Role of Environmental Pollutants in Skeletal Muscle Insulin Resistance and Mitochondrial Dysfunction

Lucia Chehade[1,2]*, Audrey Caron[1,2]* & Céline Aguer[1,2]

[1] Institut de recherche de l'Hôpital Montfort, Ottawa, ON K1K 0T2, Canada

[2] Faculty of Medicine, Biochemistry, Microbiology and Immunology Department, University of Ottawa, Ottawa, ON K1H 8L1, Canada

Correspondence: Céline Aguer, Institut de recherche de l'Hôpital Montfort, Ottawa, ON K1K 0T2, Canada. Tel: 613-746-4621, Ext. 6047. Email: celineaguer@montfort.on.ca

* These authors contributed equally to this work

Abstract

In the last decade, the incidence of diabetes in Canada has nearly doubled and is now estimated to affect one in three individuals. Type 2 diabetes (T2D) is a serious public health problem: governments and health care professional are working to control its propagation, offer better treatment alternatives and reduce its impact on patient quality of life. Insulin resistance is an early event in the development of T2D. Due to its mass and important role in the maintenance of glucose homeostasis, skeletal muscle is believed to play a central role in the development of insulin resistance. The development of this metabolic disorder is multifaceted with obesity, physical activity and diet receiving the most research interest. It is recognized that mitochondrial dysfunction, increased oxidative stress and inflammation are implicated in the development of insulin resistance in muscle. Recently, the environmental hypothesis has been advanced to explain the increased number of patients with T2D. Various persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), bisphenol A (BPA) and p, p-dichlorodiphenylchloroethane (DDT) are being investigated in their relation to T2D. However, despite the importance of skeletal muscle in the development of insulin resistance and T2D, very few studies have focused on the effect of POPs on skeletal muscle energy metabolism. This review will highlight the implication of POPs in the development of diabetes and present work being done to asses POPs' involvement in observed metabolic disarrangements, specifically at the level of skeletal muscle.

Keywords: Glucose metabolism, Inflammation, Insulin signaling, Mitochondrial function, Oxidative stress, Persistent organic pollutants, Skeletal muscle, Type 2 Diabetes

1. Introduction

Diabetes is a chronic disease affecting over 382 million people worldwide, now considered an epidemic level (Carvalho-Santos et al., 2015). Its prevalence is only expected to rise, projecting numbers to reach 592 million individuals affected by 2035 (Guariguata et al., 2014). It has been declared as a major threat to public health in the United States and worldwide (Centers for Disease Control and Prevention [CDC] 2014; World Health Organization [WHO], 2016). Characterized by insulin resistance (IR) in targeted tissues followed by a decrease in insulin production due to β-cell failure (Carvalho-Santos et al., 2015), type 2 diabetes (T2D) is responsible for 90% of all diabetes cases (Cameron et al., 2010). While it has been shown that lifestyle, exercise, and food intake are main causes behind these staggering statistics; some studies have considered the impact of environmental pollution as a contributor to metabolic disarrangements leading to the development and advancement of T2D. Today, accumulating evidence is indeed pointing to an association between environmental contaminants and the increased risk of T2D.

Persistent organic pollutants (POPs) are organic compounds that resist photolytic, biological and chemical degradation (Ngwa, Kengne, Tiedeu-Atogho, Mofo-Mato, & Sobngwi, 2015). The chemical structure of these pollutants allows them to be soluble in lipids leading to a facilitated bioaccumulation of these compounds in adipose tissue (Pavlikova, Smetana, Halada, & Kovar, 2015). Human exposure to POPs such as polychloro-dibenzopara dioxins (PCDD) and polychloro-dibenzo furans (PCDF), bisphenol A (BPA), polychlorinated

biphenyls (PCBs) and organo-chlorine pesticides such as dicholordiphenyltrichloroethane (DDT) and 1,1-dichloro- 2,2-bis(p-chlorophenyl)ethylene (DDE) occurs primarily through the consumption of animal fat found in dairy products, fish and meats (Ngwa, Kengne, Tiedeu-Atogho, Mofo-Mato, & Sobngwi, 2015). In fact, POPs remain in fatty tissues for many years because they are highly resistant to metabolic degradation (Kiviranta, Vartiainen, & Tuomisto, 2002). Many of existing POPs are still used in agriculture around the world while the potential risk to human exposure is under-evaluated. While the usage of some POPs such as DDT and PCBs have been banned in North America since the late 1970s, traces are still found in animal and plant tissue in countries that have stopped the usage of the product. In Canada, due to its resistance and semi-volatile properties, DDT has managed to be introduced into the Arctic marine ecosystems, thus increasing local human exposure (Riseborough, Walker, Schmidt, De Lappe, & Connors, 1976). Based on the Canadian Quality Soil Guidelines for the Protection of Environmental and Human Health, Herbet et al. (1994) reported concentrations of DDT and DDE, a metabolite of DDT, that range from 1.7 to 131.7 mg.kg^{-1} and 4.0 to 342.6 mg.kg^{-1}, respectively in Ontario, Canada soil. Concentrations of DDT and DDE have also been detected in maternal blood plasma, umbilical cord plasma and human breast milk in the Canadian population (D'Amour, Lye, & Murray, 2011). Similarly, a report issued by Health Canada in 2000 showed that in Ottawa, 0.2 to 12 428.6 ppt per day of PCBs (a chemical that was used mainly in electrical equipment) were found in consumable foods (Health Canada, 2013). Likewise, the public is exposed to Bisphenol A (BPA), a chemical used in the manufacture of food and beverage containers such as water bottles, storage containers, and infant bottles, through migration of the chemical from food packaging and the exposure rates in Canada for the general population range from 0.06 to 4.30 μg/kg body weight/day (D'Amour, Lye, & Murray, 2011).

