Review

Is mitochondrial dysfunction a cause of insulin resistance?

Nigel Turner and Leonie K. Heilbronn

Diabetes and Obesity Research Program, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia

Insulin resistance is a key defect associated with obesity and type-2 diabetes. The precise factors that lead to insulin resistance have not been elucidated fully, but there is a strong association between insulin resistance and inappropriate lipid accumulation in insulin-target tissues. Over the past decade, several studies have reported changes in markers of mitochondrial metabolism in insulin-resistant individuals. These observations have led to the theory that compromised mitochondrial oxidative function, particularly in skeletal muscle, causes excess lipid deposition and the development of insulin resistance. Here, we review the latest findings regarding the link between mitochondrial metabolism and insulin action and, in particular, highlight several recent studies that call into question the cause-and-effect relationship between mitochondrial dysfunction and insulin resistance.

Introduction

Over the past three decades, there has been an explosive increase in the prevalence of type-2 diabetes (T2D), and current estimates suggest that by the year 2030, over 350 million people worldwide will be afflicted with this disease [1]. The complications associated with diabetes (e.g. cardiovascular disease, stroke, neuropathy and nephropathy) are a major cause of premature morbidity and mortality, and the rising epidemic of T2D represents one of the most serious threats to human health. An early and important defect associated with obesity and T2D is insulin resistance, which is defined as 'a relative impairment in the ability of insulin to exert its effects on glucose and lipid metabolism in target tissues'. The precise factors that lead to decreased insulin action are still not completely resolved; however, there is considerable work that indicates that inappropriate lipid deposition in non-adipose tissues (e.g. muscle or liver) contributes to the development of insulin resistance (Box 1).

The rate at which lipid accumulates in tissues is determined by several factors, such as the rate of lipid uptake from the circulation and the utilization of lipid within the tissues. Several studies show that increased efficiency of fatty acid uptake probably contributes to the lipid overload observed in insulin-resistant states [2,3]. Another popular theory that has gained momentum in recent years suggests that defects in mitochondrial function might lead to obesity and lipid accumulation, thereby playing an important part in the pathogenesis of insulin resistance and T2D [4]. Most studies investigating the potential relationship between mitochondrial dysfunction and insulin resistance have concentrated on skeletal muscle, with some work also indicating that mitochondrial dysfunction might exist in association with insulin resistance in other tissues [5,6]. In this review, we examine recent developments in the literature that study the link between muscle mitochondrial metabolism and insulin resistance.

Regulation of mitochondrial biogenesis and function

In eukaryotic cells, the mitochondrion is the major platform for energy transduction, producing ATP via oxidative metabolism of nutrients. ATP production within the mitochondrion involves two major steps: (i) the oxidation of reducing equivalents (NADH or $FADH_2$) that are produced by enzymatic pathways involved in the metabolism of glucose, fatty acids and other substrates; and (ii) the phosphorylation of ADP to ATP (i.e. oxidative phosphorylation) (Figure 1).

With such an elaborate series of processes involved in oxidative metabolism of fuel substrates, it is not surprising that the transcriptional programs involved in regulating mitochondrial biogenesis and function are also complex. Induction of mitochondrial biogenesis occurs rapidly (i.e. within hours) in response to environmental stimuli (e.g. exercise) and involves the coordinated action of both nuclear- and mitochondrial-encoded genomes. The conductors of this orchestrated program of mitochondrial biogenesis are the peroxisome proliferator-activated receptor gamma (PPARγ) coactivator (PGC-1) family of inducible transcriptional coactivators, which interact with and activate an array of transcription factors (e.g. NRF-1, PPARα and ERRα) to promote transcription of genes involved in all aspects of mitochondrial metabolism and function. As such, the PGC-1 coactivators are considered key regulators of metabolic homeostasis within cells.

Links between mitochondrial dysfunction and insulin resistance

The hypothesis that abnormalities in oxidative metabolism contribute to the development of insulin resistance came to prominence approximately a decade ago with several groups reporting that oxidative enzyme activity and lipid oxidation were reduced in muscle of obese and insulin-resistant subjects [7–9]. Closely following these reports, Kelley *et al.* observed lower NADH:O_2 oxidoreductase and reduced mitochondrial size in obese subjects with insulin resistance and/or T2D [10]. Two microarray studies were also published that showed a coordinated downregulation of genes involved in mitochondrial biogenesis and

Corresponding author: Turner, N. (n.turner@garvan.org.au).

1043-2760/$ – see front matter doi:10.1016/j.tem.2008.08.001 Available online 17 September 2008

Box 1. Insulin signalling, lipid overload and insulin resistance

Insulin action is mediated through a complex signalling network downstream of the insulin receptor. In brief, insulin binds to its receptor on the plasma membrane, which increases insulin receptor tyrosine kinase activity, resulting in the phosphorylation of insulin receptor substrates (e.g. IRS-1) on tyrosine residues. IRS protein phosphorylation results in the activation of two main signalling pathways: the phosphatidylinositol-3-kinase (PI3K)-Akt/protein kinase B (PKB) pathway, which mediates most of the actions of insulin on nutrient metabolism, and the Ras-mitogen-activated protein kinase (MAPK) pathway, which is largely responsible for the effects of insulin on growth, mitogenesis and differentiation.

