Mitochondria as target of endocrine-disrupting chemicals: implications for type 2 diabetes

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Abstract

Type 2 diabetes is a chronic, heterogeneous syndrome characterized by insulin resistance and pancreatic β-cell dysfunction or death. Among several environmental factors contributing to type 2 diabetes development, endocrine-disrupting chemicals (EDCs) have been receiving special attention. These chemicals include a wide variety of pollutants, from components of plastic to pesticides, with the ability to modulate endocrine system function. EDCs can affect multiple cellular processes, including some related to energy production and utilization, leading to alterations in energy homeostasis. Mitochondria are primarily implicated in cellular energy conversion, although they also participate in other processes, such as hormone secretion and apoptosis. In fact, mitochondrial dysfunction due to reduced oxidative capacity, impaired lipid oxidation and increased oxidative stress has been linked to insulin resistance and type 2 diabetes. Herein, we review the main mechanisms whereby metabolism-disrupting chemical (MDC), a subclass of EDCs that disturbs energy homeostasis, cause mitochondrial dysfunction, thus contributing to the establishment of insulin resistance and type 2 diabetes. We conclude that MDC-induced mitochondrial dysfunction, which is mainly characterized by perturbations in mitochondrial bioenergetics, biogenesis and dynamics, excessive reactive oxygen species production and activation of the mitochondrial pathway of apoptosis, seems to be a relevant mechanism linking MDCs to type 2 diabetes development.

Introduction

Type 2 diabetes (T2D) is a chronic, lifelong condition characterized by hyperglycaemia resulting from persistent insulin resistance (IR) and/or insufficient insulin production due to β-cell dysfunction or death (Prentki & Nolan 2006). T2D is a heterogeneous, multi-factorial syndrome in which genetic predisposition and environmental factors account for its development. Over the last years, a class of chemical pollutants named endocrine-disrupting chemicals (EDCs) has been associated with several metabolic diseases, including obesity and T2D (Alonso-Magdalena et al. 2011, Neel & Sargis 2011, Gore et al. 2015, Braun 2016, Heindel et al. 2017). This association seems plausible as these chemicals can alter energy metabolism by affecting multiple cellular events in different organelles, including...

Mitochondria are essential for the normal cellular function in eukaryotic organisms. Although mitochondria are best known as the powerhouse of the cell due to their crucial role in cellular energy production via oxidative phosphorylation (OXPHOS), these organelles are also key players in other critical cellular processes. For instance, mitochondria are implicated in hormone secretion (Chow et al. 2017), generation of reactive oxygen species (ROS) (Brand 2016) and cell death (Fulda et al. 2010).

Such involvement in a wide variety of processes places the mitochondria at the epicenter of many disorders, including aging and metabolic diseases (Fosslien 2001). A growing body of evidence has linked mitochondrial dysfunction to T2D (reviewed in: Patti & Corvera 2010, Szendroedi et al. 2011, Gonzalez-Franquesa & Patti 2017, Fex et al. 2018). Essentially, alterations in mitochondrial bioenergetics, biogenesis and dynamics, as well as excessive ROS production, can impact metabolic homeostasis, which might contribute to the establishment of IR and, subsequently, T2D (Petersen et al. 2003, 2004, Houëtis et al. 2006, Anderson et al. 2009, Jheng et al. 2012).

Thus, due to its vital relevance for the proper physiology and survival of eukaryotic cells, it seems logical that pollutants targeting mitochondria would lead to harmful effects, especially on metabolic homeostasis. In fact, the term metabolism-disrupting chemical (MDC) has been recently proposed to refer to a particular class of EDCs that disturb energy homeostasis and, therefore, affects the susceptibility to metabolic disorders (Heindel et al. 2017). Bisphenol A (BPA), chlorpyrifos, tributyltin and 2,3,7,8-tetrachlorodibenzodioxin (TCDD) are some of the most common MDCs identified up to date.

In this review, we first highlight the recent evidence correlating EDCs with T2D development. We then outline how mitochondria dysfunction in different tissues involved in metabolic homeostasis is linked to IR and T2D development. Finally, we examine the deleterious effects of MDCs on different mitochondrial processes and discuss how MDC-induced mitochondria dysfunction may lead to IR and T2D. Herein, we indicate doses and type of exposure (e.g. perinatal or during adulthood) for most examples cited, as this information is critical for understanding MDCs effects. To contextualize the reader, we provide a table with the reference doses (RfD) provided by the United States Environmental Protection Agency (EPA) (Table 1) as well as two reports providing comprehensive information on EDC biomonitoring data (CDC 2009, CDC 2018).

### Introduction to mitochondria

Before discussing the evidence connecting mitochondrial dysfunction to T2D, and how MDCs affect mitochondria, we first summarize some key mitochondrial processes (Fig. 1).