2. Link Between Type 2 Diabetes and Persistent Organics Pollutants Exposure: Epidemiological Studies

With the increase in T2D incidence at a globally alarming rate, researchers have sought to elucidate whether the exposure to POPs has a physiological impact contributing to the development of IR-associated metabolic diseases. Various POPs such as PCBs, BPA and DDT have been linked to the development of IR and T2D (Neel & Sargis, 2011; Everett et al., 2007; Sargis, 2014). Epidemiological studies have reported 62% higher levels of POPs in people living with T2D compared to the rest of the population (Fierens et al., 2003; Rignell-Hydbom, Rylander, & Hagmar, 2007; Wang & Wang, 2005). In a nested case-control study, low dose POPs predicted incident T2D and it was concluded that simultaneous exposure to various POPs such as organo-chlorine pesticides and PCBs may contribute to development of obesity, dyslipidemia and IR, which are common precursors of T2D (Lee et al., 2011). Within this same study, both p,p'-DDE and PCB178 showed positive significant associations with body mass index, triglycerides, and negative association with HDL-cholesterol (Lee et al., 2011). Furthermore, based on the Nurses' Health Study, it was found that hexachlorobenze and PCB concentrations were associated with diabetes (Wu et al., 2013).

High exposure to POPs is a result of the rapid agricultural and industrial development. For example, in India, a country with high DTT usage, prevalence of diabetes has increased from 5.8% in 2000 to 8.6% in 2013 (Jaacks & Staimez, 2015). Similarly, abnormal glucose regulation has been reported in Chinese factory workers exposed to pesticides compared to unexposed people (Jaacks & Staimez, 2015). A recent cross-sectional study conducted in South Korea also showed increased risk of diabetes with increasing DTT concentration in visceral adipose tissue (Kim et al., 2014). The U.S. National Toxicology Program Workshop in 2011 also concluded that evidence is sufficient to support a positive association between DDE exposure and T2D (Kuo, Moon, Thayer, & Navas-Acien, 2013). Furthermore, many other epidemiological studies have made an association between DDE, DDT or DDD (Dichlorodiphenyldichloroethane, another metabolite of DDT) and diabetes. An analysis of workers engaged in agricultural tasks in Michigan, Hawaii, and California showed that there was an apparent association between high serum organochlorine pesticides and the appearance of hypertension, arteriosclerotic cardiovascular disease and diabetes (Morgan, Lin, & Saikaly, 1980). Also, a study performed on a cohort of Great Lakes Sport Fish Consumers established in the early 1990s and followed through 2005, tested serum for DDE and assessed diabetes diagnosis, demographics, and fish consumption. This study noted consistent dose-related associations of DDE with incident diabetes and that DDE and PCBs were higher in participants who subsequently developed diabetes, thus confirming previous studies and suggesting the possibility of a casual relationship (Turyk, Anderson, Knobeloch, Imm, & Persky, 2009).

Similarly, several studies have shown an association between PCB exposure and the development of T2D. A cross-sectional study conducted on a Native American population residing in the provinces of Ontario and Quebec in Canada, concluded that there is a significant association between PCBs and hexachlorobenzene exposure with an increased prevalence of diabetes (Aminov et al., 2016). Another study conducted on a Japanese population exposed to these pollutants (PCBs, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like-

polychlorinated biphenyls), showed a significant association with metabolic syndrome, in particular, the highest quartiles of PCB126 and PCB105 (Uemura et al., 2008). Furthermore, clinical examinations of elderly residing on dozen islands in the Northern Atlantic between Norway and Iceland showed a high prevalence of T2D and those with T2D or impaired fasting glycaemia also had higher circulating PCB concentrations (Grandjean et al., 2011).

Based on a cross-sectional study, a strong association was also made between exposure to PCDDs and PCDFs and IR (Chang, Chen, & Lee, 2016). In a similar study, conducted in non-diabetic individuals, a significant association was found between high levels of PCDD/Fs and other metabolic syndromes (Chang et al., 2009). Finally, the National Health and Nutritional Examination Survey conducted between 2003 and 2008, indicated a positive association between BPA levels in urine and T2D (Shankar & Teppala, 2011; Lang et al., 2008).

In summary, based on the numerous epidemiological studies conducted, the link between exposure to POPs (such as PCBs, DDT, DDE, PCDDs, and BPA) and the development or increased risk of T2D is clear, suggesting that POP exposure is, at least in part, responsible for the increased prevalence of T2D development in the last decades.

3. Skeletal Muscle, Insulin Resistance and Type 2 Diabetes

3.1 Importance of Skeletal Muscle in the Development of Insulin Resistance and Type 2 Diabetes

Insulin resistance is a characteristic feature of T2D and is largely implicated in the pathogenesis of the disease. In the early stages, pancreatic cells adapt to IR by increasing mass and β-cell function. While β-cell failure is considered to be ultimately responsible for the development of T2D, skeletal muscle IR is likely the primary defect, evident before hyperglycaemia develops or β-cell failure (DeFronzo & Tripathy, 2009). Indeed, skeletal muscle plays a crucial role in the maintenance of glucose homeostasis (DeFronzo & Tripathy, 2009) since it accounts for 40% of total body weight in lean individuals. Furthermore, muscle cells are the primary site of glucose storage in the form of glycogen following physical exercise or in the postprandial period accounting for 80% of whole-body glucose disposal (Yang, 2014; Kersten, 2014).

Following the consumption of a meal, blood glucose levels rise and insulin production increases to allow for the storage of glucose in tissues. Insulin activates glucose transporter 4 (GLUT4) translocation to the plasma membrane thus increasing glucose uptake into muscle cells. When muscle cells are resistant to insulin, glucose uptake is disturbed leading to hyperglycaemia (Yoshida et al., 2008). Being the major organ for glucose disposal, skeletal muscle IR leads to compensatory systems within the body, including increased production of insulin by β-cells. However, if IR and resulting hyperglycaemia persists, β-cells can no longer cope and may die through apoptosis, causing a decrease in insulin production and hyperglycaemia, establishing T2D. Thus, impaired muscle glucose uptake is thought to play a central role in the development or initiation of T2D (Gaster, Petersen, Hojlund, Poulsen, & Beck-Nielsen, 2002). A great importance therefore lies in understanding the mechanisms responsible for the development of IR in this tissue. Most studies have looked at the consequences of the exposure to POPs in tissues such as the brain, liver and pancreas. However, while skeletal muscle is largely implicated in the development of IR and T2D, studies on the effect of POPs on skeletal muscle IR are lacking.

