Chapter 4

Mitochondrial Metabolism and Insulin Action

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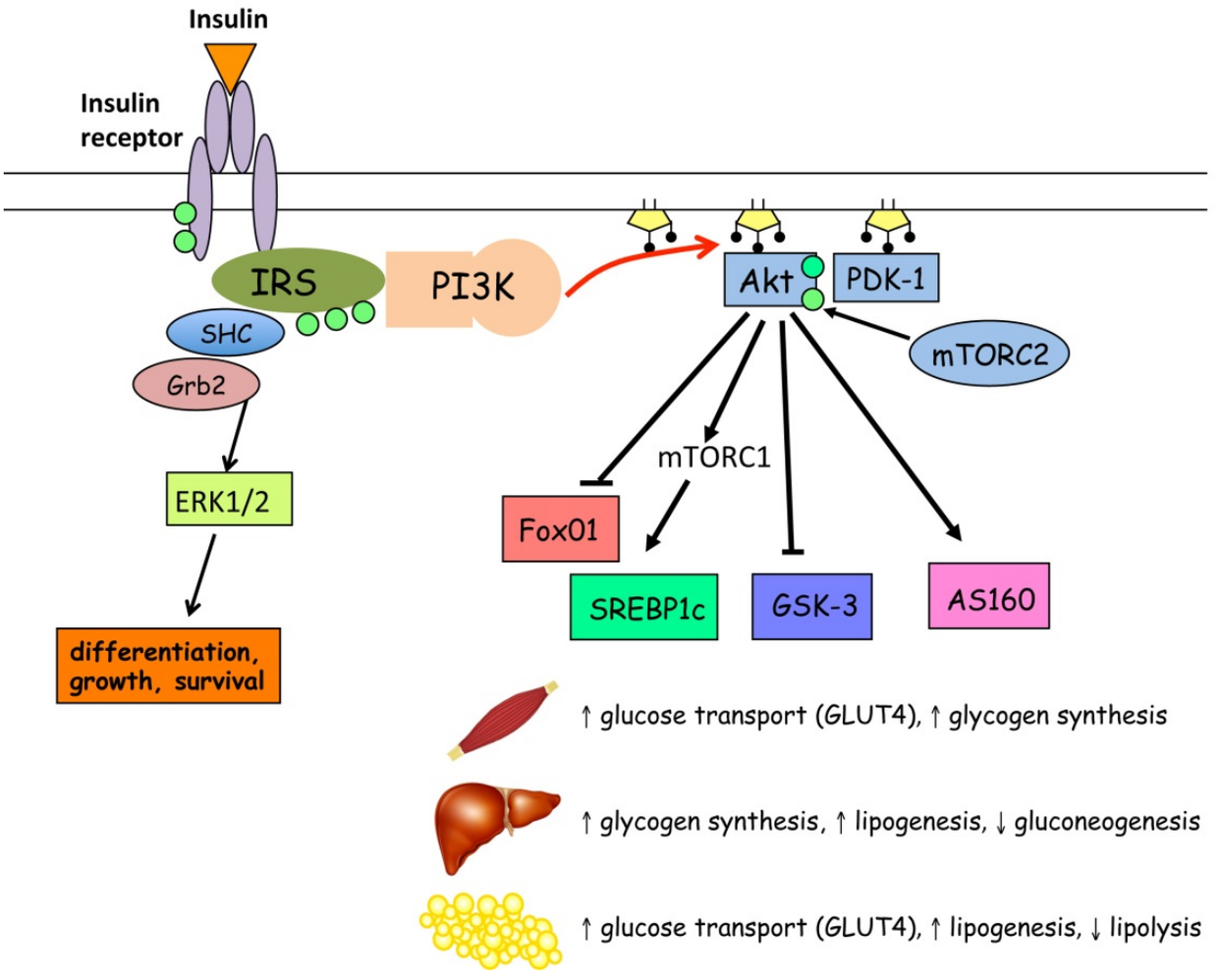
1. Introduction

