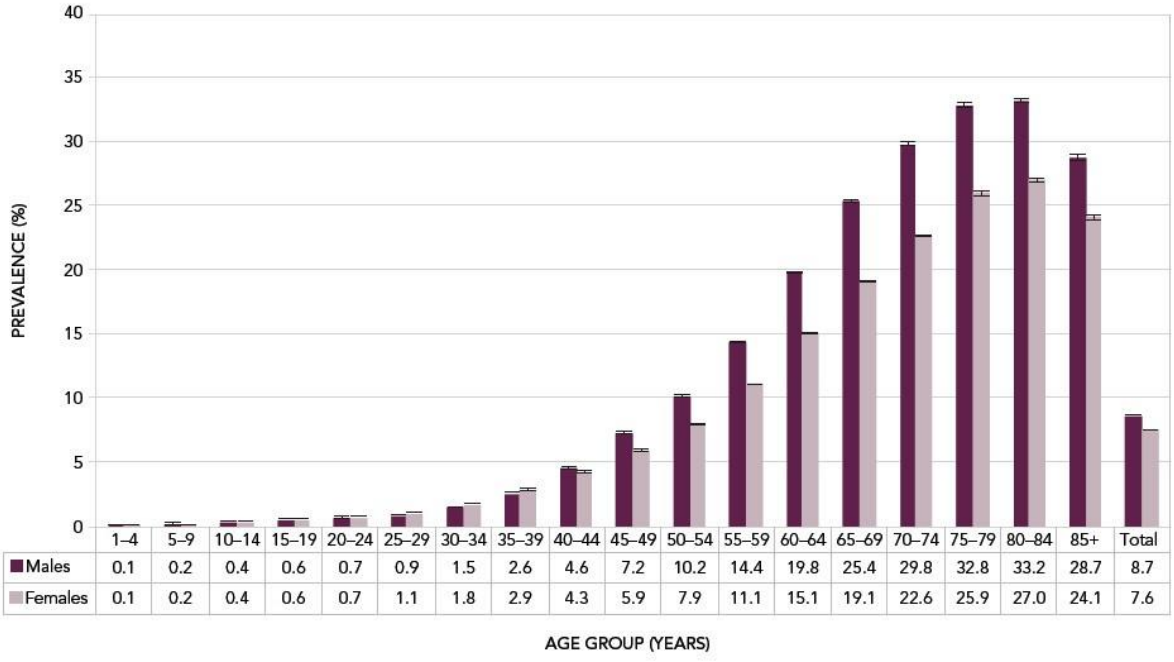
# 1. Introduction

## 1.1. Type 2 Diabetes

### 1.1.1. Epidemiological Importance

In the past decades, the number of Canadians with diabetes as nearly doubled and it is estimated that by 2020, one in three Canadian will suffer from this disease [1]. In 2013-14, the overall prevalence of type 2 diabetes (T2D) was around 8%, but it reached 30-35% in certain groups (Figure 1) [2]. In the past, T2D was called "late onset diabetes" because it mainly affected adults over 50. But its prevalence in children and teenagers has been increasing in the last 30 years [3], reaching 1 in 300 in 2013-14 [2].




| | 1–4 | 5–9 | 10–14 | 15–19 | 20–24 | 25–29 | 30–34 | 35–39 | 40–44 | 45–49 | 50–54 | 55–59 | 60–64 | 65–69 | 70–74 | 75–79 | 80–84 | 85+ | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | 0.1 | 0.2 | 0.4 | 0.6 | 0.7 | 0.9 | 1.5 | 2.6 | 4.6 | 7.2 | 10.2 | 14.4 | 19.8 | 25.4 | 29.8 | 32.8 | 33.2 | 28.7 | 8.7 |
| Females | 0.1 | 0.2 | 0.4 | 0.6 | 0.7 | 1.1 | 1.8 | 2.9 | 4.3 | 5.9 | 7.9 | 11.1 | 15.1 | 19.1 | 22.6 | 25.9 | 27.0 | 24.1 | 7.6 |

**Figure 1: Prevalence of diagnosed type 2 diabetes in Canada in 2013-14, depending on age and sex.**

This figure was obtained from the Canadian Chronic Disease Surveillance System (Public Health Agency of Canada) [2].

A similar increase in prevalence is also seen worldwide, especially in low- and middle-income groups [4], [5], which warrants new research orientation and more efficient policies. In 2014, 422 million people had been diagnosed with diabetes [4], [6], and it is estimated that about 24% of people with diabetes are undiagnosed [5]. T2D is often associated with different comorbidities and complications [4], [5], [7], such as heart disease, obesity, stroke, reduced healing capacities, neurological diseases, kidney failure, retinopathy and blindness, and high blood pressure [5], [8]. In the United States, 87,5% of adults with diagnosed diabetes are also overweight or obese (BMI > 25kg/m2), while more than 58% were on lipid-lowering medication and >73% had high blood pressure (>140mmHg/90mmHg) [5]. In 2012, diabetes caused 1.5 million deaths worldwide, while complications related to the dysregulation of glucose homeostasis caused another 2.2 million deaths [9]. Because of its health as well as its economic impacts, T2D is now considered one of the most important public health problem in multiple countries [1], [4], [5], [10]. Therefore, it is primordial to better understand the causes and mechanisms involved in the development of T2D. Since the discovery of diabetes, our knowledge about this disease has dramatically increased. We now have tools for early detection, prevention and treatment. Multiple risk factors have been associated with the development of T2D, such as unhealthy diet (quality and quantity), lack of physical activity, age, and obesity. Prevention programs rely on our actual knowledge of the risk factors and mechanisms leading to the development of diabetes. Yet, our prevention strategies have not been able to stop the diabetes pandemic. To increase their reach and effects, we need to further our understanding of the mechanisms leading to insulin resistance and abnormal energy metabolism that are associated with diabetes.

### 1.1.2. Definitions

#### 1.1.2.1. Diabetes

Diabetes is a chronic disease characterized by chronic hyperglycemia, which is defined by blood glucose >200 mg/dL, and impaired carbohydrate, lipid, and protein metabolism. It is caused by a decrease in insulin production and/or insulin action [11]. Insulin is a hormone involved in glucose homeostasis, but it is also a key player in the regulation of different metabolic pathways, including mitogenic processes and lipid metabolism [12]. Different types of diabetes exist depending on the mechanisms involved in their development and their consequences. The three major types of diabetes are type 1 diabetes (T1D) and T2D, as well as gestational diabetes. T1D is often called insulin dependent diabetes, while T2D and gestational diabetes are non-insulin dependent diabetes. In T1D, the pancreas cannot produce insulin due to gradual destruction of β-cells [13]. It is believed that T1D is due to dysregulation in the immune system that starts to attack and kill insulin producing pancreatic β-cells. Gestational diabetes develops during pregnancy possibly due to an imbalance in hormones secreted by the placenta, such as estrogen, cortisol, and human placental lactogen that can inhibit the activity of insulin causing an increase in blood glucose [14]. Finally, T2D is defined by insulin resistance at the whole-body level. T2D accounts for about 85% of diabetes cases worldwide [15], [16].

#### 1.1.2.2. Prediabetes and Insulin Resistance

The underlying mechanisms of T2D are quite complex and multiple causes have been studied. T2D develops gradually, starting by the development of insulin resistance and prediabetes. Insulin resistance is defined by a reduced response to insulin by its target tissues, such as the

liver, skeletal muscles and adipose tissue. Under healthy condition, the pancreas secretes insulin, when sensing hyperglycemia, or glucagon, under hypoglycemic conditions, to regulate blood glucose levels. Insulin is a hormone that regulates lipid, carbohydrate and protein metabolism via stimulation of glucose uptake in target tissue, while glucagon induces glucose release and/or production by the liver and the kidneys [17], [18].

Following glucose ingestion, blood glucose levels rise, which stimulates insulin secretion from the pancreatic β-cells. Insulin increases glucose uptake by peripheral tissues, such as skeletal muscle, adipose tissue and gut. Moreover, insulin suppresses endogenous glucose production by the liver. Thus, insulin secretion regulates glucose homeostasis and maintains glycemia. However, when cells are insulin resistant, the insulin response is decreased, which causes a reduction in cellular glucose uptake. Furthermore, insulin cannot repress efficiently endogenous glucose production. Insulin resistance will therefore result in hyperglycemia. In the prediabetes phase, the pancreas can further increase its insulin production to regulate glucose homeostasis and compensate the decreased response to insulin [17]–[20].

#### 1.1.2.3. What is Type 2 Diabetes?

T2D is characterized by prolonged hyperglycemia due to decreased function and mass of pancreatic β-cells, as well as insulin resistance in tissues such as liver, adipose tissue, and skeletal muscles [18]. Hyperglycemia and insulin resistance result in compensatory insulin secretion by the pancreas leading to β-cell fatigue and dysfunction. Chronically, high blood glucose leads to glucotoxicity which alters expression of genes involved in apoptosis of pancreatic β-cells, insulin production and oxidative stress [21]–[23]. Moreover, reduced insulin response is involved in increased serum free fatty acids (FFA) since adipose tissue

lipolysis is not efficiently inhibited by insulin. High levels of circulating FFA are involved in increased dysfunction, reduced proliferation and increased apoptosis of pancreatic β-cells [24]. T2D develops gradually, and it manifests only once pancreatic β-cells cannot maintain their compensatory response to insulin resistance.

#### 1.1.2.4. Causes of Types 2 Diabetes

T2D results from a complex interaction between environmental/behavioral factors and genetics. In a review of genetic risk factors of T2D, it was estimated that T2D heritability ranges between 20 and 80% [11] and over 150 genes have been associated with increased risk of T2D. Moreover, accumulation of genetic variants increases an individual risk to develop T2D. However the overall contribution of genetics to T2D is estimated to be less than 15% [25]. Other risk factors such as obesity and lifestyle show a much stronger association with T2D. In a report from Public Health England, 90% of adults with T2D were also overweight or obese [26]. Furthermore, accumulation of visceral fat is a known cause of insulin resistance development [27], [28]. Underlying factors related to obesity have recently been investigated for their potential role in insulin resistance development. It is now known that oxidative stress and inflammation are increased in obesity and involved in the pathogenesis of insulin resistance [27]–[29]. Unhealthy diet and lack of physical activity are also risk factors for the development of T2D. Excess energy intake and reduced energy expenditure have been associated with hyperglycemia and hyperlipidemia, which may be involved in the development of insulin resistance in peripheral tissues [11], [30]. In the past decades, these risk factors have been used to explain the increased prevalence of T2D. However, the increase in incidence of T2D and obesity has been too rapid to be only attributed to well established

risk factors such as genetics, reduced physical activity and/or unhealthy diet [31]. Hence, more recently the environmental hypothesis has been proposed to explain the rapid increase in the number of individuals with T2D.

## 1.2. Polychlorinated Biphenyls (PCBs) and Type 2 Diabetes

### 1.2.1. Persistent Organic Pollutants (POPs)

Since the publication of *Silent Spring* by Rachel Carson, in 1962, we have recognized that environmental pollutants are an important concern for human health and ecosystem preservation. Multiple studies and reviews have proposed that the increased prevalence of T2D and obesity could be in part attributed to endocrine disrupting chemicals (EDCs) and persistent organic pollutants (POPs) [31]–[39]. These epidemiological studies have shown a moderate-to-strong association between POP exposure and T2D in different populations from different cultural backgrounds and located in various countries. Other studies have shown a positive correlation between serum POP levels and metabolic disorders [40]. These results demonstrate the strength of the relationship between pollutants and metabolic diseases while establishing POP exposure as a worldwide threat.

POPs are man-made chemicals that are "environmentally persistent, leading to bioaccumulation and biomagnification in the food chain, an important exposure route for humans" [41]. Most POPs have a lipophilic character leading to their accumulation in adipose tissue as well as fatty compartments. This characteristic has led to the "environmental obesogen hypothesis" stating that "prenatal and/or lifetime exposure to environmental pollutants plays a role in the global obesity epidemic [42]". There is a variety of different POPs, including 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), polychlorinated

biphenyls (PCBs), and 2,3,7,8-tetrachlorodibenzo-p-diaoxin (TCDD), that have been studied *in vivo* and *in vitro* for their effects on development, metabolism, and diseases.

### 1.2.2. Polychlorinated Biphenyls

#### 1.2.2.1. Environmental Prevalence

Polychlorinated biphenyls (PCBs) were chemically engineered in the 1920s and were used in different products, such as electric equipment, ink, flames retardants, and adhesives. PCBs were never produced in Canada and were banned in the late 1970s. However, Canada imported products containing PCBs and destruction of these products resulted in PCBs being released in the environment [43]. PCBs are highly resistant to environmental and chemical degradation, and these lipophilic compounds are bioaccumulated in fat-rich food [43], [44]. They are thus still present in our food as shown in a report from Health Canada published in 2000. Exposure to PCBs from different food were measured to range from 0.2 to 12 428 part per trillion/day in Ottawa [45]. Similarly, a recent study measured that PCB levels in fishes from Ontario and Manitoba ranged from 8.98 to 63.7 ng/g of fish [37]. Due to exposure through food and the environment, significant amounts of PCBs are detected in the serum, placenta, adipose tissue, breast milk, etc. in humans [35], [46], [47].

#### 1.2.2.2. Structure of Polychlorinated Biphenyls

There are 209 different PCB congeners, each named by the position and number of chlorine substituents linked to the two phenyl rings. Figure 2A shows the basic structure of PCBs. PCBs can be separated in two classes depending on their chemical resemblance with dioxin: dioxin-like and non-dioxin-like PCBs. Moreover, PCBs can be differentiated by the arrangement of their phenyl rings. Coplanar PCBs are characterized by phenyl rings that are

in the same plane, while in non-coplanar PCBs the rings are in different planes. The phenyl rings and/or chlorine substituents arrangements dictate PCB mechanism of action. Dioxin-like PCBs (coplanar) and non-dioxin-like PCBs (non-coplanar) differ by the pathways they induce. Dioxin-like PCBs are associated with a wide range of toxic effects, such as reproductive dysfunction, immunotoxicity, liver damages, metabolic dysregulation, and developmental defects [48]. Their toxicity is mediated through the activation of aryl hydrocarbon receptor (AhR). Non-dioxin-like PCBs are also associated with liver damages, developmental and neurological effects, but their toxicity is not based on AhR activity [49]. Out of the 209 different PCB congener, only 12 are categorized as dioxin-like PCBs.

Polychlorinated biphenyl 126 (PCB126 (3,3',4,4',5-Pentachlorobiphenyl)) is a dioxin-like coplanar PCB congener. Its structure is shown in Figure 2B. PCB126 is of interest because it is one of the most common and potent PCBs. In fact, it represents 26% of toxic equivalent (TEQ) intake of all dioxin-like compounds in humans, and 60% of TEQ intake of all dioxin-like PCBs [50], [51]. Moreover, PCB126 has the highest toxic equivalency factor (TEF = 0.1) of all PCBs which is just below the highest ranked dioxin-like pollutant TCDD [52], [53].

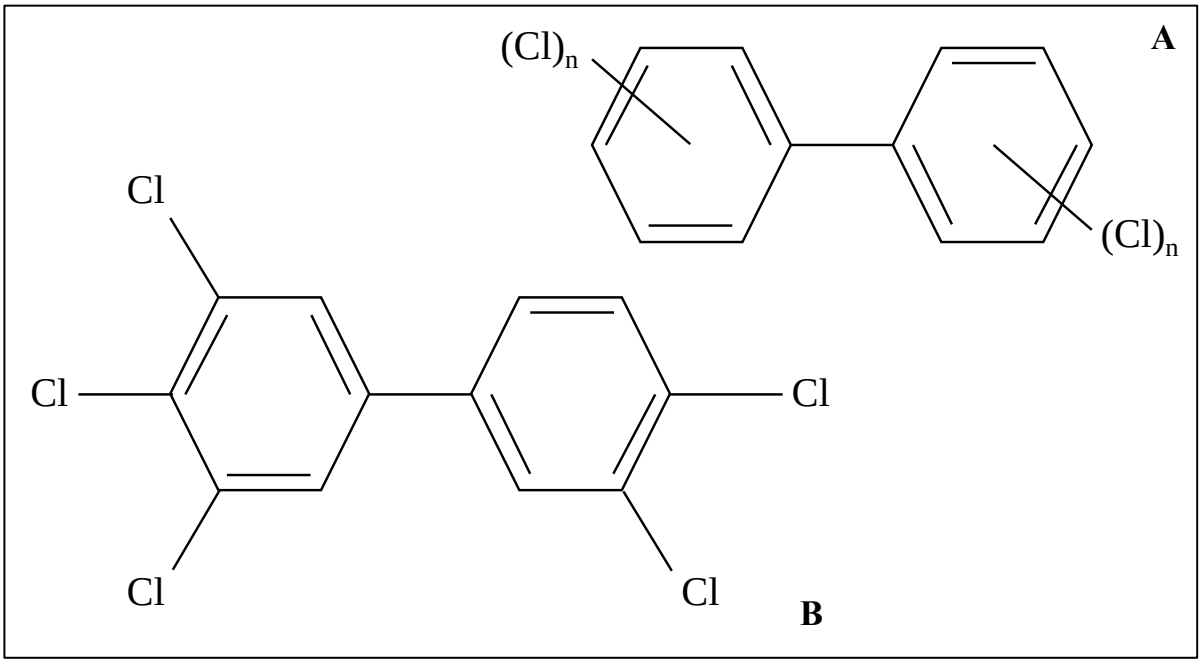



**Figure 2: Chemical structure of (A) polychlorinated biphenyls (PCBs) and (B) dioxin-like PCB126.**

PCBs are man-made chemicals made of two phenyls rings with different number and arrangements of chlorine substituents (**A**). PCB126 is a coplanar PCB with five chlorine substituents (**B**).

### 1.2.3. Mechanisms of Action of Dioxin-Like Polychlorinated Biphenyls

PCB126 toxicological effects are mediated by its binding to AhR. AhR is an intracellular receptor, located in the cytosol and the nucleus or on the mitochondrial inner-membrane, whose activation by ligands is possible if they are sufficiently lipophilic to cross the plasma membrane [54]. Cytosolic/nuclear AhR is a "ligand-activated receptor that is a member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH-PAS) transcription factor family" [55]. Once PCB126 binds AhR, the receptor translocates to the nucleus where it dimerizes with the AhR nuclear translocator protein (ARNT) and binds xenobiotic responsive elements (XREs) in the promoter and/or enhancer region of target genes, leading to upregulated expression of multiple genes. Most of these upregulated genes encode enzymes that metabolize xenobiotics, such as

the cytochrome P450 (CYP1A1, 1B1 & 1A2) and aldehyde dehydrogenase (ADH), which is an adaptive mechanism to detoxify chemicals [55]–[57]. AhR activation by xenobiotics is also involved in regulation of development, cell cycle and apoptosis, metabolism, neurogenesis, and stress response [56]. Multiple studies have proposed that this transcription factor plays a role in immune system regulation, cell proliferation, and cholesterol and glucose metabolism [55], [58]. Because AhR activation induces CYP1A1 gene expression, it has been suggested that it may be involved in increased production of reactive oxygen species (ROS) [57]. It has been shown that PCB126 activation of AhR induces oxidative stress and increases nuclear factor kappa b (NF-κB)-dependent transcriptional activity in endothelial cells [59]. NF-κB is a transcription factor involved in cell survival, cytokine production and DNA transcription. AhR interacts with the inflammatory signaling pathway, increasing inflammatory cytokines such as tumor necrosis alpha (TNF-α) [54], interleukin 6 and 8 (IL-6 and IL-8) [59], and monocyte chemoattractant protein 1 (MCP-1) [60], [61]. Moreover, AhR may activate protein kinases leading to altered phosphorylation of proteins that might be involved in metabolism, cell proliferation, and differentiation [62]. For example, coplanar PCB77 increases the phosphorylation of Akt leading to endothelial nitric oxide synthase (eNOS) phosphorylation (activation) and nitric oxide (NO) production in endothelial cells [63]. Increased NO is associated with endothelial cell dysfunction and cellular toxicity. Finally, mitochondrial AhR activation has been associated with altered cellular respiration and mitochondrial proteome, and metabolic dysfunctions [64]. The mechanisms of action of AhR and the different pathways that can be altered by its activation are shown in Figure 3.

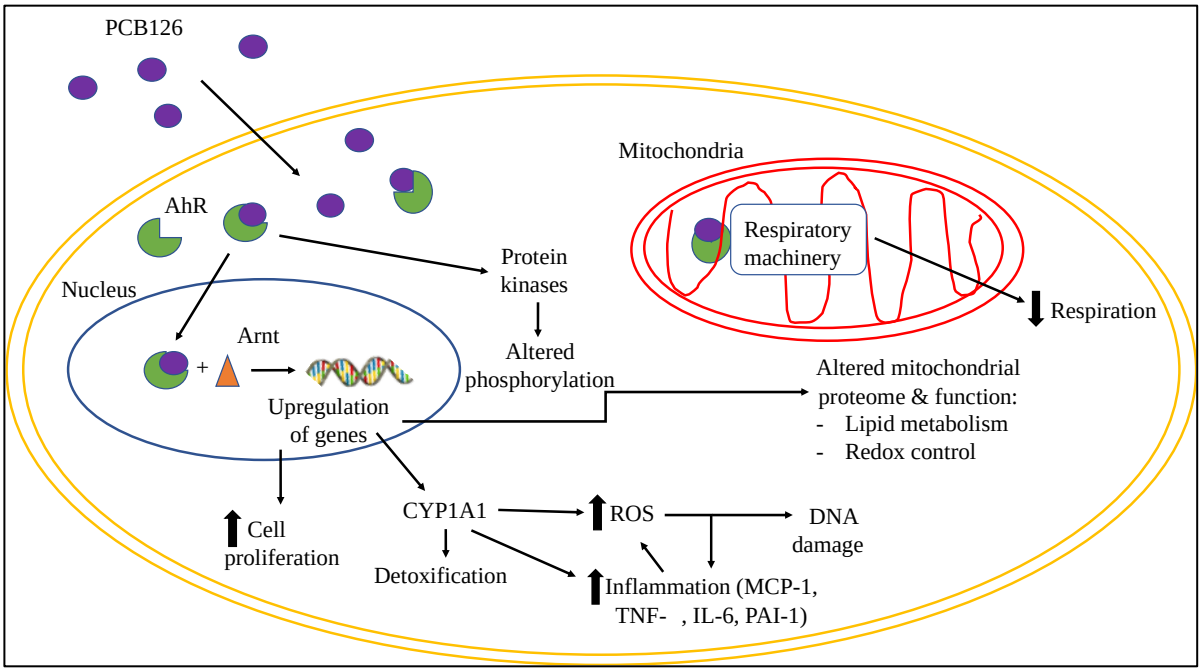



**Figure 3: Mechanisms of action and impacts of PCB126-induced activation of AhR.**

Different molecular mechanisms activated by dioxin-like PCB126. PCB126 activates AhR leading to increased inflammation and ROS production, as well as reduced cellular respiration. (Refer to text for details)

### 1.2.4. Polychlorinated Biphenyls and Type 2 Diabetes

Various epidemiological studies have measured a strong association between the prevalence of T2D and increased PCB levels [32], [36], [47], [65]. *In vivo* and *in vitro* work has also investigated whether PCB exposure results in insulin resistance development and altered energy metabolism. It has been shown that exposure to PCB153, a non-coplanar PCB, inhibits insulin-stimulated glucose uptake in hepatocytes and 3T3L1 adipocytes [66]. Moreover,

Aroclor 1254 which contains 111 different PCB congeners, such as PCB77, PCB126 and PCB153[1], can induce hyperinsulinemia and exacerbate insulin resistance in mice [67].