3.2 Insulin Signaling Pathway and Insulin Resistance in Skeletal Muscle

Insulin is responsible for maintaining blood sugar levels by activating glucose uptake into cells using specialized glucose transporters. Insulin secretion and action can be affected by various factors including hyperglycaemia, hyperlipidemia, genetic factors and exposure to POPs (Evans, Goldfine, Maddux, & Grodsky, 2003). At the cellular level, IR develops when there is a problem in the insulin signaling pathway. Mutations in genes coding for kinases or proteins, such as Akt (also known as protein kinase B (PKB)), PDK (phosphoinositide-dependant kinase 1), the insulin receptor, and imbalances in the number of phosphoinositide 3-kinase (PI3K) subunits, may hinder the insulin signaling pathway and lead to IR in muscle (Sargis, 2014; Saini, 2010).

A predisposing factor in the development of T2D is the development of muscle IR. Interestingly, many studies have demonstrated that offspring with normal glucose tolerance of two parents with T2D exhibit moderate to severe skeletal muscle IR (Vaag & Beck-Nielsen, 1992; Gulli, G., Ferrannini, Stern, Haffner, & DeFronzo, 1992; Tripathy et al., 2003). The implication of skeletal muscle IR in the development of T2D was further elucidated by a study demonstrating that genetically predisposed T2D individuals had increased IRS (Insulin Receptor Substrate)-1 serine phosphorylation and decreased PI3K activity in skeletal muscle (Morino et al., 2005). This observed increased Ser/Thr phosphorylation of IRS-1 was previously shown to impair insulin signaling. Indeed, in skeletal muscle, phosphorylation of IRS-1 on serine residues generates a negative feedback that inhibits the tyrosine phosphorylation of both insulin receptor and IRS-1 which in consequence inhibits the insulin signaling pathway and GLUT4 translocation (Morino et al., 2005).

3.3 Mitochondrial Dysfunction and Insulin Resistance in Muscle

Muscle IR is thought to develop as a result of mitochondrial dysfunction leading to disarrangement in the insulin signaling pathway. In a study done by Morino et al. (2005), it was observed that the rate of insulin-stimulated glucose uptake in muscle was 60% lower in IR offspring of parent with T2D than the control subjects associated with a 38% lower mitochondrial density (Morino et al., 2005). This study confirms the results of many others that have shown that mitochondrial function and/or number is altered in individuals diagnosed with T2D (Petersen et al., 2004; Befroy et al., 2007, Kelley et al., 2002, Ritov et al., 2005). Alteration of mitochondrial function results in intramyocellular accumulation of lipids, which has been associated with a decrease in insulin sensitivity in the rodent model and patients with T2D or obesity (Krssak, Falk Petersen, Dresner, DiPietro, Vogel, Rothman, et al., 1999; Pan et al., 1997; G. Perseghin et al., 1999; An et al., 2004). Inefficient fatty acid β-oxidation is also linked to increased muscle accumulation of lipid intermediaries such as ceramides, diacylglycerol, acyl-CoA and acylcarnitines (Zhang, Keung, Samokhvalov, Wang, & Lopaschuk, 2010). These metabolites participate in the development of muscle IR by interfering with the insulin signaling pathway (Coll et al., 2008; Weigert et al., 2004; Aguer et al., 2015). Inversely, an elevation of plasma free fatty acids induces mitochondrial dysfunction by decreasing oxidative phosphorylation and increasing IR (Brehm, Krssak, Schmid, et al., 2006).

3.4. Muscle Oxidative Stress and Insulin Resistance in Muscle

Oxidative stress is the accumulation of reactive oxygen species (ROS) deemed to be damaging. While ROS act as important signaling molecules, their effects are dose dependant and high levels could exert negative effects and alter gene expression affecting their ability to grow, differentiate, and adapt (Musarò, Fulle, & Fanò, 2010). Emerging evidences have assigned an important role to oxidative stress in muscle homeostasis and in the physiopathology of skeletal muscle (Musarò, Fulle, & Fanò, 2010). Mitochondria are a large producer of ROS under basal conditions, and when dysfunctional can produce even higher levels of ROS (Czajka et al., 2011). Skeletal muscle is a major source of oxidants in the body, since it contains a large number of mitochondria. Usually, there is a balance between the production of oxidants and antioxidants. However, if there is an imbalance in the ratio oxidants/antioxidants, ROS and other oxidants will accumulate and cause oxidative stress and cell damage. In fact, ROS can cause mutations in the DNA, lipid peroxidation and disrupt the function and expression of proteins, leading to dysfunctions in cellular processes (Matsuda & Shimomura, 2013). Generally, under conditions leading to metabolic disorders such as IR and T2D, there is an imbalance between ROS and antioxidant production by mitochondria, leading to harmful ROS accumulation. ROS can damage the components of mitochondria, directly leading to mitochondrial dysfunction. In fact, according to Bonnard et al. (2008), mitochondrial defects do not appear before IR development but is the result of oxidative stress that affects both the insulin signaling pathway and mitochondrial function. This claim is supported by data showing an increase in muscle ROS production in mice exposed to a high-fat / high-sucrose diet (Bonnard et al., 2008). Those effects were similarly observed *in vitro* when exposure of muscle cells to high glucose and high fatty acid concentrations induced ROS production and altered mitochondrial density and function (Bonnard et al., 2008). Interestingly, treatment with antioxidants decreased muscle ROS levels and restored mitochondrial integrity in both the mouse model as well as the *in vitro* muscle cell model (Bonnard et al., 2008).

Further, it has been noted that the establishment of IR in skeletal muscle causes a decrease in electron transport chain efficiency (lower ATP production rate) occurring with elevated mitochondrial ROS production (Zabielski et al., 2015). Furthermore, with decreasing muscle mitochondrial function and fatty acid β-oxidation, there is an increase in skeletal muscle accumulation of ceramides and acylcarnitines which contribute to an increase in ROS production, mitochondrial integrity alteration and IR (Zabielski et al., 2015; Aguer et al., 2015; Schmitz-Peiffer et al., 1999; Chavez & Summers, 2012; Bastie et al., 2004; Pickersgill et al., 2007).

