ASIA-PACIFIC JOURNAL OF ENDOCRINOLOGY REVIEW ARTICLE

Insulin Resistance and Mitochondrial Dysfunction in Muscle: Is there a Link?

Lauren E Wright[1], Gregory J Cooney[1,2] and Nigel Turner[1,2]

Affiliations: *[1]Diabetes & Obesity Research Program, Garvan Institute of Medical Research, Darlinghurst, Sydney, Australia and [2]St Vincent's Hospital Clinical School, University of New South Wales, Sydney, Australia*

ABSTRACT

Insulin resistance is a key defect associated with obesity and type 2 diabetes. Although a number of different factors have been proposed to contribute to the development of insulin resistance, studies in humans and rodents indicate that excess accumulation of lipid in insulin-target tissues is strongly associated with impaired insulin action. In recent times, a number of investigations have reported that various markers of mitochondrial metabolism are impaired in skeletal muscle from insulin-resistant individuals. These findings have led to the theory that defective mitochondrial function is an important factor leading to inappropriate lipid accumulation and the development of insulin resistance. In this contribution, we: (1) review the latest literature supporting a role for dysregulated mitochondrial metabolism in the development of insulin resistance; (2) highlight several recent studies that call into question the cause and effect relationship between mitochondrial dysfunction and impaired insulin action; and (3) discuss whether targeting mitochondrial function is an effective strategy for the treatment of insulin resistance and type 2 diabetes.

Keywords: insulin resistance, mitochondrial function, type 2 diabetes, skeletal muscle, lipid metabolism

Correspondence: Nigel Turner, Diabetes & Obesity Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia. e-mail: n.turner@garvan.org.au

INTRODUCTION

In recent times, there has been a dramatic increase in the prevalence of type 2 diabetes (T2D), with more than 350 million people predicted to have this disorder by the year 2030 [1]. T2D is associated with a number of comorbidities including cardiovascular disease, dislipidemia, obesity, and inflammation, often collectively referred to as the metabolic syndrome. A common aspect of these disorders is insulin resistance; defined as a reduced ability of peripheral tissues to respond to physiological levels of insulin. While the exact factors leading to impaired insulin action are not fully elucidated, experimental evidence suggests that one of the earliest defects associated with insulin resistance and T2D is lipid accumulation in non-adipose tissues, particularly muscle and liver [2–6]. Several mechanisms linking lipid metabolites to reductions in insulin action 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 antagonize insulin signaling and glucose uptake, inhibition of enzymes of glucose metabolism, and altered gene transcription [5, 7].

The level of lipid deposition within any given tissue is determined by several factors. Under conditions of elevated lipid availability, increased uptake of fatty acids contributes to enhanced lipid accumulation within tissues [8, 9]. Any impairment in the utilization (oxidation) of lipids should also lead to increased partitioning of lipids into storage pools. Indeed, a popular theory that has emerged in recent years suggests that defects in mitochondrial oxidative metabolism lead to obesity and lipid accumulation, and thus may play an important role in the pathogenesis of insulin resistance. The majority of the studies investigating the potential relationship between mitochondrial dysfunction and insulin resistance have examined skeletal muscle; however, other reports have also shown links between insulin resistance and defects in markers of mitochondrial function (e.g., respiration, mtDNA content) in other tissues such as the heart, adipose tissue, and peripheral blood cells [10–13]. In this review, we will focus on studies in skeletal muscle and highlight recent developments in the literature examining the link between mitochondrial function and insulin action in this tissue.

MITOCHONDRIAL STRUCTURE AND FUNCTION

The mitochondrion is the key site for energy production in cells, providing a platform for the oxidation of

nutrients to produce adenosine triphosphate (ATP) (**Figure 1**). Mitochondria are not static organelles, but instead exist as a dynamic network capable of fusion, fission, and movement throughout the cell. In muscle cells, the mitochondrial network is arranged into two distinct (yet interconnected) pools—the subsarcolemmal (SS) pool near the cell surface and the intermyofibrillar (IMF) pool in the interior of the cell (for reviews, see [14, 15]). It has been suggested that this assembly is important for efficient mitochondrial function; SS mitochondria have greater access to oxygen and metabolic substrates, and the proton gradient generated through substrate oxidation in the SS pool may potentially contribute fuel for ATP synthesis in the IMF pool, where energy demands are highest [15].

The processes regulating mitochondrial function are complex. Mitochondrial biogenesis can be induced rapidly (i.e., within hours) in response to environmental stimuli (e.g. exercise), and this involves a coordinated interaction between the nuclear and mitochondrial genomes. The peroxisome proliferator-activated receptor gamma (PPARγ) coactivator (PGC-1) family of transcriptional coactivators are considered master regulators of mitochondrial metabolism, as they interact with and coactivate key transcription factors (e.g., NRF-1, ERRα, PPARα/δ) that regulate the expression of genes involved in mitochondrial substrate oxidation, fiber-type determination, mitochondrial biogenesis, and mitochondrial function [16, 17].

MITOCHONDRIAL DYSFUNCTION AND ITS ASSOCIATION WITH INSULIN RESISTANCE

Approximately a decade ago, several groups demonstrated that oxidative enzyme activity and lipid oxidation were reduced in the muscle of obese and insulin-resistant subjects [18–20], suggesting that defects in mitochondrial metabolism may be involved in the development of insulin resistance. In 2002, Kelley *et al* [21] showed lower NADH:O_2 oxidoreductase activity and reduced mitochondrial size in the muscle of obese subjects with insulin resistance and/or T2D compared with control subjects. A year later, two important microarray studies reported a coordinated downregulation of genes involved in mitochondrial biogenesis and oxidative phosphorylation in subjects with T2D and non-diabetic individuals with a family history (FH+) of T2D [22, 23], further advancing the mitochondrial dysfunction theory of insulin resistance.