Abnormalities in the insulin-signalling pathway have been reported in many insulin-resistant states. Defects in insulin signalling often occur in association with excess intracellular lipid accumulation, indicating a link between the two [75]. Indeed, increased levels of intracellular lipid metabolites and defects in insulin signalling have been found in established insulin-resistant states, such as obesity, lipodystrophy and T2D [75], and also in conjunction with insulin resistance that is produced acutely during lipid infusions over several hours [76,77]. With regards to the lipid moieties thought to be responsible for reducing insulin action, elevated triglycerides are probably the most frequently reported lipid abnormality in muscle and liver of insulin-resistant humans and rodents [75,78]. However, excess triglycerides are now generally considered more of a marker of lipid oversupply to tissues, whereas accumulation of metabolically active long-chain acyl-CoAs and other cytosolic lipid metabolites, such as ceramides and diacylglycerol (DAG), are thought to be more directly associated with insulin resistance [75,78]. Several mechanisms that link these lipid metabolites to reductions in insulin signalling have been proposed, including activation of pathways and factors (e.g. protein kinase C, c-jun N-terminal kinase [JNK], reactive oxygen species, the nuclear factor κB [NFκB] pathway, protein phosphatase A2 [PPA2] and cytokines) that ultimately antagonize insulin signalling by reducing the levels of activating phosphorylation of insulin-signalling proteins [75,78]. Other suggested mechanisms by which lipid species might induce insulin resistance include inhibition of enzymes of glucose metabolism and altered gene transcription [75,78]. Thus, although a direct defect linking excess tissue lipid levels with insulin resistance still requires delineation, identifying the molecular mechanisms associated with lipid accumulation is crucial in the search for strategies to treat insulin resistance.

oxidative phosphorylation in non-diabetic individuals with a family history of T2D and in subjects with overt diabetes [11,12]. These landmark studies advanced the theory of a potential role for mitochondrial dysfunction in insulin resistance and T2D.

Since this time, a growing list of studies have reported abnormalities in markers of mitochondrial metabolism in various insulin-resistant states, including obesity, aging, T2D and polycystic ovary syndrome (PCOS) (Table 1). Several of these investigations analyzed muscle biopsies from insulin-resistant subjects and demonstrated reductions in mRNA levels for mitochondrial genes [11–15], decreased mitochondrial DNA (mtDNA) [16,17], lower protein expression of respiratory chain subunits [14], reduced oxidative enzyme activities [10,14,17] and decreases in mitochondrial size and density (by electron microscopy) [10,13,17]. In addition to the defects observed in muscle biopsies, several groups have conducted more functional mitochondrial analyses in muscle from insulin-resistant subjects. These studies either used non-invasive magnetic resonance spectroscopy (MRS) with ^{31}P or ^{13}C to measure *in vivo* ATP synthesis rates, phosphocreatine resynthesis rates or TCA-cycle activity as an index of mitochondrial function, or used *ex vivo* measurements of mitochondrial respiration or substrate oxidation. *In vivo* studies using MRS reported impaired basal and insulin-stimulated mitochondrial metabolism in insulin-resistant populations of elderly subjects [18], patients with T2D [19,20] and first-degree relatives of subjects with T2D [21–23]. *Ex vivo* studies that measured rates of respiration or substrate oxidation in isolated mitochondria or in permeabilized muscle fibres from insulin-resistant populations show that functional capacity per mitochondrion seems to be similar [16,24,25] or only mildly reduced [26] compared to control subjects. However, when mitochondrial capacity is expressed per unit mass of skeletal muscle, a substantial reduction is seen in insulin-resistant subjects [16,24,25], which indicates that the defects observed in mitochondrial function *in vivo* with MRS might be more strongly related to decreased mitochondrial number, rather than to substantial intrinsic mitochondrial defects [16,24–26].

Despite the notable findings of the aforementioned studies, the cross-sectional nature of these investigations means that they cannot decipher whether the observed mitochondrial dysfunction was a primary cause of insulin resistance or a consequence of insulin resistance. Several recent intervention studies in humans and rodents have provided evidence that supports a role for aberrant mitochondrial dysfunction in the development of insulin resistance. In one study, healthy subjects treated for one month with a nucleoside reverse-transcriptase inhibitor (part of a highly active antiretroviral therapy used to suppress human immunodeficiency virus infection) displayed a reduction in mitochondrial DNA copy number in muscle and reduced insulin sensitivity [27]. Other studies have infused fatty acids into humans for 6–48 h and reported a robust induction of whole-body insulin resistance along with reduced expression of mRNA encoding PGC1α and other mitochondrial genes in muscle, and lower rates of insulin-stimulated ATP synthesis [28–30]. Consistent with these findings, one group reported that three days of high-fat feeding reduced mRNA levels of PGC1α, PGC-1β and several other mitochondrial genes in muscle of healthy male subjects [31]. Several rodent studies have also reported reductions in mitochondrial gene expression, protein expression and mitochondrial respiration in skeletal muscle collected under conditions associated with reduced insulin action (i.e. high-fat feeding for 3–16 weeks or genetic obesity) [31–34]. Collectively, these studies indicate that defects in mitochondrial function in muscle occur in association with the induction of insulin resistance.