## 1.3. Adipose Tissue and Muscle Insulin Resistance

### 1.3.1. Importance of Muscle in Insulin Resistance and Type 2 Diabetes Development

Multiple tissues, such as the liver, skeletal muscle, brain, adipose tissue, and pancreas are involved in glucose homeostasis [68]. Skeletal muscle represents about 40% of total body weight in a healthy person. Moreover, muscle cells are responsible for 80-90% of glucose uptake and glycogen synthesis after a meal or physical exercise [12], [69]–[71]. When muscle glucose uptake or metabolism are altered the repercussions are seen at the whole-body level [69]. Understanding the mechanisms involved in muscle insulin resistance development has thus become the focus of multiple research groups. Although the development of T2D is usually associated with pancreatic β-cell dysfunction, it has been suggested that muscle insulin resistance is one of the first step toward T2D, before chronic hyperglycemia and β-cell failure [72], [73].

#### 1.3.1.1. Muscle Insulin Signaling

Under healthy conditions, insulin signaling is an important player in muscle glucose uptake [12]. The first step in muscle insulin signaling is the binding of insulin to its receptor [74] leading to the phosphorylation of the different proteins involved in the insulin response. The

[1]Different batches of Aroclor 1254 were produced at different moment, and their composition varies. The "earlier" batches contained: PCB 2, 6, 8, 15-18, 22, 26, 28, 31-33, 37, 40-42, 44, 45, 47-49, 52, 53, 56, 59, 60, 63, 64, 66, 70, 71, 74, 76, 77, 82-89, 91, 92, 94-97, 99, 101-103, 105, 109, 110, 114, 115, 117-119, 122-126, 128-139, 141, 144, 146, 147, 149, 151, 153, 154, 156-158, 163, 164, 166, 167, 170-172, 174, 176-180, 183, 187, 189, 190, 193, 194, 199, 203, 206, 208 [262]

action of insulin requires the phosphorylation of key proteins, such as insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB/Akt), leading to the translocation of the glucose transporter type 4 (GLUT4) to the plasma membrane triggering insulin-mediated glucose uptake. The detailed pathway is shown in Figure 4. The increased glucose uptake in cells as well as insulin itself also promote glucose oxidation and/or storage in muscle by activating key enzymes involved in glycolysis, glycogenesis, and/or lipogenesis [75].

Multiple factors, such as hyperglycemia, dyslipidemia and mutations of key proteins in the insulin signaling pathway, may alter the response to insulin [67], [75]. For example, it has been shown that mutations in kinase genes, including Akt or the insulin receptor, as well as imbalance in the number of PI3K subunits can alter the insulin signaling pathway [32], [74]. Moreover, increased phosphorylation of serine/threonine residues on IRS-1 results in the inhibition of the phosphorylation of IRS-1 tyrosine residues leading to an inhibition of the insulin signaling pathway [74], [76]. Interestingly, it has been shown that individuals with a family history of T2D have an increased phosphorylation of IRS-1 on serine/threonine residues, a reduced activity of PI3K [76], and have normal glucose tolerance but moderate to severe insulin resistance in skeletal muscle [77]–[79]. Taken together these results suggest that insulin resistance in muscle comes before chronic hyperglycemia and pancreas dysfunction [70].

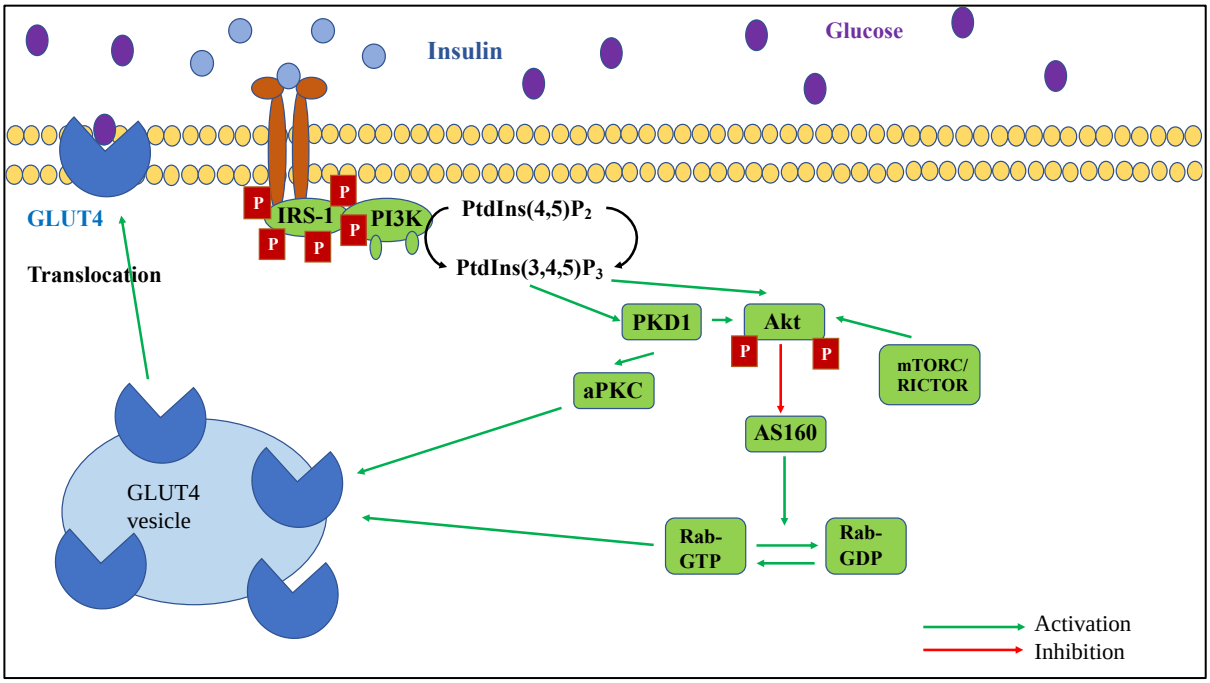



**Figure 4: Muscle insulin signaling pathway and GLUT4 translocation under insulin sensitive conditions.**

First, insulin binds to its receptor. The tyrosine kinase domain of the insulin receptor then phosphorylates IRS-1 and IRS-2. Once activated, IRS-1 activates PI3K subunits. These convert the phosphatidylinositol 4,5 P2 (PtdIns 4,5 P2) into phosphatidylinositol 3,4,5 P3 (PIP3). PIP3 can bind to phosphoinositide-dependant kinase 1 (PKD1) that activates PKB, also called Akt. Activated Akt is responsible for the inhibition of AS160 causing the activation of Rab. Once Rab is activated it releases GLUT4. GLUT4 then translocates to the membrane and triggers insulin-mediated glucose uptake. Under basal condition, GLUT4 is mostly found in intracellular vesicles but it is continuously cycled from the membrane to the vesicles [12], [74], [80].

#### 1.3.1.2. Skeletal Muscle Mitochondrial Function and Insulin Resistance

It has been suggested that muscle mitochondrial dysfunction could lead to altered insulin signaling pathway causing muscle insulin resistance. Skeletal muscle cells contain a high number of mitochondria in order to respond to increased adenosine triphosphate (ATP) demands during contraction. Under healthy conditions, mitochondria are responsible for lipid oxidation (β-oxidation) and oxidative phosphorylation. However, it has been shown that

patients with T2D have a reduced muscle mitochondrial function [81]–[84]. Interestingly, there was also a decreased mitochondrial function in relatives and offspring of patients with T2D which was associated with reduced insulin signaling activity [76], [82], [85], [86]. This suggests that mitochondrial dysfunction appears before T2D establishment and might be an important factor in the development of T2D. However, others have suggested that mitochondrial function is not altered in insulin resistant patients, and that reduced mitochondrial content would be responsible for decreased oxidative phosphorylation and electron transport chain capacity [87]. In this sense, it has been demonstrated that mitochondrial density is reduced in insulin resistant individuals, and is associated with increased phosphorylation of serine residues of IRS-1 and decreased insulin-stimulated activation of Akt [76]. Due to its role in skeletal muscle metabolism and association with T2D, mitochondrial dysfunction might be one of the early signs of insulin resistance development. Figure 5 summarizes the role of muscle mitochondrial dysfunction in the development of muscle insulin resistance.

#### 1.3.1.3. Reactive oxygen species and oxidative stress

During oxidative phosphorylation by the electron transport chain, mitochondria generate ROS, such as $H_2O_2$, and superoxide [28], [29], [88]. Due to a high mitochondrial content, skeletal muscle is an important source of ROS. ROS are often seen as toxic by-products of metabolism, however they are also essential to cell signaling and regulation under healthy conditions [89]. In fact, ROS are involved in the regulation of intracellular redox states and in oxidative modification of proteins, as well as in immune system response to pathogens and cell survival [90].

Usually, there is tightly regulated balance between the production of oxidants and anti-oxidants. However, when there is an imbalance in the oxidants/antioxidants ratio, ROS and other oxidants accumulate in cells and cause oxidative stress [29]. Increased ROS production leads to cell damage by causing DNA mutation, lipids peroxidation and altered protein expression and/or function [27], [75], [91]. Under metabolic dysfunctions, such as insulin resistance and T2D, there is an imbalance in ROS and antioxidants production by mitochondria [92]. It has been shown that in diet-induced diabetic-mice, there is an increase in ROS production in skeletal muscle cells in association with muscle mitochondrial dysfunction, reduced mitochondrial density and insulin resistance development [93]. It seems that ROS accumulation induces oxidative stress which directly alters the insulin signaling pathway [94]–[97] reducing cellular glucose uptake [98], [99]. In fact, it has been shown that increased ROS production can increase the Ser/Thr phosphorylation of IRS-1, or the degradation of IRS, reducing the insulin pathway activity in muscle cells [68]. To demonstrate that ROS were involved in muscle mitochondrial defects and insulin resistance, the effect of antioxidants has also been tested. Antioxidants reduced muscle ROS production, restored mitochondrial biogenesis and function, and increased insulin sensitivity [93], [98], [100].

#### 1.3.1.4. Muscle Mitochondrial Lipid Oxidation

Besides increased oxidative stress, decreased mitochondrial biogenesis and/or function leads to altered lipid metabolism in skeletal muscle, via decreased FA oxidation. An altered muscle mitochondrial function has been associated with decreased insulin sensitivity in patients with T2D and/or obesity due to inefficient lipid oxidation [101]. A decrease in lipid oxidation can lead to intramyocellular accumulation of lipid intermediates, such as ceramides, acyl-CoAs,

acylcarnitines, and diacylglycerol (DAG) [75]. It has been demonstrated that some of these intermediates can alter the insulin signaling pathway by inducing the phosphorylation of IRS-1 on serine/threonine residues causing a reduced muscle insulin sensitivity [102], [103]. Moreover, incomplete muscle lipid oxidation results in acylcarnitine accumulation leading to a decrease phosphorylation of Akt and reduced insulin-stimulated glucose uptake in muscle cells [104]. It has also been shown that lipid accumulation alone is not to blame for the development of insulin resistance. In fact, athletes can also store lipid droplets in their muscle cells despite being highly insulin sensitive [105]. This is often called the "athletes paradox". The mechanisms involved in the protection against intramyocellular lipid accumulation and insulin resistance are being investigated. A recent study has demonstrated the role of a specific protein, perilipin 5 (PLIN5), in the pattern of lipid storage in muscle cells (droplet size and number). This study shows that PLIN5 could be associated with the oxidative capacity of skeletal muscle cells [105]–[107]. These results concord with others showing that an increase in PLIN5 promoted the interaction between lipid droplets and mitochondria, as well as oxidative gene expression [108]. These new findings, taken together with the results showing the effect of lipid intermediates on insulin sensitivity support the role of inefficient β-oxidation in the development of muscle insulin resistance.

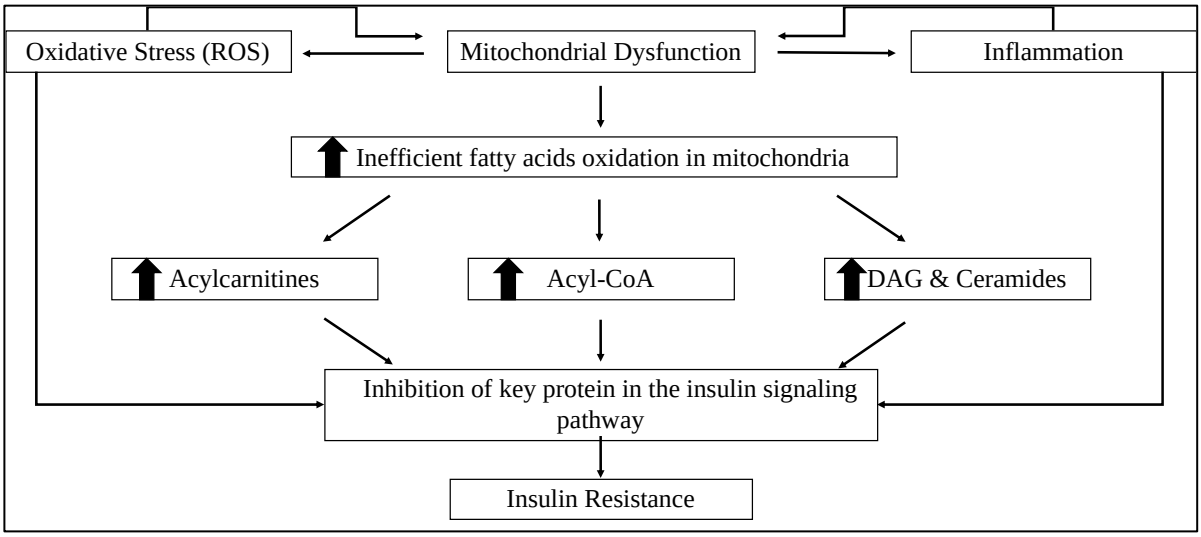



**Figure 5: Role of muscle mitochondrial dysfunction in the development of insulin resistance and T2D.**

In dysfunctional mitochondria, FA oxidation is inefficient leading to the accumulation of harmful lipids intermediates (acylcarnitines, DAG, ceramides & acyl-CoAs). These lipid intermediates can inhibit the activity of the insulin signaling pathway. This leads to insulin resistance, reduced glucose uptake and hyperglycemia. Mitochondrial dysfunction also causes increased inflammation and ROS production, which may further reduce mitochondrial function. Inflammation and ROS also inhibit key proteins in the insulin signaling pathway leading to insulin resistance.

### 1.3.2. Obesity and Adipose Tissue Dysfunction in the Development of Insulin Resistance and Type 2 Diabetes

Obesity is a well-known risk factor in the development and establishment of T2D. The National Health and Nutrition Examination Survey (NHANES) (2013–2014) showed that 1 in 3 adults, while 1 in 6 children and adolescents (2-19 years old) have obesity [109]. Individuals with overweight have a body mass index (BMI) between 25 and 29.9 kg/m2, whereas obesity is defined by a BMI over 30 kg/m2 [9], [109], [110]. The prevalence of overweight and obesity has increased in the past decades, reaching 70.2% in the US [109] and 50.4% in Canada in 2014 [9]. Obesity is characterized by an excessive expansion of adipose tissue mass in the different fat depots [111]. Under healthy condition, fatty acids (FA) are

stored in adipose tissue in the form of triglycerides (TG) [109], [112], [113]. Moreover, adipose tissue plays a role in the regulation of glucose homeostasis. Excess glucose is taken up and converted into long chain FA and TG by adipocytes in response to insulin [113]. Insulin also causes an increase in adipogenic response in adipocytes by increasing the cellular levels of peroxisome proliferator-activated receptor gamma (PPARγ), which activates adipogenesis, glucose and FFA uptake, and TG storage in adipose tissue [114]. Apart from its role in lipid storage, adipose tissue also has an endocrine function through the secretion of adipokines [104], [115]. This endocrine function of adipose tissue is detailed in the next chapter.

Under excessive energy intake, "mature adipocytes start to enlarge, undergo cellular hypertrophy and store more fat [116]". If this energy imbalance (energy intake >> energy expenditure) is prolonged, adipocyte hypertrophy leads to altered cytokine and hormone secretion to promote hyperplasia (increased number of cells) in adipose tissue. This helps to maintain healthy blood levels of glucose and FFA. However, adipocytes have a saturation point with a limited capacity to store more lipids [112], [116]. Once this threshold is reached by excessive lipid accumulation, adipocytes initiate a stress response, leading to increased local inflammation and disturbed adipokine secretion [111], [112], [116]–[118], as well as increased FFA released from lipolysis, affecting the function of other organs and tissues [69], [118].

Excessive fat accumulation in adipose tissue has also been associated with glucose intolerance and insulin resistance [69], [119], [120], and increased oxidative stress [121]. Adipose tissue insulin resistance further increases adipocyte dysfunction. Moreover, other studies have shown that increased adiposity can alter gene transcription and mitochondria-associated

protein expression in different tissues [122], including skeletal muscle and liver [123]. These effects are often associated with increased adipose tissue lipolysis leading to a rise in circulating FFA levels and increased FFA intake in tissues, such as the liver and skeletal muscle [69], [117], [118]. FFA can also be associated with an increase in inflammatory mediators secreted by dysfunctional adipocytes [69], [117], [118], [124], which may promote inflammation or metabolic dysfunction in other tissues, such as the liver and skeletal muscle [124], [125]. Furthermore, a recent study has also looked at the role of cell-free DNA released from dysfunctional adipocytes going through apoptosis or necrosis. They showed that obesity-induced DNA secretion was increased in obese mice and in patients with obesity and/or insulin resistance in association with increased macrophage recruitment and insulin resistance [126]. Taken together these results demonstrate that adipose tissue is more than a lipid storage compartment and that its dysfunction can alter the metabolism at the whole-body level.

### 1.3.3. Role of Adipose Tissue Dysfunction in Muscle Insulin Resistance and Type 2 Diabetes

It has been demonstrated that different factors secreted by adipose tissue, such as FFA and adipokines, may alter insulin sensitivity in other tissues, especially in skeletal muscle [124], [127]–[131]. As mentioned by Steinberg (2007), "obesity and [T2D] are causally linked through their association with the development of skeletal muscle insulin resistance [124]". Since it has been proposed that insulin resistance in muscle is one of the first step in T2D development, the study of the crosstalk between adipose tissue and skeletal muscle is necessary to our understanding of the pathophysiology of T2D. Various research groups have studied the impact of increased adiposity on skeletal muscle metabolism, via altered adipokine and FFA secretion.

#### 1.3.3.1. Adipokines and inflammation

Under normal conditions, adipose tissue secretes various cytokines and adipokines that can regulate the metabolic function of other tissues. When faced with abnormal lipid accumulation, cellular stress and/or harmful chemicals, adipose tissue becomes inflamed which results in alteration of cytokine secretion [69], [126]. Adipose tissue dysfunction leads to increased secretion of pro-inflammatory cytokines, such as TNF-α, IL-6 and MCP-1, and to decreased secretion of anti-inflammatory adipokines, like adiponectin. In humans, these signalling molecules may be involved in impaired insulin sensitivity and signaling, and may alter the secretion of other cytokines at the whole-body level [129].

##### 1.3.3.1.1. Adiponectin

Identified in the late 90s, adiponectin is an hormone secreted by adipocytes [117], [130], [132]. Adiponectin was initially believed to be secreted specifically by adipose tissue. However, in the past decade, it has been debated whether adiponectin could also be secreted by other tissues, such as skeletal muscle [133]. Its structure has homology to TNF-α [132], however it is an anti-inflammatory cytokine [117], [133]. It has been shown that adiponectin plays a role in maintaining energy homeostasis, regulating glycemia and lipid metabolism [44], [117], [127], [134].

Interestingly, even if most of adiponectin in circulation comes from adipose tissue, plasma levels of this adipokine are reduced in patients with T2D and/or obesity, as well as in individuals with other diseases associated with impaired insulin response [117], [135]. This decrease is also seen at the mRNA levels of adiponectin genes [136]–[138], which are usually indicative of insulin resistance in mice. Furthermore, patients with diabetes show higher

prevalence of different mutations and polymorphisms in adiponectin gene [117]. It has also been shown that in skeletal muscle cells from individuals with obesity or T2D there is a decreased mRNA expression of the adiponectin receptor 1 (AdipoR1) in response to adiponectin or leptin compared to healthy subjects [139], suggesting a decreased response to adiponectin and leptin in insulin resistant muscle cells.

In skeletal muscle, adiponectin promotes FA oxidation [134], hence a decrease in adiponectin signaling may lead to increased accumulation of lipid intermediates in muscle cells. It has also been demonstrated that adiponectin can improve whole-body insulin sensitivity making it a potential target to treat insulin resistance [117], [127], [134], [140], [141]. Adiponectin may promote insulin sensitivity and glucose uptake mainly through activation of the AMP-activated protein kinase (AMPK) [130], [140], [142]–[144] and peroxisome proliferator-activated receptor alpha (PPARα) or PPARγ [117], [124], [145], but also through other pathways described in Figure 6. On one hand, activation of AMPK causes an increase in muscle FA oxidation [146], [147], and promotes oxidative metabolism in mitochondria [148], [149]. Furthermore, it reduces oxidative stress [150] and increases mitochondrial biogenesis in skeletal muscle [151], [152]. PPARα is also involved in FA metabolism and it increases FA oxidation in mitochondria [114]. On the other hand, adiponectin may activate PPARγ, which is involved in adipocyte differentiation, and the expression of genes involved in FFA and TG uptake and storage. PPARγ agonist, such as thiazolidinediones, are used to treat T2D and other diseases associated with insulin resistance, because they increase insulin sensitivity in muscle via decreased inflammatory cytokines secretion (TNF-α & MCP-1) and increased

FA oxidation [114], [153]. Finally, adiponectin also facilitates the interaction of IRS-1/2 with the insulin receptor [154].