Another study suggests that a large redox imbalance in muscles leads to the activation of proteolysis and massive oxidation of proteins, which become more susceptible to degradation (Pellegrino et al., 2011). NADPH oxidase and electron transport chain complexes I and III (found in mitochondrial inner membrane) are main cellular sources of ROS (Aguer & Harper, 2012). A cross talk between mitochondria and NADPH oxidases exists and may represent a feed-forward vicious cycle of ROS production (Dikalov, 2011). When nutrients such as glucose or lipids are present in excess or a metabolic disarrangement is established, ROS production is increased (Henriksen, Diamond-Stanic, & Marchionne, 2011). This production of ROS in skeletal muscle mitochondria was shown to inhibit the insulin signaling pathway by acting on glycogen synthase kinase-3β (Henriksen, Diamond-Stanic, & Marchionne, 2011). Oxidative stress is also inducing a number of stress-sensitive signaling pathways (NF-κβ (nuclear factor-kappa B, transcription factor used in mediating immune and inflammatory responses), JNK (Jun N terminal Kinase) / SAPK (Stress-activated protein kinases) (SAPK/ JNK are members of the MAP Kinase family and are activated by a variety of environmental stresses, inflammatory cytokines, and growth factors), and p38 MAPK) leading to IR by interfering with IRS-1 and/or Akt (Rains & Jain, 2011; Henriksen, Diamond-Stanic, &

Marchionne, 2011) (Figure 1). The activation of NF-κβ was also shown to regulate pro-inflammatory cytokines such as tumour necrosis factor (TNF-α), which directly affects insulin signaling pathways by inhibiting IRS-1 (Wang et al., 2015). Also other serine/threonine kinases such as PKC (protein kinase C) are activated by ROS, which disrupt the ability to recruit and activate molecules that contain SH-2 (Src Homology 2 domain) hence disrupting the interaction of IRS protein with the insulin receptor (Rains & Jain, 2011).
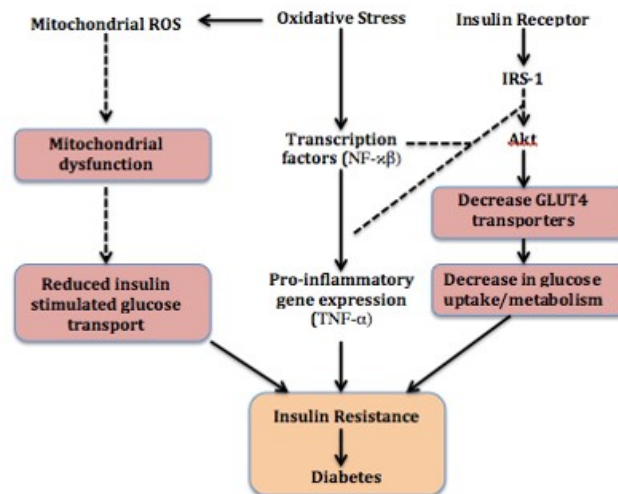

Figure 1. The effect of oxidative stress on the insulin signaling pathway and on mitochondrial dysfunction and its link to type 2 diabetes

4. Adipose Tissue and Skeletal Muscle Crosstalk in the Development of Insulin Resistance

Obesity is a risk factor in the etiology of T2D, demonstrating that there could be a link between the increase in body fat and IR development. Adipose tissue plays a role in storing excess energy as well as in the synthesis and secretion of hormones and cytokines/adipokines. The secretion of these adipokines by adipose tissue is altered by an excessive accumulation of fat, exposure to pollutants or in response to oxidative stress, consequentially affecting the function of other tissues including skeletal muscle. Moreover, cytokines and adipokines secreted by adipose tissue may have extrinsic effects on the development of IR in skeletal muscle (Kadowaki et al., 2006; Steinberg, 2007; Gadupudi et al., 2015; Quinn et al., 2005). Dysfunctional adipose tissue generally increases the secretion of pro-inflammatory cytokines and reduces that of anti-inflammatory cytokines. These mediators have various effects on the insulin signaling pathway and insulin sensitivity, as well as the expression of other cytokines.

4.1 Implication of Adipokines in the Development of Insulin Resistance

Among the adipokines secreted by adipose tissue, adiponectin is a hormone that plays a role in energy homeostasis, glucose and lipid metabolism (Yamauchi et al., 2002; Imbeault, 2007). Adiponectin sensitizes the body to insulin, and as such could be a target to fight against the development of IR (Kadowaki et al., 2006; Saini, 2010). In muscle cells, adiponectin activates AMPK (AMP-activated protein kinase) and PPAR-α (peroxisome proliferator-activated receptor) (Preedy & Hunter, 2011; Yoon et al., 2006). Upon AMPK activation, mitochondrial fatty acid oxidation and glucose uptake are increased independently of insulin signaling. Similarly, PPAR-α, a transcription factor, promotes muscle fatty acid oxidation, via upregulation of genes involved in the catabolism of lipids (Burri, Thoresen, & Berge, 2010; Kersten, 2014). As a result, adiponectin stimulates muscle fatty acid oxidation and mitochondrial oxidative capacities (Civitarese et al., 2006; Iwabu et al. 2010). It was also shown that adiponectin corrects mitochondrial dysfunction in high-fat fed mice and restores glucose homeostasis (Liu et al., 2013). Adiponectin also has a beneficial effect on insulin activity via indirect reduction of inflammation and oxidative stress (Jorlay et al. 2012).

Leptin, the first adipokine to be discovered, mediates long-term regulation of nutrient intake and energy expenditure. The amount of leptin secreted is dependent on adipose tissue mass, with higher leptin secretion when adipose tissue mass increases. In skeletal muscle, leptin regulates fatty acid transport, fatty acid oxidation, and mitochondrial function through AMPK-dependent pathways (Steinberg, Parolin, Heigenhauser, & Dyck, 2002;

McClelland et al., January 2004; Henry, Andrews, Rao, & Clarke, 2011). In non-obese individuals, leptin may also increase insulin activity, inhibit hepatic glucose production and increase cellular glucose uptake (Preedy & Hunter, 2011). However, obesity increases adipose tissue mass and thus, the secretion of leptin, resulting in leptin resistance in peripheral tissues (Steinberg, Parolin, Heigenhauser, & Dyck, 2002). However, when cells present leptin resistance, the positive impact of leptin on muscle metabolism is decreased (Aguer & Harper, 2012; Steinberg, Parolin, Heigenhauser, & Dyck, 2002). For example, in obese rats, activation of AMPK by leptin was reduced which decreased muscle fatty acid metabolism and mitochondrial content (Steinberg et al., 2006). It was also shown that chronic administration of leptin reduces muscle fatty acid uptake, via decreased expression of the muscle fatty acid transporters fatty acid translocase (FAT)/CD36 and FABPpm (plasma membrane fatty acid-binding protein) (Steinberg et al., 2001).