In the subsequent period since these landmark studies, abnormalities in mitochondrial metabolism in muscle have been reported in numerous insulin-resistant states, including obesity, aging, and T2D. In muscle biopsies obtained from insulin-resistant subjects, several markers of mitochondrial function have been reported to be reduced compared with control

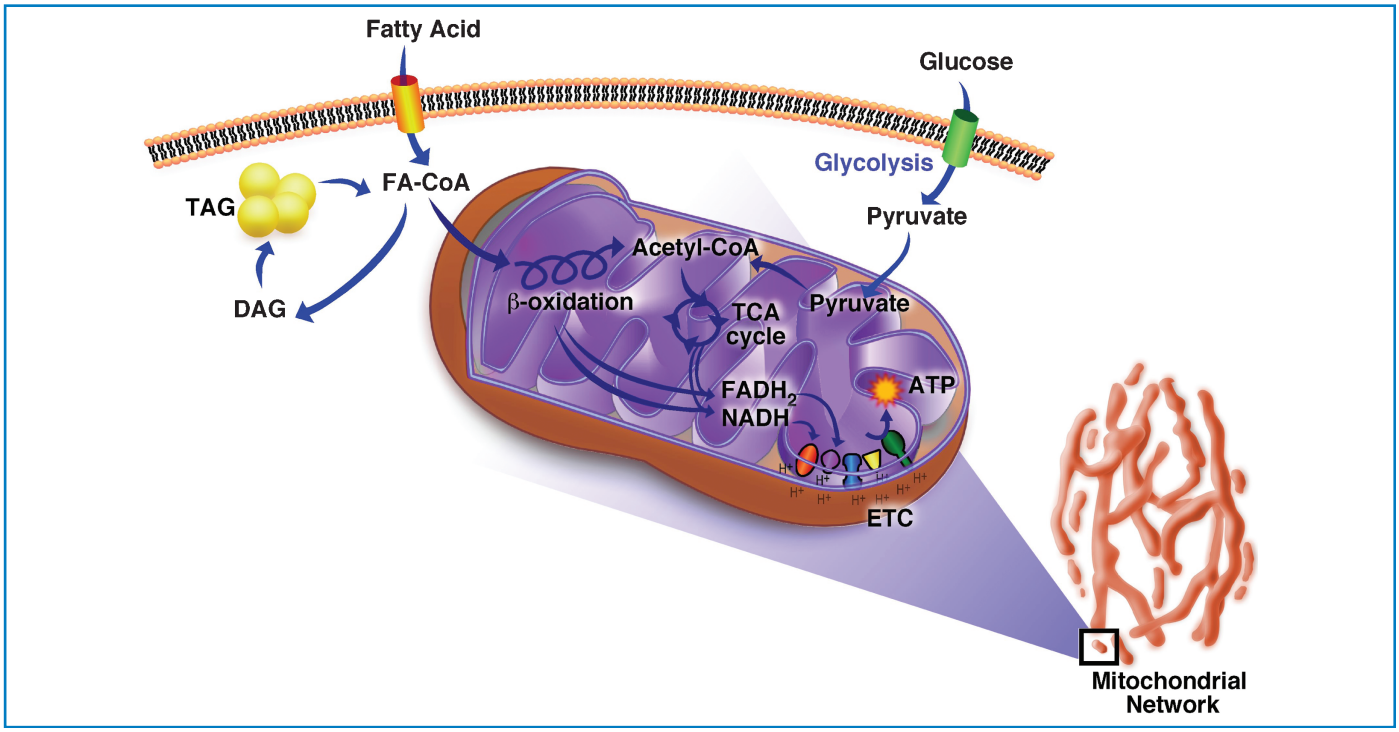

Figure 1. Pathways involved in mitochondrial energy metabolism. During the oxidative metabolism of glucose and fatty acids, reducing equivalents (NADH or $FADH_2$) are generated from glycolysis, the TCA cycle, and β-oxidation. The oxidation of NADH and $FADH_2$ to NAD^+ or FAD drives the movement of electrons along the mitochondrial respiratory chain, while protons are pumped into the intermembrane space. The electrochemical gradient produced by these protons is used by the ATP synthase (complex V of the electron transport chain) to produce ATP

subjects, including mitochondrial DNA (mtDNA) [24, 25], mRNA levels for mitochondrial genes [22, 23, 26–28], oxidative enzyme activities [21, 25, 27], protein expression of respiratory chain subunits [27], and mitochondrial size and density (by electron microscopy) [21, 25, 26]. Several investigators have also measured *in vivo* ATP synthesis rates, phosphocreatine resynthesis rates, or TCA cycle activity with non-invasive magnetic resonance spectroscopy (MRS) to obtain a more functional measure of mitochondrial activity. These *in vivo* studies have shown impaired basal and insulin-stimulated mitochondrial metabolism in insulin-resistant populations of elderly subjects [29], patients with T2D [30–32], and in first-degree relatives of subjects with T2D [33–35]. Interestingly, several studies have shown that, compared with control subjects, functional capacity per mitochondrion appears to be similar [24, 36, 37] or only mildly reduced [38] in insulin-resistant individuals, but when mitochondrial capacity is expressed per unit mass of skeletal muscle, a substantial reduction is seen in insulin-resistant subjects [24, 36, 37]. These studies have therefore suggested that the defects observed in mitochondrial function *in vivo* with MRS may be more strongly related to reductions in mitochondrial number than to substantial intrinsic mitochondrial defects [24, 36, 37]. However, in an elegant recent study, Phielix *et al* [32] measured *in vivo* mitochondrial function (with MRS) and *ex vivo* mitochondrial respiration in muscle from the same T2D subjects and observed that, in this population of subjects, the *in vivo* mitochondrial dysfunction was largely explained by defective mitochondrial substrate oxidation.

