Developmental programming of obesity and insulin resistance: does mitochondrial dysfunction in oocytes play a role?

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ABSTRACT: Insulin resistance is a key defect associated with obesity, type 2 diabetes and other metabolic diseases. While a number of factors have been suggested to cause defects in insulin action, there is a very strong association between inappropriate lipid deposition in insulin target tissues and the development of insulin resistance. In recent times, a large number of studies have reported changes in markers of mitochondrial metabolism in insulin-resistant individuals, leading to the theory that defects in mitochondrial substrate oxidation are responsible for the buildup of lipid intermediates and the development of insulin resistance. The primary support for the mitochondrial theory of insulin resistance comes from studies in skeletal muscle; however, there is recent evidence in murine models that mitochondrial dysfunction in oocytes may also play a role. Oocytes from obese or insulin-resistant mice have been shown to exhibit abnormalities in many different mitochondrial parameters, including mitochondrial morphology and membrane potential. Here we review the findings regarding the link between mitochondrial dysfunction and insulin resistance, and propose that abnormalities in mitochondrial metabolism in oocytes may predispose to the development of obesity and insulin resistance and thus contribute to the inter-generational programming of metabolic disease.

Key words: mitochondria / obesity / oocyte / insulin resistance

Introduction

The prevalence of obesity and type 2 diabetes (T2D) has increased dramatically in recent times, with these conditions now among the most significant health challenges facing modern society. The World Health Organization estimates that there are >1.4 billion people who are overweight globally (WHO, 2012), while close to 400 million people worldwide have T2D (http://www.idf.org/diabetesatlas/5e/Update2012). Obesity and T2D are frequently associated with a number of comorbidities, including dyslipidemia, cardiovascular disease and inflammation, collectively referred to as the metabolic syndrome. A central facet of the disorders comprising the metabolic syndrome is insulin resistance, defined as a reduced capacity for insulin to regulate carbohydrate and lipid metabolism in target tissues.

In this review we will discuss the potential relationship between mitochondrial dysfunction and the development of insulin resistance. Evidence for this association will be presented and we will explore emerging studies that show obesity and insulin resistance are linked with abnormalities in mitochondrial function in oocytes. These findings of mitochondrial defects preconception raise the possibility that dysfunctional energy metabolism in oocytes may partially contribute to the inter-generational transmission of metabolic disease.

Insulin resistance

Insulin is a key hormone regulating glucose and lipid homeostasis. Under normal physiological conditions, insulin is secreted in response to a meal to promote the uptake and storage of nutrients, with skeletal muscle being a major site for insulin-stimulated glucose disposal (DeFronzo et al., 1985). In addition, insulin effectively suppresses glucose output from the liver to help maintain blood glucose levels within a tight range. In the insulin resistant state, the effect of insulin on the above pathways is compromised, leading to hypersecretion of insulin from the pancreatic beta cells. The ensuing hyperinsulinemia can adequately compensate for the insulin resistance in most of the population; however in genetically susceptible individuals the beta cells ultimately fail in the face of the increased workload and this leads to elevated blood glucose levels and T2D. Thus insulin resistance can be considered
a very early and important factor in the pathogenesis of T2D (Samuel et al., 2010).

At the molecular level, the precise mechanisms responsible for insulin resistance are not fully elucidated. Overactivation of stress-related and inflammatory pathways has been reported in tissues of insulin-resistant humans and rodents (Ozcan et al., 2004, 2006; Houssis et al., 2006; Schenk et al., 2008; Anderson et al., 2009b; Hoehn et al., 2009; Gregor and Hotamisligil, 2011), but whether alterations in these pathways are a cause or consequence of insulin resistance remains unresolved. One factor that is among the earliest defects associated with insulin resistance and T2D is lipid accumulation in non-adipose tissues. When nutrients are in excess, fatty acids and their metabolites accumulate at high levels in tissues such as skeletal muscle, liver and the heart, precipitating defects in insulin action (Pan et al., 1997; Itani et al., 2002; Adams et al., 2004; Kraegen and Cooney, 2008; Samuel and Shulman, 2012; Turner et al., 2013). More specifically, while triacylglycerols can be considered a marker for excess lipid storage into tissues, it is the accumulation of metabolically active lipid metabolites including ceramides, diacylglycerol and long chain acyl-CoAs that is thought to be responsible for the induction of insulin resistance (Kraegen and Cooney, 2008; Samuel and Shulman, 2012). Mechanistically, the accumulation of these lipid species activates many pathways and factors (e.g. protein phosphatase 2A, c-jun N-terminal kinase, reactive oxygen species, protein kinase C, the nuclear factor kB pathway and cytokines) that directly antagonize insulin signal transduction and glucose metabolism (Kraegen and Cooney, 2008; Samuel and Shulman, 2012).