In addition to studies that show a correlation between mitochondrial dysfunction and the development of insulin resistance, several interventions that improve insulin sensitivity also enhance mitochondrial function. The most obvious of these treatments is exercise, which has been shown to stimulate mitochondrial function in muscle and improve insulin action [35–37]. Caloric restriction has also been reported to improve insulin sensitivity [38,39] and robustly stimulate mitochondrial biogenesis in muscle [40,41]. Another recent study showed that treatment of

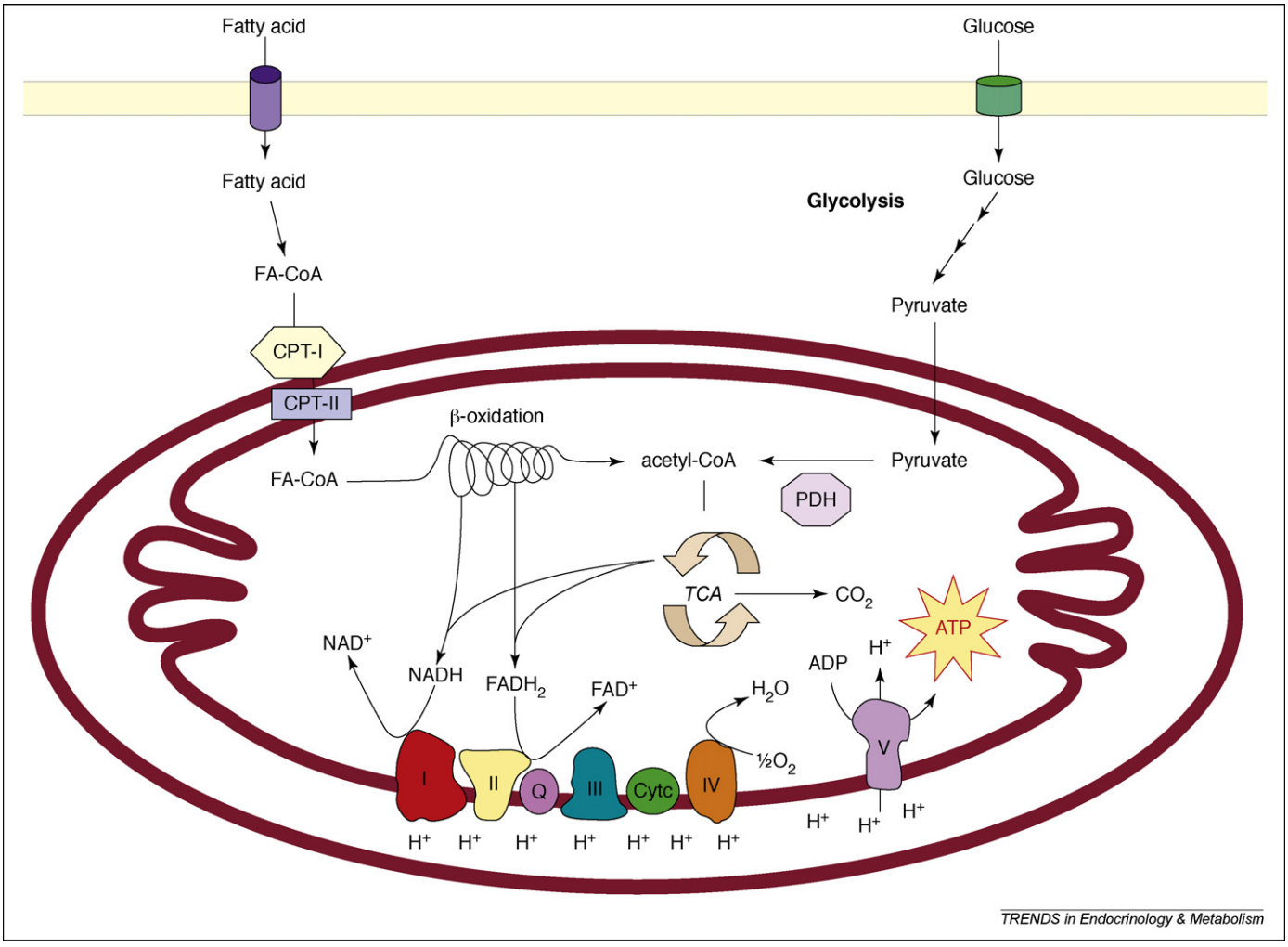

Figure 1. Pathways involved in mitochondrial energy metabolism. During the oxidative metabolism of glucose and fatty acids, reducing equivalents (NADH or $FADH_2$) are generated from glycolysis, the TCA cycle and β-oxidation. When NADH and $FADH_2$ are oxidized to NAD^+ or FAD, electrons pass along the mitochondrial respiratory chain while proteins are pumped into the intermembrane space through complex I, complex II and complex IV. The electrons are transferred to oxygen at complex IV to produce H_2O. The pumped protons generate an electrochemical gradient across the inner mitochondrial membrane, which is used as the driving force for ATP synthase (complex V) to produce ATP.