Resistin is another adipokine that plays a role in the development of IR in the liver and adipose tissue, but also in skeletal muscle, due to its inhibitory action on AMPK (Satoh et al., 2004; McTernan, Kusminski, & Kumar, 2006; Saini, 2010), which decreases muscle glucose transport (Steinberg et al., 2007). Furthermore, resistin inhibits the insulin signaling pathway in skeletal muscle, possibly via the activation of toll-like receptor 4 (TLR-4), which further increases blood glucose levels (Steppan et al., 2001; Benomar et al., 2013). Other studies have shown that resistin induces the expression of SOCS-3 (suppressor of cytokine signaling-3) and PTP-1B (protein-tyrosine phosphatase 1B), both inhibitors of the insulin signaling pathway, by decreasing the phosphorylation and expression of the insulin receptor (Kusminski, Mcternan, & Kumar, 2005; Benomar et al., 2013). The role of resistin in IR development was further demonstrated by the removal of visceral fat that reduced the serum levels of resistin and led to reversal of IR in rats (Borst, Conover, & Bagby, 2005).

4.2 Secretion of Inflammatory Factors and the Development of Muscle Insulin Resistance

During obesity, increased inflammation in adipose tissue is related to the infiltration of macrophages within adipocytes. Those macrophages secrete the pro-inflammatory cytokines IL-6 (interleukin 6) and TNF-α (tumor necrosis factor α) (Wiseberg et al., 2003). TNF-α and IL-6 were shown to negatively impact the regulation of muscle glucose and lipid metabolism. For example, chronic exposure of muscle cells to TNF-α or IL-6 promotes IR (Plombard et al., 2005; Peraldi & Spiegelman, 1998; Bhatnagar et al., 2010; Wang, Xiaowen, & Du, 2010; Preedy & Hunter, 2011; Nieto-Vazquez, Fernandez-Veledo, de Alvaro, & Lorenzo, 2008). In muscle cells, it has been shown that TNF-α not only increases the phosphorylation of IRS-1 on serine residues which results in the inhibition of Akt phosphorylation and decreased GLUT4 translocation (Plomgaard et al., 2005; Steinberg, 2007; Saini, 2010; Bhatnagar et al., 2010), but also reduces the expression of key proteins of the insulin signaling pathway such as the insulin receptor, IRS-1, and GLUT4 (Cawthorn & Sethi, 2008). Similarly, chronic exposure to IL-6 impairs GLUT4 translocation due to an increased phosphorylation of IRS-1 on serine residues. Taken together these effects of TNF-α and IL-6 on muscle cells result in decreased muscle glucose uptake. This might be done through increased inflammation and oxidative stress (Bhatnagar et al., 2010; Nieto-Vazquez et al., 2008), which activate JNK and NFκB signaling pathways (Cawthorn & Sethi, 2008; Nieto-Vazquez, Fernandez-Veledo, de Alvaro, & Lorenzo, 2008). It has also been shown that IR might be caused by the effect of TNF-α on the transcriptional activity through downregulation of proteins in the insulin signaling pathway (Cawthorn & Sethi, 2008). In addition, TNF-α reduces the activity of AMPK by upregulating PP2C (protein phosphatase 2 C) gene expression (Steinberg, 2007; Cawthorn & Sethi, 2008), therefore reducing glucose uptake in muscle cells but also muscle fatty acid oxidation leading to fatty acid intermediaries' accumulation involved in the development of muscle IR. Furthermore, some targets of TNF-α decrease the expression of genes that regulate fatty acids and glucose uptake in adipose cells (Preedy & Hunter, 2011). TNF-α also induces inducible nitric oxide synthase (iNOS) and stimulates nitrite production by muscle cells causing a reduction in insulin action (Bédard, Marcotte, & Marette, 1997). Moreover, TNF-α, as well as interleukin-6 (IL-6) decrease adiponectin secretion by the adipose tissue, which reduces insulin sensitivity and mitochondrial biogenesis, and increases ROS production via mitochondrial dysfunction in muscle cells (Fasshauer et al., 2003; Nieto-Vazqquez et al., 2008).

During an inflammatory state, MCP-1 (monocyte chemoattractant protein 1) is another mediator that is secreted by the adipose tissue, which usually attracts immune cells to the physiologically wounded sites. It has been shown that macrophage recruitment plays a role in the development of T2D (Patsouris et al., 2014) and that mice lacking one of the MCP-1 receptor, CCR2 (C-C chemokine receptor type 2), are partially protected against the development of obesity-related IR (Shoelson, Lee, & Goldfine, 2006).

In conclusion, multiple studies have demonstrated a link between adipose tissue function, regulation of its production of cytokine/adipokines and the development of IR in skeletal muscle, showing that the communication between adipose tissue and skeletal muscle is central in the development of IR in patients with obesity.

5. Role of POPs in the Development of Insulin Resistance and Mitochondrial Dysfunction

5.1. Effects of POPs on Insulin Sensitivity

Animal studies have previously associated POPs with the development of IR and glucose intolerance. For example, it has been shown that certain mixtures of PCBs (i.e. Aroclor) diminish the action of insulin in adipose cells (Park et al., 2013). Similarly, a chronic exposure (20 wk) of 36 mg/kg/wk of Aroclor 1254, which contains several PCBs induced hyperinsulinemia and exacerbated IR in mice (Gray et al., 2013). Moreover, Ibrahim et al. (2011) demonstrated that mice on a high fat diet with salmon fillet intake (containing POPs) exhibited resistance to insulin more than mice fed a diet with reduced levels of POPs. In a similar study in rats, intake of purified salmon oil had low impact on the development of IR and obesity, whereas rats that consumed crude salmon oil containing POPs over a period of 10 weeks (including PCBs and DDT) showed exaggerated obesity and IR development (Midtbo et al., 2013).