### Bioenergetics

Mitochondria are often recognized as the powerhouse of the cell, as they are responsible for most part of the cellular energy produced. It is in the mitochondria that sugars, proteins and fats are converted into energy in the form
of ATP via two metabolic processes, namely tricarboxylic acid cycle (TCA) and OXPHOS. The free energy carried by the coenzymes NADH and flavin adenine dinucleotide (FADH2) is used to transport electrons in the electron transport chain (ETC, located in the inner mitochondrial membrane). Most part of the free energy coming from the electron transfer is used to form an electrochemical gradient ($\Delta \psi_m$) that drives the synthesis of ATP by the ATP synthase. Q and c: ubiquinone and cytochrome c, respectively. (B) Mitochondrial dynamics: mitochondrial fusion (left), which generates large, interconnected mitochondrial networks, is mainly governed by the dynamin-related proteins, mitofusins 1 and 2 (MFN1 and MFN2) and optic atrophy 1 (OPA1). On the other hand, mitochondrial fission (right) leads to fragmented, separated mitochondria. This process is regulated by several proteins, including dynamin-related protein 1 (DRP1), mitochondrial fission protein 1 (FIS1), and the mitochondrial fission factor (MFF). (C) Mitochondria ROS generation and antioxidant activity. Due to its redox activity, the ETC generates reactive oxygen species (ROS) as a consequence of electron leak during the oxidative phosphorylation. The figure depicts the main sites of ROS production in the ETC, i.e. complexes I and III. Of note, there are at least 11 different sites linked to ROS production in the mitochondria, such as the α-ketoglutarate dehydrogenase and the glycerol 3-phosphate dehydrogenase. Complexes I and III generate superoxide radical ($O_2^\cdot$), which can be dismutated to hydrogen peroxide ($H_2O_2$) by the enzymes superoxide dismutase (SOD) 1 (CuZnSOD, located in the intermembrane space) and 2 (MnSOD, located in the mitochondrial matrix). Other antioxidant enzymes, such as catalase (CAT) and glutathione peroxidase (GPx), can decompose $H_2O_2$ into $H_2O$ and/or $O_2$. (D) Mitochondrial pathway of apoptosis. After an apoptotic stimulus, activated BH3-only proteins translocate to mitochondria where inactivate anti-apoptotic BCL-2 proteins and activate pro-apoptotic BAX and BAK. BAX oligomerization in the outer mitochondrial membrane (OMM) leads to OMM permeabilization and release of cytochrome c (c) and SMAC/DIABLO (S) from the intermembrane space into the cytosol. Under certain conditions (e.g. glucose deprivation and ischemia/reperfusion injury), long-lasting opening of the mitochondrial PTP may also contribute to cytochrome c release. In these situations, prolonged PTP opening leads to mitochondrial dysfunction (e.g. depolarization, and inhibition of oxidative phosphorylation and ATP synthesis) and matrix swelling, which, in turn, causes outer mitochondrial membrane rupture and release of pro-apoptotic factors, including cytochrome c (consecutive arrows). Once in the cytosol, cytochrome c drives caspase activation, which will culminate in activation of apoptosis.

Biogenesis

Mitochondrial biogenesis is a tightly regulated process whereby cells increase their mtDNA content, mitochondrial
mass and activity in response to different physiological conditions.

Under certain physiological or stressful conditions, activation of different signaling cascades culminates with the activation of transcription factors and co-regulators encoded by both nucleus and mitochondria. From the nuclear side, nuclear respiratory factors 1 and 2 (NRF1 and NRF2) are the two major transcription factors, directly modulating the expression of the mitochondrial transcription factor A (TFAM) and transcription factor B proteins (TFBs), two key regulators of the transcription and replication of mtDNA (Gleyzer et al. 2005, Scarpulla 2008).

Mitochondrial biogenesis is coordinated by members of the peroxisome proliferator-activated receptor (PPAR) coactivator-1 (PGC-1) family of coactivators, namely PGC-1α (PGARYcoactivator-1α), PGC-1β (PGARYcoactivator-1β) and PRC (PGC-1 related coactivator) (Puigserver et al. 1998, Wu et al. 1999, Andersson & Scarpulla 2001, Lin et al. 2002). PGC-1α is considered the master regulator of mitochondrial biogenesis, as its activation leads to activation of several transcription factors, such as PPARs, NRF1/NRF2, estrogen-related receptors (ERRα, ERRβ, ERRγ) and thyroid hormone receptors (TRα and TRβ) (Puigserver & Spiegelman 2003, Scarpulla 2011).

Dynamics

Mitochondria are very dynamic organelles, exhibiting a wide variety of shapes, size and location that can change within a few seconds or minutes (Twig et al. 2010, Picard et al. 2016). These characteristics are related to active, regulated processes called mitochondrial fission and fusion (also known as mitochondrial dynamics), as well as the ability of mitochondria to build extensive intracellular networks through the formation of a tubular reticulum (Bereiter-Hahn 1990, Scott & Youle 2010, Prasai 2017). A proper control of mitochondrial dynamics is very important for several biological processes, such as regulation of neuronal development (Choi et al. 2013, Burté et al. 2015, Denton et al. 2018), ROS production (Yu et al. 2006, Huang et al. 2016, Ježek et al. 2018) and apoptosis (Frank et al. 2001, Olichon et al. 2003, Suen et al. 2008).

Mitochondrial fission promotes fragmented, separated mitochondria in a process regulated by several proteins, including dynamin-related protein 1 (DRP1), a master regulator of mitochondrial division in eukaryotic cells and mitochondrial fission protein 1 (FIS1). Conversely, mitochondrial fusion generates large, interconnected mitochondrial networks. Three dynamin-related proteins, namely mitofusins 1 and 2 (MFN1 and MFN2), and optic atrophy 1 (OPA1), mediate mitochondrial fusion in mammals (Suárez-Rivero et al. 2016, Williams & Ding 2017). Both processes contribute to keep a healthy pool of mitochondria by actively participating in mitophagy, the mitochondrial quality control process by which damaged or dysfunctional mitochondria are eliminated by selective autophagy (Lemasters 2005, Williams & Ding 2017). The mitophagic process is mainly orchestrated by two main proteins, namely phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and Parkin (PARK2). More recently, other proteins participating in mitophagy have been identified, including the E3 ubiquitin ligase ariadne RBR E3 ubiquitin protein ligase 1 (ARHI) and the inner mitochondrial membrane protein, prohibitin 2 (PHB2) (Palikaras & Tavernarakis 2014, Villa et al. 2017, Wei et al. 2017).