mice with resveratrol induced PGC-1α activity, with subsequent improvement in mitochondrial metabolism [42]. In parallel with these changes, mice also exhibited enhanced insulin sensitivity. These studies provide further evidence of a close link between mitochondrial function and insulin action.

What factors might cause defects in mitochondrial function?

Overall, the studies detailed above indicate that diminished mitochondrial function in muscle is commonly associated with insulin resistance. That several of these studies have been conducted in lean individuals with a family history of T2D indicates that, at least in those populations, mitochondrial defects might be among the earliest factors involved in the pathogenesis of insulin resistance. An important question, therefore, is 'What factor(s) contribute to these mitochondrial defects?'

Genetic factors

Mitochondrial proteins are encoded by both nuclear and mitochondrial genomes, and there is some evidence that mtDNA deletions or mutations in nuclear-encoded genes (e.g. *PGC-1α* and *NDUFB6*) are linked with insulin action and T2D [43–45]. Additionally, muscle cell culture studies provide evidence that genetic programming is a strong determinant of the metabolic phenotype of human muscle; primary human skeletal muscle cells grown for approximately five weeks in culture display similar metabolic characteristics (e.g. gene expression and lipid partitioning between oxidation and storage) to the *in vivo* phenotype of the donor subject [46,47].

Sedentary lifestyle

Physical activity is a major regulator of mitochondrial function in muscle, with exercise potently activating mitochondrial biogenesis and chronic inactivity associated with reduced mitochondrial number [48]. Obesity and other metabolic disorders are linked with reduced activity levels and increased sedentary behaviour [49–51]. Thus, it is likely that some mitochondrial defects reported in overweight or obese insulin-resistant subjects can be explained, in part, by low levels of physical activity. Consistent with such a notion, Rimbert *et al.* [35] showed that physical

Table 1. Studies investigating markers of mitochondrial function in insulin-resistant humans

Insulin-resistant population	Marker of mitochondrial function	Refs
Lean elderly	↓ ATP synthesis (^{31}P-MRS)	[18]
Lean FH$^+$	↓ ATP synthesis (^{31}P-MRS)	[22]
Lean FH$^+$	↓ mRNA, ↓ mitochondrial density, ↓/↔ protein, ↔ mtDNA	[13]
Lean FH$^+$	↓ insulin-stimulated ATP synthesis (^{31}P-MRS)	[23]
Lean FH$^+$	↓ TCA cycle activity (^{13}C-MRS)	[21]
Overweight or obese ND	↓ mRNA, ↓ protein, ↓ enzyme activity	[14]
Obese ND	↓ fatty acid oxidation (per muscle)	[25]
Obese ND (PCOS)	↓ mRNA	[15]
Overweight or obese FH$^+$ and T2D	↓ mRNA	[11]
Overweight ND and T2D	↓ mRNA	[12]
Obese ND and T2D	↓ enzyme activity, ↓ mitochondrial size	[10]
Obese ND and T2D	↓ enzyme activity, ↓ mitochondrial density, ↓ mtDNA	[17]
Obese ND and T2D	↓ fatty acid oxidation (per muscle)	[24]
Overweight T2D	↓ phosphocreatine recovery (^{31}P-MRS)	[19]
Overweight T2D	↓ basal and insulin-stimulated ATP synthesis (^{31}P-MRS)	[20]
Obese T2D	↓ mitochondrial respiration (per mitochondrion), ↔ enzyme activity	[26]
Obese T2D	↓ mitochondrial respiration (per muscle), ↓ mtDNA, ↔ enzyme activity	[16]
Asian-Indian ND and T2D	↑ mRNA, ↑ mtDNA, ↑ enzyme activity, ↑ mitochondrial ATP synthesis	[60]
Obese T2D	↔ phosphocreatine recovery (^{31}P-MRS)	[61]

Reduced (↓), increased (↑) or no change (↔) relative to control group. T2D, type-2 diabetes; FH$^+$, family history of T2D; ND, non-diabetic; PCOS, polycystic ovary syndrome.

activity levels were a major determinant of mitochondrial fatty acid oxidative capacity and insulin sensitivity in trained and non-trained individuals, regardless of age.