The major disease epidemics of modern society are not those of contagion, but are the result of lifestyle imposed upon our genetic pre-disposition. Unrestricted access to calorie-dense food, along with a reduction in physical activity, has resulted in a rapid rise in metabolic disorders. One such condition, type 2 diabetes (T2D), has increased dramatically in recent times, with the International Diabetes Foundation estimating that 371 million people world-wide have T2D, with this number expected to increase to greater than 550 million by 2030 (http://www.idf.org/diabetesatlas/5e/Update2012). T2D is characterized by fasting blood glucose levels higher than 7.0 mM or two-hour blood glucose levels higher than 11.1 mM after a glucose tolerance test. T2D rarely occurs in isolation and is frequently associated with a number of comorbidities, including obesity, dyslipidemia, cardiovascular disease, and inflammation, collectively referred to as the metabolic syndrome.

A central aspect of the disorders comprising the metabolic syndrome is insulin resistance; defined as an impaired ability for insulin to regulate fuel metabolism in target tissues. With respect to glucose homeostasis the main insulin-responsive tissues involved are skeletal muscle, liver and adipose tissue. Under normal physiological conditions, insulin is released into the circulation from the beta cells in the islets of Langerhans in the pancreas in response to the ingestion of a meal. Upon binding to its receptor, insulin stimulates a well-described signaling cascade [1] involving the phosphorylation, docking and translocation of a series of signaling molecules, ultimately leading to alterations in specific endpoints of glucose and lipid metabolism (Figure 1):

- In skeletal muscle, insulin promotes the translocation of the glucose transporter GLUT4 to the plasma membrane to increase glucose uptake and also stimulates glycogen synthesis.
- The major hepatic actions of insulin are the promotion of glycogen and lipid synthesis and the suppression of gluconeogenesis.

- In adipose tissue, insulin stimulates GLUT4-mediated glucose uptake and lipid synthesis, and additionally represses lipolysis, leading to net lipid accumulation.



IRS, insulin receptor substrate; SHC, Src Homology 2 domain; GRB2, growth factor receptor-bound protein 2; ERK, extracellular-signal-regulated kinases or classical MAP kinases; PI3K Phosphoinositide 3-kinase; PDK1, phosphoinositide-dependent protein kinase 1; mTORC mammalian target of rapamycin complex; FoxO1 Forkhead box protein O1; SREBP1c sterol regulatory element binding protein 1c; GSK-3, glycogen synthase kinase 3; AS160, 160 kDa Akt substrate.

Figure 1. Insulin signaling pathway. Binding of insulin to the insulin receptor initiates a signaling cascade that involves multiple phosphorylation events (green circles) and leads to alterations in glucose and lipid metabolism.

In the insulin resistant state, the effect of insulin on the above pathways is compromised, leading to insufficient uptake of glucose into tissues and an impaired suppression of hepatic glucose output. To overcome the diminished effectiveness of insulin, the pancreatic beta cells secrete more insulin. The ensuing hyperinsulinemia can adequately compensate for the insulin resistance in most of the population, however in genetically susceptible individuals, the beta cells ultimately fail in the face of the increased workload and this leads to elevated blood

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glucose levels and T2D. Thus insulin resistance can be considered a very early and important player in the pathogenesis of T2D.

At the molecular level, the precise mechanisms responsible for insulin resistance are not fully elucidated. Studies have reported overactivation of stress-related and inflammatory pathways in tissues of insulin resistant humans and rodents. For example, ER stress was shown by the Hotamisligil lab to be present in the liver of obese mice and subsequent studies using chaperones that reduce ER stress revealed improvements in metabolic homeostatsis [2,3]. Oxidative stress has also been implicated in the development of insulin resistance, with studies showing elevated reactive oxygen species generation in insulin resistant cell models, rodents and humans [4-6]. Finally, inflammation in adipose tissue and liver (and to some extent muscle) has been reported in obese, insulin-resistant humans and rodents [7,8]. While the above factors are often described as causative players in the development of insulin resistance, it still remains unresolved whether they are the primary factors leading to diminished insulin action, or if they arise as a consequence of insulin resistance.