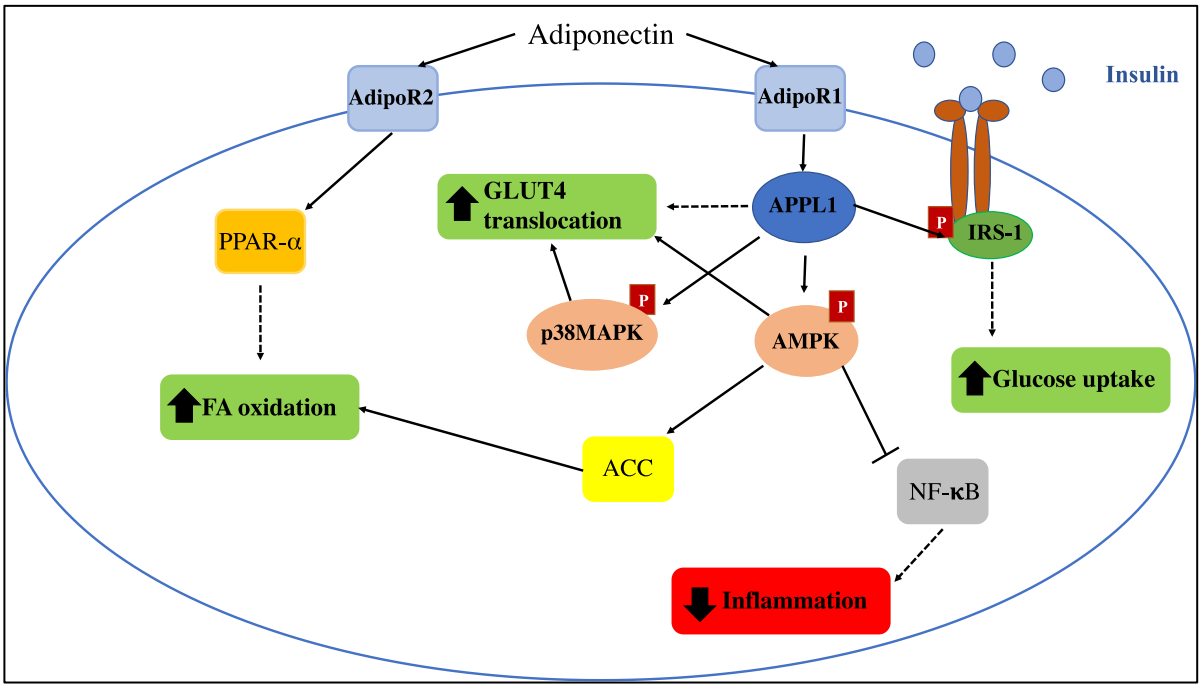



**Figure 6: Signaling pathways affected by adiponectin in adipose tissue and skeletal muscle.**

Adiponectin from adipose tissue increases glucose uptake in adipocytes and muscle cells, by facilitating the interaction between IRS1/2 and the insulin receptor. It may also activate GLUT4 translocation by activating different pathways, such as p38MAPK or the AMPK pathways. Adiponectin also promotes FA oxidation, via PPARα and AMPK activation. Moreover, adiponectin inhibits NF-κB, reducing inflammation, which may also promote glucose metabolism.

### 1.3.3.2. Leptin

Leptin is also a hormone secreted by adipose tissue and involved in maintaining energy balance, via regulation of energy intake and energy expenditure. Another important role of leptin is the regulation of FA oxidation in nonadipocyte cells. In fact, leptin increases the action of PPARα and/or AMPK promoting lipid oxidation and reducing ectopic lipid

accumulation in tissues, such as the pancreatic islets. Hence, leptin is involved in FA homeostasis [142].

Circulating levels of leptin, but not leptin activity, are related to adipose tissue mass. It has been shown that individuals with obesity have higher levels of circulating leptin but do not respond to leptin, which is called leptin resistance [155]–[158]. On the other hand, in leptin sensitive people, leptin can increase insulin sensitivity by inhibiting liver glucose production and increasing glucose uptake by various cell types, including muscle cells [159]. In skeletal muscle, leptin directly increases the activity of IRS-1 leading to GLUT4 translocation [160], which promotes insulin-stimulated glucose uptake [161]. Moreover, leptin can also promote FA oxidation in skeletal muscle through the activation of AMPK [142]. Interestingly, it has been shown that the activation of AMPK signaling and FA oxidation by leptin was reduced in muscle cells from subjects with obesity [162]. Figure 7 illustrates how leptin may improve glucose uptake and FA oxidation in muscle cells.

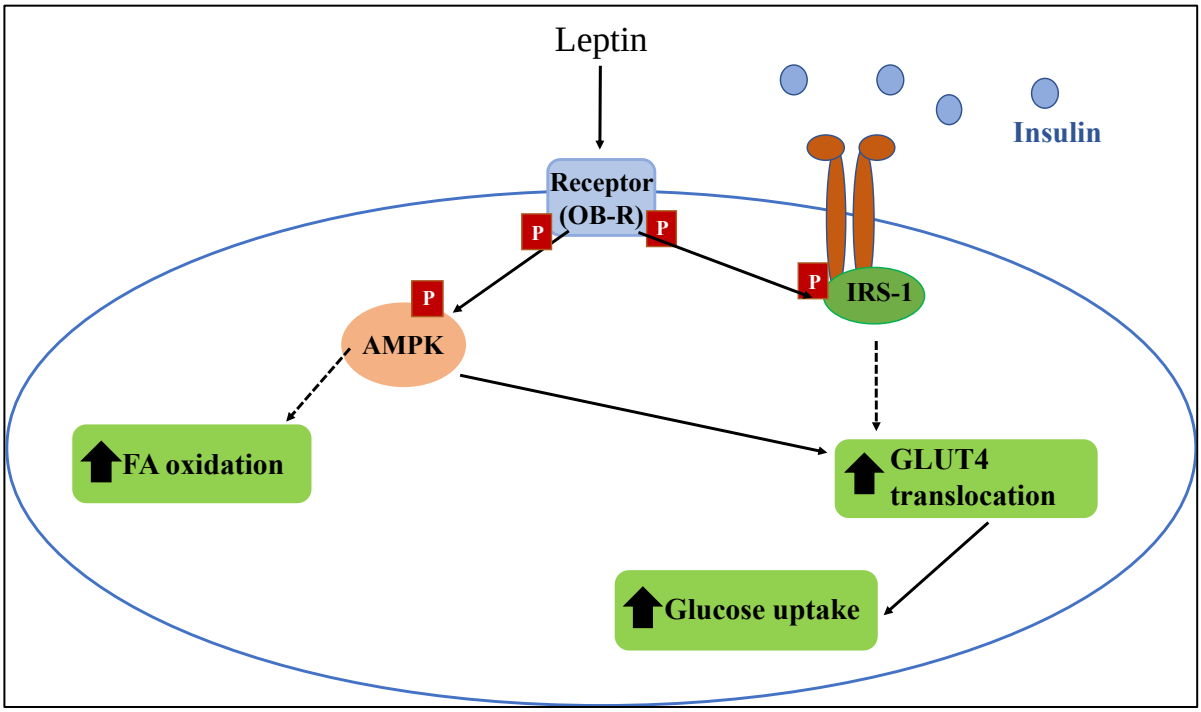



**Figure 7: Signaling pathways that may be involved in the positive effects of leptin on skeletal muscle energy metabolism.**

In skeletal muscle, leptin promotes FA oxidation and glucose uptake, via AMPK activation and increased IRS-1 activity.

#### 1.3.3.3. Tumor necrosis factor alpha

One of the most studied cytokine, TNF-α has multiple pro-inflammatory effects in various tissues. TNF-α is secreted mostly by macrophages during the acute phase reaction but it can also be produced by other cell types [163]. It is involved in the secretion of other pro-inflammatory cytokines, such as IL-6 [164]. It has been shown that individuals with obesity and/or T2D have increased circulating levels of TNF-α, and circulating levels of TNF-α are positively correlated with BMI and hyperinsulinemia [111], [165], [166]. Interestingly, after injection of TNF-α in human, circulating TG concentration was increased [167], whereas

insulin-stimulated glucose uptake was decreased in peripheral tissues [167], [168], showing a direct effect of TNF-α in insulin resistance development.

When secreted by adipocytes, TNF-α is also associated with insulin resistance development in adipose tissue [169] [131], [170], [171], which would further increase adipose tissue dysfunction and inflammation. Furthermore, in adipocytes, TNF-α phosphorylates IRS-1 on Ser/Thr residues leading to decreased glucose uptake [172]. Moreover, TNF-α reduces the expression of multiple genes involved in FA and glucose oxidation, especially in adipocytes [145], [173]. TNF-α can increase lipolysis in 3T3L1 adipocytes, while inhibiting lipoprotein lipase (LPL) [174] that hydrolyzes TG from lipoprotein to FA and monoacylglycerol. This increased circulating FFA and TG levels in response to TNF-α might increase the risk of insulin resistance in other tissues [175].

Recent studies have also demonstrated that TNF-α is involved in the development of insulin resistance in skeletal muscle. In healthy individuals, a TNF-α infusion inhibited whole-body glucose disposal and increased phosphorylation of different proteins involved in insulin signaling (IRS-1, JNK) in skeletal muscle resulting in decreased insulin signal transduction [176]. This was confirmed *in vitro*, with insulin resistance development in TNF-α-treated muscle cells [164], [176]. It seems that TNF-α induces muscle insulin resistance via increased serine phosphorylation on IRS-1 and decreased expression of GLUT4 [74], [124], [164]. Furthermore, TNF-α induction of muscle insulin resistance might be due to its role in the production of NF-κB, an important pro-inflammatory transcription factor [164] which could increase inflammation in muscle and promote mitochondrial dysfunction. Finally, TNF-α can inhibit AMPK [124], which in turn may lead to a reduced FA oxidation and glucose uptake.

These different pathways altered by increased TNF-α secretion in adipose tissue and other target tissues, such as skeletal muscle, are shown in Figure 8.

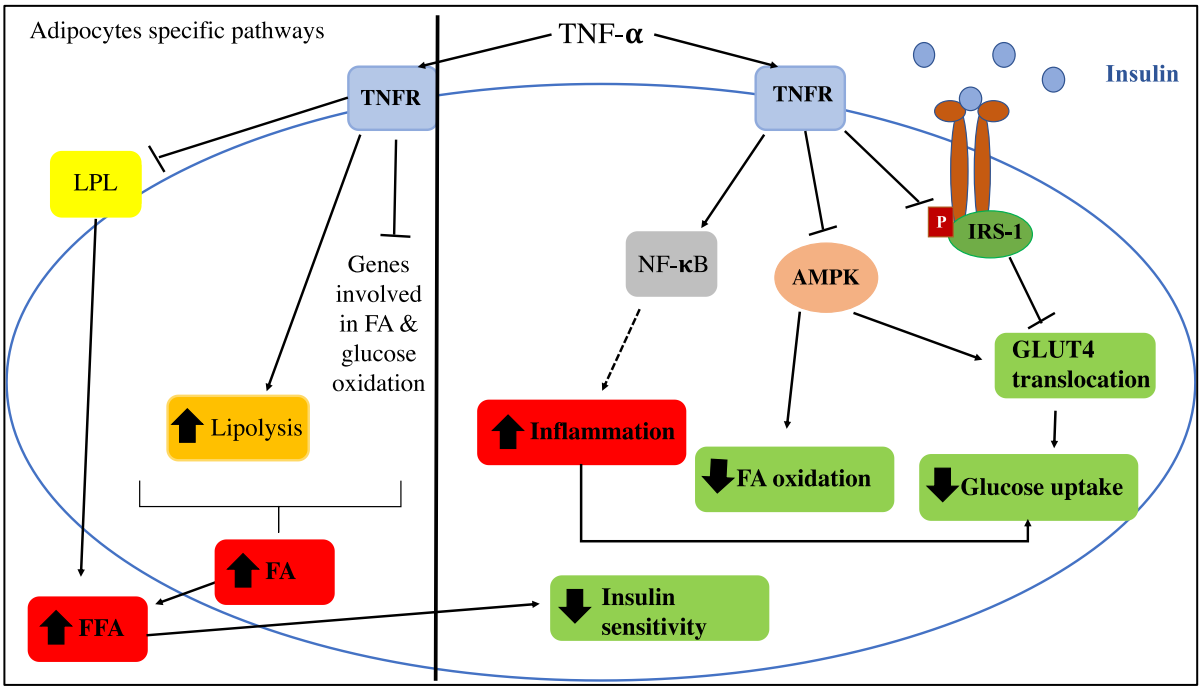



**Figure 8: TNF-α alters multiple signaling pathways involved in metabolism.**

In adipose tissue, TNF-α inhibits the expression of genes involved in FA and glucose oxidation, and LPL, and it increases lipolysis. Thus, there is an increased secretion of FFA from adipose tissue. Circulating FFA might reduce insulin sensitivity in target tissues, such as skeletal muscle. In adipose tissue and skeletal muscle, TNF-α promotes inflammation and reduces FA oxidation and glucose uptake.

### 1.3.3.4. Interleukin 6

Interleukin 6 is secreted by multiple cell types, such as immune cells (T cells and macrophages), adipocytes, skeletal muscle cells and epithelial cells. In human, it has been demonstrated that IL-6 is abundantly expressed in adipose tissue [111] and muscle from individuals with obesity [162]. The role of IL-6 depends on the cause and source of its secretion. On one hand, it acts as a pro-inflammatory cytokine, especially when secreted by

adipose tissue and immune cells, and on the other hand, it is an anti-inflammatory myokine, when secreted by muscle during contraction [131], [177]. Diverging results have led to different hypothesis on the role of IL-6 in obesity and T2D. IL-6 expression and secretion are increased in adipocytes from individuals with obesity [111], [178] and circulating IL-6 concentration is associated with FFA levels [111]. It has been shown that IL-6 inhibits LPL, but does not affect lipolysis [168], [179].

Interestingly, co-culture of 3T3L1 adipocytes with L6 muscle cells caused an increased expression of IL-6 mRNA and a decreased insulin-stimulated phosphorylation of Akt in muscle cells [180]. The authors have thus suggested that increased secretion of IL-6 by adipocytes might be associated with the development of muscle insulin resistance. Finally, it has been demonstrated that acute IL-6 exposure enhances FA oxidation and glucose transport while a prolonged exposure promotes insulin resistance development in skeletal muscle cells [131], [181]. How IL-6 secreted by adipose tissue can alter muscle energy metabolism is described in Figure 9.

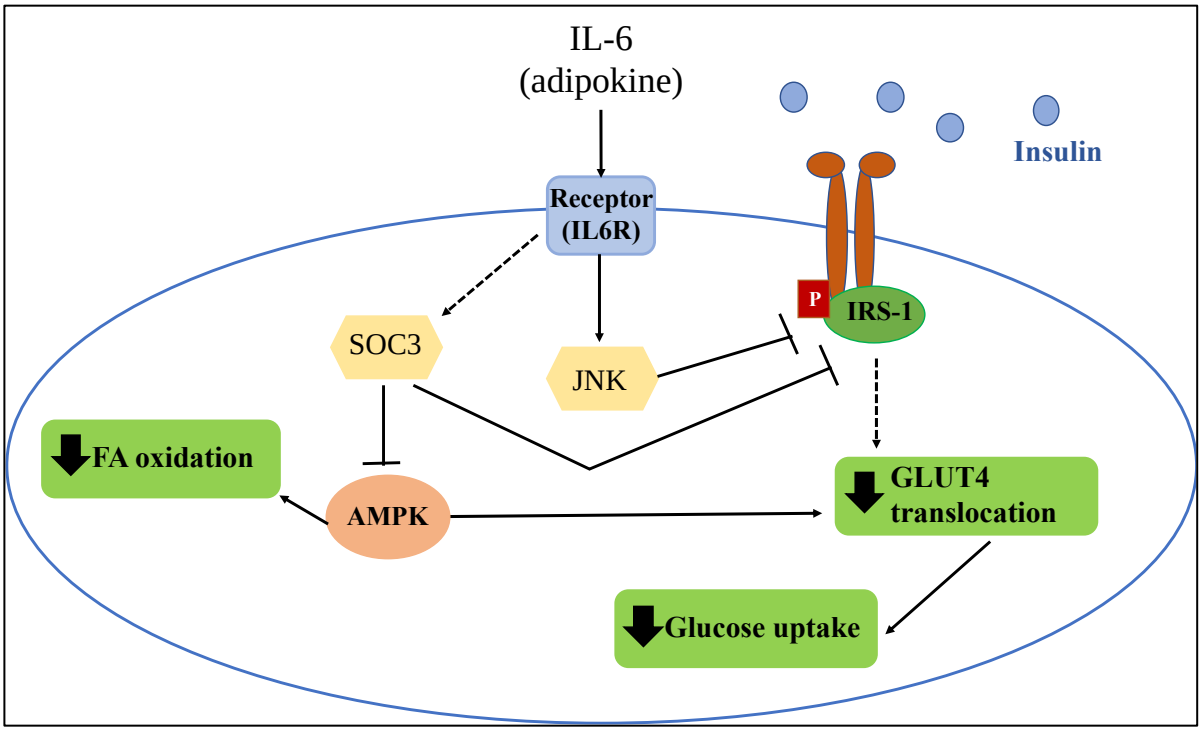



**Figure 9: Role of the adipokine IL-6 in skeletal muscle regulation of energy metabolism.**

When IL-6 is secreted by adipose tissue, it can inhibit IRS-1 activity, by JNK and/or SOC3 phosphorylation of Ser/Thr residues on IRS-1, leading to decreased glucose uptake [182], [183]. IL-6 also inhibits AMPK, which reduces FA oxidation and GLUT4 translocation.

### 1.3.3.5. Other adipocytokines

Multiple other adipocytokines have been identified in the past decades but their role in glucose homeostasis and muscle metabolism are not well understood yet.

MCP-1 is an important chemokine responsible for monocytes/macrophages recruitment [184]. MCP-1 is produced constitutively by different cells types, including adipocytes. MCP-1 may be induced by oxidative stress, cytokines, such as TNF-α and IL-1, and/or growth factors [184]. Overexpressing MCP-1 in adipose tissue of transgenic mouse models resulted in increased macrophage infiltration in adipose tissue, as well as increased TNF-α and IL-6 mRNA levels in adipose tissue and increased circulating FFA. These mice were also insulin

resistant [185]. Finally, it has been shown that MCP-1 can decrease insulin-stimulated glucose uptake in myotubes [186], [187].

Another adipose tissue-specific secreted factor, resistin, is a hormone that might be involved in obesity and T2D. Controversy exists on the main source of resistin. It seems that the predominant source would be macrophages that have infiltrated adipose tissue. However, resistin can also be expressed in human adipocytes [188], [189], especially if they share features with macrophages [131]. Serum concentrations of resistin are associated with obesity in human [188]. However, it was shown that resistin can reduce adipogenesis in rodents [190]. In adipose tissue, resistin has been associated with impaired insulin signaling in part due to its effect on other cytokines but the mechanism remains unclear [191]–[193]. In rodents, resistin has also been shown to play a role in insulin resistance development in the liver and skeletal muscle [193].

Beside these most common adipocytokines, another player, plasminogen activator inhibitor-1 (PAI-1) has been investigated for its potential role in obesity and insulin resistance development. PAI-1 is involved in tissue remodeling and the fibrinolytic process [194]. However, it has also been shown that high circulating levels of PAI-1 are associated with the metabolic syndrome and insulin resistance [194]. In a Japanese population, BMI was the strongest determinants for serum levels of PAI-1, and obesity seemed to play an important role in PAI-1-related insulin resistance development [195]. However, the mechanisms have not yet been investigated.

Taken together, these results show the relationship between adipose tissue function, the regulation of adipokine secretion and insulin resistance development in skeletal muscle.

#### 1.3.3.6. Free Fatty Acids and Lipolysis

Adipose tissue dysfunction, caused by excessive enlargement of adipocytes, impairs its capacity to properly store and metabolize lipids. Obesity and adipocyte dysfunction are associated with increased lipolysis due to insulin resistance in adipocytes, leading to increased serum FFA. These FFA further increase insulin resistance in adipose tissue via increased inflammation [118]. Moreover, adipose tissue dysfunction has been associated with increased lipid droplets in muscle which can promote the accumulation of harmful lipid intermediates responsible for the development of mitochondrial dysfunction and/or insulin resistance as previously discussed [196], [197]. In this sense, it has been shown that long chain saturated FFA can reduce insulin-stimulated glycogenesis and glucose uptake via increased DAG and ceramide accumulation in C2C12 muscle cells [198].

## 1.4. Polychlorinated Biphenyls and Metabolic Dysfunctions

### 1.4.1. Polychlorinated Biphenyls and Adipose Tissue Inflammation

In adipose tissue, it seems that different PCBs can have different outcomes. Certain PCBs, including PCB126, inhibit adipogenesis [128], [199], while others, like PCB77, actually activate adipocyte differentiation [200]. It has been suggested that PCB126 activates AhR which inhibits PPARγ transcription leading to decreased adipogenesis [128].

Beside differentiation, PCBs also alter adipose tissue inflammation profile by activating AhR leading to increased CYP1A1 expression [58]. It has been shown that PCB77 and PCB126 can activate inflammatory pathways in adipocytes and endothelial cells via increased production of pro-inflammatory adipokines, including TNF-α, IL-6 and MCP-1, and decreased anti-inflammatory adipokines [60], [199], [201], [202]. Different mechanisms have

been proposed to explain how PCB77 increases inflammation. It has been shown that PCB77 activates NF-κB in HUVEC which could be associated with increased secretion of TNF-α [201]. PCB77- or PCB126-induced activation of AhR and its downstream targets, including caveolin-1, CYP1A1, mitogen activated protein kinase (MAPK) and c-Jun-K-terminal kinase (JNK), could be associated with increased oxidative stress and MCP-1 secretion in endothelial cells [61].