Oxidative stress

Another factor that might contribute to the mitochondrial dysfunction observed in insulin resistance is oxidative stress. Oxidative stress refers to an imbalance between the production of reactive species and antioxidant defences that leads to the damage of proteins, lipids and DNA. Mitochondria are a major source of reactive oxygen species (ROS), which are generated as a by-product of metabolic reactions within this organelle [52]. Mitochondria have also been shown to be a primary target for oxidative attack [53,54]. Under conditions of glucose and fatty acid oversupply, nutrient overflow into cells favours a state of increased ROS production [55,56], with elevated ROS levels potentially leading to oxidative damage within mitochondria and compromised function. In support of such a relationship, Bonnard *et al.* [34] recently provided evidence that oxidative stress is a major factor causing mitochondrial dysfunction in mice fed a diet rich in fat and sugar.

Insulin resistance

Several recent studies have shown that insulin itself has a marked effect on mitochondrial function, giving rise to the hypothesis that mitochondrial defects might be secondary to insulin resistance. For instance, an 8-h insulin infusion in humans increased mitochondrial oxidative capacity in skeletal muscle, as determined by increases in mitochondrial mRNA transcript levels, mitochondrial protein synthesis rates, enzyme activities of cytochrome c oxidase and citrate synthase, and ATP production [57]. This response, however, was blunted in patients with T2D. Recently, Asmann *et al.* [58] used a 7-h low- and high-dose insulin infusion (0.25 and 1.5 mU/kg of fat-free mass per min, respectively) with euglycemia in subjects with T2D and in age-, sex- and BMI-matched controls. At low insulin levels, ATP synthesis rates were similar between diabetic and non-diabetic subjects; however, at high insulin doses, non-diabetic individuals showed increased mitochondrial ATP production rate, but this response was impaired in diabetic individuals. These diabetic individuals also had reduced glucose disposal and diminished expression of mitochondrial genes [58]. These same investigators also recently examined the effect of acute insulin removal from subjects with type-1 diabetes and found that insulin deficiency caused reductions in mitochondrial ATP production and in the expression of mitochondrial genes in skeletal muscle [59]. Overall, these studies provide evidence that insulin can affect mitochondrial gene expression and function and, therefore, support the idea that decreased mitochondrial capacity might arise, in part, as a consequence of impaired insulin action. However, it should be noted that these results have been obtained using extended periods (7–8 h) of high insulin; therefore, further research is required to determine whether normal postprandial insulin excursions (3–4 h) have a similar effect on mitochondrial metabolism.

Dissociation of mitochondrial dysfunction and insulin resistance

The concept that dysfunction of mitochondria in skeletal muscle might be a major factor leading to insulin resistance is gaining wide acceptance. However, conflict still exists in the field. In fact, a substantial number of recent studies in both humans and rodents directly challenge the notion that a reduction in mitochondrial oxidative capacity is an essential part of the link between lipid accumulation (obesity) and insulin resistance.

Nair *et al.* [60] recently reported that Asian Indians displayed higher mtDNA content, elevated expression of genes involved in oxidative phosphorylation, increased oxidative enzyme activity and greater mitochondrial ATP-production rates in muscle, despite being more insulin resistant than age-, sex- and BMI-matched North American counterparts. Furthermore, even though Asian-Indian individuals with T2D exhibited reduced insulin sensitivity and higher muscle lipid levels compared to Asian Indians without T2D, markers of mitochondrial oxidative capacity were not different between these two groups [60]. This study, therefore, indicates that insulin

resistance observed in Asian Indians compared to that in North Americans of European descent cannot be explained by mitochondrial dysfunction in skeletal muscle. Another recent study examined whether deficits in mitochondrial function were present in muscle from obese patients in either early or advanced stages of T2D. Using post-exercise phosphocreatine recovery kinetics as an index of mitochondrial function, this study found no differences in mitochondrial function between either group of T2D patients and normoglycemic controls matched for age, body composition and habitual physical activity levels [61]. These findings, then, indicate that defective mitochondrial metabolism in muscle was not responsible for the insulin resistance and T2D observed in these subjects.

Several intervention studies in humans have also reported findings that are discordant with the mitochondrial dysfunction theory of insulin resistance. In insulin-resistant subjects with a family history of T2D, treatment for one week with the anti-lipolytic agent acipimox improved insulin sensitivity but resulted in decreased mitochondrial gene expression in muscle [62]. In addition, overweight and obese subjects who lost weight via dietary restriction displayed improved insulin sensitivity in the absence of any measurable change in mtDNA, cardiolipin content or NADH-oxidase activity and, in fact, displayed a subtle decrease in mitochondrial size [63]. Another study found that in overweight patients with T2D, eight weeks of treatment with the anti-diabetic agent rosiglitazone induced a significant improvement in insulin sensitivity without altering *in vivo* mitochondrial function (phosphocreatine recovery rates) in muscle [64]. The above studies show that improvements in insulin sensitivity can occur without enhanced mitochondrial function in muscle.