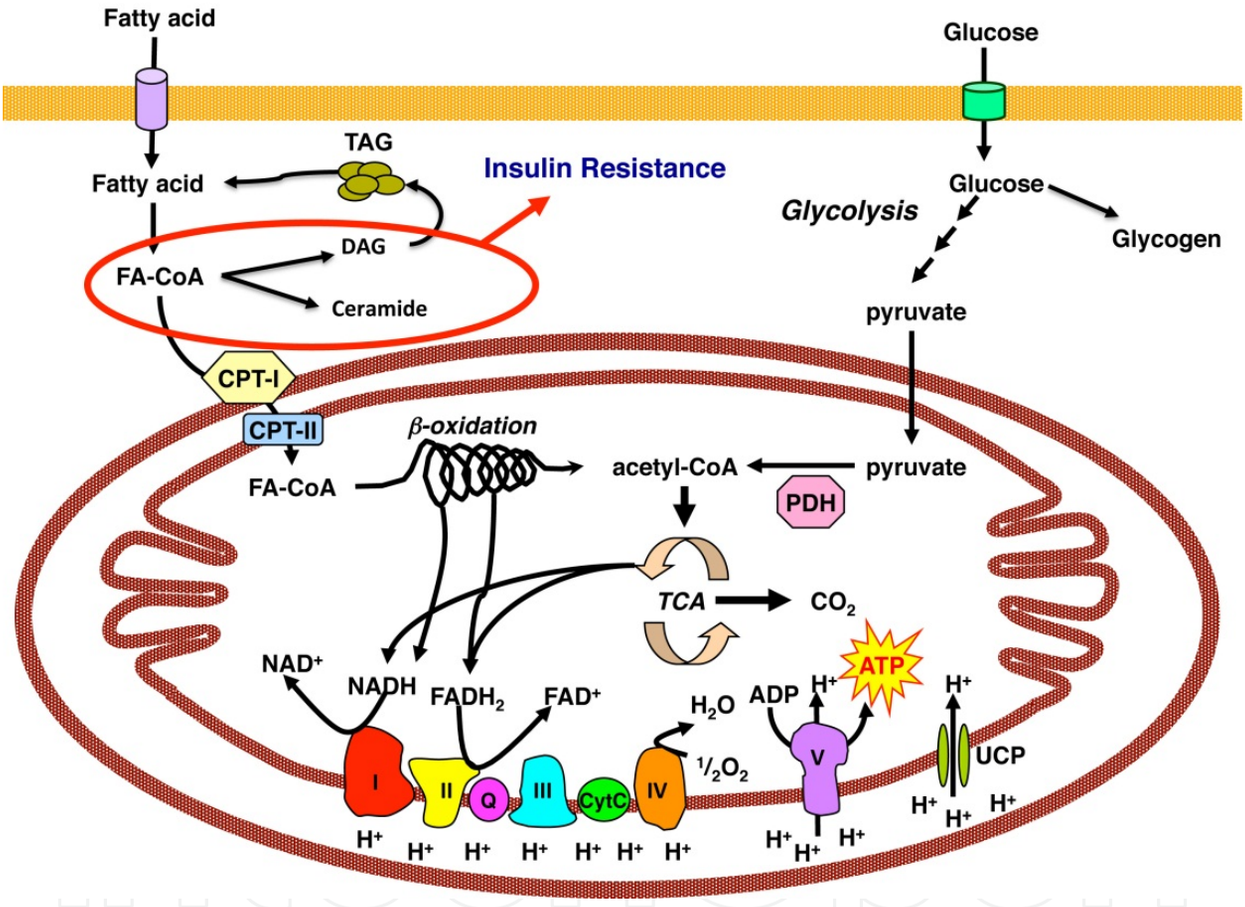
One factor that is one of the earliest defects associated with insulin resistance and T2D is lipid accumulation in non-adipose tissues [9-13]. Under conditions of excess nutrient supply, fatty acids and their metabolites inappropriately spillover into tissues such as skeletal muscle, liver and the heart, precipitating defects in insulin action. More specifically, while elevated triglycerides are frequently reported in tissues of insulin resistant humans and rodents, the accumulation of metabolically active long chain acyl-CoAs (LCACoAs) and other cytosolic lipid metabolites, such as ceramides and diacylglycerol (DAG), are considered to be more directly linked with insulin resistance [9,10]. In support of this, the above lipid metabolites can activate many 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 directly antagonize insulin signal transduction and glucose metabolism pathways [9,10].