Interestingly, different groups have investigated the effects of pollutants, including PCB126, in diet-induced rodent models of obesity. They have demonstrated that pollutants exaggerate diet-induced insulin resistance, adiposity, and adipose tissue inflammation in rats fed a high-fat diet compared to rats fed a regular diet [203]. Furthermore, in mice with obesity-induced insulin resistance, PCBs exacerbate hyperinsulinemia and insulin resistance compared to lean insulin sensitive mice [33]. These results suggest that rodents with pre-established metabolic disorder, such as obesity and insulin resistance, have an increased sensitivity to PCBs.

Taken together, the above-mentioned studies show that the increased inflammation and reduced differentiation induced by PCBs, including PCB126, results in adipose tissue dysfunction further increasing inflammation. Since increased inflammation is closely linked to the development of peripheral insulin resistance and mitochondrial dysfunction, it is thus possible that through increased inflammation, PCB could cause mitochondrial dysfunction and insulin resistance in other tissues, such as skeletal muscle.

### 1.4.2. Polychlorinated Biphenyls and Mitochondrial Dysfunction

AhR activation has been associated with reduced cellular respiration [58], [60], [61] and might be responsible for PCB126-induced mitochondrial dysfunction in different cell types. In

endothelial cells, TCDD which has mechanism of action similar to PCB126 disrupts the interaction between mitochondrial AhR and ATP synthase reducing its efficiency [204]. ATP synthase is the last complex of the electron transport chain (ETC) and its activity is primordial to ATP synthesis. Besides, CYP450 1A1 is activated by AhR when it binds PCB126 leading to decreased mitochondrial function in the liver and adipose tissue [64], [128], [205], as shown in Figure 3. It has also been shown in the liver that PCB126 increases mitochondrial size [206], which is known to result in mitochondrial dysfunction [207]. The effect of PCB126 on mitochondrial function could also be induced by its accumulation in mitochondria. Once in the mitochondria, PCB126, like TCDD [208], could directly cause damage to constitutive proteins or ETC complexes. Beside these effects, it has been demonstrated that PCB126 promotes ROS production in hepatocytes, chondrocytes and endothelial cells [59], [209]. This increased ROS production might be responsible for the PCB126-induced oxidative stress [59], [210], and could also be involved in mitochondrial defects [211] and/or reduced ATP production [212] caused by PCB126.

### 1.4.3. Polychlorinated Biphenyls, Muscle Insulin Resistance and Mitochondrial Dysfunction

Despite the role of skeletal muscle in glucose homeostasis regulation, PCB effects on skeletal muscle metabolism has not been investigated thoroughly. Recent results from our laboratory showed that direct PCB126 exposure in L6 rat skeletal muscle cells induced a 20% decrease in glucose uptake and glycolysis rate but had no effect on oxidative stress [211]. The reduction in glucose uptake concords with another report showing a decrease in GLUT4 translocation and glucose disposal in rat muscles after exposure to a PCB mixture [213]. Other results from our laboratory also demonstrated that exposure of rats to a single injection of PCB126 for a

week resulted in a 40% reduction mitochondrial respiration with complex I substrates in permeabilized muscle fibers [214]. Interestingly, no mitochondrial dysfunction was measured *in vitro* in muscle cells exposed directly to PCB126 [211]. Skeletal muscle response to PCB126 is therefore different *in vivo* (whole organism) and *in vitro*. It therefore seems that the mitochondrial dysfunction in skeletal muscle of rats exposed to PCB126 was not the result of a direct effect of the pollutant on muscle mitochondria.

## 2. Hypothesis and Objectives

Because PCBs are mostly accumulated in adipose tissue, we hypothesized that PCB126 might first induce adipose tissue dysfunction resulting in altered adipokine secretion and inflammation. This altered adipokine profile/inflammation could then be responsible for skeletal muscle mitochondrial dysfunction. Hence, the overall aim of this project was to study the role of adipose-to-muscle communication in PCB126-induced metabolic defects as illustrated in Figure 10. The specific objectives of this study were 1) To determine the effect of PCB126 exposure on adipocyte cytokine/adipokine production; 2) To study whether the communication between adipose and muscle tissues may explain muscle abnormal glucose metabolism when exposed to PCB126; 3) To study whether the communication between adipose and muscle tissues may explain muscle mitochondrial dysfunction when exposed to PCB126, and 4) To study whether pre-established insulin resistance in adipocytes alters the metabolic responses to PCB126 in adipocytes and the communication between adipocytes and muscle cells.

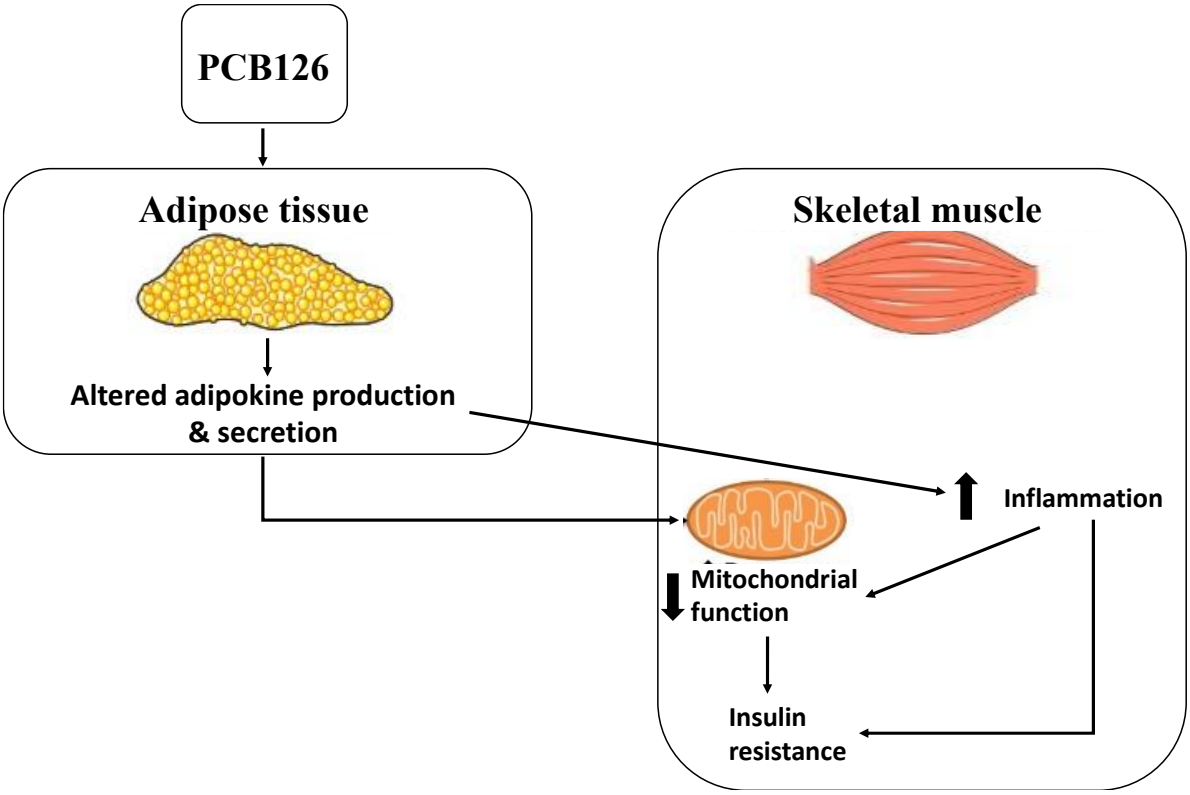



**Figure 10: PCB126 might alter skeletal muscle metabolism via increased adipokine secretion by adipose tissue.**

Hypothesis: PCB126 might first induce adipose tissue dysfunction altering adipokine secretion. This altered adipokine profiles could then be responsible for skeletal muscle mitochondrial dysfunction and insulin resistance development.

# 3. Methods

## 3.1. Cell culture

All cell lines were kept at -80°C or liquid nitrogen until needed. Cells were cultured in a humidified incubator at 37°C with 5% CO2.

### 3.1.1. 3T3L1 adipocytes

3T3L1 were grown in low glucose DMEM (1.0 g/L glucose, 4 mM L-glutamine and 110 mg/L sodium pyruvate, Wisent), 10% CS (Calf Serum, Wisent) and 1X antimycotic-antibiotic (AA) (Wisent). The medium was refreshed every two days until cells reach ≈90% confluence. For differentiation, three different media were used at during specific time as described in table 2. Different insulin concentrations and durations were used to induced insulin resistance in adipocytes (IR). The adipocytes were differentiated for 10 days. 3T3-L1 cells were kindly provided by Dr. Ella Atlas, at the Environmental Health Centre (Ottawa, ON, Canada).

**Table 1: Adipocytes (3T3L1) differentiation protocol and schedule.**

| Adipocytes insulin sensitivity status | Differentiation Media | | |
|---|---|---|---|
| | **ADM1** | **ADM2** | **ADM3** |
| **Insulin sensitive adipocytes (IS)** | DMEM low glucose (1 g/L) | DMEM low glucose (1 g/L) | DMEM low glucose (1 g/L) |
| | 10% FBS | 10% FBS | 10% FBS |
| | 0.5 mM 3-Isobutyl-1-methylxanthine | 100 nM Insulin | 1X Antibiotic-Antimycotic |
| | 1 $\mu$M Dexamethasone | 1X Antibiotic-Antimycotic | |
| | 100 nM Insulin | | |
| | 1X Antibiotic-Antimycotic | | |
| **Insulin resistant adipocytes (IR)** | DMEM low glucose (1 g/L) | MDA2 | |
| | 10% FBS | DMEM low glucose (1 g/L) | |
| | 0.5 mM 3-Isobutyl-1-methylxanthine | 10% FBS | |
| | 1 $\mu$M Dexamethasone | 500 nM Insulin | |
| | 500 nM Insulin | 1X Antibiotic-Antimycotic | |
| | 1X Antibiotic-Antimycotic | | |

| Differentiation Schedule | | | | | |
|---|---|---|---|---|---|
| **Adipocytes** | **Day 0 to 2** | **Day 2 to 4** | **Day 4 to 6** | **Day 6 to 8** | **Day 8 to 10** |
| **IS Adipocytes** | ADM1 | ADM2 | ADM1 | ADM2 | ADM3 |
| **IR Adipocytes** | ADM1 | ADM2 | ADM1 | ADM2 | ADM2 |

### 3.1.2. C2C12 muscle cells

C2C12 myoblasts were grown with DMEM low glucose, 10% FBS and 1X AA. The medium was refreshed every other day until cells reach ≈90% confluence. For differentiation, the medium contained DMEM low glucose, 2% FBS and 1X AA and was refreshed every 2 or 3 days. Muscle cells were differentiated for 7 days. C2C12 muscle cells were purchased from Sigma-Aldrich.

### 3.1.3. Mouse primary muscle cells

Mouse primary myoblasts derived from gastrocnemius and tibialis muscles of wild-type mice with a C57BL/6J background were used to test the effect of our PCB126 treatments on insulin sensitivity because C2C12 do not respond well to insulin in term of glucose uptake due to high levels of basal glucose uptake [215]. Mouse primary myoblasts were cultured and grown in a DMEM:F12 1:1 medium (Wisent) supplemented with 20% FBS, 1X AA, 3 µg/ml gentamicin (Wisent) and 5 ng/ml recombinant mouse fibroblast growth factor basic (FGF-b) (Sigma). At ≈90% cell confluence, growth medium was replaced by a differentiation medium composed of DMEM low glucose supplemented with 2% FBS, 1X AA and 3 µg/ml gentamicin. Cells were differentiated into myotubes for 7 days. Cells were cultured on Matrigel matrix (1X in DMEM) (Corning) coated equipment. Primary mouse cells were a kind gift from Dr. Marc Foretz (Institut Cochin, Paris, France).

## 3.2. PCB126 treatment

Cells were exposed to different concentration of PCB126 (0, 1, 10, 100 nM). These concentrations are equivalent to the concentration previously used in our laboratory in our *in vivo* study. These concentrations (0 to 100 nM) were chosen to represent physiological

PCB126 concentration. In fact, exposure to PCB126 in Canadian Inuit population is between 0.05 nM to 27 nM [216], while the daily intake of PCB126 is estimated to 12 pg/day [51].

On day 10 of differentiation, adipocytes were exposed for 24hrs to 0, 1, 10 or 100 nM of PCB126 dissolved in 0.1% dimethyl sulfoxide (DMSO, Sigma). Control medium (0 nM) contained 0.1% DMSO. After the 24hrs PCB126 treatment, the conditioned medium of adipocytes (CM) was used to treat C2C12 myotubes or mouse primary muscle cells, in order to determine whether a change in some factors secreted by adipocytes upon exposure to PCB126 affected the metabolism of myotubes. CM was transferred to C2C12 myotubes or mouse primary muscle cells at day 7 of differentiation, for 24hrs. As a control condition, myotubes were also directly treated with the same concentrations of PCB126 for 24hrs, on day 7 of differentiation. After the 24hr-treatments, all cells were prepared for the different experiments described below.

## 3.3. Cell Viability

To determine whether the different treatments had an effect on cell survival, viability was measured using the PrestoBlue method (ThermoFisher) according to manufacturer's instructions. 3T3L1 and C2C12 were grown and differentiated in 96-well plates (20 000 cells/well) and treated with PCB126 or 3T3-L1 CM for 24hrs as described above. Cells were then incubated for 30 min in 1X PrestoBlue® reagent and absorbance was measured at 570 nm and 600 nm (reference wavelength). Each independent experiment (n=3) was done in triplicates for 3T3-L1 adipocytes and in six replicates for C2C12 myotubes.

## 3.4. Lipid accumulation

To determine whether the treatments had an effect on 3T3-L1 and C2C12 intracellular lipid accumulation, lipid droplet accumulation was measured by Oil Red O staining (Sigma). Lipid accumulation is an indicator of adipocyte differentiation. It also measures intramyocellular lipids, which are believed to be related to insulin resistance development [106], [217]–[219]. For these experiments, 3T3-L1 were grown and differentiated in 6-well plates, while C2C12 were grown and differentiated in 12-well plates. The cells were treated with PCB126 and/or 3T3-L1 CM as described above, and then fixed with 4% paraformaldehyde in PBS for 10 min. Oil Red O (0.5%) was dissolved in isopropanol. This solution was then diluted in three volumes of water for two volumes of 0.5% Oil Red O and filtered to remove undissolved Oil Red O residues (0.2 μM Supor Membrane). Cells were then incubated with 0.5 mL (12-wells plates) or 1 mL (6-wells plates) of Oil Red O for 20 minutes. Cells were washed three times with distilled water and visualized by light microscope using a constant light intensity and with a 40X objective. Finally, to quantify lipid accumulation, Oil Red O was extracted with 70% isopropanol and absorbance read at 490 nm [220]. Each independent experiment (n=3) was done in duplicates and Oil Red O extraction was read in triplicate.

## 3.5. Cytokine and adipokine measurements

### 3.5.1. Protein concentration measurements

To determine whether the different treatments affected cytokine and adipokine secretion, 3T3-L1 were grown and differentiated in 6-well plates, whereas C2C12 were grown and differentiated in 12-well plates followed by 24hr-treatments as described above. At the end of the treatments, culture media were collected and stored at -80°C until further

cytokine/adipokine measurements. Adipokine (IL-6, TNF-α, leptin, adiponectin, MCP-1, PAI-1, and resistin) concentrations in the different culture media were determined by the Bio-Plex Multiplex Immunoassay System (Bio-Rad) using the mouse adipocyte magnetic bead panel kit (Millipore, MADCYMAG-72K-07), according to manufacturer's instructions [56]. 10 µL of each sample and standards were added to the detection beads and incubated overnight at 4°C with gentle agitation, washed 3 times followed by addition of secondary antibodies coupled to biotin for 30 minutes. After 3 washes, SA-PE (streptavidin-Phycoerythrin conjugate) was added to each well for 10 minutes at room temperature and washed 3 times. Beads were resuspended in drive fluid (Bio-Rad) and read by the Bio-Plex. Each independent experiment (n=3) was done in duplicate.

### 3.5.2. mRNA quantification by RT-qPCR

To determine whether the different treatments affected mRNA expression, 3T3-L1 were grown and differentiated in 6-well plates followed by 24hrs-treatments as described above. At the end of the treatments, cell lysed using RLT buffer with 0.01% mercaptoethanol (Sigma) from the RNeasy Mini Kit (Qiagen) and lysates were stored at -80°C. Total RNA was extracted from cell lysates using the RNease Mini Kit where genomic DNA was removed using the RNase-Free DNase Kit (Qiagen) following the manufacturer's recommendations [221]. RNA concentration and extraction quality were measured using a NanoDropTM 1000 Spectrophotometer (ThermoFisher). Quality was determined using the 260/280 ratio and the 260/230 ratio. These ratios permit assessment of RNA purity. Ratio ranging from 1.8-2.0 represents a high level of RNA purity. If the 260/280 ratio is lower than 2.0 it indicates the presence of contaminants such as proteins or DNA, while a 260/230 ratio lower than 1.8

indicates contamination of RNA by organic compounds, such as phenols or thiocyanates. Then 0.5 μg RNA was reverse transcribed with iScript cDNA Synthesis Kit (Bio-Rad) following the manufacturer's protocol [222] in a CFX96-PCR Detection System. Samples presenting ratios of $2.0 \pm 0.75$ were used to perform the fallowing steps. Complementary DNA (cDNA) was amplified and quantified in a CFX96-PCR Detection System using the iQSYBR SsoFast EvaGreen Supermix (Bio-Rad). Primers for each target genes were design and chosen by Dr Vian Peshdary and are summarized in Table 2. All genes were normalized to β-actin levels and analyzed using the comparative CT (ΔΔCT) method, as described previously [223]. β-actin was chosen as internal control due to its high stability in adipocytes. Each independent experiment (n=3) was done in triplicates.

**Table 2: Primer sequence used for RT-qPCR.**

| Target gene | Primer sequence | |
|---|---|---|
| **IL-6** | Forward | 5'-GCCTTCTTGGGACTGATGCT-3' |
| | Reverse | 5'-TGCCATTGCACAACTCTTTTC-3' |
| **Adiponectin** | Forward | 5'- TGACGACACCAAAAGGGCTC-3' |
| | Reverse | 5'- CACAAGTTCCCTTGGGTGGA-3' |
| ***β*-actin** | Forward | 5'- GACTTCGAGCAAGAGATGGC -3' |
| | Reverse | 5'- CCAGACAGCACTGTGTTGGC -3' |

## 3.6. Lipolysis

To determine whether PCB126 or insulin sensitivity status had an impact on the lipolysis rate in adipocytes, glycerol and FFA were quantified in the adipocyte media by using a lipolysis quantification kit (LIP-3-NC-L1, ZenBio) following the manufacturer's protocol. Briefly, 3T3-L1 adipocytes were differentiated and treated with PCB126 as described above, in 96-well plates. Then, cells were incubated for 3h in assay buffer with PCB126. The media was

used to quantify FFA and glycerol secretion. PCB126 was also used directly in assay buffer to test its interaction with reagents from the assay kit. Glycerol or FFA released to the medium were assessed by measuring the absorbance at 540 nm. The increase in absorbance at 540 nm is directly proportional to glycerol or FFA concentration of the sample. Each independent experiment (n=3) was done in triplicate.

## 3.7. Glucose uptake

To determine whether the different treatments affected glucose uptake in cells, 3T3-L1, C2C12 and primary mouse muscle cells were grown and differentiated in 24- or 48-well plates, followed by 24hr-treatment as described above. Glucose uptake was measured as in Klip et al. (1986) [224]. Cells were starved for 3hrs at 37°C in serum-free DMEM low glucose. During the last 20 min of the starvation period, 100 nM insulin was added in half of the wells. Cells were then washed three times with Hepes Buffered Saline (HBS, 140 mM NaCl, 20 mM Hepes-Na, pH 7.4, 5 mM KCl, 2.5 mM MgSO4 and 1 mM CaCl2). For specific glucose uptake 200 μL of transport solution (TS) was added (HBS with 10 μM 2-Deoxy-Glucose and 0.5 μCi/mL 3H 2-Deoxy-glucose, 25 Ci/mmol Perkin Elmer). For non-specific glucose uptake TS with 10 μM Cytochalasin B (Sigma) was added to three wells. Cells were incubated at room temperature for 5 min. The cells were then washed three times with ice cold PBS. Cells were lysed in 0.5 mL of 0.05 M NaOH and 0.4 mL were measured by scintillation counting with a Tri-Carb2910TR counter (Perkin Elmer, Canada). The remaining 0.1 mL cell lysate was used to determine protein content using a Bradford protein assay. The volumes mentioned here were adapted to perform the experiments with mouse primary muscle cells in 48-well plates.

Each independent experiment (n=4 for 3T3L1, n=3 for C2C12 and n=4 for primary mouse muscle cells) was done in triplicate.

## 3.8. Mitochondrial respiration and glycolysis

To determine whether the different treatments affected 3T3-L1 and C2C12 energy metabolism, mitochondrial function was measured by determination of oxygen consumption rates (OCR), and glycolysis by determination of extracellular acidification rates (ECAR), using an extracellular flow analyzer (Seahorse XF-96, Agilent). The protocols provided by Agilent was followed for these experiments with slight modifications [225]–[227]. At the end of the 24hr-treatments, cells were rinsed three times with assay buffer (8.3 g/L DMEM, 2 mM sodium pyruvate, 5 mM dextrose, and 0.75 mM L-glutamine, at pH 7.4, Sigma-Aldrich). Cells were then incubated at 37°C, without $CO_2$, in 180 µL of assay buffer for 30-45 minutes. OCR and ECAR were first measured at baseline for 4 cycles comprising: 2 min measurement, medium mixing for 2 min and 2 min pause before starting the next cycle. Then, inhibitors of the respiratory chain were injected into each well in the following order: oligomycin, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and antimycin A (all from Sigma-Aldrich). After each injection, OCR and ECAR measurements were made for 3 cycles (measuring, mixing, rest, 2 min each). Final inhibitor concentrations used were 600 ng/mL oligomycin, 1 µM FCCP and 2 µM antimycin A for 3T3-L1 and 600 ng/mL oligomycin, 1 µM FCCP and 4 µM antimycin for C2C12. At the end of the experiment, cells were lysed with 50 µL of 0.05 M NaOH and proteins were quantified by the Bradford method (Bio-Rad). OCR and ECAR values are expressed per µg of total cellular protein. Each independent experiment (n=4) was done in 5 or 6 replicates.