Several recent studies using gene-manipulated mice have directly tested whether tissue-specific alterations in mitochondrial function influence insulin sensitivity. In mice with the deletion of mitochondrial transcription factor A, there is marked impairment in mitochondrial oxidative capacity in muscle; however, these mice showed improved glucose clearance during a glucose tolerance test and normal insulin-stimulated glucose uptake in isolated muscle strips [65]. In another study, conditional deletion of apoptosis-inducing factor in muscle resulted in a pattern of mitochondrial oxidative phosphorylation deficiency that closely resembles that observed in human insulin resistance [11,12], yet these mice were lean, insulin sensitive and protected against high-fat-diet-induced insulin resistance [66]. Mice with either muscle-specific deletion of PGC-1α or with a loss-of-function mutation of PGC-1β also show defects in markers of mitochondrial function in muscle; however, in these animals, insulin sensitivity in muscle is preserved or, in fact, slightly improved compared to control mice [67,68]. In another study, mice with muscle-specific transgenic overexpression of PGC-1α displayed improved exercise capacity and increased mitochondrial gene expression, mtDNA and mitochondrial enzyme activity compared with control animals, but this increased mitochondrial capacity did not alter glucose and insulin tolerance in these mice [69]. Overall, these studies in gene-manipulated mice have failed to demonstrate a clear effect of altering mitochondrial function on insulin action. These studies, however, must be interpreted with some caution because they represent an extreme situation in which there is a complete lack or substantial overexpression of a specific protein; therefore, it is possible that the phenotype (or lack thereof) might be partially explained by compensatory adaptations (e.g. activation of AMP-activated protein kinase) induced by these manipulations [65].

We and others have also used dietary animal studies to show that high-fat feeding significantly increases mitochondrial fatty acid oxidative capacity, enzyme activity and protein expression, despite inducing insulin resistance at the whole-body and muscle level [70–72]. In line with these findings, Koves *et al.* [73] reported increased fatty acid oxidation in homogenates and mitochondria isolated from muscle of high-fat-fed insulin-resistant rodents. Their findings led them to put forth a provocative theory, which suggests that lipid excess results in an increase in fatty acid flux through β-oxidation in the absence of a coordinated increase in capacity of other oxidative pathways. This process generates incomplete fatty acid oxidation products, which then contribute to the insulin-resistant state. Although it is not totally incongruous with some of the studies mentioned above, further experimental evidence is needed to substantiate this theory (in particular, defining the mechanism by which incomplete fatty acid oxidation products have a deleterious effect on insulin action).

The studies detailed in this section illustrate that despite the findings showing an association between mitochondrial dysfunction and insulin resistance in lean and obese subjects, there are instances in which an uncoupling between muscle mitochondrial dysfunction and insulin resistance is observed. It should be acknowledged, however, that a myriad of experimental factors could account for differences observed between studies, such as the patient population studied (e.g. ethnicity and fitness level), the particular muscle group examined (e.g. vastus lateralis versus soleus), the specific measure of mitochondrial metabolism employed and in rodent studies, the composition and content of the high-fat diet, in addition to the length of feeding.

Concluding remarks and future directions

In recent years, abnormal mitochondrial metabolism has been observed in insulin-resistant states, which has led to the theory that mitochondrial dysfunction is a key factor contributing to insulin resistance. Although this hypothesis is appealing, there are still several unresolved issues. For example, it is unclear whether defects in mitochondrial function observed in insulin-resistant individuals are inherited, are the result of environmental factors (e.g. low physical activity and caloric excess) or are a consequence of insulin resistance itself. Indeed, even though mitochondrial defects have been shown to be among the earliest defects observed in lean individuals with a family history of T2D [13,21–23], these subjects were tested when insulin resistance was already present and, therefore, it is unclear whether mitochondrial abnormalities were a primary defect or occurred secondary to or in parallel with this insulin resistance. Determining the cause-and-effect relationship between mitochondrial dysfunction and insu-

lin resistance in humans is challenging but could potentially be resolved with a long-term longitudinal study that examines changes in mitochondrial function and insulin sensitivity in individuals over time. Another important issue is whether the decrease in mitochondrial function observed in insulin-resistant humans (i.e. ~30%), would limit the ability of muscle to oxidize fatty acids and lead to lipid accumulation as proposed [4]. Under resting conditions, the rate of oxygen utilization in muscle is low; however, when energy demands are high, such as during maximal exercise, muscle has an enormous capacity to increase substrate oxidation over basal levels [74]. Considering there is such a substantial 'spare' capacity to elevate substrate oxidation in muscle, it is questionable whether mitochondrial deficiencies observed in insulin-resistant subjects would have any impact on the rate of fatty acid oxidation under normal, free-living conditions in which energy requirements would be relatively low.

Despite these unanswered questions and several recent reports that show little or no indication of mitochondrial dysfunction in some states of insulin resistance, it seems that in certain populations, defects in mitochondrial function are probably involved in the development and/or maintenance of the insulin-resistant state. An important challenge for future research is to determine whether strategies aimed at specifically upregulating mitochondrial function might have therapeutic potential in the treatment of insulin resistance and T2D in such individuals.