The extent of lipid accumulation within any given tissue is determined by several factors. Under conditions of elevated lipid availability, enhanced uptake of fat into tissues contributes to greater lipid deposition [14,15]. This increased uptake is associated with greater expression and/or translocation of fatty acid transport proteins (e.g. CD36). Any impairment in the utilization (oxidation) of lipids would also be predicted to increase partitioning of lipids into storage pools. Indeed, over the last decade a popular theory has emerged suggesting that defects in mitochondrial oxidative metabolism, particularly in skeletal muscle, lead to obesity and lipid accumulation and thus may play an important role in the pathogenesis of insulin resistance and T2D [16].

2. Mitochondrial structure and function

The mitochondrion is the key site for energy production in cells, providing a platform for the oxidation of fuel substrates to produce ATP. During the oxidative metabolism of nutrients (primarily glucose and fatty acids under normal circumstances), 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 electron transport chain coupled to the pumping of protons into the intermembrane space through complex I, III and IV. The electrons are transferred to oxygen at complex IV to produce H_2O. The pumped protons generate an electrochemical gradient across the inner mitochondrial membrane, which is used as the driving force for the ATP synthase (complex V) to produce ATP. The electrochemical gradient may also dissipate through uncoupling proteins (UCP), producing heat in a process referred to as thermogenesis.



TAG Triacylglycerol; DAG Diacylglycerol; PDH Pyruvate dehydrogenase; CPT Carnitine palmitoyltransferase; UCP Uncoupling Protein

Figure 2. 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 protons are pumped into the intermembrane space through complex I, III and 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. Protons can also enter the matrix through uncoupling proteins. Deficiencies in mitochondrial fatty acid oxidation can lead to the buildup of bioactive lipid intermediates (red circle) that can cause insulin resistance.

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2.1. Mitochondrial biogenesis

Mitochondrial function within a given tissues is regulated at a number of different levels, including the number or density of mitochondria. The biogenesis of new mitochondria involves a coordinated interaction between the nuclear and mitochondrial genomes [17]. The mitochondrial genome encodes for 13 protein subunits of the mitochondrial respiratory complexes, as well tRNAs and rRNAs necessary for the translation of the mitochondrial-encoded proteins. The nuclear genome therefore encodes the vast majority of mitochondrial proteins and also encodes the transcription factor responsible for controlling mitochondrial transcription, namely TFAM. Proteins encoded by the nucleus are translated in the cytosol and imported into the appropriate mitochondrial compartments via a suite of import complexes [18]. Thus it is obvious that mitochondrial biogenesis is an extremely complex process, reliant upon the exquisite orchestration of separate genomes and multiple cellular processes.

The master regulators of the mitochondrial biogenic program are the peroxisome proliferator-activated receptor gamma (PPARγ) coactivator (PGC-1) family of transcriptional coactivators. The PGC-1 proteins are promiscuous coactivators that interact with and promote transcriptional activity in the key transcription factors (described below) that regulate the expression of genes involved in mitochondrial substrate oxidation, fibre-type determination, mitochondrial biogenesis and mitochondrial function [17,19]. The PGC-1 proteins do not bind directly to DNA, but instead recruit a wide array of chromatin-remodelling cofactors to transcriptional complexes. PGC-1α was the first described member of this family, initially identified in a screen for activators of PPARγ in brown adipocytes [20]. The other members of the family, PGC-1β and PRC were identified based on sequence homology to PGC-1α [21,22]. Overexpression and knockout studies for PGC-1 proteins have provided clear evidence that these coactivator proteins induce increases in mitochondrial oxidative capacity and promote a switch to a more oxidative fibre type in muscle [23-27].