Exposure to 1.7 mg/kg body weight to prenatal DDT from gestational day 11.5 to postnatal day 5 in rats impaired energy expenditure and metabolism in adults by increasing adiposity, impairing insulin secretion, decreasing glucose tolerance and elevating gluconeogenesis (La Merrill et al., 2014). While the analyses were performed on the liver, the results suggest that exposure to DDT during development increases the risk for developing metabolic syndrome (La Merrill et al., 2014).

BPA may have similar effects on insulin sensitivity. Long-term exposure to BPA leads to metabolic dysfunctions in adult male mice including glucose intolerance, decreased secretion of adiponectin and muscle IR without affecting β-cell function (Moon et al., 2015). Furthermore, exposure to BPA during pregnancy mimicked the effects of a high-fat diet (Garcia-Arevalo et al., 2014). These groups reported fasting hyperglycaemia, glucose intolerance and high levels of non-essential fatty acids in plasma of BPA-exposed mice compared to controls. Furthermore, glucose stimulated insulin release was also disrupted.

In humans, it was demonstrated that metabolically abnormal obese individuals had higher plasma concentrations of POPs (PCBs, trans-nonachlor) compared to metabolically healthy obese people, suggesting a link between circulating POP concentrations and the development of the metabolic syndrome (Gauthier et al., 2014). Also, insulin-stimulated glucose uptake was impaired in adipocytes (3T3L1) when treated with a mixture of POPs (Gauthier et al., 2014).

Taken together, these studies strongly suggest that exposure to POPs induce glucose intolerance and the development of IR in multiple models. The mechanisms by which POPs affect insulin action, including increased oxidative stress, inflammation and mitochondrial dysfunction will be discussed below.

5.2 Effects of POPs on Oxidative Stress

As discussed previously, POPs such as DDT, DDE, BPA and PCBs impair glucose homeostasis in animal models. These observed effects could be the result of increased ROS production induced by POP exposure. In *Hypostomus commersoni,* an important catfish to study both ecological impacts and a vehicle to human exposure, POPs were found at significant concentrations in the liver and muscle (Bussolaro et al., 2012). Results of that study associated banned pesticides such as aldrin, dieldren and DDT with various histopathological findings in the liver and gills (Bussolaro et al., 2012). Interestingly, a negative correlation between the concentration of several POPs and glutathione S-transferase and glucose-6-phosphatate dehydrogenase were also observed (Bussolaro et al., 2012). Glucose-6-phosphate dehydrogenase is an enzyme involved in the pentose phosphate pathway which produces NADPH and pentoses. NADPH maintains the levels of glutathione which helps protect cells against oxidative damages (Mazulis et al., 2015). Exposure to POPs may therefore increase oxidative damage through modulating glutathione levels.

POPs are present and metabolized in the liver of exposed organisms and are known to cause liver damage via oxidative stress. As expected, hepatocytes exposed to DTT showed elevated ROS content and in return ROS increased NF-kB activation in those cells (Jin et al., 2014). Following NF-kB activation, caspase-dependent cell death is triggered causing an increase in mitochondrial potential and apoptosis. This confirms that DDT intoxication contributes to the generation of free radicals in cells and tissues (Jin et al., 2014).

Similarly, exposure to PCB126 in endothelial cells, hepatocytes and chondrocytes increased ROS levels (Hennig et al., 2002; Lee & Yang, 2012), oxidative stress (Hennig et al., 2002; Ramadass et al., 2003), and caused mitochondrial defects (Park et al., 2013). Mitochondrial electron transport chain defects also result in an increase in the production of ROS (Park et al., 2013). ROS production and oxidative stress by coplanar PCBs for example happen through the activation of AhR, which in turn activates the transcription of genes of cytochrome P450 (CYP1A1/2 and CYP1B1) and enzyme ligands metabolism (Park et al., 2013; Baker et al., 2012). For instance, PCB77 induces oxidative stress by acting as an agonist to AhR, which subsequently activates JNK/SAPK and eventually leads to glucose and insulin

impairment (Slim, Toborek, Robertson, Lehmler, & Hennig, 2000). *In vivo* and *in vitro* studies have shown that oxidative stress induces inflammation through the activation of NF-κβ (Hennig et al., 2002). Hennig's further studies demonstrated that PCB exposure induces oxidative stress, activating inflammatory pathways involved in a number of pathologies such as atherosclerosis and cardiovascular disease (Hennig et al., 2002; Hennig et al., 2005). The role of oxidative stress in endothelial cell damage mediated by PCBs was demonstrated by treating endothelial cells with antioxidants such as vitamin E, which rescued PCB-induced oxidative stress and endothelial cell dysfunction (Hennig, Hammock, Slim, Toborek, Saraswathi, & Robertson, 2002).

An oral chronic exposure in adult female rats to a representative mixture of POPs composed of endosulfan, chlorpyrifos, naphthalene and benzopyrane caused an increase in mitochondrial superoxide dismutase (SOD) (enzyme that alternately catalyzes the partitioning of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2)) and other mitochondrial oxidative parameters suggesting the POP mixture induced oxidative stress particularly in mitochondria (Lahouel et al., 2016). In another study, a mixture of POPs containing perfluorinated (PFC), brominated mixture, PCBs, DDT, hexachlorobenzene, chlordanes, thexachlorocyclohexanes and dieldrin, not only induced the production of ROS but also induced apoptosis in a human hepatocarcinoma cell line (Wilson et al., 2016).

5.3 Effects of POPs on Inflammation

POP exposure is therefore linked to an increase in ROS production and oxidative stress, which in turn activates inflammatory pathways. Indeed, for example, PCB77 treatment in adipocytes and endothelial cells (5 μmol/L for 16 hours) activated inflammatory pathways, increased the production of pro-inflammatory adipokines (TNF-α, IL-6 and MCP-1) and reduced the expression of anti-inflammatory adipokines (adiponectin and leptin) (Wang, Xiaowen, & Du, 2010; Arsenescu et al., 2008). In addition, it has been demonstrated that PCB77 blocks Akt activation by insulin in endothelial cells, due to an increased secretion of TNF-α; while the increased secretion of IL-6 may be responsible for the phosphorylation of IRS-1 on serine residues (Wang, Xiaowen, & Du, 2010). Finally, it has been demonstrated *in vivo,* in mice, and *in vitro* in human multipotent adipose-derived stem cells, that TDCC, PCB126 and PCB153 increase the activity of the inflammatory response by binding to the AhR (aryl hydrocarbon receptor) (Kim et al., 2012). The same observations were made when adipocytes were exposed to POPs and the proposed mechanism involves the AhR receptor, inflammation and mitochondrial dysfunction (Imbeault et al., 2012). In conclusion, it is proposed that POPs increase proinflammatory messengers and decrease anti-inflammatory mediators (Imbeault et al., 2012), which in turn have an impact on the insulin signaling pathway and therefore lead to the development of IR.