## 3.9. Bradford assay for protein quantification

As mentioned above, the Bradford method was used to quantify proteins after the different experiments. Briefly, standards (0 to 200 µg/mL) were prepared using bovine serum albumin (BSA, Sigma) with Bradford reagent and water. Sample were lysed in 0.05 M NaOH. Protein samples and standards were prepared as recommended in the protocol from Bio-Rad [228]. Sample and standards were loaded in triplicate in a 96-well clear plate and read at 595 nm using a spectrophotometer (Fisher Scientific).

## 3.10. Statistical Analysis

Data shown are the means ± standard error of the mean (SEM) of at least 3 independent experiments. These experiments were performed at different moment when possible and using cells from different source tubes. A one- or two-way ANOVA with Fisher's protected least significant difference (PLSD) post-hoc test were used to determine statistical differences. A $p<0.05$ was considered significant.

# 4. Results

We first optimized our adipocytes differentiation protocol and confirmed the induction of insulin resistance in 3T3L1 cells. Using different conditions to differentiate adipocytes we measured glucose uptake to determine the insulin response, and lipid staining with ORO to study the effects of the conditions on differentiation itself. As shown in figure 11A and B, the IR condition reduced insulin-stimulated glucose uptake in adipocytes compared to IS condition ($p=0.0269$ compared to basal glucose uptake, Figure 11A, and $p=0.0438$ compared to IS condition, Figure 11B). Moreover, there was no difference in lipid accumulation between the IS and IR condition suggesting that the two differentiation protocols did not affect adipogenesis (Figure 12).

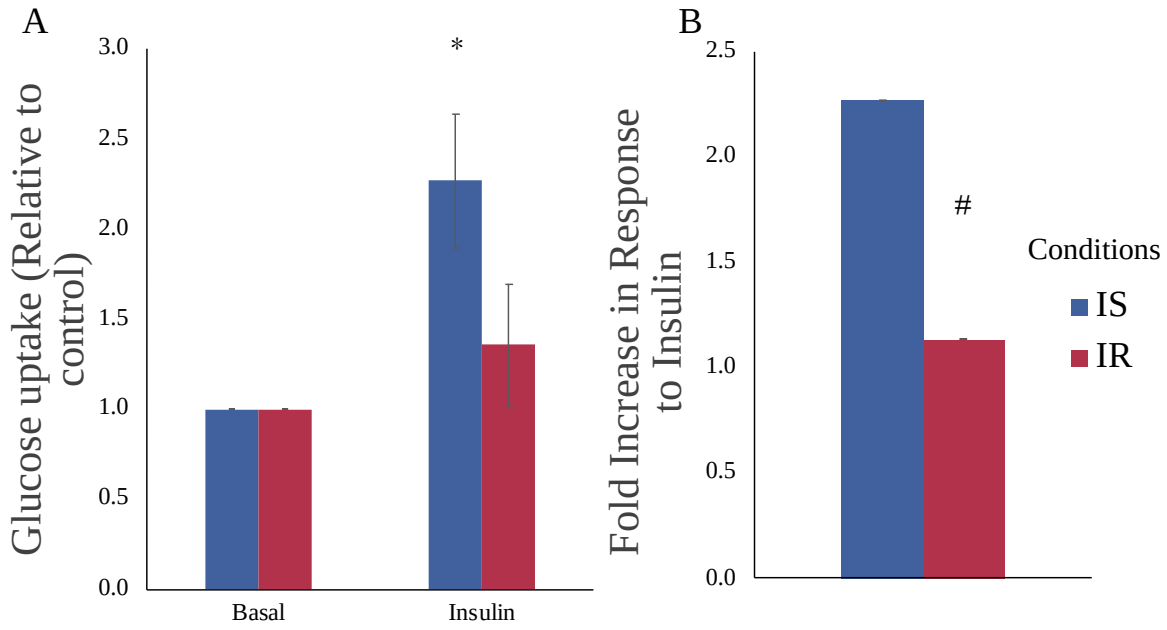



**Figure 11: Glucose uptake measure in adipocytes differentiated using IS and IR conditions.**

Optimization of the differentiation protocols was performed, and conditions IS an IR were chosen for this project. Glucose uptake (A) and insulin response (B) were measured to

determine the effect of the condition on glucose metabolism and insulin sensitivity. (*: p≤0.05, compared to basal, #: p≤0.05, compared to IS).


**Figure 12: Micrographs of ORO staining of neutral lipid from differentiated IS and IR adipocytes.**

The IS and IR differentiation protocols did not alter differentiation in adipocytes.

## 4.1. A 24hr-exposure to PCB126 or CM has no effect on cell viability or lipid droplet accumulation

It has previously been shown that 24hr-treatment with 0.01 to 10 μM PCB126 induced apoptosis in chondrocytes [209]. However, it has also been demonstrated that 0 to 2.5 μM PCB126 for 24hrs did not alter cell viability in L6 myotubes [211]. The effect of PCB126 on cell death might vary depending on cell type. Therefore, we first investigated the impact of 24hr-treatment to 0-100 nM PCB126 on cell viability in 3T3L1 adipocytes and C2C12 myotubes. We also determined if 3T3L1 CM exposure for 24hr would alter cell viability in C2C12 myotubes. Cell viability was measured using the PrestoBlue method with live cells after the 24hr-treatments. PCB126 did not alter cell survival in adipocytes or myotubes exposed directly to PCB126 both in IS and IR conditions (Figure 13A and B). Cell viability was surprisingly significantly increased in myotubes exposed to CM from IS adipocytes treated with 100 nM PCB126, compared to the control condition (CM 0 nM PCB126) (p=0.0005, Figure 13A).

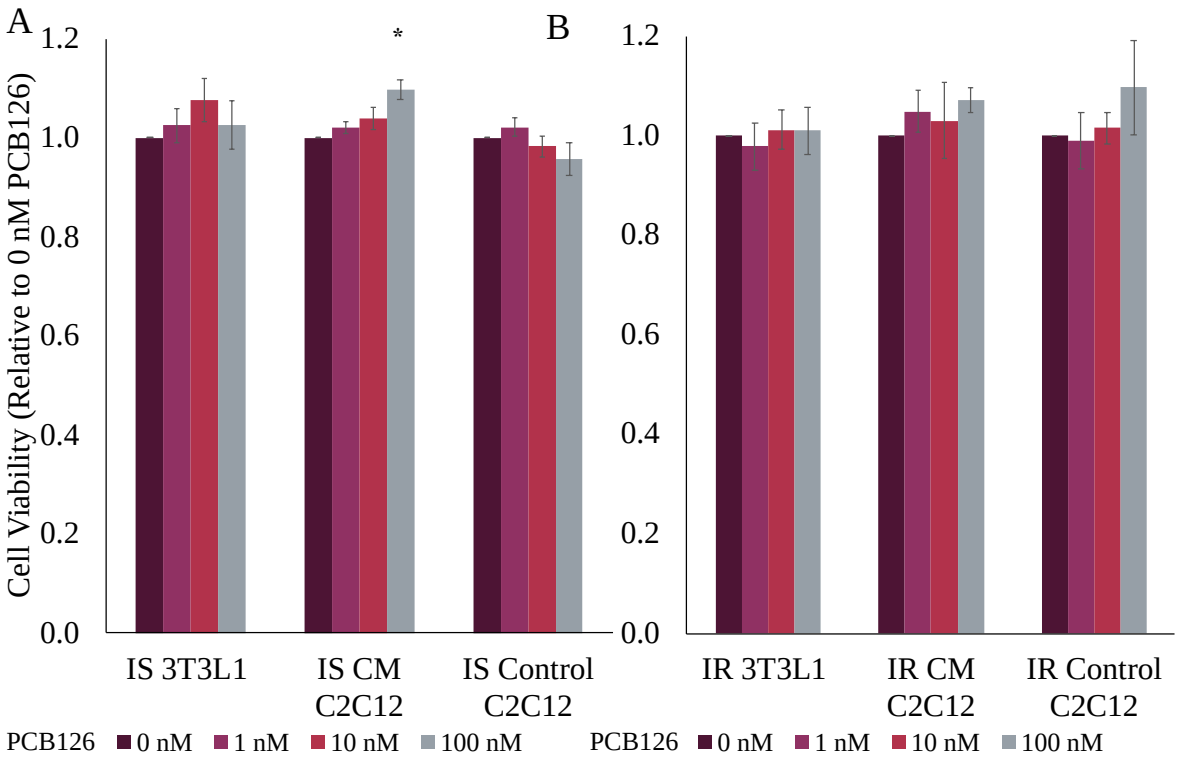



**Figure 13: A 24hr-exposure to PCB126 or conditioned media of adipocytes does not influence cell survival in IS and IR 3T3L1 adipocytes and C2C12 myotubes.**

IS (A) and IR (B) 3T3L1 adipocytes and control C2C12 myotubes were exposed to different concentrations of PCB126 for 24hrs. The conditioned media from adipocytes was subsequently transferred to IS (A) or IR (B) CM C2C12 myotubes. After treatment, cells were incubated with PrestoBlue for 30 min. Plates were read at 570 nm and 600 nm (reference wavelength). Average of normalized absorbance is presented relative to control (no PCB) ±SEM. (n=3 independent experiments, each independent experiment was done at least in triplicate, * represents a $p<0.05$ compared to 0 nM PCB126).

Beside its effect on cell survival, PCB126 has also be associated with decreased adipogenesis [128]. Therefore, we determined whether PCB126 exposure altered lipid accumulation and differentiation in adipocytes. We also determined if CM from IS or IR adipocytes altered intramyocellular lipid accumulation in myotubes. Oil Red O staining assesses differentiation in preadipocytes and adipocytes [229], and it has also been used to demonstrate lipid droplet accumulation in muscle cells [230]. 24hr-treatments with different concentrations of PCB126 had no effect on lipid accumulation in IS and IR 3T3L1 adipocytes and in control C2C12 myotubes (Figure 14). Lipid accumulation in C2C12 myotubes (Figure 14) was not altered by a 24hr-exposure to CM from IS or IR 3T3L1 adipocytes.

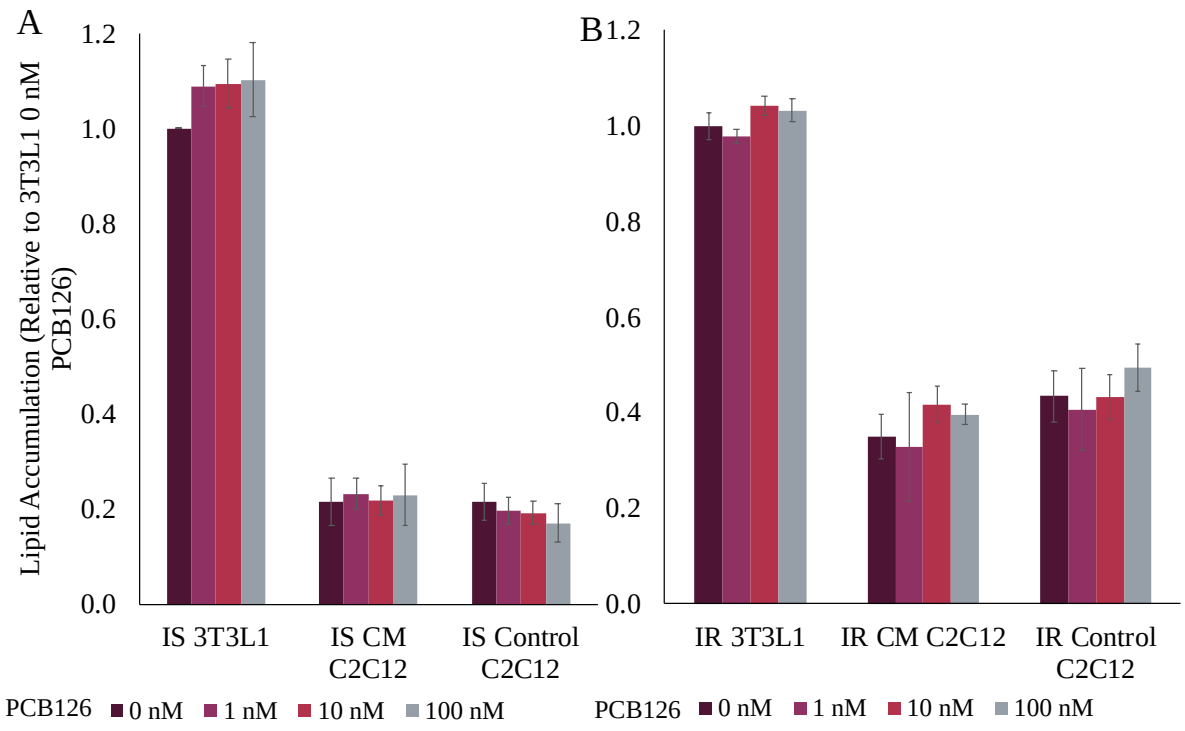



**Figure 14: A 24hr-exposure to PCB126 or conditioned media does not influence neutral lipid content in IS and IR 3T3L1 adipocytes, nor in C2C12 myotubes.**

IS (A) and IR (B) 3T3L1 adipocytes and control C2C12 myotubes were exposed to different concentrations of PCB126 for 24hrs. The conditioned media from adipocytes was subsequently transferred to IS (A) or IR (B) CM C2C12 myotubes. After treatment, cells were fixed, and lipid droplets stained with Oil Red O. Stained lipid droplets were extracted with isopropanol and a triplicate for every well was read at 492 nm. Data are presented as mean relative to 0 nM PCB126 in adipocytes ± SEM (n=4 independent experiments, each independent experiment was done at least in triplicate).

## 4.2. PCB126 altered adipocytokine expression and secretion by adipocytes

Adipose tissue inflammation is one of the key players in adipose-to-muscle communication [231]. Since PCBs activate AhR leading to increased inflammation [58], [201], we determined whether a 24hr-PCB126 exposure altered inflammatory profile in IS and IR 3T3L1 adipocytes by assessing expression and secretion of adipokines. Adiponectin and IL-6 mRNA expression was measured by RT-qPCR in IS and IR adipocytes. After a 24hr-PCB126 treatment, adiponectin mRNA expression was significantly reduced in IS adipocytes exposed to 1 and 10 nM PCB126 compared to the control condition (0 nM IS adipocytes) ($p=0.0492$ and 0.0397 respectively, Figure 15A). PCB126 did not alter mRNA expression of adiponectin in IR adipocytes. There was a significant decrease in adiponectin mRNA expression in IR adipocytes compared to IS adipocytes ($p=0.0003$, Figure 15A). IL-6 mRNA expression in IS and IR adipocytes was however not altered by PCB126-exposure (Figure 15B). There was also a trend toward increased IL-6 mRNA expression in IR adipocytes compared to IS adipocytes, which was not significantly different ($p=0.08$, Figure 15B).

Adipokine concentration was also measured in cell culture media after the 24hr-treatments. Using the Bio-Plex Multiplex Immunoassay System, we quantified six different adipokines in the media: adiponectin, IL-6, TNF-α, leptin, MCP-1 and PAI-1. A 24hr-treatment with PCB126 did not significantly alter adipokine secretion in IS 3T3L1 adipocytes (Figure 16). However, a 100 nM PCB126 treatment for 24hrs significantly increased the secretion of adiponectin ($p=0.0098$, Figure 17A), leptin ($p<0.0001$, Figure 17B), IL-6 ($p=0.0002$, Figure 17C), MCP-1 ($p=0.0018$, Figure 17D), and TNF-α (<0.0001, Figure 17F) in IR adipocytes.

The concentration of PAI-1 in IR adipocytes was also increased but it did not reach statistical significance ($p=0.0967$, Figure 17E).

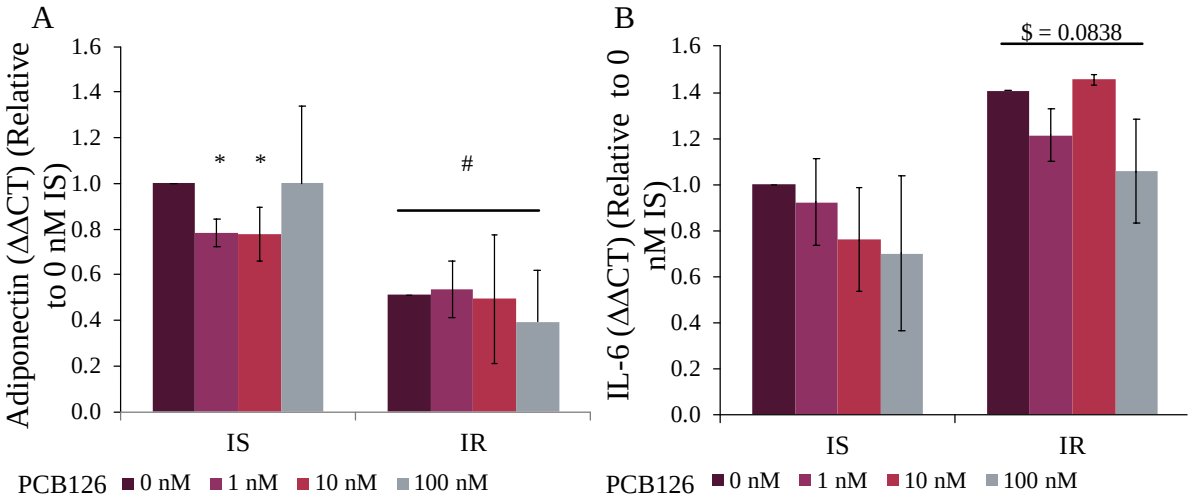



**Figure 15: A 24hr-exposure to PCB126 decreased adiponectin mRNA expression in IS 3T3L1 adipocytes.**

IS and IR 3T3L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. After treatment, cells were lysed, and total RNA was extracted. RNA was reverse transcribed to cDNA. cDNA was amplified and quantified in a CFX96-PCR Detection System using the iQSYBR SsoFast EvaGreen Supermix (Bio-Rad) and appropriate primers (Table 2). Adiponectin (A) and IL-6 (B) were normalized to β-actin levels and analyzed using the ΔΔCT method. Average of normalized ΔΔCT is presented relative to control (IS, no PCB) ±SEM. (n=3 independent experiments, each independent experiment was done at least in triplicate, * represents a $p<0.05$ compared to 0 nM PCB126, # represents a $p<0.05$ compared to IS adipocytes).

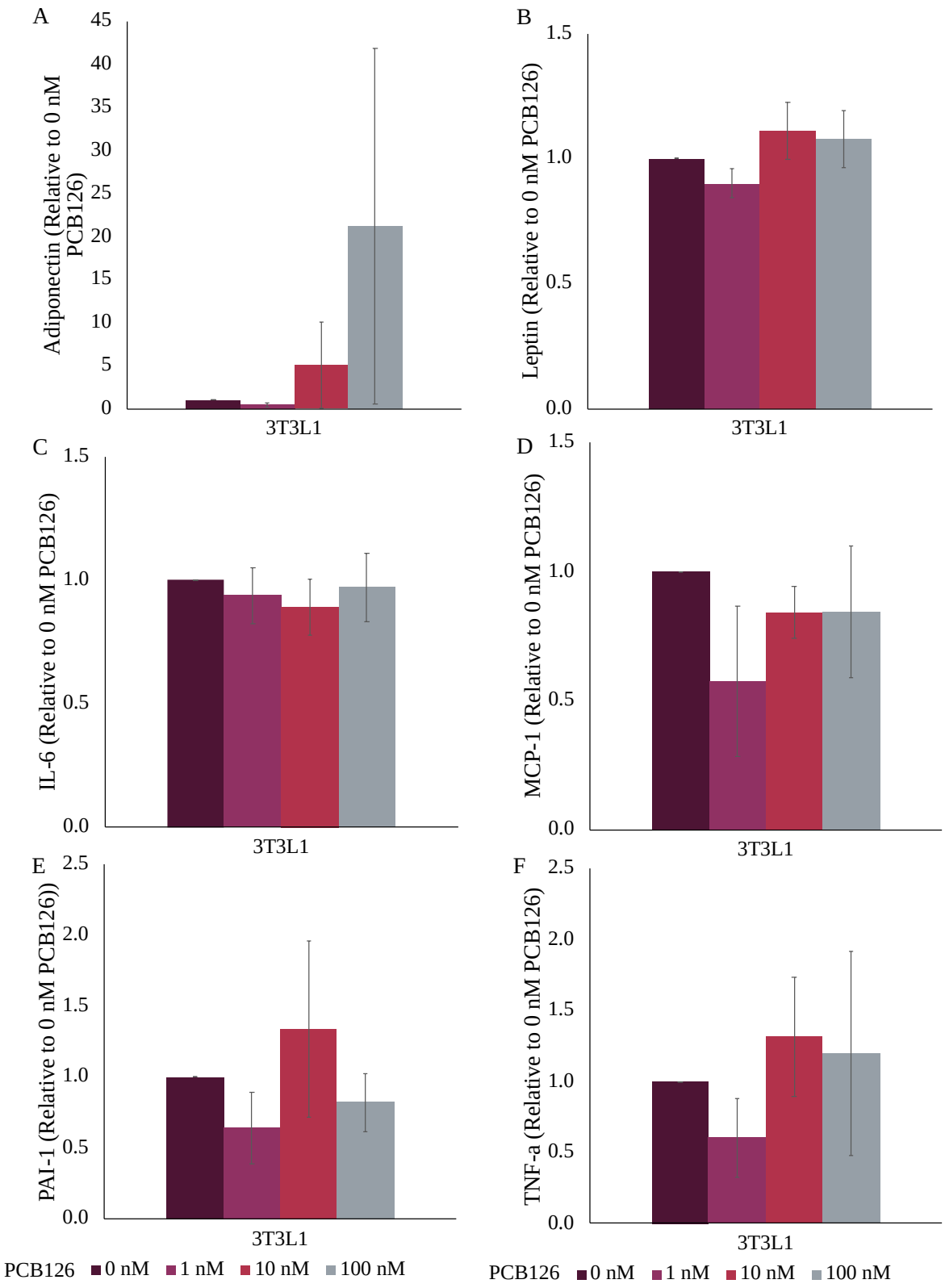

A
Adiponectin (Relative to 0 nM PCB126)
45
40
35
30
25
20
15
10
5
0
3T3L1
B
Leptin (Relative to 0 nM PCB126)
1.5
1.0
0.5
0.0
3T3L1
C
IL-6 (Relative to 0 nM PCB126)
1.5
1.0
0.5
0.0
3T3L1
D
MCP-1 (Relative to 0 nM PCB126)
1.5
1.0
0.5
0.0
3T3L1
E
PAI-1 (Relative to 0 nM PCB126))
2.5
2.0
1.5
1.0
0.5
0.0
3T3L1
F
TNF-a (Relative to 0 nM PCB126)
2.5
2.0
1.5
1.0
0.5
0.0
3T3L1
PCB126 0 nM 1 nM 10 nM 100 nM
PCB126 0 nM 1 nM 10 nM 100 nM

**Figure 16: Adipocytokine concentration in media from insulin sensitive 3T3L1 adipocytes exposed to PCB126 for 24hrs.**

IS 3T3L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. After treatment, media was collected and used to determine adiponectin (A), leptin (B), IL-6 (C), MCP-1 (D), PAI-1 (E) and TNF-α (F) concentration using a Bio-Plex Multiplex Immunoassay System (Bio-Rad) and a specific kit (# MADCYMAG-72K, Millipore). Data are presented as mean ± SEM (n=3 independent experiments, each independent experiment was done in 2 replicates).