PGC-1α appears to be the most responsive member of this family, with PGC-1β proposed to be important in the regulation of basal mitochondrial content [28]. Environmental stimuli such as exercise, fasting and cold exposure can induce a rapid increase in PGC-1α expression and activity [19]. PGC-1α also promotes its own expression through a feed-forward mechanism [29]. The activity of PGC-1α is regulated by a number of post-translational mechanisms including acetylation and phosphorylation. The relative level of PGC-1α acetylation is determined by the balance between the activity of the acetyltransferase GCN5 and the NAD^+ dependent deacetylase SIRT1 [30,31]. With respect to phosphorylation, the energy sensing kinase AMPK has been shown to directly phosphorylate PGC-1α and alter its transcriptional activity [32]. Thus in responsive to specific stimuli, changes in the concentrations of key molecules within the intracellular milieu (e.g. NAD^+, adenine nucleotides) results in stimulation of upstream regulators of PGC-1α activity and initiaition of the mitochondrial biogenic cascade. While the role of PGC-1α in regulating mitochondrial biogenesis is well established, recent work from the Spiegelman lab has described a novel splice isoform of PGC-1α that also regulates skeletal muscle hypertrophy [33], indicating there is still much to learn about the biology of the PGC-1 transcriptional coactivator proteins.

2.2. Nuclear transcription factors involved in mitochondrial biogenesis

PGC-1 proteins orchestrate the mitochondrial biogenic program by promoting transcriptional activity through a variety of transcription factors. Nuclear transcription factors bind to specific sequences in gene promoter regions to regulate transcription of a subset of specific genes. The key transcription factors that regulate many of the genes involved in the respiratory chain and other mitochondrial pathways are described below.

2.2.1. Nuclear Respiratory Factor 1 (NRF-1)

NRF-1 plays a crucial role in coordinating nuclear and mitochondrial gene expression. It induces the expression of TFAM, as well as other components of the mitochondrial transcriptional machinery (e.g. TFB1M and TFB2M) [34,35]. NRF-1 also promotes the expression of mitochondrial import proteins that are involved in transporting nuclear-encoded proteins into mitochondria. Forced overexpression of NRF-1 in skeletal muscle in mice, results in increased expression of a subset of mitochondrial proteins, but no net increase in mitochondrial oxidative capacity [36]. Deletion of NRF-1 in mice on the other hand has been shown to be embryonic lethal, due to disruption of mitochondrial function [37].

2.2.2. Nuclear Respiratory Factor 2 (NRF-2/GABP)

Nuclear respiratory factor-2 (NRF-2 humans/GABP mice) is a second critical transcription factor that regulates the expression of proteins involved in mitochondrial function and biogenesis. The target genes of NRF-2 include all respiratory complex IV subunits, TFAM and a range of other proteins involved in mitochondrial transcription and replication [38]. Consistent with the findings in NRF-1 knockout animals, NRF-2 deletion also causes a lethal phenotype, underlying the crucial importance of this transcription factor [39].

2.2.3. Estrogen-Related Receptors (ERR)

The estrogen related receptor family contains 3 members, ERRα, ERRβ, ERRγ. ERRs regulate the expression of a wide array of genes involved in substrate uptake, the TCA cycle, fatty acid oxidation (FAO), oxidative phosphorylation and mitochondrial fusion [40,41]. ERRα knockout mice only display a mild phenotype [42], however deletion of the other two isoforms results in a lethal phenotype [43,44].