ROS and oxidative stress

Mitochondria are considered the major source of intracellular ROS, which are mainly produced as consequence of electron leak from the ETC during the OXPHOS. Mitochondria present at least 11 different sites associated with ROS generation; it is generally well accepted that complexes I and III are the two major sites (Brand 2016).

ROS act as a double-edged sword for the cells. Physiological concentrations of ROS act as second messengers in diverse cellular and mitochondrial processes and signaling pathways (Sena & Chandel 2012). Conversely, excessive ROS can react with lipids, nucleic acids (including mtDNA) and proteins, causing oxidative damage and, eventually, cell death (Circu & Aw 2010). To keep ROS levels under control and avoid their potentially detrimental effects, mitochondria have evolved an antioxidant defense composed by non-enzymatic (e.g. ascorbic acid and α-tocopherol) and enzymatic (e.g. catalase and superoxide dismutase, SOD) systems (Sies 1993). Moreover, it has been suggested that UCP2 and UCP3 might be involved in the control of ROS production (Arsenijevic et al. 2000, Mailloux & Harper 2011, Pons et al. 2015). When antioxidant defenses fail to cope with excessive ROS production, cells undergo oxidative stress, which has been associated with IR and T2DM (Houstitis et al. 2006, Anderson et al. 2009, Tangvarasittichai 2015).

Mitochondria and cell death

Along with their role in energy production, mitochondria are also implicated in several signaling pathways.
For instance, mitochondria play a crucial role in the intrinsic pathway of apoptosis, which requires permeabilization of the outer mitochondrial membrane (OMM). Regulation of OMM permeabilization depends on the balance between anti- and pro-apoptotic B-cell lymphoma 2 (BCL-2) family proteins. This family comprises three groups of proteins: anti-apoptotic (e.g. BCL-2 and BCL-XL), pro-apoptotic (e.g. BAX and BAK) and BH3-only proteins (e.g. PUMA, and BIM) (Youle & Strasser 2008). Upon exposure to cell death stimuli (e.g. DNA damage), BH3-only proteins translocate to mitochondria, where they bind and inactivate anti-apoptotic BCL-2 proteins. Subsequently, BH3-only proteins stimulate BAX and BAK, causing OMM permeabilization and release of pro-apoptotic proteins, such as cytochrome c and SMAC/DIABLO, from the intermembrane space. Additionally, long-lasting opening of the mitochondrial permeability transition pore (PTP) also contributes to cytochrome c release under certain conditions (e.g. glucose deprivation and ischemia/reperfusion injury). Once in the cytosol, cytochrome c binds to the apoptotic protease activating factor 1, leading to the activation of caspase-9 and -3, and, ultimately, apoptosis (Ow et al. 2008, Youle & Strasser 2008).

**EDCs and T2D**

EDCs have been defined by the Endocrine Society as ‘an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action’ (Zoeller et al. 2012). The definition includes a great variety of compounds, such as industrial and waste products, plasticizers, flame retardants, pesticides and food additives. Human exposure mainly occurs by ingestion, inhalation and dermal uptake (Gore et al. 2015). The current knowledge on the relationship between EDCs and T2D is briefly discussed below and in other comprehensive reviews (Alonso-Magdalena et al. 2011, Gore et al. 2015, Heindel et al. 2017, Mimoto et al. 2017, Nadal et al. 2017, Lind & Lind 2018).

Although initial concern on EDCs was focused on their capacity to induce reproductive abnormalities, we have learned over the years that these compounds can also exert other detrimental effects. In this regard, an enlarging body of evidence has provided a strong support for the role of EDCs in the etiology of diabetes and other metabolic disorders (Alonso-Magdalena et al. 2011, Gore et al. 2015, Heindel et al. 2017, Nadal et al. 2017). Among them, those with the most conclusive evidence of a diabetogenic role are plasticizers like BPA and phthalates, some persistent organic pollutants (POPs), such as dioxins, polychlorinated biphenyls (PCBs), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), and dichlorodiphenyltrichloroethane (DDT), as well as some heavy metals, including arsenic and cadmium (Gore et al. 2015, Cardenas et al. 2017).

Numerous studies conducted in animals have explored the metabolic effects of MDCs at different timing of exposure. In adult animals, several MDCs have been shown to decrease insulin sensitivity and promote glucose intolerance. For instance, BPA exposure (100μg/kg/day) resulted in postprandial hyperinsulinemia, impaired glucose tolerance and marked IR in mice (Alonso-Magdalena et al. 2006) along with defective insulin signaling in liver and muscle (Batista et al. 2012). Additionally, BPA exposure (50μg/kg) diminished hepatic glucokinase activity (Perreault et al. 2013). At higher doses (20 and 200μg/kg/day), BPA also impaired insulin signaling and decreased hepatic glucose oxidation and glycogen content (Jayashree et al. 2013). Analogously, the exposure to some PCBs, such as aroclor (0.5, 5 and 500μg/kg/day), PCB-118 (37.5μg/kg, PCB-138 (37.5μg/kg or PCB-126 (10μg/kg/day), has been associated to increased body weight gain, IR, hyperinsulinemia and changes in pancreatic α- (decrease) and β-cell (increase) mass (Ruzzin et al. 2009, Zhang et al. 2015a, Kim et al. 2016, Loiola et al. 2016). Importantly, complex interactions between diet and PCBs have been reported in the development of metabolic disorders. Thus, aroclor (36mg/kg/week) exacerbated high-fat diet (HFD)-induced IR (Gray et al. 2013), while other PCBs (PCB-77: 50μg/kg; PCB-126: 1.6mg/kg) provoked glucose intolerance and impaired insulin sensitivity in obese mice only when there was a switch from HFD to a less caloric diet (Baker et al. 2013, 2015).