Acknowledgements

Research on mitochondrial metabolism in the laboratories of N.T. and L.K.H. is funded by the National Health and Medical Research Council of Australia (NHMRC), the Diabetes Australia Research Trust and the Rebecca Cooper Medical Research Foundation. N.T. and L.K.H. are supported by Career Development Awards from the NHMRC. We would like to thank Gregory Cooney for his critical comments on this manuscript.

References

1 Wild, S. *et al.* (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 27, 1047–1053
2 Hegarty, B.D. *et al.* (2002) Increased efficiency of fatty acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-resistant rats. *Diabetes* 51, 1477–1484
3 Bonen, A. *et al.* (2004) Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J.* 18, 1144–1146
4 Lowell, B.B. and Shulman, G.I. (2005) Mitochondrial dysfunction and type 2 diabetes. *Science* 307, 384–387
5 Choo, H.J. *et al.* (2006) Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia* 49, 784–791
6 Bugger, H. and Abel, E.D. (2008) Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. *Clin. Sci. (Lond.)* 114, 195–210
7 Kim, J.Y. *et al.* (2000) Lipid oxidation is reduced in obese human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 279, E1039–E1044
8 Simoneau, J.A. *et al.* (1999) Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J.* 13, 2051–2060
9 Kelley, D.E. *et al.* (1999) Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am. J. Physiol.* 277, E1130–E1141
10 Kelley, D.E. *et al.* (2002) Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51, 2944–2950
11 Patti, M.E. *et al.* (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc. Natl. Acad. Sci. U. S. A.* 100, 8466–8471
12 Mootha, V.K. *et al.* (2003) PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* 34, 267–273
13 Morino, K. *et al.* (2005) Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J. Clin. Invest.* 115, 3587–3593
14 Heilbronn, L.K. *et al.* (2007) Markers of mitochondrial biogenesis and metabolism are lower in overweight and obese insulin-resistant subjects. *J. Clin. Endocrinol. Metab.* 92, 1467–1473
15 Skov, V. *et al.* (2007) Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. *Diabetes* 56, 2349–2355
16 Boushel, R. *et al.* (2007) Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia* 50, 790–796
17 Ritov, V.B. *et al.* (2005) Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 54, 8–14
18 Petersen, K.F. *et al.* (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300, 1140–1142
19 Schrauwen-Hinderling, V.B. *et al.* (2007) Impaired *in vivo* mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia* 50, 113–120
20 Szendroedi, J. *et al.* (2007) Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. *PLoS Med.* 4, e154
21 Befroy, D.E. *et al.* (2007) Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes* 56, 1376–1381
22 Petersen, K.F. *et al.* (2004) Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* 350, 664–671
23 Petersen, K.F. *et al.* (2005) Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. *PLoS Med.* 2, e233
24 Bandyopadhyay, G.K. *et al.* (2006) Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. *Diabetes* 55, 2277–2285
25 Holloway, G.P. *et al.* (2007) Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. *Am. J. Physiol. Endocrinol. Metab.* 292, E1782–E1789
26 Mogensen, M. *et al.* (2007) Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* 56, 1592–1599
27 Fleischman, A. *et al.* (2007) Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am. J. Physiol. Endocrinol. Metab.* 292, E1666–E1673
28 Brehm, A. *et al.* (2006) Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. *Diabetes* 55, 136–140
29 Hoeks, J. *et al.* (2006) Peroxisome proliferator-activated receptor-γ coactivator-1 and insulin resistance: acute effect of fatty acids. *Diabetologia* 49, 2419–2426
30 Richardson, D.K. *et al.* (2005) Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increases the expression of extracellular matrix genes in human skeletal muscle. *J. Biol. Chem.* 280, 10290–10297
31 Sparks, L.M. *et al.* (2005) A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes* 54, 1926–1933
32 Lionetti, L. *et al.* (2007) Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. *Int. J. Obes. (Lond).* 31, 1596–1604
33 Jove, M. *et al.* (2004) Impaired expression of NADH dehydrogenase subunit 1 and PPARγ coactivator-1 in skeletal muscle of ZDF rats: restoration by troglitazone. *J. Lipid Res.* 45, 113–123

34 Bonnard, C. *et al.* (2008) Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J. Clin. Invest.* 118, 789–800

35 Rimbert, V. *et al.* (2004) Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. *FASEB J.* 18, 737–739

36 Ostergard, T. *et al.* (2006) Impact of exercise training on insulin sensitivity, physical fitness, and muscle oxidative capacity in first-degree relatives of type 2 diabetic patients. *Am. J. Physiol. Endocrinol. Metab.* 290, E998–E1005

37 Toledo, F.G. *et al.* (2007) Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. *Diabetes* 56, 2142–2147

38 Larson-Meyer, D.E. *et al.* (2006) Effect of calorie restriction with or without exercise on insulin sensitivity, β-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 29, 1337–1344