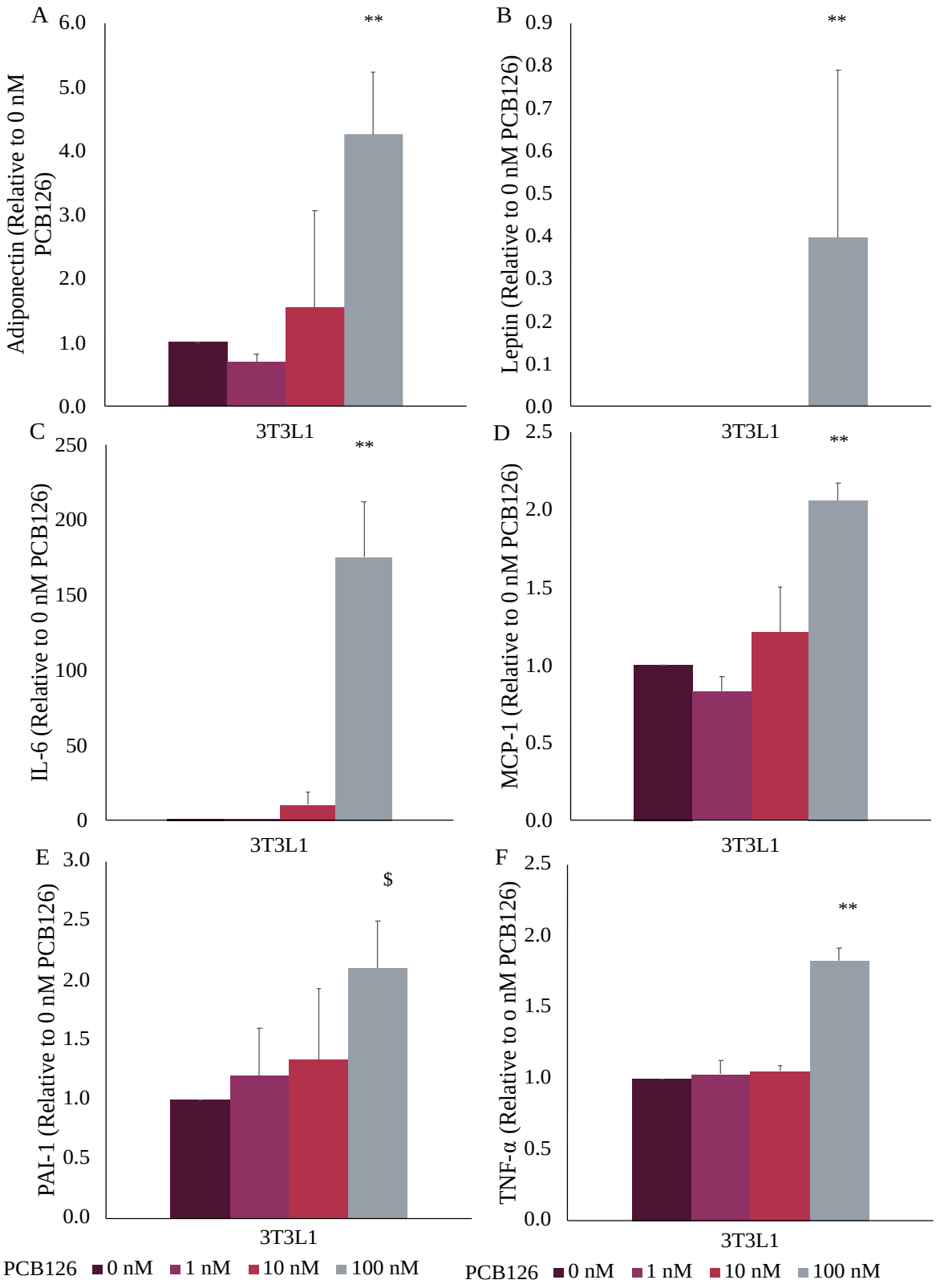

A
Adiponectin (Relative to 0 nM PCB126)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
**
3T3L1
B
Leptin (Relative to 0 nM PCB126)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
**
3T3L1
C
IL-6 (Relative to 0 nM PCB126)
250
200
150
100
50
0
**
3T3L1
D
MCP-1 (Relative to 0 nM PCB126)
2.5
2.0
1.5
1.0
0.5
0.0
**
3T3L1
E
PAI-1 (Relative to 0 nM PCB126)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$
3T3L1
F
TNF-α (Relative to o nM PCB126)
2.5
2.0
1.5
1.0
0.5
0.0
**
3T3L1
PCB126 0 nM 1 nM 10 nM 100 nM
PCB126 0 nM 1 nM 10 nM 100 nM

**Figure 17: Adipocytokine concentration in media from insulin resistant 3T3L1 adipocytes exposed to PCB126 for 24hrs.**

IR 3T3L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. After treatment, media was collected and used to determine adiponectin (A), leptin (B), IL-6 (C), MCP-1 (D), PAI-1 (E) and TNF-$\alpha$ (F) concentration using a Bio-Plex Multiplex Immunoassay System (Bio-Rad) and a specific kit (# MADCYMAG-72K, Millipore). Data are presented as mean ± SEM. (n=3 independent experiments, each independent experiment was done in 2 replicates. **: $p \leq 0.01$ compared to 0, 1 and 10 nM and $: $p = 0.0967$ compared to 0 nM).

## 4.3. Lipolysis

Another player in adipose-to-muscle communication is an altered secretion of FA by adipose tissue [75] which might be promoted by PCB exposure [38], [232]. Lipolysis is defined by the hydrolytic cleavage of TG into FFA and glycerol [233]. Moreover, "basal fat cell lipolysis […] is elevated during obesity and is closely associated with insulin resistance" [234]. Therefore, we determined if a 24hr-PCB126 exposure or insulin resistance altered the rate of lipolysis by adipocytes. Using a kit specific to 3T3L1 adipocytes, we measured FFA and glycerol concentration in cell culture media after PCB126 treatment. Exposure to 10 and 100 nM PCB126 for 24hrs significantly decreased FFA concentration in IS adipocyte media compared to control (0 nM PCB126) ($p=0.049$ and $p=0.0147$ respectively) and compared to 1 nM PCB126 ($p=0.0248$ and $p=0.0076$ respectively) (Figure 18A). A similar, but non-significant decrease in glycerol concentration was also measured in the media of IS adipocytes exposed to 10 and 100 nM PCB126 (Figure 18B). However, a 24hr-PCB126 treatment did not significantly alter lipolysis rate in IR adipocytes (Figure 18A and B). We also compared lipolysis rate in IS and IR adipocytes. There was a significant decreased concentration of FFA and glycerol in media from IR adipocytes compared to IS adipocytes ($p=0.0009$ and $p=0.0005$ respectively, Figure 18). This lower lipolysis rate in IR adipocytes was probably the result of the high insulin concentration used to induce insulin resistance in IR adipocytes, since insulin is known to inhibit lipolysis and favor TG synthesis.

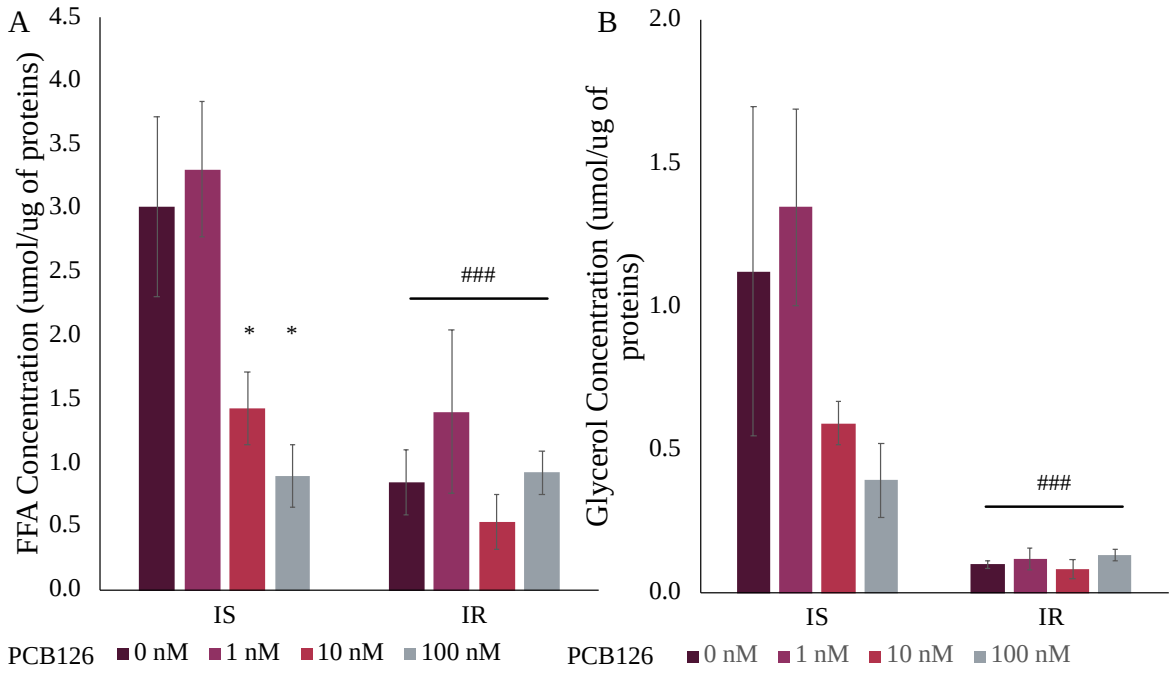



**Figure 18: A 24hr-exposure to 10 and 100 nM PCB126 reduced lipolysis rate in IS adipocytes.**

IS and IR 3T3L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. After treatment, media was collected and used to determine FFA (A) and glycerol (B) concentration using a specific lipolysis kit (ZenBio). Data are presented as mean relative to control (IS, no PCB) ± SEM. (n=3 independent experiments, each independent experiment was done in 2 replicates. *: $p \leq 0.05$ compared to 0 and 1 nM PCB126 and ###: $p \leq 0.001$ compared to IS condition).

## 4.4. PCB126 decreases glucose uptake in insulin resistant adipocytes and in myotubes exposed to conditioned media of insulin resistant adipocytes

High circulating levels of PCBs have been associated with reduced glucose uptake in skeletal muscle cells [211] and inhibition of the insulin signaling pathway activity [201]. Moreover, adipose tissue inflammation has been associated with decreased insulin sensitivity in other tissues [27], [29], [235]. Hence, we determined the effect of PCB126 on basal and insulin-stimulated glucose uptake in adipocytes and myotubes, by following the uptake of radioactively labeled [1-3H] 2-Deoxyglucose. In IS adipocytes, the absolute values of basal 2-deoxyglucose uptake (no insulin) were between 2 and 5 pMol/min/μg of protein, with a significant 2.5-3.5-fold increase in response to insulin ($p<0.0001$, Figure 19A and B). These values are similar to what has been previously published in adipocytes [236]. Whereas PCB126-exposure did not alter basal and insulin-stimulated glucose uptake in IS adipocytes (Figure 19A), a 100 nM PCB126 significantly decreased basal and insulin-stimulated glucose uptake in IR adipocytes ($p=0.0305$, Figure 19C). There was no significant increase in glucose uptake in response to insulin in IR adipocytes (Figure 19C), confirming that our IR conditions resulted in insulin resistance development.

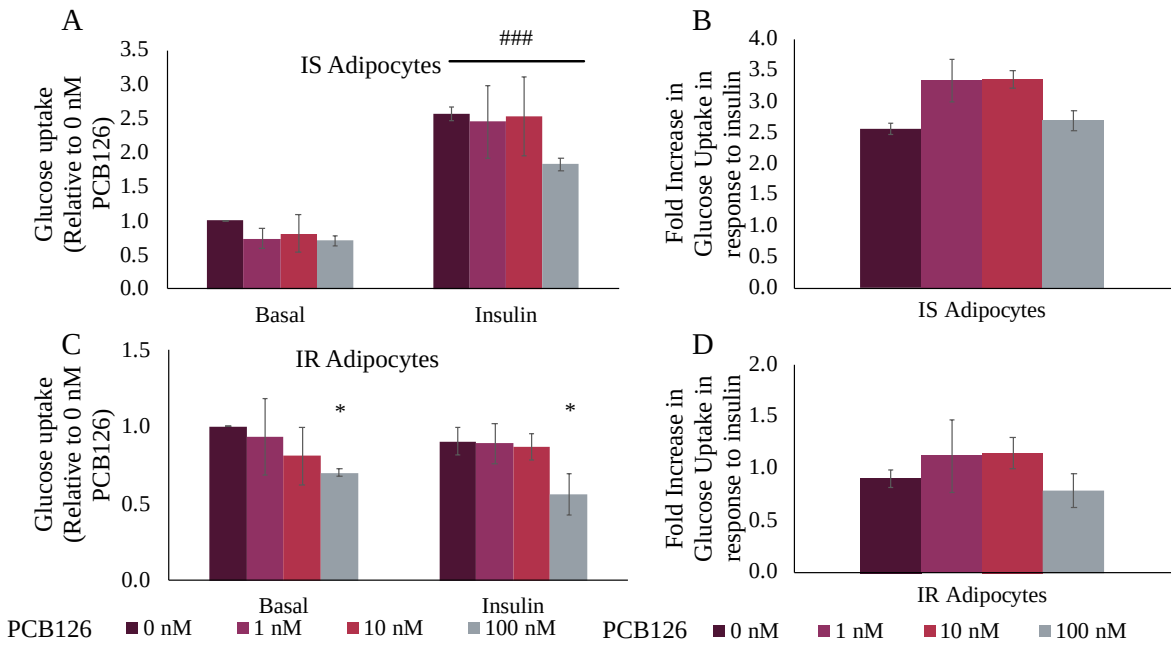



**Figure 19: A 24hr-exposure to 100 nM PCB126 decreased basal and insulin-stimulated glucose uptake in IR adipocytes.**

IS (A) and IR (C) 3T3L1 adipocytes were exposed to different concentrations of PCB126 for 24hrs. After treatment, cells were serum starved (serum-free DMEM) for 3hrs. 100 nM insulin was added for the last 20 min of starvation, followed by 5 min treatment with radio-labelled 2-deoxyglucose. Data are presented relative to control (no PCB, 0.1% DMSO) ± SEM. Fold increase of IS (B) and IR (D) adipocytes represents the increase in glucose uptake in response to insulin. (n=3 independent experiments, each independent experiment was done in 3 replicates. *: $p \leq 0.05$ compared to 0 nM PCB126 and ###: $p \leq 0.0001$ compared to basal).

While exposure of C2C12 myotubes to the CM of IS adipocytes did not alter basal glucose uptake (Figure 20A), exposure to the CM from IR adipocytes significantly decreased basal glucose uptake in C2C12 myotubes ($p=0.0349$ at 10 nM and $p=0.0006$ at 100 nM, Figure 20C). Interestingly, there was no effect of direct PCB126 exposure in IR control myotubes (Figure 20D).

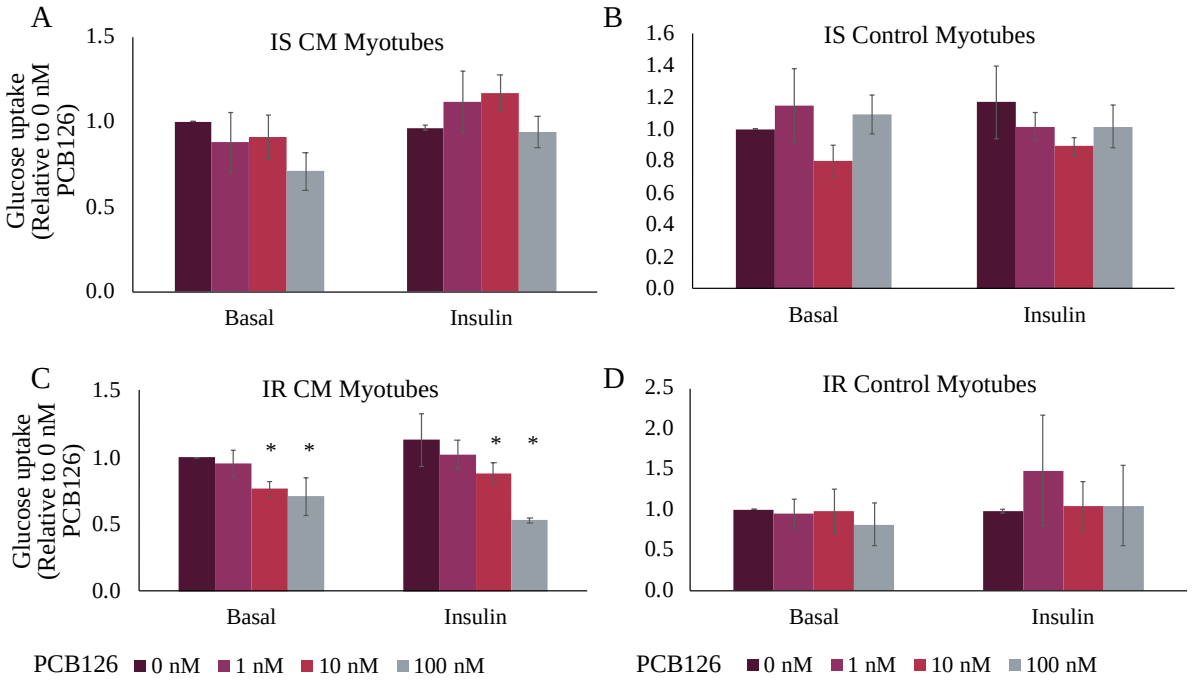



**Figure 20: CM from IR adipocytes exposed to 10 and 100 nM PCB126 decreased basal decreased basal and insulin-stimulated glucose uptake in myotubes.**

C2C12 myotubes were directly exposed to different concentrations of PCB126 for 24hrs (Control myotubes, B and D) or to conditioned media of IS (CM myotubes, A and C) adipocytes. After treatment, cells were serum starved (serum-free DMEM) for 3hrs. 100 nM insulin was added for the last 20 min of starvation, followed by 5 min treatment with radio-labelled 2-deoxyglucose. Data are presented relative to control (no PCB, 0.1% DMSO) ± SEM. (n=3 independent experiments, each independent experiment was done in 3 replicates. *: $p \leq 0.05$ compared to 0 nM PCB126).

As seen in Figure 20, C2C12 myotubes did not increase glucose uptake in response to insulin. This result was not surprising since it is known that C2C12 myotubes do not respond well to insulin in term of glucose uptake due to high levels of basal glucose uptake [215]. In order to determine whether exposure to PCB126 or CM alter the insulin sensitivity of muscle cells, we therefore tested two models of muscle cells that were responsive to insulin (L6 rat myotubes and mouse primary myotubes). Similar results between the two types of cells led us to test the effect of direct and indirect (CM) exposure to PCB126 in mouse primary muscle cells. In IS Control myotubes (0 nM PCB126), the absolute values of basal 2-deoxyglucose uptake (no insulin) were around 15 pMol/min/μg of protein, with a 1.4-fold increase in response to insulin (Figure 21C). A 24hr-exposure to CM from IS or IR adipocytes did no significantly alter basal or insulin-stimulated glucose uptake in mouse primary muscle cells (Figure 21A). Surprisingly, in IS control myotubes, direct exposure to 10 nM PCB126 tended to increase basal glucose uptake without reaching significance ($p=0.0749$), but no effect was measured in IR control myotubes (Figure 21B). Nevertheless, CM from PCB126-treated IS adipocytes significantly decreased insulin-sensitivity of myotubes (Figure 21B). The decreased fold-increase in IS myotubes exposed directly to PCB126 ($p=0.0005$, $p=0.0002$ and $p=0.0002$ respectively, Figure 21C) was more associated with an increased basal glucose uptake (Figure 21B) than a real decrease in insulin-stimulated glucose uptake (Figure 21B). PCB126 had no significant effect on insulin response in IR control myotubes or myotubes exposed to CM from IR adipocytes (Figure 21D). Interestingly, IR control myotubes had a ~1-fold increase, while IS control myotubes showed a ~1.5-fold increase in glucose uptake with insulin (Figure

21C and D, respectively). This demonstrates that IR condition also induced insulin resistance in control primary mouse muscle cells.