Cadmium and arsenic may also promote perturbations on glucose handling. Cadmium administration has been shown to promote hyperglycaemia, impaired glucose tolerance and reduced plasma insulin levels (Ithakissios et al. 1975, Merali & Singhal 1980, Kanter et al. 2003, Edwards & Prozialeck 2009, Trevino et al. 2015). Similarly, arsenic (0.05–50ppm) has been shown to alter glucose tolerance (Paul et al. 2011, Huang et al. 2015), an effect that was aggravated in diabetic mice (Liu et al. 2014) or in the presence of a metabolic stressor (e.g. HFD) (Paul et al. 2011).

Increasing attention has been focused on the exposure to MDCs during intrauterine life, which turns
out to be an extremely sensitive period. Several studies have largely demonstrated that maternal exposure to environmental pollutants during development may lead to severe metabolic perturbations. Thus, offspring from mice exposed to BPA (5–5000 μg/kg/day) during pregnancy manifested glucose intolerance, IR as well as alterations on β-cell function and mass (Alonso-Magdalena et al. 2010, Angle et al. 2013). Impairments on glucose and lipid metabolism were exacerbated when animals were challenged with HFD (Wei et al. 2011, García-Arevalo et al. 2014) and, in some cases, were accompanied by alterations in the structure of the hypothalamic energy balance circuitry (MacKay et al. 2013, 2017). At earlier stages, BPA (10 μg/kg/day and 25 mg/kg/diet) altered pancreas development (Garcia-Arevalo et al. 2016, Whitehead et al. 2016). In addition, metabolomics studies have demonstrated global changes in metabolism, including energy metabolism and brain function on BPA-exposed pups (0.025, 0.25 and 25 μg/kg/day) (Cabaton et al. 2013). Importantly, BPA-induced metabolic disorders can be transmitted to next generation (Li et al. 2014, Susiarjo et al. 2015, Bansal et al. 2017).

Notably, the adverse metabolic outcomes affect not only the offspring but also the mother. BPA-exposed dams (10 and 100 μg/kg/day) developed gestational glucose intolerance and decreased insulin sensitivity (Alonso-Magdalena et al. 2010, Susiarjo et al. 2015). Although these metabolic perturbations disappeared after parturition, the remission was only temporal and alterations reappeared months later (Alonso-Magdalena et al. 2015).

Prenatal exposure to PCB has also been linked to the programming of glucose and energy homeostasis in the offspring. Exposure to PCB-153 (0.09–1406 μg/kg/day) during gestation and lactation resulted in increased glucose levels in the male offspring while, in the female, promoted increased glucagon levels and decreased pancreatic weight (van Esterik et al. 2015). In combination with a HFD, PCB exposure provoked hepatic steatosis, alterations in the plasma adipokine profile and increased expression of genes related to lipid biosynthesis (Wahlang et al. 2013). Likewise, DDT-exposed offspring (1.7 mg/kg/day) manifested glucose intolerance, hyperinsulinemia, dyslipidemia and impaired thermogenesis (La Merrill et al. 2014).

The metabolic effects of di(2-ethylhexyl) phthalate (DEHP) have also been explored. DEHP exposure (1.25 and 6.25 mg/kg/day) throughout gestation and lactation resulted in abnormalities in pancreas development and function, which were reflected by decreased pancreatic β-cell mass and insulin content at the moment of weaning (Lin et al. 2011). When getting older (8–9 weeks of age), DEHP-exposed animals presented elevated glucose levels and impaired glucose and insulin tolerance.

Other persistent compounds, such as PFOS (16, 32 and 64 μM), have been proposed to disrupt pancreas organogenesis (Sant et al. 2017). This has been demonstrated to occur in zebrafish while, in rodents, PFOS (0.3 and 3 mg/kg/day) administration during gestational and lactational periods altered glucose metabolism not only in the offspring but also in the mother (Wan et al. 2014). Conversely, no changes on glucose tolerance were observed in the PFOA-offspring (3–3000 μg/kg/day), although the animals exhibited alterations in the hepatic structure (van Esterik et al. 2016).

Regarding human data, a number of cross-sectional studies have established a link between BPA levels and the incidence of T2D. Representative data from National Health and Nutrition Examination Survey (NHANES) (2003–2004) demonstrated a positive correlation between BPA levels and increased incidence of T2D and cardiovascular disease (Lang et al. 2008). Similar results were observed in data pooled across collection years (2003–2004/2005–2006) (Melzer et al. 2010). Studies based on NHANES 2003–2008 reported a connection between BPA levels and the incidence of diabetes (Shankar & Teppala 2011) and prediabetes (Sabanayagam et al. 2013) independently of traditional diabetes risk factors. BPA has also been linked to the prevalence of IR, hyperinsulinemia and adverse glucose homeostasis (Wang et al. 2012, Beydoun et al. 2014, Tai & Chen 2016). In children, BPA has been associated to the presence of IR regardless of BMI (Menale et al. 2017). Human exposure to some phthalate metabolites has also been associated with elevated fasting glucose and insulin levels, IR, as well as increased prevalence of diabetes in different populations (Svensson et al. 2011, James-Todd et al. 2012, Lind et al. 2012, Kim et al. 2013, Trasande et al. 2013, Huang et al. 2014, Sun et al. 2014a, Dales et al. 2018).