39 Weiss, E.P. *et al.* (2006) Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am. J. Clin. Nutr.* 84, 1033–1042

40 Civitarese, A.E. *et al.* (2007) Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med.* 4, e76

41 Nisoli, E. *et al.* (2005) Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science* 310, 314–317

42 Lagouge, M. *et al.* (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell* 127, 1109–1122

43 Ling, C. *et al.* (2007) Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. *J. Clin. Invest.* 117, 3427–3435

44 Liang, P. *et al.* (1997) Increased prevalence of mitochondrial DNA deletions in skeletal muscle of older individuals with impaired glucose tolerance: possible marker of glycemic stress. *Diabetes* 46, 920–923

45 Barroso, I. *et al.* (2006) Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. *Diabetologia* 49, 501–505

46 Ukropcova, B. *et al.* (2005) Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. *J. Clin. Invest.* 115, 1934–1941

47 Hulver, M.W. *et al.* (2005) Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metab.* 2, 251–261

48 Hoppeler, H. and Fluck, M. (2003) Plasticity of skeletal muscle mitochondria: structure and function. *Med. Sci. Sports Exerc.* 35, 95–104

49 Levine, J.A. *et al.* (2005) Interindividual variation in posture allocation: possible role in human obesity. *Science* 307, 584–586

50 Hamilton, M.T. *et al.* (2007) Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes* 56, 2655–2667

51 Levine, J.A. *et al.* (2008) The role of free-living daily walking in human weight gain and obesity. *Diabetes* 57, 548–554

52 Andreyev, A.Y. *et al.* (2005) Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc.)* 70, 200–214

53 Choksi, K.B. *et al.* (2004) Oxidatively damaged proteins of heart mitochondrial electron transport complexes. *Biochim. Biophys. Acta* 1688, 95–101

54 Lesnefsky, E.J. *et al.* (2008) Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat. *Biochim. Biophys. Acta* 1777, 1020–1027

55 Schrauwen, P. and Hesselink, M.K. (2004) Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes* 53, 1412–1417

56 Evans, J.L. *et al.* (2002) Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr. Rev.* 23, 599–622

57 Stump, C.S. *et al.* (2003) Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7996–8001

58 Asmann, Y.W. *et al.* (2006) Skeletal muscle mitochondrial functions, mitochondrial DNA copy numbers, and gene transcript profiles in type 2 diabetic and nondiabetic subjects at equal levels of low or high insulin and euglycemia. *Diabetes* 55, 3309–3319

59 Karakelides, H. *et al.* (2007) Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. *Diabetes* 56, 2683–2689

60 Nair, K.S. *et al.* (2008) Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes* 57, 1166–1175

61 De Feyter, H.M. *et al.* (2008) Early or advanced stage type 2 diabetes is not accompanied by *in vivo* skeletal muscle mitochondrial dysfunction. *Eur. J. Endocrinol.* 158, 643–653

62 Bajaj, M. *et al.* (2007) Paradoxical changes in muscle gene expression in insulin-resistant subjects after sustained reduction in plasma free fatty acid concentration. *Diabetes* 56, 743–752

63 Toledo, F.G. *et al.* (2008) Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes* 57, 987–994

64 Schrauwen-Hinderling, V.B. *et al.* (2008) The insulin sensitizing effect of rosiglitazone in type 2 diabetes mellitus patients does not require improved *in vivo* muscle mitochondrial function. *J. Clin. Endocrinol. Metab.* 93, 2917–2921

65 Wredenberg, A. *et al.* (2006) Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. *Biochem. Biophys. Res. Commun.* 350, 202–207

66 Pospisilik, J.A. *et al.* (2007) Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell* 131, 476–491

67 Handschin, C. *et al.* (2007) Abnormal glucose homeostasis in skeletal muscle-specific PGC-1α knockout mice reveals skeletal muscle-pancreatic β cell crosstalk. *J. Clin. Invest.* 117, 3463–3474

68 Vianna, C.R. *et al.* (2006) Hypomorphic mutation of PGC-1β causes mitochondrial dysfunction and liver insulin resistance. *Cell Metab.* 4, 453–464

69 Calvo, J.A. *et al.* (2008) Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. *J. Appl. Physiol.* 104, 1304–1312

70 de Wilde, J. *et al.* (2008) Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. *Physiol. Genomics* 32, 360–369

71 Turner, N. *et al.* (2007) Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 56, 2085–2092

72 Hancock, C.R. *et al.* (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7815–7820

73 Koves, T.R. *et al.* (2008) Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab.* 7, 45–56

74 Bangsbo, J. (2000) Muscle oxygen uptake in humans at onset of and during intense exercise. *Acta Physiol. Scand.* 168, 457–464

75 Savage, D.B. *et al.* (2007) Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol. Rev.* 87, 507–520

76 Holland, W.L. *et al.* (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. *Cell Metab.* 5, 167–179

77 Yu, C. *et al.* (2002) Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J. Biol. Chem.* 277, 50230–50236

78 Hegarty, B.D. *et al.* (2003) The role of intramuscular lipid in insulin resistance. *Acta Physiol. Scand.* 178, 373–383