One limitation of the above studies is that they simply provide a snapshot of different populations at a given time and therefore cannot delineate whether the observed mitochondrial dysfunction was a primary cause of insulin resistance. However, further evidence for a potential link between mitochondrial dysfunction and insulin resistance comes from intervention studies in humans, rodents, and cell culture systems, where various experimental manipulations cause both defects in mitochondrial function and reductions in insulin sensitivity. For example, in muscle cells where mitochondrial function was disrupted by either genetic (ethidium bromide) or metabolic (e.g., oligomycin) stress, there were multiple insulin signaling abnormalities, and insulin-stimulated glucose metabolism was severely compromised [39, 40]. In humans, infusion of fatty acids for 6–48 h resulted in a robust induction of whole-body insulin resistance and reduced insulin-stimulated ATP synthesis rates and expression of mRNA encoding PGC1α and other mitochondrial genes in muscle [41–43]. Similarly, several rodent studies using established models of insulin resistance have also reported reductions in mitochondrial gene expression, protein expression, ATP synthesis rates, and mitochondrial respiration in skeletal muscle from high-fat fed (3–16 weeks) or genetically obese rodents [44–49]. A further example of the link between mitochondrial dysfunction and insulin resistance comes from a report showing that antiretroviral therapy used to suppress human immunodeficiency virus (HIV) infection reduces mitochondrial DNA copy number in muscle and concomitantly causes insulin resistance [50]. Finally, a recent study has shown that treatment of rodents with atrazine, a herbicide used extensively in the USA, leads to obesity, insulin resistance, and altered mitochondrial morphology and function. This intriguing report suggests that certain environmental factors (e.g., toxins) may play an as yet unappreciated role in the current epidemic of obesity and insulin resistance, through effects on mitochondrial function [51]. Overall, the above studies demonstrate that there are many instances in which perturbations in insulin action and mitochondrial metabolism in muscle occur together.

WHAT FACTORS CONTRIBUTE TO MITOCHONDRIAL DYSFUNCTION IN INSULIN-RESISTANT MUSCLE?

It is clear that mitochondrial dysfunction is frequently linked with insulin resistance, and an important unresolved question is what factor(s) lead to these mitochondrial defects?

Genetic and Epigenetic Factors

There is some evidence in the literature that genetic factors may play a role in determining the metabolic phenotype of human skeletal muscle. For example, primary human skeletal muscle cells in culture display many similar characteristics to the *in vivo* phenotype of the donor subject (e.g., elevated lipogenic gene expression, decreased insulin-stimulated glucose metabolism, and lower lipid oxidation with obesity and T2D), despite being subjected to similar culture conditions for several weeks [52–55]. Of interest, a recent gene expression profiling study reported that, although primary human myotubes established from T2D subjects displayed reduced insulin-stimulated glucose metabolism and impaired lipid oxidation, there was no difference in expression for genes involved in oxidative phosphorylation or mitochondrial biogenesis [56]. While these findings do not support a role for mitochondrial dysfunction being an intrinsic defect in insulin-resistant muscle, it is also possible that different biochemical pathways are not affected equally when primary muscle cells are grown in culture. Further evidence that genetic factors may be involved in the mitochondrial dysfunction observed in insulin resistance comes from studies showing that mutations in mtDNA or nuclear-encoded genes (e.g., *PGC-1α*, *NDUFB6*) are linked to deficits in insulin action and T2D [57–60]. Additionally, the expression of

NDUFB6 and *ATP50*—two genes identified as down-regulated in insulin-resistant skeletal muscle—was also recently reported to be regulated by epigenetic factors (e.g., DNA methylation) [57, 61]. Collectively, the above studies support the notion that some of the metabolic defects observed in skeletal muscle of insulin-resistant and T2D subjects may, in part, be inherited.

Oxidative Stress

Oxidative stress refers to an imbalance between the production of reactive species and antioxidant defenses, leading to damage of macromolecules. Reactive oxygen species (ROS) are produced during metabolic reactions within cells and, accordingly, mitochondria are one of the major sources of ROS [62]. It has been suggested that an oversupply of glucose and fatty acids promotes a state of increased ROS production [63, 64], and as mitochondria are particularly susceptible to oxidative attack [65, 66], this may lead to defects in mitochondrial function. Although there is some support for this premise in the literature [47], Mogensen *et al* [38] reported no difference in lipid peroxidation (4-hydroxy-2-nonenal levels) in muscle biopsies of T2D patients compared with matched control subjects. Furthermore, another recent study conducted *ex vivo* measured ROS production and ATP synthesis in muscle mitochondria isolated from lean control subjects, obese, insulin-resistant subjects, and body mass index (BMI)-matched T2D subjects and could find no clear pattern, suggesting that mitochondrial ROS production was involved in the generation of mitochondrial dysfunction or insulin resistance in these individuals [67]. However, it is worth noting that there is currently no reliable method for determining oxidative stress *in vivo* and, as *ex vivo* measures of ROS production or measurement of one of the multitude of oxidative stress markers may not necessarily reflect what is occurring *in vivo*, care should be taken when interpreting the above findings.

Altered Mitochondrial Dynamics

A breakdown or inefficiency in the mitochondrial network may play a role in the pathogenesis of insulin resistance in skeletal muscle. Mitochondrial remodeling is a complex process of breaking and reforming the double membrane, which is mediated by fusion and fission proteins. Reductions in key proteins mediating these dynamic processes have been reported in insulin-resistant and obese states [68, 69]. For example, the expression of mitofusin 2 (MFN2), a fusion protein that appears to have additional roles apart from maintenance of the mitochondrial network [68, 70, 71], correlates with the glucose oxidation rate [72] and is reduced in the skeletal muscle of insulin-resistant humans and diabetic Zucker rats [68]. Repression of MFN2 also results in decreased glucose oxidation, cellular respiration, and mitochondrial membrane potential, and causes fragmentation of the mitochondrial network [68], suggesting that the reduced levels of MFN2 observed in obesity may play some role in the pathogenesis of insulin resistance.

Reduced Physical Activity

Exercise potently stimulates mitochondrial biogenesis in skeletal muscle, whereas chronic inactivity is associated with reduced mitochondrial number [73]. Therefore, the mitochondrial defects reported in overweight or obese insulin-resistant subjects could be the result of reduced levels of physical activity or an impaired response to exercise. Obesity and other metabolic disorders have been linked with decreased activity levels and increased sedentary behavior [74–76], whereas many [77–79], but not all [80], reports have shown that subjects with a family history of T2D and obese subjects with T2D have a normal response to exercise training with regard to mitochondrial biogenesis in muscle. Therefore, although it is difficult to answer definitively at present, it is likely that low levels of physical activity partially contribute to the mitochondrial dysfunction observed in insulin-resistant subjects.