Besides that, a large number of epidemiological evidence supports the role of different POPs in metabolic disorders. This is the case of some PCBs and organochlorine pesticides that have been correlated with decreased insulin sensitivity and diabetes risk (Wang et al. 2008, Lee et al. 2010, 2011, Wu et al. 2013, Suarez-Lopez et al. 2015). Some studies have also evaluated the metabolic consequences of POP exposure in early life. Thus, prenatal exposure to some PCBs has been associated with increased fasting insulin levels in girls at 5 years of age (Tang-Peronard et al. 2015), while PCBs and organochlorine exposure has been related to decreased insulin levels.
in newborns (Debost-Legrand et al. 2016). Regarding perfluorinated compounds (PFCs), PFOA exposure has been linked to elevated diabetes prevalence in adults (He et al. 2017) and elderly (Lind et al. 2014), while an association between PFOA levels and impaired β-cell function has been observed in adulthood (Domazet et al. 2016). Perfluoroalkoxy alkanes exposure during pregnancy was positively related to impaired gestational glucose tolerance (Matilla-Santander et al. 2017).

**Mitochondria and T2D**

Numerous studies have reported mitochondrial alterations in peripheral tissues that play a crucial role in the control of glucose metabolism (Kelley et al. 2002, Kusminski & Scherer 2012, Sebastian et al. 2012, Supale et al. 2012). However, whether those abnormalities are the cause or a consequence of T2D remains unclear. As this subject is outside the scope of the present review, and it has been extensively reviewed elsewhere, the interested reader is referred to some reviews on the topic (Patti & Corvera 2010, Szendroedi et al. 2011, Di Meo et al. 2017, Gonzalez-Franquesa & Patti 2017, Fex et al. 2018).

**MDCs and mitochondria**

Effects of MDCs on mitochondria have been described since the 1960s. Yet up to date, only a few studies have shown a correlation between MDC-induced mitochondrial dysfunction and IR/T2D. In this section, we discuss those mechanisms.

**MDCs and mitochondrial bioenergetics**

Numerous studies have reported that MDCs exposure affect mitochondrial bioenergetics, either via direct interaction with mitochondrial proteins or secondary to transcriptional changes (Melnick & Schiller 1982, Moreno & Madeira 1991, Shertzer et al. 2006, Walters et al. 2009, Carchia et al. 2015).

In pancreatic islets, BPA (25μg/L) induced mitochondrial swelling and decreased cytochrome c oxidase activity and ATP levels (Song et al. 2012). This reduction in ATP levels was later confirmed in rat INS-1E cells, and it was associated with a BPA-induced reduction in mitochondrial mass, dissipation of the mitochondrial membrane potential (MMP) and abnormal expression of genes involved in mitochondrial function (Lin et al. 2013). To better understand the mechanisms whereby environmental doses of BPA lead to mitochondrial dysfunction in isolated islets, Carchia and colleagues used a transcriptomic approach, which revealed that 1 nM BPA decreased the expression of 29 genes, some of which were involved in OXPHOS and mitochondrial dysfunction, such as Uqcrb, Atp1b1 and iars (Carchia et al. 2015). Interestingly, these findings were not restricted to *ex vivo* or *in vitro* studies. Pancreatic islets from F1 and F2 adult male mice offspring of mothers exposed to BPA (10mg/kg/day) presented impaired mitochondrial oxygen consumption and changes in the expression of several mitochondrial genes (Bansal et al. 2017). As mitochondria are critical for the regulation of β-cell mass and function (Kaufman et al. 2015), mitochondrial dysfunction might be related to BPA-induced β-cell impairment (Alonso-Magdalena et al. 2006, Carchia et al. 2015, Bansal et al. 2017).

Upregulation of *Ucp2* mRNA expression has been found upon BPA exposure (Wei et al. 2011, Song et al. 2012, Bansal et al. 2017). Elevated UCP2 activity might be, at least in part, responsible for the above-mentioned reduction in the MMP and ATP levels, leading to attenuated GSIS, as reported in islets from UCP2-overexpressing animals (Chan et al. 1999, 2001). However, it is important to confirm UCP2 expression at protein level before drawing any conclusion, as it has been shown that UCP2 expression is mainly regulated at the translational level (Hurtaud et al. 2007).

Multiple cellular processes could be potentially affected by mitochondrial dysfunction in hepatocytes. Augmented hepatic lipid accumulation has been found in adult rats treated with atrazine (30μg/kg/day) (Lim et al. 2009), BPA (40μg/kg/day) (Jiang et al. 2014a) and tributyltin (0.1μg/kg/day) (Bertuloso et al. 2015) and was usually accompanied by reduced activity of mitochondrial proteins (mainly respiratory complexes I and III), MMP and ATP production (Lim et al. 2009, Jiang et al. 2014a). Studies in hepatocyte cell lines and/or mitochondria isolated from liver suggest that these effects may be direct on mitochondria (Nakagawa & Tayama 2000, Gogvadze et al. 2002, Lim et al. 2009, Huc et al. 2012, Moon et al. 2012, Sagarkar et al. 2016). Altogether, these studies suggest that intrahepatic lipid accumulation derived from mitochondrial dysfunction might be partially responsible for the IR and glucose intolerance observed in atrazine- and BPA-treated animals (Lim et al. 2009, Alonso-Magdalena et al. 2010, Wei et al. 2011, García-Arevalo et al. 2014).