Some PCBs also decrease adipocyte differentiation (Gadupudi et al., 2015; Arsenescu et al., 2008) responsible for adipose tissue dysfunction. For example, PCB126 inhibits adipogenesis of human preadipocytes (Gadupudi et al., 2015). However, PCB77 does not seem to have a similar effect (Chapados et al., 2012). Furthermore, treatment of mouse adipocytes (3T3L1) with low concentrations of PCB77, and of human preadipocytes with PCB126 increases the expression and production of pro-inflammatory adipokines such as TNF-α and MCP-1, and decreases the expression of adiponectin (Arsenescu et al., 2008; Hennig et al., 2002; Kim et al., 2012). These effects may be partly caused by an increased fat cell size and were not observed by PCB153 exposure which has a different structure than PCB126 or PCB77 (PCB126 and PCB77 are coplanar PCBs whereas PCB153 is non-coplanar) (Arsenescu et al., 2008). Given the role of TNF-α, MCP-1, and adiponectin in the development of IR as previously discussed, the effects of these pollutants on the secretion of these adipokines/cytokines by adipose tissue could thus result in the perturbation of glucose homeostasis at the whole-body level.

5.4 Effects of POPs on Mitochondrial Function

POPs have shown to have a negative effect on energy metabolism and the expression and/or functioning of mitochondrial proteins. For example, in isolated rat-liver mitochondria, DDT acts as a weak ATP-ase inhibitor, which can be explained by the disruption of the integrity of the inner membrane by DDT molecules (Nishihara & Utsumi, 1985). On the other hand, Byczkowski (1976) demonstrated that DDT and its metabolites are inhibitors of the electron transfer chain in mitochondria at the site between NADH (Nicotinamide adenine dinucleotide coenzyme) and Coenzyme Q/ubiquinone pointing to mitochondrial function disturbance following exposure. Furthermore, evidence showed that a decrease in oxidative phosphorylation in brain and liver mitochondria occurred in rats treated with 600 mg of DDT for 24h due to the uncoupling of oxidative phosphorylation (Byczkowski, 1976). Rat liver mitochondria were shown to be more sensitive to the uncoupling action of DDT than rat brain mitochondria, suggesting an alternate mode of action in the brain (Byczkowski & Tłuczkiewicz, 1978). A significant decrease in repolarization potential (after a phosphorylation cycle), state 3 respiration and an increase in uncoupled respiration were also shown in testicular mitochondria after exposure to DDE (Mota et al.,

2011). Further, death of Sertoli cells (cells of the testicles part of the seminiferous tubules) by apoptosis through the mitochondrial pathway, and an increase in ROS production dependent on DDE concentration were also observed (Xiong et al., 2006). Moreover, a decrease in SOD was present confirming that oxidative stress may be an important component in DDE-induced toxicity (Shi et al., 2010). In liver and testis mitochondria, DDE also decreased oxygen consumption stimulated by ADP (State 3) as well as the activity of succinate-cytochrome c reductase (Mota et al., 2011).

Similarly, PCB exposure also causes mitochondrial dysfunction. Indeed, a variety of PCBs (tetra-chlorinated biphenyls), hydroxylated PCBs or mix of PCBs (Aroclor 1254) were shown to act as inhibitors of the electron transport chain as well as uncoupling agents (Ebner & Braselton 1987; Hasegawa, Ogata, & Tomokuni, 1982; Narasimhan, Kim, & Safe, 1991; Nishihara & Utsumi, 1987). PCB exposure likely causes mitochondrial dysfunction through AhR-mediated inflammation. Indeed, 2,3,7,8-tetrachlorodibenzodioxin (TCDD), which has a similar action to co-planoar PCBs, inhibits the interaction between mitochondrial AhR and an ATP synthase domain, which reduces the effectiveness of mitochondrial ATP synthesis in endothelial cells (Park et al., 2013). Moreover, mitochondrial function is reduced when there is an activation of cytochrome P450 1A1, which is caused by the interaction of PCB126 with AhR in adipose tissue and liver (Gadupudi et al., 2015; Song & Freedman, 2005).

BPA also affects mitochondrial function. It was shown that BPA disturbs mitochondrial function by inducing an overload of Ca^{2+} (Hughes et al., 2000; Orrenius et al., 2003). It was then confirmed that these observed effects are the result of ROS production and oxidative stress induced by BPA exposure (Wang et al., 2016).

It has also been proposed that the effect of POPs (TCCD, PCB and DDT) could be caused by a direct accumulation of pollutants in the mitochondria of endothelial cells (Park et al., 2013). Moreover, it has been observed that exposure to those POPs reduced the expression of PGC1-α, which decreased mitochondrial biogenesis and function and fatty acid oxidation at the whole-body level (Ruzzin et al., 2010).

6. What is Known About the Effects of POPs on Skeletal Muscle Energy Metabolism?

The direct effects of POPs on muscle energy metabolism have not been adequately studied. Most studies, as discussed above, have focused on the effects of these products on the liver, endothelial cells and adipose tissue. Muscle metabolism may differ from other tissues in that oxidative phosphorylation enzymes are differently expressed, making the muscle more sensitive to respiratory-chain deficiency (Rossignol, Letellier, Malgat, Rocher, & Mazat, 2000). While GLUT4 insulin-dependent transporters influence glucose uptake within the muscle and adipose tissue, the other organs such as the liver, kidneys, and brain take up glucose using non-insulin glucose transporter isotypes GLUT2 and GLUT3, respectively (Scheepers, Joost, & Schurmann, 2004). GLUT4 transporter in skeletal muscle plays a central role in whole-body metabolism and its function is a key determinant of glucose homeostasis (Huang & Czech, 2007), as previously discussed in the present review.