Insulin Resistance

A number of recent reports have shown that mitochondrial function is directly influenced by insulin, and thus it has been hypothesized that mitochondrial defects may be secondary to insulin resistance. For example, insulin infusion increases markers of mitochondrial metabolism (e.g., enzyme activity, ATP production, mitochondrial gene expression) in muscle, with this response being blunted in insulin-resistant T2D patients [81, 82]. The converse is also observed, with acute insulin removal from subjects with type 1 diabetes leading to reductions in mitochondrial ATP production and expression of mitochondrial genes in skeletal muscle [83]. These studies provide evidence that insulin can affect mitochondrial gene expression and function, perhaps indicating that decreased mitochondrial capacity may arise, in part, as a consequence of insulin resistance. It should be noted, however, that extended periods (7–8 h) of high insulin have been used in the above studies, and it is unclear whether normal postprandial insulin excursions (3–4 h) have a similar effect on mitochondrial metabolism.

MITOCHONDRIAL DYSFUNCTION IS NOT ALWAYS LINKED TO INSULIN RESISTANCE

Despite the large number of studies in which dysfunctional mitochondria have been associated with insulin resistance, conflict still exists within the field. In fact, there is a growing list of recent studies in both humans and rodents that directly challenge the notion

that defects in mitochondrial function are an essential part of the link between lipid accumulation (obesity) and insulin resistance.

Human Studies

De Feyter *et al* [84] recently examined post-exercise phosphocreatine recovery kinetics with MRS as an index of mitochondrial oxidative capacity in muscle from obese patients in either early or advanced stages of T2D. Mitochondrial function was found to be similar between both groups of T2D patients and normoglycemic control subjects matched for age, body composition, and habitual physical activity levels [85]. Similarly, in another group of well-controlled T2D patients, resting and maximal ATP turnover (measured with MRS) in muscle were not impaired compared with physical activity-, age-, and weight-matched control subjects [86]. Nair *et al* [87] recently reported that Asian Indians were more insulin resistant than their age-, sex-, and BMI-matched North American counterparts, but at the same time exhibited higher mtDNA content, elevated expression of genes involved in oxidative phosphorylation, increased oxidative enzyme activity, and greater mitochondrial ATP production rates in muscle. Furthermore, within the Asian Indians, markers of mitochondrial oxidative capacity were not different in subjects with or without T2D, even though the latter group had reduced insulin sensitivity and higher muscle lipid levels compared with individuals without T2D [87]. These studies provide evidence that, at least in these populations, insulin resistance cannot be explained by mitochondrial dysfunction in skeletal muscle.

Several human intervention studies have also shown that changes in insulin sensitivity can occur in the absence of improvements in muscle mitochondrial function. For instance, dietary restriction in overweight and obese subjects resulted in improved insulin sensitivity in the absence of any measurable change in mtDNA, cardiolipin content, or NADH-oxidase activity and, in fact, a small decrease in mitochondrial size was observed in these subjects [88]. One week of treatment with the anti-lipolytic agent acipimox improved insulin sensitivity in insulin-resistant subjects with a family history of T2D, but resulted in decreased mitochondrial gene expression in muscle [89]. Rosiglitazone treatment for 8 weeks also induced a significant improvement in insulin sensitivity without altering *in vivo* mitochondrial function (phosphocreatine recovery rates) in the muscle of overweight patients with T2D [90].

Rodent Studies

A number of investigators have used gene-manipulated mice to test directly whether alterations in mitochondrial function are linked to changes in insulin sensitivity. Mitochondrial transcription factor A plays an important role in transcription of the mitochondrial genome and, in mice with a deletion of this protein, there is a marked impairment in mitochondrial oxidative capacity in muscle [91]. However, these animals showed improved glucose clearance during a glucose tolerance test and normal insulin-stimulated glucose uptake in isolated muscle strips [91]. In another study, a gene expression pattern of mitochondrial oxidative phosphorylation deficiency similar to that observed in human insulin resistance [22, 23] was produced by conditional deletion of apoptosis-inducing factor in muscle, yet these mice displayed reduced adiposity, were insulin sensitive, and protected against the deleterious effects of a high-fat diet [92]. Defects in markers of mitochondrial metabolism in muscle have also been produced through muscle-specific deletion of PGC-1α or loss-of-function mutation of PGC-1β; however, in these animals, insulin sensitivity in muscle is preserved or in fact slightly improved compared with control mice [93, 94]. Interestingly, in independently generated mice with muscle-specific overexpression of PGC-1α, markers of mitochondrial function display the expected increase, yet these animals display impaired insulin sensitivity because of either excessive fatty acid delivery into muscle [95] or decreased GLUT4 expression [96]. In another recent study, mitochondrial function was studied during the progression from insulin resistance to T2D in Zucker diabetic fatty (ZDF) rats, with normal *in vivo* muscle oxidative capacity and improved activity of enzymes involved in lipid oxidation observed in the ZDF rats, despite the disturbances in glucose metabolism [84]. Overall, the studies in gene-manipulated mice have failed to demonstrate a clear link between mitochondrial function and insulin action. One must exercise caution when interpreting the data from these mice, however, as they represent an extreme situation in which there is a complete lack of or substantial increase in the content/function of a specific protein; therefore, it is possible that the phenotype (or lack thereof) may be partially explained by compensatory adaptations (e.g., activation of adenosine monophosphate (AMP)-activated protein kinase or increase in some other lipid metabolism pathway) induced by these manipulations [91, 95].

Several groups, including our own, have also conducted dietary studies in rodents, demonstrating that high-fat feeding significantly increases mitochondrial fatty acid oxidative capacity, enzyme activity, and protein expression, at the same time as inducing insulin resistance at the whole-body and muscle level [97–99]. Interestingly, Koves *et al* [100] have put forward the theory that, under conditions of increased lipid availability, the increase in mitochondrial fatty acid oxidation results in the generation of incomplete fatty acid oxidation products, which then contribute to the insulin-resistant state. This provocative theory

still requires further experimental substantiation, particularly in light of the recent findings of Bruce *et al* [101], in which *in vivo* skeletal muscle overexpression of CPT-1 increased fatty acid oxidation and ameliorated diet-induced insulin resistance.