The extent of lipid accumulation within any given tissue is determined by a number of factors, including the uptake and utilization of lipids, and for some tissues the rate of lipid synthesis and export. Elevations in the rate of lipid synthesis and export by a number of factors, including the uptake and utilization of lipids, and mitochondrial oxidative metabolism, particularly in skeletal muscle, may contribute to obesity and lipid accumulation, and thus might be involved in the pathogenesis of insulin resistance and T2D (Lowell and Shulman, 2005; Turner and Heilbronn, 2008).

**Mitochondrial structure and function**

The mitochondrion is the major site for energy generation in cells, providing a platform for the oxidation of fuel substrates to produce ATP (Fig. 1). Mitochondria are double membrane organelles composed of an outer membrane, intermembrane space and inner membrane that surrounds the mitochondrial matrix. The inner membrane is highly folded to increase surface area and is the site of the electron transport chain complexes. Within the matrix there are a large number of enzymes involved in many different metabolic pathways (e.g. tricarboxylic acid cycle (TCA) cycle, β-oxidation) and multiple copies of the mitochondrial genome (mtDNA). During nutrient oxidation, reducing equivalents (NADH or FADH₂) that are generated from glycolysis, the TCA cycle and β-oxidation provide electrons that pass along the mitochondrial electron transport chain, coupled to the pumping of protons into the intermembrane space through complex I, III and IV. The electrons are transferred to oxygen at complex IV to produce H₂O. The pumped protons generate an electrochemical gradient across the inner mitochondrial membrane, which is used as the driving force for the ATP synthase (complex V) to produce ATP. Uncoupling proteins may also dissipate the electrochemical gradient to produce heat in a process referred to as thermogenesis.

Mitochondrial content in cells is regulated by a coordinated interplay between the nuclear and mitochondrial genomes. Mitochondria exist largely as a reticular network and are constantly engaged in the process of fusion and fission, providing morphological plasticity to allow adjustments in response to the prevailing cellular stresses and metabolic requirements (Ferree and Shirihai, 2012). In muscle cells, the mitochondrial network is arranged into two discrete but interconnected pools—the subsarcolemmal pool near the cell surface, and the intermyofibrillar pool in the interior of the cell between myofibres (Skuła-chev, 2001; Westermann, 2008; Picard et al., 2013).

**Mitochondrial dysfunction in muscle and its association with insulin resistance**

Given the critical role of mitochondria in fuel metabolism, perturbations in any aspect of mitochondrial regulation and function have the potential to impact on metabolic homeostasis. Indeed, the basis of the mitochondrial theory of insulin resistance is that the buildup of lipid metabolites and the subsequent development of insulin resistance occurs as a result of dysfunction of mitochondrial substrate oxidation.

Defects in mitochondria have been observed in a variety of tissues (adipose tissue, heart, liver) of insulin-resistant humans and rodents (Metzler et al., 2002; Scheuermann-Freestone et al., 2003; Choo et al., 2006; Kaaman et al., 2007; Perseghin et al., 2008; Anderson et al., 2009a; Cypress et al., 2009; Szendroedi et al., 2009a; Pfannenberg et al., 2010), and there are also recent reports of mitochondrial dysfunction in oocytes in obesity and insulin resistance (discussed in detail below). However, the primary support for the mitochondrial theory of insulin resistance comes from studies in skeletal muscle.

Abnormalities in mitochondrial metabolism in muscle have been reported in a variety of insulin-resistant states, including aging, obesity and T2D. Studies in muscle biopsies from insulin-resistant human subjects have revealed that several different markers of mitochondrial function are reduced compared with control subjects, including mRNA levels for mitochondrial genes (Mootha et al., 2003; Patti et al., 2003; Morino et al., 2005; Heilbronn et al., 2007; Skov et al., 2007; Wang et al., 2010; van Tienen et al., 2012), activity of oxidative enzymes (Kelley et al., 1999, 2002; Simonneau et al., 1999; Kim et al., 2000; Ritov et al., 2005; Heilbronn et al., 2007), expression of mitochondrial proteins (Heilbronn et al., 2007; Hwang et al., 2010), mitochondrial DNA content (Ritov et al., 2005; Boushel et al., 2007) and mitochondrial size and number (by electron microscopy) (Kelley et al., 2002; Morino et al., 2005; Ritov et al., 2005). Several investigators have also used non-invasive magnetic resonance spectroscopy (MRS) to assess in vivo ATP synthesis rates, phosphocreatine synthesis rates or TCA cycle activity in skeletal muscle of human subjects, to obtain a functional readout of mitochondrial metabolism in vivo. These studies have shown impaired basal and insulin-stimulated mitochondrial metabolism in different...
Collectively, the above studies illustrate that there are many instances where defects in mitochondrial metabolism and impairments in insulin action occur in conjunction with each other in skeletal muscle. While these studies do not definitively show causality, and there are examples of a dissociation between insulin resistance and mitochondrial dysfunction (Turner and Heilbronn, 2008), it would appear that under certain circumstances, the presence of mitochondrial defects can predispose to the development of obesity and insulin resistance. Indeed, a case report using MRS has reported that a MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) patient with primary mitochondrial dysfunction due to mtDNA mutations displayed insulin resistance in muscle in association with reduced baseline and insulin-stimulated ATP synthesis capacity (Szendroedi et al., 2009b). It is also interesting to note that defects in various markers of mitochondrial metabolism have been observed in lean individuals with a family history of insulin resistance and T2D (Patti et al., 2003; Petersen et al., 2004, 2005; Morino et al., 2005; Befroy et al., 2007). Because these changes were noted before individuals were substantially obese, it suggests that there may be inherited defects in mitochondrial metabolism that predispose individuals to developing insulin resistance, which is discussed in detail below.