Atrazine exposure also leads to muscle lipid accumulation, reduced mitochondrial respiration and disruption of mitochondrial proteins (Patti & Corvera 2010, Alonso-Magdalena et al. 2006, Carchia et al. 2015, Bansal et al. 2017).
changes in mitochondrial morphology. Curiously, reduced oxygen consumption rate was not related to changes in the protein expression of several mitochondrial OXPHOS complex subunits (e.g. SDHA and UQRC2), but rather to direct effects on mitochondrial complex III. Moreover, atrazine-treated rats were insulin resistant and presented low energy expenditure (Lim et al. 2009). In L6 myotubes, atrazine treatment (50 and 100μM) resulted in the downregulation of several genes related to OXPHOS (e.g. mt-Co3 and Sdhc) and mitochondrial biogenesis (e.g. Tfam), leading to reduced ATP production (Sagarkar et al. 2016). Similar results were observed in cardiac muscle from BPA-treated male rats (50μg/kg/day). Additionally, BPA-treated animals also presented reduced MMP and activities of mitochondrial complexes I–IV (Jiang et al. 2015). Similarly, prenatal exposure to BPA (50μg/kg/day) downregulated genes related to OXPHOS pathway (e.g. Ccys), fatty acid oxidation (e.g. Acadm and Lp1) and mitochondrial biogenesis (e.g. Nrfl and Ppargc1a) as well as reduced MMP in heart of neonatal rats (Jiang et al. 2014b). TCDD (10nM) induced mitochondrial dysfunction in C2C12 skeletal myoblasts, leading to dissipation of MMP, reduced levels of mitochondrial genome-coded cytochrome c oxidase subunits I and II and increased ROS production (Biswas et al. 2008). Despite the lack of more direct evidence on IR and T2D development, the results above are in line with findings in insulin-resistant and/or T2D individuals, in which alterations in muscle mitochondrial capacity and gene expression (e.g. Nrf1, SDHC and PPARGC1A), as well as increased intramyocellular lipid accumulation, have been documented (Jacob et al. 1999, Patti et al. 2003, Petersen et al. 2004).

In human adipose-derived stem cells, tributyltin (100nM) inhibited oxygen consumption (Llobet et al. 2015). Similarly, 3T3-L1 adipocytes exposed to BPA (10nM), 4-nonylphenol (600pM) and diethylstilbestrol (0.23pM) exhibited decreased mitochondrial respiration and mitochondria-associated ATP production, as well as reduced glycolytic function (Tsou et al. 2017). These effects might be due to direct effects on the activity of mitochondrial proteins and/or modulation of mitochondria biogenesis, which are consistent with the mitochondrial impairment observed in WAT from obese/T2D individuals (Bogacka et al. 2005, Choo et al. 2006, Dahlman et al. 2006, Rong et al. 2007).

**MDCs and mitochondrial biogenesis**

A wide variety of MDCs interfere with mitochondrial biogenesis by modulation of proteins involved in this process. For instance, livers from rats exposed to sodium arsenite (25ppm) presented reduced levels of Nrf1, Nrf2 and Tfam, and Ppargc1a (PGC-1α) and diminished expression and activity of mitochondrial complexes (Prakash & Kumar 2016). In human liver HepG2 cells and C3H10T1/2 mesenchymal stem cells, the plasticizer benzyl butyl phthalate (BBP, 1nM–50μM) also downregulated the expression of NRF1, NRF2, TFAM and PPARGC1A (Zhang et al. 2015b, Zhang & Choudhury 2017). Additionally, levels of sirtuins (SIRT) 1 and 3, two NAD-dependent deacetylases with different roles in the control of mitochondrial biogenesis (Nogueiras et al. 2012), were also decreased. Under certain metabolic conditions (e.g. fasting or obesity), SIRT1 and SIRT3 can also impact hepatic gluconeogenesis and fatty acid metabolism (Milne et al. 2007, Hirschey et al. 2010), pancreatic insulin secretion and viability (Moynihan et al. 2005, Caton et al. 2013) and fatty acid and glucose metabolism in skeletal muscle and adipose tissue (Picard et al. 2004, Jing et al. 2011). Hence, downregulation of these proteins may impair metabolic function, leading to metabolic diseases. In fact, some studies have shown that Sirt1 deletion results in hyperglycaemia and IR (Wang et al. 2011), whereas SIRT1 gain-of-function prevents diabetes in mice models (Banks et al. 2008). Similarly, Sirt3-knockout mice and cultured myoblasts silenced for Sirt3 exhibited increased oxidative stress and impaired insulin signaling (Jing et al. 2011).

Reportedly, hearts from neonatal rats prenatally exposed to BPA (50μg/kg/day) had decreased expression of mitochondrial biogenesis regulators (PGC-1α, ERRα, ERRγ, PPARα, NRF1 and TFAM) (Jiang et al. 2014b). Likewise, long-term exposure to BPA (50μg/kg/day) induced PGC-1α promoter hypermethylation and reduced Ppargc1a expression in heart tissue upon 24 and 48 weeks of treatment (Jiang et al. 2015). Interestingly, similar results were found in skeletal muscle from insulin-resistant patients with T2D and obese individuals, where PGC-1α methylation was related to reduced mitochondrial biogenesis (Barrés et al. 2009, 2013). Along with decreased PGC-1α expression, BPA-treated animals also displayed reduced levels of Nrf1, Nrf2 and Tfam, and some of their target genes, including Atp5e, Atp5o, Uqcr10 and Uqcrf1. These data show that BPA-induced perturbations in mitochondrial biogenesis might be, at least partially, responsible for the mitochondrial abnormalities observed in BPA-treated animals, such as reduction in the activity of mitochondrial proteins, depolarized MMP and decreased ATP levels (Lin et al. 2013, Jiang et al. 2014b, 2015).

Another mechanism by which MDCs may induce mitochondrial perturbations was described in human
trophoblast-like JAR cells treated with 2 nM TCDD (Chen
et al. 2010). In this study, TCDD exposure decreased mtDNA copy number and increased mtDNA deletions, including a 7599-bp deletion of mtDNA encompassing genes encoding proteins involved in mitochondrial function. In addition, TCDD cells presented reduced MMP, lower ATP levels and increased oxidative stress.