Thus, although there is substantial literature showing an association between mitochondrial dysfunction and insulin resistance in lean and obese subjects, there are many instances in which alterations in muscle mitochondrial function are not accompanied by the ''predicted'' changes in insulin sensitivity. Obviously, there are many experimental variables that may explain the differences observed between studies, such as the ethnicity and fitness level of the patient population studied, the muscle group examined, the specific technique used to measure mitochondrial function, and factors relating to the dietary regime (e.g., duration of feeding, fat content, and composition).

CAN MITOCHONDRIA BE TARGETED FOR THE TREATMENT OF INSULIN RESISTANCE?

It is clear from the above section that there is still a great deal of controversy surrounding the precise role that mitochondria may or may not play in the development of insulin resistance. This does not, however, preclude mitochondria from being a potential therapeutic target for the treatment of insulin resistance. Indeed, there are many instances in which interventions improve mitochondrial function and enhance insulin sensitivity. One obvious example is exercise, which robustly stimulates mitochondrial biogenesis in muscle and improves insulin action [77, 79, 102, 103]. Caloric restriction has also been shown to have an insulin-sensitizing effect [104, 105] and to improve markers of mitochondrial function in muscle [106, 107]. Calorie restriction is thought to exert many of its beneficial effects through the stimulation of SIRT1, and activators of SIRT1 (e.g., resveratrol and more potent specific activators) have been shown to enhance mitochondrial metabolism in several tissues and to improve insulin sensitivity in a number animal models of insulin resistance and T2D [108–110]. Agonists for the nuclear receptor PPARδ have also been shown to increase mitochondrial fatty acid oxidation in muscle and improve insulin action in high-fat fed and genetically obese rodents [111, 112].

Although these studies all suggest that enhancing mitochondrial function is an effective treatment for insulin resistance, it is interesting to note that several insulin-sensitizing agents, including metformin, thiazolidinediones, and berberine, all appear to exert their effects, in part, by inhibiting mitochondrial respiratory Complex 1. Specifically, inhibition of Complex I by these agents leads to alterations in the intracellular AMP/ATP ratio and activation of AMPK in several tissues (e.g., liver, muscle, adipose) [108,113–118]. The subsequent interaction of AMPK with its multiple downstream targets [119] is then likely responsible for many, but not all, of the beneficial metabolic effects of these compounds. Chronically, one of the effects of AMPK activation is stimulation of mitochondrial biogenesis in muscle and, while this has been observed for metformin [117] and pioglitazone [120, 121], a number of studies have reported that rosiglitazone [90, 122] does not increase mitochondrial function. The reason why pioglitazone and rosiglitazone have discordant effects on mitochondrial biogenesis, while both improving insulin sensitivity, is intriguing and warrants further investigation.

CONCLUSIONS AND FINAL REMARKS

The mitochondrial dysfunction theory of insulin resistance proposes that defects in mitochondrial metabolism are important in the pathogenesis of insulin resistance. Specifically, a reduced capacity for mitochondrial lipid oxidation has been suggested to be a major factor leading to the build up of deleterious lipid intermediates, which subsequently impair insulin action. There are a number of unresolved issues with this theory, however. For example, it is unclear whether defects in mitochondrial function observed in insulin-resistant individuals are inherited, are acquired (e.g., due to low physical activity or caloric excess), or develop secondary to the insulin resistance itself. Indeed, even though mitochondrial defects are present in lean individuals with a family history of T2D [26, 33–35], these studies have been conducted in already insulin-resistant subjects and, accordingly, cannot resolve whether mitochondrial abnormalities were a primary defect or occurred secondary to or in parallel with this insulin resistance. A long-term longitudinal study in humans examining changes in mitochondrial function and insulin sensitivity in individuals over time may be the only way to determine the cause and effect relationship between mitochondrial dysfunction and insulin resistance, although such an experiment would be challenging. Another important issue that needs to be considered is whether the reported decreases 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 [123]. Under resting conditions, the rate of oxygen utilization in muscle is low; and even conservative calculations for extremely unfit individuals suggest that muscle has an enormous capacity to increase substrate oxidation over basal levels [99]. This substantial ''spare'' capacity to elevate substrate oxidation in muscle brings into question whether mitochondrial deficiencies observed in insulin-resistant subjects would have any impact on the rate of fatty acid oxidation when energy requirements are relatively low (e.g., normal free-living conditions). Despite these unanswered questions, the fact that a number of compounds that influence mitochondrial

metabolism also have beneficial effects on insulin action indicates that developing strategies to manipulate the function of this important organelle should be explored for the development of future therapies for insulin resistance and its associated metabolic disorders.

Disclosure: Research on mitochondrial metabolism in the laboratories of LEW, GJC, and NT 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. LEW is supported by a University of New South Wales Postgraduate Award. NT is supported by a Career Development Award, and GJC by a Research Fellowship from the NHMRC. LEW, GJC, and NT declare no conflicts of interest.

Disclosures: The authors have no financial conflicts of interest relevant to the article to disclose.