Developmental programming of obesity and insulin resistance: a role for mitochondria?

It is increasingly apparent that obesity and insulin resistance are developmentally programmed. Obesity and other conditions linked with insulin resistance (e.g. polycystic ovary syndrome (PCOS)) are highly prevalent in women of child-bearing age (Goodarzi et al., 2011; Ogden et al., 2014), and women who are obese prior to and during pregnancy are more likely to have obese babies (reviewed in Ruager-Martin et al. (2010)) that are at higher risk of insulin resistance in childhood. These changes are mediated by obesogenic signals from mother to offspring occurring across the continuum of developmental windows, including the stages of blastocyst implantation, placental formation, gestation, lactation and early childhood, which are each critical for offspring health. However, there are emerging data that the developmental origins of obesity and insulin resistance are established even at the time of conception. For example two independent studies reported that maternal BMI prior to pregnancy is
positively associated with child BMI at 7 and 14 years of age (Reilly et al., 2005; Lawlor et al., 2007). Even more remarkable, children conceived after dramatic maternal weight loss no longer had a predisposition for obesity and insulin resistance compared with siblings conceived when the mother was obese (Kral et al., 2006; Smith et al., 2009). Animal models demonstrate that maternal obesogenic signals are transmitted to maturing oocytes and preimplantation blastocysts. Lambs were born with increased fat mass if the mothers were overfed prior to conception, even though at the blastocyst stage they were transferred to lean surrogate recipients for gestation (Rattanatray et al., 2010). Studies in mice have also shown that metabolic differences between obese mothers and lean mothers are evident in the earliest stages of embryo development and even prior to conception. Specifically, oocytes from obese mice contain high levels of lipid (Wu et al., 2010) and exhibit delayed nuclear maturation (Jungheim et al., 2010) and are less likely to be fertilized following mating (Wu et al., 2010; Luzzo et al., 2012). Oocytes that do fertilize often fail to develop to the blastocyst stage or exhibit delayed development even when they are fertilized in vitro (Minge et al., 2008; Igosheva et al., 2010; Luzzo et al., 2012).

### Obesity-induced defects in oocyte mitochondria

While the exact mechanisms responsible for the inter-generational transmission of metabolic disease are not fully resolved, there is increasing evidence demonstrating that obesity in females causes changes to the mitochondria in their oocytes, and that these mitochondrial alterations may be, at least partially, responsible for the developmental programming of obesity and insulin resistance in the offspring. Oocytes from mice fed high fat diet for 4 weeks exhibited reduced mitochondrial membrane potential at both the germinal vesicle (GV) and metaphase II (MII) stages, as well as a clustered pattern of mitochondrial distribution, indicative of altered mitochondrial activity (Wu et al., 2010). Independently, female mice fed high fat diet for 6 weeks had oocytes with a similar pattern of clustered mitochondria but increased tetramethylrhodamine methyl ester (TMRM)-stained fluorescence, indicative of increased mitochondrial membrane potential, as well as an increased oxidized intracellular redox state in both oocytes and zygotes (Igosheva et al., 2010). Oocytes (and cumulus cells) of mice fed high fat diet for 4 weeks also had altered mitochondrial morphology by electron microscopy, specifically fewer and disarrayed cristae, decreased electron density of matrix, swelling and more vacuoles (Luzzo et al., 2012). Interestingly, these mitochondrial defects in oocytes of obese female mice are associated with a higher content of mtDNA (Igosheva et al., 2010; Luzzo et al., 2012) as well as increased expression of mitochondrial biogenesis markers Tfam and Nrf1 mRNA (Igosheva et al., 2010) and elevated PGC-1α (transcriptional coactivator regulating genes involved in energy metabolism) and Drp1 protein (which controls the final part of mitochondrial fission) (Luzzo et al., 2012) suggesting that there may be increased stimulation of mitochondrial biogenic pathways to compensate for impaired mitochondrial function in the oocytes of obese mice. Oocytes from obese mice still had similar levels of ATP as oocytes from lean controls however, suggesting that mitochondrial metabolism or function is only subtly altered, or there are changes in other pathways that help to preserve ATP levels (Luzzo et al., 2012). To our knowledge mitochondria have not been examined specifically in oocytes of obese compared with non-obese women; however, there are reports of poor oocyte and embryo quality in obese women (reviewed in Wu et al., 2011).