As alterations in the process of mitochondrial biogenesis seem to be involved in the mitochondrial dysfunction observed in insulin-responsive tissues in T2D (Gonzalez-Franquesa & Patti 2017), changes in mitochondrial biogenesis also seem to contribute to EDC-induced IR.

**MDCs and mitochondrial dynamics**

A growing body of evidence suggests that impairment of mitochondrial dynamics is related to IR and T2D (Yoon et al. 2011, Rovira-Llopis et al. 2017). Alterations in mitochondrial fission and fusion contribute to mitochondrial dysfunction (Bach et al. 2003, Jheng et al. 2012, Boutant et al. 2017), oxidative and/or ER stress (Yu et al. 2006, Sebastian et al. 2012, Wang et al. 2015) and β-cell apoptosis (Men et al. 2009, Molina et al. 2009), which, eventually, might lead to impaired glucose homeostasis and IR. In human-induced pluripotent stem cells, exposure to tributyltin (50 nM) or chlorpyrifos (30 μM) reduced MFN1 expression, leading to mitochondrial fragmentation (Yamada et al. 2016, 2017). Chlorpyrifos (25–100 μM) also caused mitochondrial dysfunction (reduced complex I activity, MMP and ATP levels), increased ROS production and activation of PINK1/Parkin-mediated mitophagy in SH-SHYSY neuroblastoma cells (Dai et al. 2015, Park et al. 2017). These data correlate with a study showing fragmented mitochondrial network in myocardium of diabetic patients, which was associated with a reduced MFN1 expression in atrial tissue. Additionally, attenuated complex I activity and higher ROS levels were also observed in their atrial myocardium (Montaigne et al. 2014).

Cadmium exposure also changes mitochondrial dynamics. Liver from cadmium-treated animals (1–2 mg/kg/day) and L02 hepatocytes (12 μM) presented excessive mitochondrial fragmentation, which preceded mitochondrial dysfunction. Moreover, cadmium augmented DRP1 expression and its recruitment into mitochondria. These changes in DRP1 expression and recruitment, as well as mitochondrial fragmentation, were associated with disturbances in Ca²⁺ homeostasis (Xu et al. 2013). The same group also showed that cadmium-induced DRP1-dependent intense mitochondrial fission and mitophagy, causing mitochondrial loss and hepatotoxicity. Interestingly, DRP1 inhibition reverted cadmium-induced effects on mitochondria (Pi et al. 2013).

Acute exposure to high doses of BPA (25–100 μM) induced mitochondrial dysfunction and loss, as well as activation of PINK1/Parkin-dependent, AMPK-mediated mitophagy in neuronal cells (Agarwal et al. 2015). Similarly, chronic exposure to BPA (40 μg/kg/day in vivo; 100 μM in vitro) increased DRP1-mediated mitochondrial fragmentation in the hippocampus of rat brains and in neuronal stem cells. Interestingly, inhibition of DRP1 reverted BPA-induced mitochondrial dysfunction and fragmentation (Agarwal et al. 2016). These findings are in line with observations in differentiated myoblasts, in which palmitate-induced mitochondrial dysfunction, fission and impaired insulin-stimulated glucose uptake were prevented by inhibition of DRP1 using pharmacological and genetic approaches (Jheng et al. 2012).

Taken together, these studies suggest that MDC-induced alterations in mitochondrial dynamics might be part of the mechanism by which mitochondrial dysfunction and exacerbated oxidative stress lead to IR. Conversely, it is worth noting that, in most cases, mitophagy acts as a protective mechanism under certain stress response circumstances (Kubli & Gustafsson 2012, Meyer et al. 2017). Therefore, mitophagy activation in response to MDCs might be a way to protect against MDC-induced cell injury.

**MDCs, ROS and oxidative stress**

Due to their ability to impair mitochondrial bioenergetics, it seems reasonable to assume that MDCs also modulate ROS generation. PFCs, especially PFOA and PFOS, induce ROS production likely as a consequence of inhibition of mitochondrial respiratory chain (mainly complexes I and II) (Panaretakis et al. 2001, Mashayekhi et al. 2015, Shabalina et al. 2016). Of note, PFOA effects on ROS production and MMP were reduced by treatment with the antioxidant N-acetylcysteine (Panaretakis et al. 2001).

More recently, Han and collaborators have shown that livers from rats treated with PFOS (1 or 10 mg/kg) presented augmented ROS levels and diminished antioxidant defense (decreased SOD and catalase activities; lower total GSH and GSH/GSSG ratio) (Han et al. 2018). These findings are in line with previous studies showing that HFD increased the H₂O₂-emitting potential of mitochondria and induced a shift of the cellular redox environment to a more oxidized state in skeletal muscle (Anderson et al. 2009). Interestingly, PFOS-induced oxidative stress was associated

Chronic exposure to arsenic affects insulin sensitivity in peripheral tissues through multiple mechanisms and signaling pathways (Diaz-Villasenor et al. 2007). A recent study showed that arsenic-induced oxidative stress dampened insulin-dependent glucose uptake and GLUT4 expression in adipocytes and myotubes. In this study, arsenic treatment (0.5–2 µM) downregulated the expression of the mitochondrial oxidative stress response protein SIRT3 and, some SIRT3-target proteins, namely FOXO3a, MnSOD and PGC-1α (Divya et al. 2015). Furthermore, overexpression of SIRT3 or MnSOD partially restored insulin sensitivity in these cells. Curiously, SIRT3 deficiency leads to pronounced oxidative stress and development of IR and metabolic syndrome in mice (Hirschey et al. 2011, Jing et al. 2011).