Acknowledgments: We would like to thank Lauren McKnight for assistance with the preparation of the figure.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047–1053.
2. Pan DA, Lillioja S, Kriketos AD, *et al*. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997;46:983–988.
3. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes*. 2002;51:2005–2011.
4. Adams JM, 2nd, Pratipanawatr T, Berria R, *et al*. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes*. 2004;53:25–31.
5. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev*. 2007;87:507–520.
6. Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol*. 2008;19:235–241.
7. Hegarty BD, Furler SM, Ye J, Cooney GJ, Kraegen EW. The role of intramuscular lipid in insulin resistance. *Acta Physiol Scand*. 2003;178:373–383.
8. Hegarty BD, Cooney GJ, Kraegen EW, Furler SM. Increased efficiency of fatty acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-resistant rats. *Diabetes*. 2002;51:1477–1484.
9. Bonen A, Parolin ML, Steinberg GR, *et al*. 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*. 2004; 18:1144–1146.
10. Choo HJ, Kim JH, Kwon OB, *et al*. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. *Diabetologia*. 2006;49: 784–791.
11. Bugger H, Abel ED. Molecular mechanisms for myocardial mitochondrial dysfunction in the metabolic syndrome. *Clin Sci (Lond)*. 2008;114:195–210.
12. Song J, Oh JY, Sung YA, Pak YK, Park KS, Lee HK. Peripheral blood mitochondrial DNA content is related to insulin sensitivity in offspring of type 2 diabetic patients. *Diabetes Care*. 2001;24:865–869.
13. Park KS, Lee KU, Song JH, *et al*. Peripheral blood mitochondrial DNA content is inversely correlated with insulin secretion during hyperglycemic clamp studies in healthy young men. *Diabetes Res Clin Pract*. 2001;52:97–102.
14. Westermann B. Molecular machinery of mitochondrial fusion and fission. *J Biol Chem*. 2008;283:13501–13505.
15. Skulachev VP. Mitochondrial filaments and clusters as intracellular power-transmitting cables. *Trends Biochem Sci*. 2001; 26:23–29.
16. Arany Z. PGC-1 coactivators and skeletal muscle adaptations in health and disease. *Curr Opin Genet Dev*. 2008;18:426–434.
17. Scarpulla RC. Transcriptional paradigms in Mammalian mitochondrial biogenesis and function. *Physiol Rev*. 2008;88: 611–638.
18. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;279:E1039–1044.
19. Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *Faseb J*. 1999;13:2051–2060.
20. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol*. 1999;277: E1130–1141.
21. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*. 2002;51:2944–2950.
22. Patti ME, Butte AJ, Crunkhorn S, *et al*. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA*. 2003;100:8466–8471.
23. Mootha VK, Lindgren CM, Eriksson KF, *et al*. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genet*. 2003;34:267–273.
24. Boushel R, Gnaiger E, Schjerling P, Skovbro M, Kraunsoe R, Dela F. Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. *Diabetologia*. 2007;50:790–796.
25. Ritov VB, Menshikova EV, He J, Ferrell RE, Goodpaster BH, Kelley DE. Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes*. 2005;54:8–14.
26. Morino K, Petersen KF, Dufour S, *et al*. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J Clin Invest*. 2005;115:3587–3593.
27. Heilbronn LK, Gan SK, Turner N, Campbell LV, Chisholm DJ. Markers of mitochondrial biogenesis and metabolism are lower in overweight and obese insulin-resistant subjects. *J Clin Endocrinol Metab*. 2007;92:1467–1473.
28. Skov V, Glintborg D, Knudsen S, *et al*. Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. *Diabetes*. 2007;56:2349–2355.
29. Petersen KF, Befroy D, Dufour S, *et al*. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science*. 2003;300:1140–1142.
30. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, *et al*. Impaired *in vivo* mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia*. 2007;50:113–120.
31. Szendroedi J, Schmid AI, Chmelik M, *et al*. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. *PLoS Med*. 2007;4:e154.
32. Phielix E, Schrauwen-Hinderling VB, Mensink M, *et al*. Lower intrinsic ADP-stimulated mitochondrial respiration underlies *in vivo* mitochondrial dysfunction in muscle of male type 2 diabetic patients. *Diabetes*. 2008;57:2943–2949.
33. Befroy DE, Petersen KF, Dufour S, *et al*. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. *Diabetes*. 2007;56:1376–1381.

34. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004;350:664–671.
35. Petersen KF, Dufour S, Shulman GI. Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. *PLoS Med*. 2005;2:e233.
36. Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. 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*. 2006;55:2277–2785.
37. Holloway GP, Thrush AB, Heigenhauser GJ, *et al*. Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. *Am J Physiol Endocrinol Metab*. 2007;292:E1782–1789.
38. Mogensen M, Sahlin K, Fernstrom M, *et al*. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes*. 2007;56:1592–1599.
39. Lim JH, Lee JI, Suh YH, Kim W, Song JH, Jung MH. Mitochondrial dysfunction induces aberrant insulin signalling and glucose utilisation in murine C2C12 myotube cells. *Diabetologia*. 2006;49:1924–1936.
40. Park SY, Choi GH, Choi HI, Ryu J, Jung CY, Lee W. Depletion of mitochondrial DNA causes impaired glucose utilization and insulin resistance in L6 GLUT4myc myocytes. *J Biol Chem*. 2005;280:9855–9864.
41. Brehm A, Krssak M, Schmid AI, Nowotny P, Waldhausl W, Roden M. Increased lipid availability impairs insulin-stimulated ATP synthesis in human skeletal muscle. *Diabetes*. 2006; 55:136–140.
42. Hoeks J, Hesselink MK, Russell AP, *et al*. Peroxisome proliferator-activated receptor-gamma coactivator-1 and insulin resistance: acute effect of fatty acids. *Diabetologia*. 2006;49: 2419–2426.
43. Richardson DK, Kashyap S, Bajaj M, *et al*. 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*. 2005;280:10290–10297.
44. Sparks LM, Xie H, Koza RA, *et al*. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes*. 2005;54:1926–1933.
45. Lionetti L, Mollica MP, Crescenzo R, *et al*. Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. *Int J Obes (Lond)*. 2007;31:1596–1604.
46. Jove M, Salla J, Planavila A, *et al*. Impaired expression of NADH dehydrogenase subunit 1 and PPARgamma coactivator-1 in skeletal muscle of ZDF rats: restoration by troglitazone. *J Lipid Res*. 2004;45:113–123.
47. Bonnard C, Durand A, Peyrol S, *et al*. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest*. 2008;118:789–800.
48. Yerby B, Deacon R, Beaulieu V, Liang J, Gao J, Laurent D. Insulin-stimulated mitochondrial adenosine triphosphate synthesis is blunted in skeletal muscles of high-fat-fed rats. *Metabolism*. 2008;57:1584–1590.
49. Laurent D, Yerby B, Deacon R, Gao J. Diet-induced modulation of mitochondrial activity in rat muscle. *Am J Physiol Endocrinol Meta*b2007;293:E1169–1177.
50. Fleischman A, Johnsen S, Systrom DM, *et al*. Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am J Physiol Endocrinol Metab*. 2007;292:E1666–1673.
51. Lim S, Ahn SY, Song IC, *et al*. Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. *PLoS One*. 2009;4:e5186.
52. Ukropcova B, McNeil M, Sereda O, *et al*. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. *J Clin Invest*. 2005;115:1934–1941.
53. Hulver MW, Berggren JR, Carper MJ, *et al*. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. *Cell Metab*. 2005;2:251–261.
54. Gaster M, Petersen I, Hojlund K, Poulsen P, Beck-Nielsen H. The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity. *Diabetes*. 2002;51:921–927.
55. Gaster M, Rustan AC, Aas V, Beck-Nielsen H. Reduced lipid oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin: evidence from cultured myotubes. *Diabetes*. 2004;53:542–548.
56. Frederiksen CM, Hojlund K, Hansen L, *et al*. Transcriptional profiling of myotubes from patients with type 2 diabetes: no evidence for a primary defect in oxidative phosphorylation genes. *Diabetologia*. 2008;51:2068–2077.
57. Ling C, Poulsen P, Simonsson S, *et al*. Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. *J Clin Invest*. 2007;117:3427–3435.
58. Barroso I, Luan J, Sandhu MS, *et al*. Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. *Diabetologia*. 2006;49:501–505.
59. Lindroos MM, Majamaa K, Tura A, *et al*. m.3243A>G mutation in mitochondrial DNA leads to decreased insulin sensitivity in skeletal muscle and to progressive beta-cell dysfunction. *Diabetes*. 2009;58:543–549.
60. Szendroedi J, Schmid AI, Meyerspeer M, *et al*. Impaired mitochondrial function and insulin resistance of skeletal muscle in mitochondrial diabetes. *Diabetes Care*. 2009;32:677–679.
61. Ronn T, Poulsen P, Tuomi T, *et al*. Genetic variation in ATP5O is associated with skeletal muscle ATP50 mRNA expression and glucose uptake in young twins. *PLoS One*. 2009;4:e4793.
62. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc)*. 2005;70:200–214.
63. Schrauwen P, Hesselink MK. Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes. *Diabetes*. 2004;53: 1412–1417.
64. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev*. 2002;23:599–622.
65. Choksi KB, Boylston WH, Rabek JP, Widger WR, Papaconstantinou J. Oxidatively damaged proteins of heart mitochondrial electron transport complexes. *Biochim Biophys Acta*. 2004;1688:95–101.
66. Lesnefsky EJ, Hoppel CL. Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat. *Biochim Biophys Acta*. 2008;1777:1020–1027.
67. Abdul-Ghani MA, Jani R, Chavez A, Molina-Carrion M, Tripathy D, Defronzo RA. Mitochondrial reactive oxygen species generation in obese non-diabetic and type 2 diabetic participants. *Diabetologia*. 2009;52:574–582.
68. Bach D, Pich S, Soriano FX, *et al*. Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. *J Biol Chem*. 2003;278:17190–17197.
69. de Brito OM, Scorrano L. Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. *Antioxid Redox Signal*. 2008;10:621–633.
70. Chen KH, Guo X, Ma D, *et al*. Dysregulation of HSG triggers vascular proliferative disorders. *Nature Cell Biol*. 2004;6:872–883.
71. Neuspiel M, Zunino R, Gangaraju S, Rippstein P, McBride H. Activated mitofusin 2 signals mitochondrial fusion, interferes with Bax activation, and reduces susceptibility to radical induced depolarization. *J Biol Chem*. 2005;280:25060–25070.
72. Mingrone G, Manco M, Calvani M, Castagneto M, Naon D, Zorzano A. Could the low level of expression of the gene encoding skeletal muscle mitofusin-2 account for the metabolic inflexibility of obesity? *Diabetologia*. 2005;48:2108–2114.