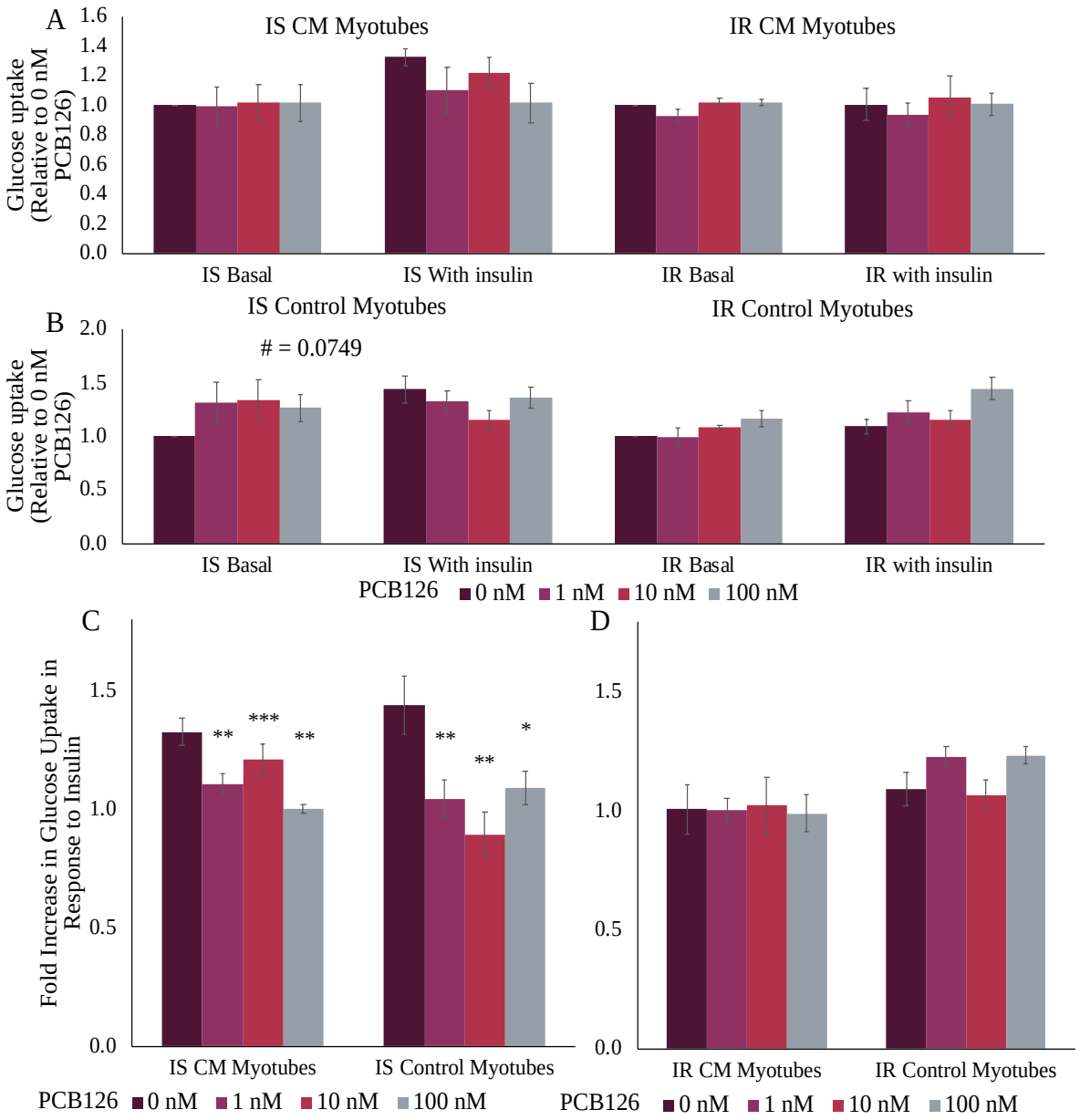



**Figure 21: Decreased insulin-stimulated glucose uptake in IS myotubes exposed directly to PCB126 or to CM of adipocytes.**

Differentiated primary mouse muscle cells were directly exposed to different concentration of PCB126 for 24hrs (B), or to conditioned media of IS or IR adipocytes exposed to PCB126 (A). After treatment, cells were serum starved (serum-free DMEM) for 3hrs. 100 nM insulin was added for the last 20 min of starvation, followed by 5 min treatment with radio-labelled 2-deoxyglucose. Data are presented as mean relative to control (IS, no PCB, 0.1% DMSO) ± SEM. Fold increase of IS (C) and IR (D) CM or control myotubes represent the increase in glucose uptake in response to insulin. (n=4 independent experiments, each independent

experiment was done in 3 replicates. *: p≤0.05, **: p≤0.01 and ***: p≤0.001 compared to 0 nM PCB126).

## 4.5. PCB126 decreases basal glycolysis rate and maximal glycolytic capacity in IR adipocytes

To further study the effect of 24hr-exposure to PCB126 on glucose metabolism, we determined if the reduced glucose uptake in C2C12 myotubes exposed to CM from IR adipocytes was related to a decrease in glycolysis rate (measurement of extracellular acidification rate (ECAR) a marker of glycolysis). We and others previously showed that direct exposure to PCBs reduces glycolysis in L6 skeletal muscle cells [211] and in neuroblastoma cells [237]. A 24hr-exposure to PCB126 did not alter glycolysis rate in IS adipocytes (Figure 22A), nor in myotubes exposed directly to PCB26 (Figure 22C and F) or to CM from IS or IR adipocytes (Figure 22B and E). Hence, CM from IR adipocytes exposed to PCB126 did not decrease glycolysis rate in C2C12 myotubes even if it induced a decrease in glucose uptake. However, resting glycolysis rate and maximal glycolytic capacity were significantly decreased in IR adipocytes exposed to 1 to 100 nM PCB126 (for 1 nM p=0.0008 and 0.0007, for 10 nM p=0.0012 and 0.0018, and for 100 nM p=0.0029 and 0.0077, respectively) (Figure 22D).

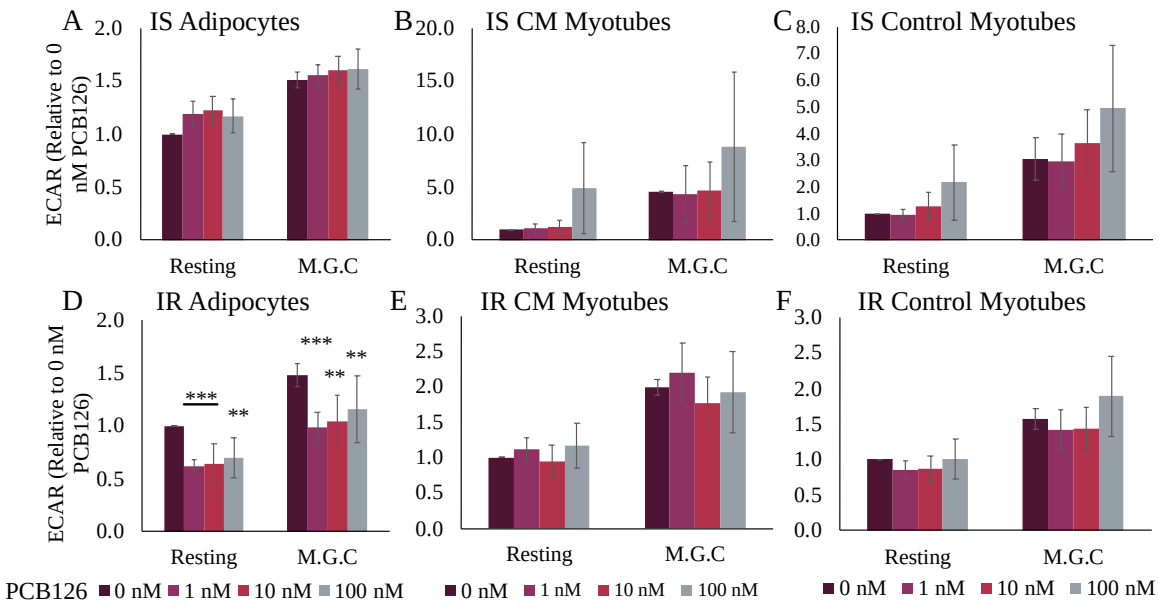



**Figure 22: A 24hr-exposure to PCB126 decreased glycolysis rate in IR adipocytes but not in IS adipocytes.**

IS and IR 3T3L1 adipocytes (A, D) and C2C12 (C, F) myotubes were directly exposed to different concentrations of PCB126 for 24hrs, or to conditioned media of IS or IR adipocytes (B, E), in XF-96 well plate (Agilent). Basal extracellular acidification rate (ECAR) was first measured, followed by treatment with 600 ng/mL oligomycin to measure maximal glycolytic capacity (M.G.C) with a Seahorse analyzer (Agilent). Data are presented as mean ±SEM. (n=4 independent experiments, each independent experiment was done in 5 replicates. **: $p \leq 0.01$ and ***p: 0.001 compared to 0 nM PCB126).

## 4.6. PCB126 decreases resting and proton leak-associated oxygen consumption in IR adipocytes

Finally, one of our objectives was to determine if adipose-to-muscle communication was responsible for the decreased muscle mitochondrial function measured in *in vivo* in rats exposed to PCB126 [214]. Oxygen consumption rate (OCR) was not affected by a 24hr-exposure to PCB126 in IS adipocytes (Figure 23A), while OCR was significantly decreased in IR adipocytes (Figure 23D) exposed to 1-10 nM PCB126 (for 1 nM p=0.0279 and 0.0429, and for 10 nM p=0.0480 and 0.0448, respectively). Direct 24hr-treatment with 100 nM PCB126 increased resting OCR and maximal mitochondrial capacity in IS control myotubes (In IS p=0.0555 and 0.0438, respectively, Figure 23C), and increased resting and proton leak associated OCR in IR control myotubes (p=0.0037 and 0.0302, respectively, Figure 23F). However, treatment of C2C12 myotubes with CM from IS or IR adipocytes exposed to PCB126 did not alter mitochondrial function (Figure 23B and E). Therefore, even in PCB126 has a negative effect on adipocyte mitochondrial function, it seems that direct or indirect exposure to PCB126 does not alter negatively mitochondrial function in C2C12 myotubes.

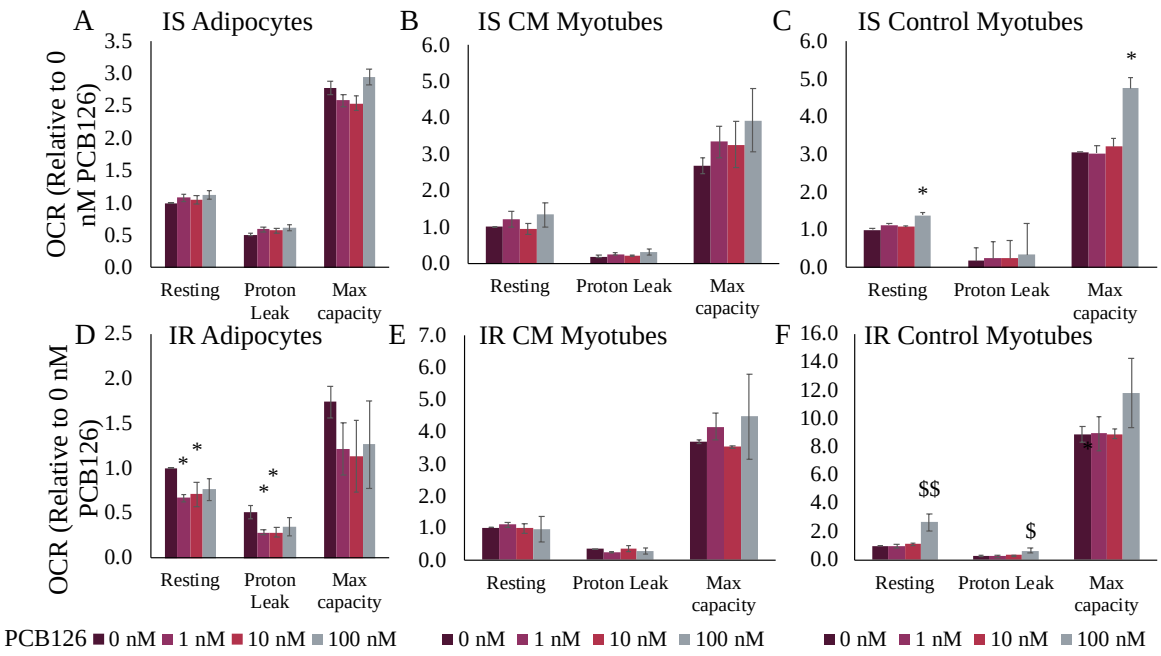



**Figure 23: A 24hr-exposure to PCB126 decreased oxygen consumption rate in IR 3T3L1 adipocytes, but PCB126 increased oxygen consumption rate in IR and IS control myotubes.**

IS and IR 3T3L1 adipocytes (A, D) and C2C12 myotubes (C, F) were directly exposed to different concentration of PCB126 for 24hrs, or to conditioned media of IS or IR adipocytes (B, E), in XF-96 well plate (Agilent). Oxygen consumption rate (OCR) was measured with a Seahorse analyzer (Agilent). (n=4 independent experiments, each independent experiment was done in 5 replicates. *: $p \leq 0.05$ compared to 0 nM; $= $p \leq 0.05$ and $$: $p \leq 0.01$ compared to 0, 1 and 10 nM).

# 5. Discussion

Exposure to PCB126 has been associated with insulin resistance development and mitochondrial dysfunction [206], [207], [211], [214], [238]. We previously showed that a one-week exposure to PCB126 resulted in mitochondrial dysfunction in permeabilized muscle fibers from rats [214]. However, direct PCB126-exposure of L6 skeletal muscle cells did not altered mitochondrial function [211]. PCB126 is a lipophilic compound that increases inflammation in adipose tissue [128], [239], and increased adipose tissue inflammation is one potential cause of insulin resistance and mitochondrial dysfunction in skeletal muscle [64], [164]. We therefore hypothesized that skeletal muscle mitochondrial dysfunction in rats exposed to PCB126 was the result of an alteration of adipocytokine secretion by adipose tissue. The main objective of the present study was thus to determine the role of adipose-to-muscle communication in the development of mitochondrial dysfunction and glucose metabolism alterations in skeletal muscle when exposed to PCB126. The specific objectives were 1) To determine the effect of PCB126 exposure on adipocyte cytokine/adipokine production; 2) To study whether the communication between adipose and muscle tissues may explain muscle abnormal glucose metabolism when exposed to PCB126; 3) To study whether the communication between adipose and muscle tissues may explain muscle mitochondrial dysfunction when exposed to PCB126, and 4) To study whether pre-established insulin resistance in adipocytes alters the metabolic responses to PCB126 in adipocytes and the communication between adipocytes and muscle cells. To do so, we first determined the effect of PCB126 exposure in IS vs. IR adipocytes on different metabolic parameters. We then used the CM from these IS or IR adipocytes to expose C2C12 or primary mouse myotubes for 24

hrs to the different mediators secreted by IS or IR adipocytes exposed to PCB126, and explored whether the adipocyte secretome could influence muscle mitochondrial function and glucose metabolism. The different results of the present study are summarized in Table 3.

**Table 3: Summary of results obtained from this study**

| | Effect of PCB126 exposure | | Effect of IR (independently of PCB126) |
|---|---|---|---|
| | IS condition | IR condition | |
| **3T3L1 adipocytes** | | | |
| Lipolysis | ↓ | - | ↓ |
| Adiponectin mRNA levels | ↓ | - | ↓ |
| IL-6 mRNA levels | - | - | ↑(trend) |
| Adipokine secretion | - | ↑ | ND |
| Glucose uptake | - | ↓ | ND |
| Insulin sensitivity | - | - | ↓ |
| Glycolysis | - | ↓ | ND |
| Mitochondrial function | - | ↓ | ND |
| **Myotubes exposed to CM** | | | |
| Glucose uptake | - | ↓ | ND |
| Insulin sensitivity | ↓ | - | ↓ |
| Glycolysis | - | - | ND |
| Mitochondrial function | - | - | - |
| **Myotubes directly exposed to PCB126 (controls)** | | | |
| Glucose uptake | - or ↑ | - | ND |
| Insulin sensitivity | - | - | ↓ |
| Glycolysis | - | - | ND |
| Mitochondrial function | ↑ | ↑ | ND |

## 5.1. Metabolic dysfunction and inflammation induced by PCB126 in adipocytes

Due to its lipophilic character, the role of PCB126 in adipose tissue dysfunction development is of interest. It has previously been demonstrated that exposure of human preadipocytes to PCB126 decreased differentiation [128]. The decreased adipogenesis was associated with PCB126-induced AhR activation, which inhibits PPARγ [199]. In the present study, we exposed fully differentiated 3T3L1 adipocytes to PCB126 for 24hrs. Unlike when adipocytes were exposed to PCB126 for the all differentiation [128], a 24hr-exposure in mature adipocytes did not alter neutral lipid content, a marker of adipocyte differentiation. This suggests that timing of PCB126-exposure (before, during or after differentiation) plays a role in its effect on adipogenesis.

Besides decreased adipogenesis, others have also shown that PCB126 and PCB77 induce pro-inflammatory cytokines, such as MCP-1, TNF-α, IL-6 and PAI-1 [58], [128], [199], while reducing anti-inflammatory adipokines, such as adiponectin and leptin [60], [128], [199]. Inflammation might be altered through PCB126-AhR activation in adipose tissue, as it has been suggested with PCB77 [58]. In accordance with previous reports [128], [239], in the present study, PCB126 exposure resulted in decreased expression of adiponectin at the mRNA level in IS adipocytes, and in increased secretion of inflammatory adipokines, including TNF-α, IL-6 and MCP-1, by IR adipocytes. It has previously been shown that exposure to PCB126 before differentiation decreases adiponectin expression and secretion [128], but here we only measured a reduced adiponectin mRNA expression in IS adipocytes. It is possible that the different timing of PCB126 exposure are responsible for these differences. It is also possible that a longer treatment might be needed to reduce the secretion of adiponectin by adipocytes.

It has previously been shown that AhR activation leads to a delay inflammatory response after exposure to PCB126 [239], which could explain that we only measured an effect of our PCB126 treatment on adiponectin mRNA levels.

To the best of our knowledge, the effects of PCB126-exposure on glucose metabolism and mitochondrial function in adipocytes have not been studied yet. The present study thus provides new insight into the role of PCB126 in adipose tissue metabolic dysfunction. We have demonstrated that PCB126 exposure decreased basal glucose uptake, glycolysis rate and mitochondrial respiration only in IR adipocytes. Taken together these results suggest that environmentally relevant levels of PCB126 induced metabolic dysfunctions in IR adipocytes. This concord with previous results obtained in other metabolically active cells, such as hepatocytes and skeletal muscle cells [205], [211].

One of the objectives of this project was to determine the effects of pre-established insulin resistance on the metabolic response to PCB126-exposure. Insulin resistance is associated with reduced metabolic flexibility [30], [240], increased risk of cancer [241], and infection [242]. Furthermore, other metabolic disorders, especially obesity and high-fat diet, have been associated with increased negative impact of pollutants on metabolism [33], [203]. For example, it has been shown that PCBs exacerbate hyperinsulinemia and insulin resistance in obesity-induced IR mice compared to non-IR mice [33]. These results suggest an increased sensitivity to stressors when mice are already insulin resistant. However, how insulin resistance affects the metabolic response to PCB126 exposure in adipocytes had not been investigated before. Thus, we investigated whether the metabolic response to PCB126 treatment was different in IR vs. IS adipocytes. Our results suggest that IR adipocytes were

more sensitive to PCB126-induced inflammation than IS adipocytes. This might be due to a better regulation of inflammatory response in metabolically healthy adipocytes. Furthermore, this increased inflammation in IR adipocytes exposed to PCB126 was associated with decreased mitochondrial function, glycolysis rate and glucose uptake. Interestingly, these effects were not measured in IS adipocytes. As far as we know, the present study is the first one showing that insulin sensitivity status may influence the response of adipocytes to PCB126 exposure.

It has previously been shown that increased inflammation promotes mitochondrial dysfunction and alters glucose metabolism [74], [121], [164], [235], [243]. It is therefore possible that increased adipokine secretion in IR adipocytes was responsible for their decreased mitochondrial respiration and glycolysis rate. However, PCB126 exposure has also been directly associated with decreased cellular respiration in different cell types [64], [214]. It is thus also possible that mitochondria from IR adipocytes are more sensitive to PCB126. Moreover, IS adipocytes might have better metabolic flexibility and/or compensation mechanisms leading to reduced negative effects of PCB126.

It is important to note that in the insulin resistant condition, high insulin levels were present in the media during PCB126-treatment. This could have led to the activation/inhibition of different metabolic pathways and also altered adipokine profile and metabolic response. Hyperinsulinemia is also associated with increased ROS production in adipose tissue [244]. However, our control IR adipocytes (0 nM PCB126) were exposed to the same concentration of insulin than the IR adipocytes treated with 1-100 nM PCB126, but only the IR adipocytes exposed to PCB126 presented a decreased mitochondrial function. This suggests that the

presence of insulin in the media was not responsible for the difference in mitochondrial function and glycolysis rate between IS and IR adipocytes.

We also determined whether the insulin sensitivity status of adipocytes resulted in alteration of lipolysis rate. Interestingly, FFA and glycerol concentration was significantly lower in the media of IR compared to IS adipocytes. This was unexpected since adipose tissue lipolysis is usually increased with insulin resistance [234]. However, in our study, insulin resistance in adipocytes was induced by exposing adipocytes to high concentration of insulin during the whole-differentiation process. Since insulin is known to inhibit lipolysis in adipocytes, it is probable that the presence of high insulin concentration in our IR condition resulted in an inhibition of lipolysis.

## 5.2. Alteration of metabolism induced directly or indirectly by PCB126 in myotubes

Despite the role of skeletal muscle in glucose homeostasis, the effect of PCB126 on skeletal muscle metabolism has not yet been deeply studied. We previously measured a decreased glycolytic function and glucose uptake in L6 muscle cells exposed to PCB126 for 24hrs [211]. Moreover, it has been shown that exposure of rats to a PCB mixture (Aroclor 1254) altered the insulin signaling pathway and reduced GLUT4 translocation in muscle [213]. In the present study, we showed that in C2C12 myotubes, direct exposure to PCB126 (IS and IR controls) did not negatively alter glucose uptake and glycolysis rate. The different effects of PCB126 on glucose metabolism (glucose uptake and glycolysis rate) might be due to physiological difference between L6 rat muscle cells, C2C12 mice muscle cells and primary mouse muscle cells. It is known that C2C12 lack the machinery (Insulin-responsive GLUT4

vesicles) required to present a significant insulin-stimulated glucose uptake [245], while L6 and primary mouse muscle cells possess these vesicles [245], [246]. Another factor that could impact the response of muscle cells to PCB126 could be the origin of the cells. Our primary mouse muscle cells were isolated from fast twitch muscles (gastrocnemius and tibialis) [247], which have a glycolytic phenotype, while C2C12 were derived from leg muscle of dystrophic mice [248] and their phenotype is not clearly defined. In fact, C2C12 may differentiate into different metabolic phenotypes depending on their environment (glucose concentration, FBS levels, stresses, etc.) [249]. Under conditions similar to ours (1 g/L glucose and 2% FBS), C2C12 should express a greater proportion of slow myosin heavy chains [250]–[252], and thus might have a more oxidative metabolism. Since our primary mouse muscle cells were isolated from glycolytic muscles, and it is known that mouse primary myotubes display a myosin heavy chain phenotype that corresponds to the fibre from which they originated [250], [253], they should express a greater proportion of fast myosin heavy chains. These physiological differences between L6, C2C12 and primary mouse muscle cells could explain the different results obtained in this study compared to our previous work.