It is likely that the defects observed in oocytes of obese mice are, at least in part, due to the hyperinsulinaemia that results from insulin resistance, because mouse models of hyperinsulinaemia show remarkably similar mitochondrial alterations. Mice treated with either insulin to cause insulin resistance, or insulin plus hCG to cause insulin resistance and hyperandrogenemia (similar to women with PCOS), exhibited oocytes with poor morphology, reduced fertilization rates and impaired developmental competence (Ou et al., 2012), similar to mice with diet-induced obesity (Minge et al., 2008; Igosheva et al., 2010; Wu et al., 2010; Luzzo et al., 2012). Mitotracker staining showed altered mitochondrial localization in the oocytes from insulin-resistant mice that was clumped and dispersed rather than peri-nuclear and organized around the spindle in GV and MII oocytes, respectively (Ou et al., 2012). JC-1 staining showed that MII oocytes from insulin-resistant mice had fewer active/high membrane potential mitochondria and this was associated with higher levels of reactive oxygen species and H$_2$O$_2$ (Ou et al., 2012). Interestingly, in contrast to mice with high fat diet-induced obesity, oocytes from insulin-resistant mice had reduced mtDNA content and lower ATP in MII oocytes compared with oocytes from metabolically normal controls (Ou et al., 2012). This suggests that high fat diets cause mitochondrial biogenesis in oocytes, as occurs in response to high fat diet in other tissues such as muscle (Turner et al., 2007; Hancock et al., 2008), while insulin resistance results in impaired mitochondrial function and metabolism.

These mitochondrial alterations in oocytes of obese animals can have lasting consequences on offspring growth and metabolism (Fig. 2). If blastocysts from obese female mice are transferred to non-obese recipients for gestation, the pups derived from obese embryo donors are born smaller (Luzzo et al., 2012). This is a similar low birthweight phenotype as offspring from mice that were obese and fed high fat diet throughout pregnancy (Jungheim et al., 2010) and that went on to become heavier than offspring from control females and exhibit increased body fat and glucose intolerance, the characteristics of T2D. Recent work also shows that genetically induced maternal insulin resistance in mice, in the absence of obesity, is sufficient to cause defective mitochondria, including insulin resistance and increased hepatic lipogenesis in offspring (Isagainties et al., 2014). Lipid accumulation and disrupted insulin signaling also occurs in muscle of adult offspring born to overfed sheep (Yan et al., 2011), having been established during fetal development (Yan et al., 2010).

Mitochondrial metabolism defects are also apparent in offspring from obese mothers. Rats fed obesogenic diets from prior to conception through lactation have offspring with reduced liver and kidney mtDNA (Taylor et al., 2005; Burgueno et al., 2013), altered mitochondrial protein expression (Bruce et al., 2009) and predisposition for metabolic disease (Bruce et al., 2009; Burgueno et al., 2013). Importantly, restricting maternal overnutrition to the peri-conception window resulted in similar offspring phenotypes. Male rat offspring from mothers that were overfed from 3 weeks prior to conception to 1 week post-mating exhibited decreased energy expenditure and increased respiratory exchange ratio indicative of an impaired ability to use fatty acids (Borengasser et al., 2011). Markers of hepatic mitochondrial function (Pgc1a mRNA, mitochondrial Sirt3 mRNA and protein, electron transport chain complexes and ATPase) were reduced in the livers of these offspring from obese mothers (Borengasser et al., 2011) and so may be the underlying basis of reduced hepatic fatty acid oxidation and insulin resistance.
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Future directions

Oocyte-derived mitochondria give rise to the entire complement of mitochondria in offspring tissues and their integrity and health is thus vital for optimal embryo formation and fetal development. Obesity and insulin resistance in females causes alterations to oocyte mitochondria and it is likely that these defects contribute to the metabolic reprogramming that is observed in offspring from obese mothers. We propose that future studies should concentrate on identifying the specific pathways leading to the metabolic derangements in oocyte mitochondria, as well as determining whether these defects can be reversed by interventions in the mother prior to conception.

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Authors’ roles

N.T. and R.L.R conceptualized and wrote the manuscript together.

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Conflict of interest

None declared.

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