73. Hoppeler H, Fluck M. Plasticity of skeletal muscle mitochondria: structure and function. *Med Sci Sports Exerc*. 2003;35:95–104.
74. Levine JA, Lanningham-Foster LM, McCrady SK, *et al*. Interindividual variation in posture allocation: possible role in human obesity. *Science*. 2005;307:584–586.
75. Levine JA, McCrady SK, Lanningham-Foster LM, Kane PH, Foster RC, Manohar CU. The role of free-living daily walking in human weight gain and obesity. *Diabetes*. 2008;57:548–554.
76. Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*. 2007;56:2655–2667.
77. Ostergard T, Andersen JL, Nyholm B, *et al*. 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*. 2006;290:E998–1005.
78. Mogensen M, Vind BF, Hojlund K, Beck-Nielsen H, Sahlin K. Maximal lipid oxidation in patients with type 2 diabetes is normal and shows an adequate increase in response to aerobic training. *Diabetes Obes Metab*. 2009. Epub ahead of print
79. Bruce CR, Kriketos AD, Cooney GJ, Hawley JA. Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type 2 diabetes. *Diabetologia*. 2004;47:23–30.
80. De Filippis E, Alvarez G, Berria R, *et al*. Insulin-resistant muscle is exercise resistant: evidence for reduced response of nuclear-encoded mitochondrial genes to exercise. *Am J Physiol Endocrinol Metab*. 2008;294:E607–614.
81. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci USA*. 2003;100:7996–8001.
82. Asmann YW, Stump CS, Short KR, *et al*. 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*. 2006;55:3309–3319.
83. Karakelides H, Asmann YW, Bigelow ML, *et al*. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. *Diabetes*. 2007;56: 2683–2689.
84. De Feyter HM, Lenaers E, Houten SM, *et al*. Increased intramyocellular lipid content but normal skeletal muscle mitochondrial oxidative capacity throughout the pathogenesis of type 2 diabetes. *Faseb J*. 2008;22:3947–3955.
85. De Feyter HM, van den Broek NM, Praet SF, Nicolay K, van Loon LJ, Prompers JJ. Early or advanced stage type 2 diabetes is not accompanied by *in vivo* skeletal muscle mitochondrial dysfunction. *Eur J Endocrinol*. 2008;158:643–653.
86. Trenell MI, Hollingsworth KG, Lim EL, Taylor R. Increased daily walking improves lipid oxidation without changes in mitochondrial function in type 2 diabetes. *Diabetes Care*. 2008; 31:1644–1649.
87. Nair KS, Bigelow ML, Asmann YW, *et al*. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. *Diabetes*. 2008;57:1166–1175.
88. Toledo FG, Menshikova EV, Azuma K, *et al*. Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes*. 2008;57:987–994.
89. Bajaj M, Medina-Navarro R, Suraamornkul S, Meyer C, DeFronzo RA, Mandarino LJ. Paradoxical changes in muscle gene expression in insulin-resistant subjects after sustained reduction in plasma free fatty acid concentration. *Diabetes*. 2007;56:743–752.
90. Schrauwen-Hinderling VB, Mensink M, Hesselink MK, Sels JP, Kooi ME, Schrauwen P. 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*. 2008;93:2917–2921.
91. Wredenberg A, Freyer C, Sandstrom ME, *et al*. Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. *Biochem Biophys Res Commun*. 2006;350:202–207.
92. Pospisilik JA, Knauf C, Joza N, *et al*. Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell*. 2007;131:476–491.
93. Handschin C, Choi CS, Chin S, *et al*. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. *J Clin Invest*. 2007;117:3463–3474.
94. Vianna CR, Huntgeburth M, Coppari R, *et al*. Hypomorphic mutation of PGC-1beta causes mitochondrial dysfunction and liver insulin resistance. *Cell Metab*. 2006;4:453–464.
95. Choi CS, Befroy DE, Codella R, *et al*. Paradoxical effects of increased expression of PGC-1alpha on muscle mitochondrial function and insulin-stimulated muscle glucose metabolism. *Proc Natl Acad Sci USA*. 2008;105:19926–19931.
96. Miura S, Kai Y, Ono M, Ezaki O. Overexpression of peroxisome proliferator-activated receptor gamma coactivator-1alpha down-regulates GLUT4 mRNA in skeletal muscles. *J Biol Chem*. 2003;278:31385–31390.
97. de Wilde J, Mohren R, van den Berg S, *et al*. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. *Physiol Genomics*. 2008; 32:360–369.
98. Turner N, Bruce CR, Beale SM, *et al*. 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*. 2007;56:2085–2092.
99. Hancock CR, Han DH, Chen M, *et al*. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc Natl Acad Sci USA*. 2008;105:7815-20
100. Koves TR, Ussher JR, Noland RC, *et al*. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. *Cell Metab*. 2008;7:45–56.
101. Bruce CR, Hoy AJ, Turner N, *et al*. Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high fat diet-induced insulin resistance. *Diabetes*. 2009; 58:550-8.
102. Rimbert V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B. Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. *Faseb J*. 2004;18:737–739.
103. Toledo FG, Menshikova EV, Ritov VB, *et al*. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. *Diabetes*. 2007;56:2142–2147.
104. Larson-Meyer DE, Heilbronn LK, Redman LM, *et al*. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care*. 2006;29:1337–1344.
105. Weiss EP, Racette SB, Villareal DT, *et al*. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. *Am J Clin Nutr*. 2006;84:1033–1042.
106. Civitarese AE, Carling S, Heilbronn LK, *et al*. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. *PLoS Med*. 2007;4:e76.
107. Nisoli E, Tonello C, Cardile A, *et al*. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science*. 2005;310:314–317.
108. Turner N, Li JY, Gosby A, *et al*. Berberine and its more biologically available derivative, dihydroberberine, inhibit mitochondrial respiratory complex I: a mechanism for the action of berberine to activate AMP-activated protein kinase and improve insulin action. *Diabetes*. 2008;57:1414–1418.
109. Milne JC, Lambert PD, Schenk S, *et al*. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature*. 2007;450:712–716.

110. Feige JN, Lagouge M, Canto C, *et al*. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab*. 2008;8:347–358.
111. Tanaka T, Yamamoto J, Iwasaki S, *et al*. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA*. 2003;100:15924–15929.
112. Lee CH, Olson P, Hevener A, *et al*. PPARdelta regulates glucose metabolism and insulin sensitivity. *Proc Natl Acad Sci USA*. 2006;103:3444–3449.
113. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J*. 2000;348(Pt 3): 607–614.
114. Brunmair B, Staniek K, Gras F, *et al*. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? *Diabetes*. 2004;53: 1052–1059.
115. LeBrasseur NK, Kelly M, Tsao TS, *et al*. Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. *Am J Physiol Endocrinol Metab*. 2006;291:E175–181.
116. Saha AK, Avilucea PR, Ye JM, Assifi MM, Kraegen EW, Ruderman NB. Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue *in vivo*. *Biochem Biophys Res Commun*. 2004;314:580–585.
117. Suwa M, Egashira T, Nakano H, Sasaki H, Kumagai S. Metformin increases the PGC-1alpha protein and oxidative enzyme activities possibly via AMPK phosphorylation in skeletal muscle *in vivo*. *J Appl Physiol*. 2006;101:1685–1692.
118. Lee YS, Kim WS, Kim KH, *et al*. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. *Diabetes*. 2006;55:2256–2264.
119. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nature Rev Mol Cell Biol*. 2007;8: 774–785.
120. Coletta DK, Sriwijitkamol A, Wajcberg E, *et al*. Pioglitazone stimulates AMP-activated protein kinase signalling and increases the expression of genes involved in adiponectin signalling, mitochondrial function and fat oxidation in human skeletal muscle *in vivo*: a randomised trial. *Diabetologia*. 2009; 52:723–732.
121. Skov V, Glintborg D, Knudsen S, *et al*. Pioglitazone enhances mitochondrial biogenesis and ribosomal protein biosynthesis in skeletal muscle in polycystic ovary syndrome. *PLoS One*. 2008;3: e2466.
122. Pagel-Langenickel I, Schwartz DR, Arena RA, *et al*. A discordance in rosiglitazone mediated insulin sensitization and skeletal muscle mitochondrial content/activity in type 2 diabetes mellitus. *Am J Physiol Heart Circ Physiol*. 2007;293: H2659-66.
123. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science*. 2005;307:384–387.