It has been demonstrated that high circulating levels of pollutants in humans are associated with decreased mitochondrial enzyme activity in muscle [254]. Furthermore, we previously showed that PCB126 exposure in rats was associated with decreased mitochondrial function in muscle fibers (*in vivo*) [214]. However, PCB126 did not alter mitochondrial function in rat L6 myotubes (*in vitro*) [211] which was confirmer here in C2C12 myotubes exposed directly to PCB126. One of our objectives was thus to determine if the adipose-to-muscle communication in the context of PCB126 exposure could induce mitochondrial dysfunction

in skeletal muscle. However, CM from PCB126-treated IS or IR adipocytes did not alter mitochondrial function or glycolysis rate in C2C12 muscle cells. Therefore, with the present model, we cannot confirm that the adipose-to-muscle communication is responsible for the decreased mitochondrial function in muscle fibers from rats exposed to PCB126.

However, indirect exposure to PCB16 through CM adipocytes decreased myotube glucose uptake and insulin sensitivity. Interestingly, this decrease was not measured in control myotubes directly exposed to the pollutant. This suggests that PCB126 exposure alters the adipocyte secretome, which in turn may negatively affect glucose uptake in muscle cells. As previously discussed, PCB126 induced a greater inflammatory response in IR adipocytes compared to IS adipocytes. This increased inflammation could explain the decreased basal glucose uptake that was measured only in myotubes exposed to CM from PCB-126 treated IR adipocytes. The effect of CM from IR adipocytes on myotube insulin response was however not possible to determine since our IR conditions inhibited insulin response in mouse muscle cells independently of PCB126 exposure. In another hand, PCB126 treatment in IS adipocytes altered mRNA adiponectin expression but did not affect the secretion of adiponectin or other adipokines, suggesting that the effect of IS CM on myotube insulin sensitivity might be due to other secreted factors. We thus confirmed that PCB126 negatively alters insulin response in skeletal muscle, as it has previously been suggested at the whole-body level in *in vivo* studies [33], [238], and *in vitro* work with coplanar PCB77 in endothelial cells [201], [255]. However, our results suggest that this only happens in the presence of mediators secreted by IS adipocytes.

Besides altered adipokine profile, IS and IR adipocytes also secreted different levels of FFA which might alter muscle metabolism. IR adipocytes had a decreased lipolysis rate compared to IS adipocytes and PCB126 treatment did not significantly affect FFA secretion by IR adipocytes. This suggests that the decreased glucose uptake in C2C12 exposed to the CM of PCB126-treated IR adipocytes was not due to increased FFA exposure. Similarly, the decreased insulin sensitivity in myotubes exposed to CM from PCB126-treated IS adipocytes was probably not due to an effect of FFA since lipolysis rate was decreased rather than increased in IS adipocytes exposed to PCB126. Taken together, these results suggest that the effect of the CM of PCB126-treated adipocytes on muscle cell glucose uptake and insulin sensitivity was not due to an increased FFA availability and probably more the results of other secreted factors induced by PCB126 exposure in adipocytes. Since we were unable to detect any significant difference in the measured adipokines in response to PCB126 treatment in IS adipocytes, further research is needed to determine which factor(s) secreted by PCB126-exposed adipocytes affect myotube insulin sensitivity. The potential candidates include adipocytokines not measured in our study (IL-1, IL8, keratinocyte chemoattractant-1 (KC-1)), other metabolites that could be differently secreted when exposed to PCB126, as well as miRNA or DNA.

## 5.3. Adipose-to-muscle communication

For decades, adipose tissue was considered only as a storage tissue. However, there has been a paradigm shift when its endocrine role was brought to light [117]. Adipose tissue plays an important role in regulation of metabolic function in other tissues, such as skeletal muscle and liver [112], [113]. Adipocytes secrete adipokines that are involved in autocrine/paracrine and

endocrine functions. Adipokines alter metabolic response locally in adipose tissue, as well as in distant tissues, including skeletal muscle [113], [117], [130]. Under healthy condition, adipokines maintain energy homeostasis but dysregulation of adipokine secretion causes lipotoxicity in skeletal muscle [130]. In this sense, adipose tissue dysfunction has been associated with increased inflammation, which may explain the association between obesity and the increased risk to develop insulin resistance and T2D.

Here, we have demonstrated that PCB126 promotes inflammation in adipose tissue, especially in IR adipocytes. Using the CM from adipocytes exposed to PCB126, we demonstrated that adipocyte secretome decreased insulin response and glucose uptake in skeletal muscle cells. Similar results have been obtained when muscle cells were treated directly with different adipokines, such as TNF-α, MCP-1 and IL-6 [187], [231]. Taken together, the results from the present study and others suggest that PCB126-induced inflammation in adipose tissue might be responsible for the decreased insulin response and reduced glucose uptake in muscle cells [128], [213], [239]. Interestingly, treatment with CM from hypoxia-treated 3T3L1 adipocytes also induced insulin resistance in C2C12 myotubes [243], suggesting that different stressors might impact adipokine secretion which in turn alter muscle insulin sensitivity.

## 5.4. Limitations and Future direction

In most research projects, limitations arise from experimental designs and the choice of the studied model. In this project, we used the conditioned media to reproduce *in vitro* the adipose-to-muscle communication. This model is different from the co-culture model, which tries to replicate the crosstalk between tissues (communication between muscle and adipose tissue in the two directions) [256], [257]. This limits our interpretation since muscle-to-adipose

communication may also regulate metabolism [231], [256], [258]. However, our goal was to determine the role of adipokines secreted by adipocytes after PCB126 exposure on muscle energy metabolism. Our model was therefore appropriate to study the adipose-to-muscle communication but needs to be considered as an isolated system that possesses several limitations.

On one hand, *in vitro* cell culture does not take into account the interaction between the different systems and organs. At the whole-body level, PCBs will also alter the secretion of cytokines and the production of ROS from other tissues and cell types, such as the liver, immune cells and endothelial cells [203], [214], [237], which can alter muscle metabolism. Moreover, PCBs might be metabolized by the liver *in vivo* which is not taken into account in our model. Thus, *in vitro* models, such as ours, do not represent what is happening in the organism. However, *in vitro* studies are needed to investigate specific parts of this complex system.

On the other hand, cell lines are not a perfect representation of their respective mature tissue/organ. Here, we used 3T3L1 adipocytes, C2C12 myotubes and primary mouse muscle cells. 3T3L1 adipocytes do not represent mature adipose tissue, which is composed of pre-adipocytes and matured adipocytes, blood vessels, and other types of cells, including immune cells (i.e. macrophages). Adipokines secreted by adipose tissue can recruit other cells, such as keratocytes and macrophages, to respond to different stresses [117], [259], [260]. In cell culture, these interactions cannot be reproduced. However, 3T3L1 adipocytes are a good *in vitro* model to study the effects of different compounds (drugs, POPs, EDC, etc) or nutrients on specific molecular mechanisms involved in adipogenesis, ROS production and glucose

metabolism [257]. Similar limitations exist with the muscle cell lines we used. C2C12 myotubes form a monolayer of cells and they "progressively acquire a structured pattern up to the appearance of sarcomeres and myofibrils [249]". They are often used to study the characteristics of skeletal muscle because they can contract under electric pulse stimulation and express specific proteins [248]. Moreover, as previously mentioned, it is difficult to investigate insulin response in C2C12 myotubes since they do not respond well to insulin in term of glucose uptake due to high levels of basal glucose uptake and lack of insulin-responsive GLUT4 vesicle [245]. Mouse primary muscle cells are a better model to study insulin response in muscle. Furthermore, they retain the characteristics of their fiber type of origin and may develop into contracting myotubes [253]. However, they are still limited to a monolayer and do not represent exactly physiological responses of mature skeletal muscle.

Furthermore, most of our experiments with IS and IR adipocytes were done at different moments, making hard to compare their metabolic responses. We were able to determine directly the effects of pre-established IR on lipolysis rate and mRNA adipokine expression in adipocytes, but not on other parameters. While we cannot make conclusions on the association of pre-established IR and increased metabolic sensitivity to PCB126-exposure, our results strongly suggest that IR adipocytes are more sensitive to PCB126 exposure than IS adipocytes.

We have demonstrated that direct exposure to PCB126 does not alter muscle glucose metabolism, while CM from adipocytes exposed to PCB126 did decrease glucose uptake and insulin response in muscle cells. Even if our results suggest that increased secretion of inflammatory markers from adipocytes might be responsible for abnormal glucose uptake in myotubes, it is not possible to conclude from our results that it is actually the case. Future

studies are thus needed to determine whether one or several adipokines for which the secretion has been increased in response to PCB126 treatment are responsible for abnormal glucose transport in myotubes. This could be done by reproducing glucose uptake assay with specific antibodies that inhibit these different adipokines.

Our results demonstrate that PCB126 induces mitochondrial dysfunction in adipocytes. To our knowledge, adipose tissue mitochondrial function in the context of PCB-exposure had never been studied before. In the future, new studies should determine whether exposure to this pollutant *in vivo* in rodents also affect adipose tissue mitochondrial function, and whether mitochondrial function is altered in adipose tissue from individuals known to be highly exposed to this type of pollutant. If this altered mitochondrial function is confirmed in *in vivo* models, it would then be interesting to deeply study the cause of mitochondrial dysfunction in PCB126-exposed adipocytes. For example, we could determine if PCB126 has an effect on super-complex formation, mitochondrial content and/or mitochondrial structure.

Finally, our results do not support our hypothesis that PCB126 might first induce adipose tissue dysfunction resulting in altered adipokine secretion and inflammation, leading to skeletal muscle mitochondrial dysfunction. In the present study, the 24hr-treatment with PCB126 represents an acute exposure, while in the humans PCB126 exposure is chronic in combination with other POPs. Multiple studies have shown that exposure to PCB126 pre-differentiation and/or longer treatments induced a significant reduction in adipogenesis and promoted a strong inflammatory response [128]. It would be interesting to investigate how early exposure to PCB126 affects inflammatory response in adipocytes and if this could lead to decreased mitochondrial function in skeletal muscle. Furthermore, in our model, PCB126

increased the secretion of MCP-1 by adipocytes. MCP-1 secretion is known to cause macrophage infiltration in adipose tissue, further increasing adipose tissue inflammation [184], [261]. It would be interesting to investigate the effects of PCB126 on adipocytes co-cultured with macrophages, and to use CM from these co-cultured cells to treat muscle cells. This model might be closer to the physiological communication between adipose tissue and muscle in the context of inflammation induced by PCB126 exposure.

# 6. Conclusion

In summary, we demonstrated that PCB126 promotes inflammation and metabolic defects in adipocytes particularly when those cells were already insulin resistant before exposing them to the pollutant. Moreover, we also showed that the adipose-to-muscle communication is needed for alteration of glucose uptake and insulin response in skeletal muscle in response to PCB126 treatment. However, there was no clear evidence from our model that adipose-to-muscle communication is responsible for muscle mitochondrial defects when rats are exposed *in vivo* to PCB126.

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Audrey Caron
Gatineau, Qc. Ca.

## Education

- **Master in Biochemistry** – University of Ottawa, Ottawa, On. (Expected Summer 2018)
- **Honors Bachelor of Health Sciences** – University of Ottawa, Ottawa, On. (Diploma received in May 2016, *Magna cum laude*)
- **Programme préuniversitaire : sciences de la nature** – Cégep de Sherbrooke, Sherbrooke, Qc. (2010-2012)
- **Programme préuniversitaire : sciences Humaines** – Cégep de Victoriaville, Victoriaville, Qc. (2005-2007)
- **High school (DES+)** – Polyvalente la Samare, Plessisville, Qc. (2000-2005)

## Research Experience

### Presentation

- **Poster Presentation**
  **BMI Poster Day** – *Ottawa, On.* (May 3, 2018). Garneau, L., Bloomfield, S., **Caron, A**. & Aguer, C. Role of Myokines in the Development of Metabolic Defects During Type 2 Diabetes and the Effect of Muscle Contraction on the Secretion of these Myokines.
- **Poster Presentation**
  **Biomedical science Poster Day** – *Ottawa, On.* (April 27, 2018). Bloomfield, S. **Caron, A**. & Aguer, C. Optimizing an in vitro model of muscle contraction to study myokine secretion
- **Poster Presentation**
  **Poster Day, Interdisciplinary School of Health Sciences** – *Ottawa, On.* (April 3, 2018), Kpade, V., Jafar, H., **Caron, A**. & Aguer, C. Comorbidité entre le diabète de type 2 et la dépression.
- **Abstract submitted**
  **Société française de diabète** – *Nantes, France.* (March 20-23, 2018). Garneau, L., Bloomfield, S., **Caron, A.** & Aguer, C. Validation d'un modèle in vitro de l'exercice pour l'étude du rôle des myokines dans les défauts métaboliques musculaires au cours du diabète de type 2

- **Poster Presentation**

**Cells Symposia: Next Gen Immunology** – *Rehovot, Israel.* (February 11-14, 2018). **Caron, A.** & Aguer, C. Role of inflammation and crosstalk in PCB126-induced metabolic defects.

- **Oral Presentation**
  **Réunion scientifique annuelle 2018 de la Société québécoise de lipidologie, de nutrition et de métabolisme (SQLNM), du Réseau de recherche en santé cardiométabolique, diabète et obésité (CMDO) du FRQS et du Congrès COLosUS** – *Magog, Qc.* (February 7-9, 2018). **Caron, A.** & Aguer, C. Le dialogue entre le tissu adipeux et le muscle squelettique pourrait expliquer le développement d'anomalies métaboliques musculaires induites par les BPC
- **Poster Presentation**
  **Canadian Lipoprotein Conference** – *Ottawa, On.* (October 19-22, 2017): Role of the adipose tissue and skeletal muscle crosstalk in PCB-induced skeletal muscle metabolic defects. **Caron, A**. & C. Aguer.
- **Oral & Poster Presentation**
  **Bengt Saltin International Graduate Course** – *Gatineau, Qc.* (September 18-21, 2017): **Caron, A.** & Aguer, C. Role of the adipose tissue-skeletal muscle crosstalk in PCB126-induced metabolic defects
- **Poster Presentation**
  **BMI Poster Day** – *Ottawa, On.* (May 11, 2017): Adipose tissue and skeletal muscle crosstalk in PCB-induced skeletal muscle metabolic defects. **Caron, A**. & C. Aguer.
- **Poster Presentation**
  **Biomedical science Poster Day** – *Ottawa, On.* (April 29, 2017): Murray, M., **Caron, A**. & C. Aguer. Effects of PCB77 on Skeletal Muscle Energy Metabolism and Insulin Sensitivity.
- **Poster Presentation**
  **Réunion scientifique annuelle 2017 de la Société québécoise de lipidologie, de nutrition et de métabolisme (SQLNM), du Réseau de recherche en santé cardiométabolique, diabète et obésité (CMDO) du FRQS et du Congrès COLosUS** – *Sherbrooke, Qc.* (February 8-10, 2017): Adipose tissue and skeletal muscle crosstalk in PCB-induced skeletal muscle metabolic defects. **Caron, A**., Murray, M. & C. Aguer.
- **Poster Presentation**
  **Keystone Symposia: Diabetes** – *Keystone, CO.* (January 21-27, 2017): Adipose tissue and skeletal muscle crosstalk in PCB-induced skeletal muscle metabolic defects. **Caron, A**., Murray, M. & Dre. C. Aguer.

- **Poster Presentation**

**Annual Conference of the Canadian Nutrition Society** – *Gatineau, Qc.* (May 5–7, 2016): Extrinsic mechanisms involved in the metabolic defaults of skeletal muscle exposed to PCB126. **Caron, A**. & C. Aguer.

- **Poster Presentation**
  **Réunion scientifique annuelle 2016 de la Société québécoise de lipidologie, de nutrition et de métabolisme (SQLNM), du Réseau de recherche en santé cardiométabolique, diabète et obésité (CMDO) du FRQS et du Congrès COLosUS** – *Sherbrooke, Qc.* (February 10-12, 2016): Mécanismes extrinsèques impliqués dans les défauts métaboliques du muscle squelettique exposé aux BPC126. **Caron, A**. & C. Aguer.
- **Poster Presentation**
  **Conference of the Society of Epidemiology and Biostatistics** – *Toronto, On.* (June 21-22, 2015): Incidence rates of lymphoma and leukemia in rheumatoid arthritis patients using adalimumab: a literature review. **Caron, A**. & Dusseault, A.
- **Poster Presentation**
  **Research Day – Faculty of Health Sciences** – *Ottawa, On.* (April 11, 2015: The relationship between leukemia and lymphoma in rheumatoid arthritis patients and tumor necrosis factor alpha antagonist (TNF-α) drugs: a literature review. **Caron, A**. & Dusseault, A.

## Publications

- BPA and BPS exposure in L6 myotubes alters skeletal muscle energy metabolism. Chehade, L., **Caron, A**., Chapados, NA. & Aguer, C. (In preparation, submission in July 2018, Toxicological Sciences)
- DDT and DDE exposure in L6 myotubes alters skeletal muscle energy metabolism. Chehade, L., **Caron, A**., Chapados, NA. & Aguer, C. (In preparation, submission in July 2018, Toxicological Sciences)
- Adipose-to-muscle communication in PCB126-induced skeletal muscle metabolic defects. **Caron, A**., Peshdary, V., Atlas, E. & Aguer, C. (In preparation, submission in July 2018, FASEB Journal)
- Mechanisms Involved in the Metabolic Defects of Skeletal Muscle Exposed to PCB77. **Caron, A***., Murray, M*. & Aguer, C. (In preparation, submission in June 2018, BBA Molecular basis of disease) * co-first authors

- IL-15 improves skeletal muscle oxidative metabolism and glucose uptake in association with increased respiratory chain supercomplex formation and AMPK pathway activation.

Nadeau, L., **Caron, A.**, Patten, DA., Garneau, L., Foretz, M., Haddad, P., Anderson, BG., Quinn, L., Harper ME. & Aguer, C. (Submitted to BBA – General Subjects on April 30th, 2018)

- Chechade, L*., **Caron, A*.** & Aguer, C. (December 2016). Persistent organic pollutants: potential role in the development of insulin resistance and mitochondrial dysfunction in skeletal muscle. *Environment and Natural Resources Research*, 6(4). (*co-first authors)
- Mauger, J-F., Nadeau, L., **Caron, A.,** Chapados, N. A. & C. Aguer. (2015). Polychlorinated biphenyl 126 exposure in L6 myotubes alters glucose metabolism: a pilot study. *Environmental Science and Pollution Research*
- **Caron. A**. (July 2, 2015) Ebola en Afrique de l'Ouest : l'impact des déterminants sociaux. *Revue interdisciplinaire des sciences de la santé*. http://ijhs2.deonandan.com/wordpress/archives/2464

## Professional Experience

- **Faculty of Sciences, University of Ottawa** – *Ottawa, On.* (January 2018 – May 2018)
  Corrector (BCH1540 – Introduction à la biologie)
- **Faculty of Sciences, University of Ottawa** – *Ottawa, On.* (September 2017 – December 2017)
  Teaching Assistant and Lab. Demonstrator (BCH3526 – Microbiologie)
- **Institut du Savoir Montfort, Hôpital Montfort** — *Ottawa, On.* (March 2017 – February 2018)
  Teaching Assistant
- **Student Supervision, University of Ottawa** – *Ottawa, On.*
  - Dalibard, O. (March 2018 – June 2018)
  - Bloomfield, S. (May 2017 – April 2018)
  - Girres, N. (March 2017 – June 2017)
  - Murray, M. (August 2016 – April 2017)
- **Faculty of Medicine, University of Ottawa** — *Ottawa, On.* (July 2017 – May 2018)
  Proctor for different courses (ANP1506, MED Unité I, MED Unité II, etc)
- **Faculty of Sciences, University of Ottawa** — *Ottawa, On.* (January 2017 – April 2017)
  Teaching Assistant and Lab. Demonstrator (BCH2733 – Introduction à la biochimie)
- **Faculty of Sciences, University of Ottawa** — *Ottawa, On.* (January 2017 – April 2017)
  Corrector (BCH3520 – Métabolisme)
- **Department of chemistry, University of Ottawa** — *Ottawa, On*. (September 2015 – April 2016)
  Teaching Assistant and Lab. Demonstrator (CHM 1711 & 1721 – Chimie générale et organique)

- **Department of Biology, University of Ottawa** — *Ottawa, On*. (January 2016 – April 2016)
  Teaching Assistant (BIO 2533 - Génétique)
- **Academic Writing Help Centre, University of Ottawa** — *Ottawa, On*. (Sept. 2014 – December 2015)
  Writing advisor (French)
- **Institut de recherche clinique de Montréal** — *Montréal, Qc*. (April 2014 – Sept. 2014)
  Research Internship (Beta-Thalassemia, PKD, anemia falciform)

## Scholarships and Awards

- **Seahorse Travel Award – Agilent Technology, USA** (January 2018)
- **Master Scholarship – Fond de recherche en santé du Québec** (2017-18 & 2018-19)
- **Excellence Scholarship, University of Ottawa** (2017-2018)
- **Travel Grant – Faculty of Medicine, Department of Biochemistry, Microbiology and Immunology, University of Ottawa** (2016-2017)
- **Seahorse Travel Award – Agilent Technology, USA** (January 2017)
- **Travel Grant – Faculty of Graduated Studies, University of Ottawa** (2016-2017)
- **CGS-M, Natural Sciences and Engineering Research Council of Canada (NSERC Master Scholarship)** (2016-2017)
- **Excellence Scholarship, University of Ottawa** (2016-2017)
- **Admission Scholarship, University of Ottawa** (2016-2018)
- **Research Scholarship: Institut de recherche clinique de Montréal** (April 2014 – September 2014)

## Extracurricular Activities

- **Journée Conférence des étudiants, Institut du Savoir Montfort – Recherche** – *Ottawa, On.* (3 April 2018)
  Organizer and moderator
- **Consultative Committee, Institut du Savoir Montfort** – *Ottawa, On.* (October 2017 – Ongoing)
  Graduate Student Representative
- **Sauvetage Bénévole Outaouais – Ottawa Volunteer Search and Rescue** – *Ottawa, On.* (July 2017 – Ongoing)
  Ground Searcher

- **Student Committee, Institut du Savoir Montfort** – *Ottawa, On*. (June 2017 – May 2018)
  Member
- **St-John's Ambulance** – *Ottawa, On*. (March 2015 – June 2017)
  Medical First Responder
- **Hôpital Montfort** – *Ottawa, On.* (Summers 2015 & 2016)
  Research volunteer
- **Fondation le Pont vers l'Autonomie** — *Victoriaville, Qc*. (June 2014 – June 2016)
  Translator, Communication and social media