ROS play a critical role in pancreatic β-cell dysfunction and death (Evans et al. 2003). In general, MDC-induced ROS contributes to β-cell death. In INS-1E cells, BPA (25–100 µM) increased ROS production and depleted intracellular GSH, which was followed by DNA damage and p53 activation (Xin et al. 2014). At 1 nM, BPA-treated islets showed increased ROS levels as well as reduced expression of two ROS-scavenging genes, glutathione peroxidase 3 (Gpx3) and superoxide dismutase 2 (Sod2), resulting in NF-κB activation (Carchia et al. 2015). In both studies, treatment with N-acetylcysteine partially rescued BPA-induced changes, reinforcing that oxidative stress is part of the mechanism underlying BPA effects on β-cells.

As aforementioned, BPA exposure increases Ucp2 expression in β-cells. As UCP2 activation protects β-cells from ROS deleterious effects (Chan et al. 2004), this increment might be a defensive response against BPA-induced β-cell stress. However, there is no evidence supporting this statement yet.

MDCs and cell death

A common feature of T2D development is the reduction in β-cell mass secondary to increased rates of β-cell death. In this context, exposure to certain metabolic conditions (e.g. chronic hyperglycemia and hyperlipidemia) activates several stress responses that trigger β-cell apoptosis (Cnop et al. 2005, Rhodes 2005). Likewise, many MDCs cause β-cell apoptosis via activation of responsive mechanisms known to be associated with β-cell demise in T2D, such as ER and oxidative stress (Lu et al. 2011, Chang et al. 2013, Lin et al. 2013, Sun et al. 2014b, Carchia et al. 2015, Suh et al. 2017a,b). For instance, INS-1 cells exposed to DEHP (25–625 µM) presented excessive ROS generation as well as inhibited nuclear factor erythroid 2-related factor 2 (NRF-2)-dependent antioxidant response. Furthermore, DEHP treatment induced Ca²⁺ depletion and activation of ER stress response via PKR-like ER kinase (PERK) pathway. Ultimately, DEHP-induced persistent oxidative and ER stress caused β-cell dysfunction and apoptosis (Sun et al. 2014b).

In response to oxidative and ER stress, several mitogen-activated protein kinases (MAPK) involved in cell survival responses are activated (McCubrey et al. 2006, Darling & Cook 2014). In β-cells, exposure to arsenic, cadmium or tributyltin resulted in the activation of some MAPK, namely c-Jun N-terminal kinase 1/2 (JNK1/2), extracellular signal-regulated kinases 1/2 (ERK1/2) and p38 (Lu et al. 2011, Chang et al. 2013, Huang et al. 2018). Interestingly, only JNK signaling was directly implicated in apoptosis, which is in agreement with findings suggesting a pro-apoptotic role for JNK1 in β-cells (Marroqui et al. 2014, Dos Santos et al. 2017).

Several MDCs regulate PTP opening and expression of BCL-2 proteins (Panaretakis et al. 2001, Gogvadze et al. 2002, Yang et al. 2012, Xia et al. 2014), though little is known about PTP contribution to β-cell apoptosis. Regarding the modulation of BCL-2 members, available studies show decreased Bcl2 expression, and either increased (Lin et al. 2013, Carchia et al. 2015) or unchanged (Lu et al. 2011, Chang et al. 2013) Bax expression. These changes potentiated the BAX/BCL-2 ratio, favoring the pro-apoptotic pathway.

Altogether, some possible mechanisms for MDC-induced, oxidative and ER stress-mediated β-cell apoptosis include (1) induction of the transcription factor C/EBP homologous protein (CHOP), which modulates the expression of some BCL-2 members, such as BCL-2, PUMA and BIM (McCullough et al. 2001, Wall et al. 2014); (2) JNK1 activation, which activates BAX and BIM by phosphorylation (Kim et al. 2006, Marroqui et al. 2014), upregulates DPS and PUMA (Gurzov et al. 2009, Cunha et al. 2016) and downregulates MCL-1 (Allagnat et al. 2011), and (3) activation of proinflammatory pathways, such as NF-κB and TNFα, which, among other effects, modulate the expression of BCL-2 members (Cnop et al. 2005, Gurzov & Eizirik 2011).

Concluding remarks

Besides the great number of studies comprising MDCs and mitochondria, little is known about the relationship between MDC-induced mitochondria dysfunction and...
IR/T2D, especially in tissues such as skeletal muscle and adipose tissue. In this review, we have summarized some of the effects promoted by MDCs on mitochondria. Essentially, MDC-induced mitochondrial dysfunction is characterized by perturbations in mitochondrial bioenergetics, biogenesis and dynamics, excessive ROS production and activation of the mitochondrial pathway of apoptosis. These alterations might be relevant for insulin-responsive tissues, in which emerging evidence implicate mitochondrial dysfunction as a contributor mechanism linking MDCs to T2D development (Di Meo et al. 2017, Fex et al. 2018). Furthermore, it is important to keep in mind that humans are exposed to a mixture of MDCs that might affect mitochondrial function and metabolic homeostasis simultaneously, leading to alterations in insulin sensitivity (adipose tissue, skeletal muscle and liver) as well as cell dysfunction and death (pancreatic β-cells) (Fig. 2).

Recent advances in the field of omics will be of great advantage for MDC research. For instance, the use of mitochondriomics technology, which is the study of the properties of mitochondrial DNA, has been suggested to identify molecular ‘fingerprints’ in MDC research (Messerlian et al. 2017). Application of mitochondriomics along with other omics technologies (e.g. genomics, transcriptomics and epigenomics), and integration with more classical approaches will certainly strengthen our knowledge of MDC exposome. Nevertheless, we believe it is still too soon to tell whether all these omics-based information will allow us to know if interactions between exposome and genomics will predict a T2D phenotype.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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