However, knowing that skeletal muscle is the largest glucose consumer in the body and participates to glucose homeostasis, the effects of POPs such as DDT, PCBs and BPA on muscle glucose metabolism and oxidative stress must be understood in order to evaluate their implication in the development of IR and T2D. An interesting study has shown that POP exposure in mice through the consumption of farmed salmon decreased muscle insulin-stimulated glucose uptake in association with a decrease in insulin-stimulated Akt phosphorylation and an increase in intramyocellular lipid accumulation (Ibrahim et al., 2011). Furthermore, rats exposed to a mixture of PCBs showed a decrease in muscle glucose uptake in association with a decrease in GLUT4 translocation (Williams et al., 2013). These results are in accordance with recent data from our laboratory showing that exposure to PCB126 induced a 20% decrease in glucose uptake and glycolysis in L6 muscle cells (Mauger, Nadeau, Caron, Chapados, & Aguer, 2015). Similarly, an exposure to BPA for 8 days at 100 µg/kg in mice caused skeletal muscle insulin-stimulated tyrosine phosphorylation of the β subunit on the insulin receptor to be impaired associated with reduced Akt phosphorylation. Muscle and liver also showed upregulation of IRS-1 expression, while MAPK pathway was impaired in muscle (Batista et al., 2012).

Some data also suggest that exposure to some POPs results in muscle mitochondrial dysfunction and disrupts skeletal muscle energy metabolism. For example, it was demonstrated that an increase in circulating levels of pollutants following weight loss in humans was linked to a decrease in mitochondrial enzymatic activity in skeletal muscle (Imbeault, Tremblay, Simoneau, & Joanisse, 2002). Furthermore, preliminary results from our laboratory demonstrated that exposure to DDT in rats significantly decreased muscle mitochondrial function (Chapados, Tremblay, Haddad, & Aguer, 2014). Rats exposed to 40 mg/kg of DDT by a single intraperitoneal injection for a week have shown a significant reduction in oxygen consumption rate measured with complex I substrates (glutamate, malate and pyruvate) in permeabilized muscle fibers (Chapados, Tremblay, Haddad, & Aguer, 2014). Interestingly so, exposure of rat L6 muscle cells *in vitro* to DDE at 1 µM and 10 µM showed a shift from oxidative metabolism to

glycolytic metabolism and both DDT and DDE have shown a general tendency to increase glycolysis and glucose uptake (unpublished results). Preliminary studies from our laboratory have also shown that rats exposed to 1.05 µg/kg PCB126 have decreased mitochondrial respiration as measured in permeabilized muscle fibers (Chapados, Tremblay, Haddad, & Aguer, 2014). However, exposition of L6 muscle cells to PCB126 *in vitro* did not result in major mitochondrial respiration defects (Mauger, Nadeau, Caron, Chapados, & Aguer, 2016), suggesting that the effect of PCB126 *in vivo* on muscle mitochondrial function does not involve a direct mechanism since it was not reproduced *in vitro*. This could be explained by extrinsic mechanisms, which are not observed in the absence of other tissues, such as adipose tissue. Since PCBs are stored in adipose tissue and PCB exposure alters adipokine secretion by adipose tissue (Arsenescu et al., 2008; Hennig et al., 2002; Kim et al., 2012), we propose that PCB126 exposure affects not only skeletal muscle mitochondrial function, but also muscle insulin sensitivity through increased inflammatory cytokines (such as TNFα) or altered adipokine secretion from adipose tissue (Figure 2). Further studies are thus needed to explore this hypothesis.
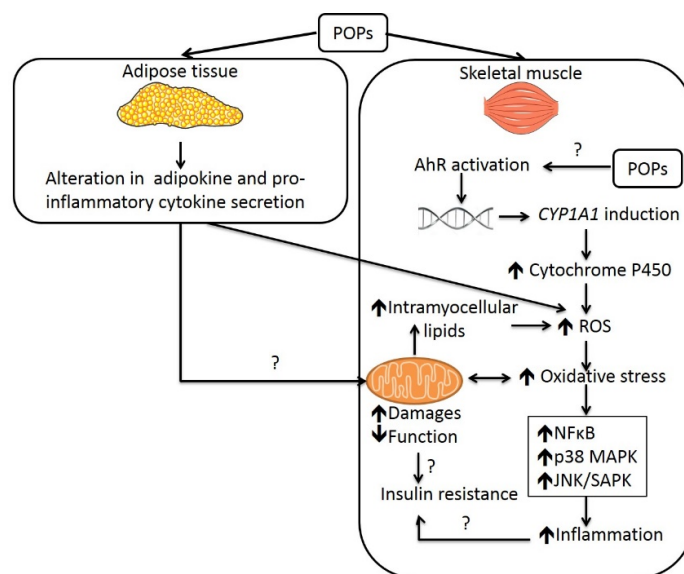

Figure 2. Schematic representation summarizing the direct effects of POPs on adipose tissue and skeletal muscle, with subsequent communication between adipose tissue and muscle in the development of insulin resistance. Question marks represent areas that are still under investigation. The hypothesis that exposure to POP alters adipose tissue cytokine/adipokine release leading to muscle mitochondrial dysfunction and IR is still under investigation

7. Conclusion

Due to its mass, skeletal muscle is largely implicated in the maintenance of glucose homeostasis and is thus a major player in the development of IR and T2D. Exposure to POPs is known to alter whole-body insulin sensitivity in rodents and to be associated with T2D in humans. This effect of POPs is probably through an increased oxidative stress and inflammation, which also alters mitochondrial function in different tissues and cells. Despite the important role of skeletal muscle in the pathogenesis of IR and T2D, the effect of POPs on muscle insulin sensitivity and mitochondrial function has been underappreciated. Mechanisms responsible for muscle mitochondrial dysfunction and glucose uptake disarrangement following exposure to DDT, DDE, PCB126, or BPA (and potentially other POPs) need to be studied in order to develop strategies (e.g. anti-oxidant or anti-inflammatory treatments) to improve muscle protection against POP aggressions and ultimately prevent and treat the development of IR and T2D due to POP exposure.

Acknowledgments

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