2.2.4. Peroxisome-Proliferator Activated Receptors (PPAR)

Similar to the ERR family, PPARs nuclear receptors that exist as 3 separate isoforms PPARα, PPARδ, PPARγ. The expression of PPARs varies markedly across different tissues, with PPARα being highly expressed in liver, PPARδ in skeletal muscle and PPARγ in adipose tissue [45]. PPARs are activated by long-chain polyunsaturated fatty acids and a range of lipid derivatives, and several mitochondrial genes, particularly those involved in fatty acid oxidation, are amongst their gene targets [46,47]. Several lipid lowering (e.g. fibrates) and anti-diabetic drugs (e.g. thiazolidinediones) target the PPAR proteins.

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2.2.5. Yin Yang 1 (YY1)

A range of mitochondrial genes are also regulated by the transcription factor YY1. Puigserver's group have shown that the regulation of mitochondrial oxidative function by mTOR is regulated through YY1 [48] and recently it has been shown that mitochondrial function and morphology are abnormal in mice with muscle-specific YY1 knockout, highlighting an important role for this transcription factor in regulating mitochondrial function [49].

2.3. Mitophagy

In addition to the biogenesis of new organelles, mitochondrial content is also partly determined by the rate of degradation. Indeed, mitochondrial autophagy (or mitophagy) is now recognized as a key quality control process regulating mitochondrial homeostasis [50]. Autophagy is a conserved cellular event in which damaged organelles and proteins are degraded in a two-step process, that first involves the formation of a double membrane structure called the 'autophagosome', followed by the fusion of the autophagosome with lysosomes and the subsequent degradation of the enveloped contents. Mitophagy can be inititiated by a number of events that signal stress within mitochondria, such as opening of the permeability transition pore or fragmentation of mitochondria [51,52]. The list of proteins that are thought to be involved in the mitophagy process (e.g. PINK1, Parkin, Nix) is increasing rapidly [50]. From a physiological perspective, mitophagy plays important roles in several developmental processes, such as red blood cell maturation and the removal of paternal mitochondria following fertilization of the oocyte [53-55]. However, recent evidence also suggests that abnormal mitophagic activity may contribute to the aging process, as well as a number of different diseases, such as neurodegenerative disorders [56].

2.4. Intrinsic factors regulating mitochondrial function

While the number of mitochondria is obviously an important determinant of the oxidative capacity of different tissues, variations in the intrinsic properties of mitochondria are also critical. Mitochondria from different sites in the body can have different capacities for the same process. For example, mitochondria from red slow-twitch and white fast-twitch muscle display very different rates of fatty acid oxidation [57]. This difference is in line with the functional requirements of these muscles, and is likely related to the differences in protein expression of key enzymes in this pathway [58]. In addition to differential expression of proteins within specific pathways, another emerging factor that may influence mitochondrial oxidative capacity is post-translational modifications of mitochondrial enzymes. Following translation, many different facets of protein function (activity, subcellular localization, protein-protein interactions) can be altered by the addition of functional groups to specific residues in the protein. The most well-described PTM is likely phosphorylation, and proteomics screens have revealed widespread phosphorylation of mitochondrial proteins [59]. The activity of specific mitochondrial proteins, such as uncoupling protein 3, has been shown to be directly regulated by phosphorylation [60]. Other recent work has shown that perhaps the most abundant PTM in mitochondria is lysine acetylation. Acetylation involves the transfer of an acetyl group from acetyl-CoA to a lysine residue in specific proteins. More than 30% of mitochondrial proteins

have been shown to be acetylated, and reversible lysine acetylation/deacetylation has been shown to impact on the activity of a large range of mitochondrial enzymes involved in virtually all metabolic pathways within this organelle [61]. In addition to phosphorylation and acetylation, numerous other PTMs (e.g. glutathionylation, nitrosylation, succinylation) have been described as being present in mitochondria [62-64], and their functional importance remains to be determined.

2.5. Mitochondrial dynamics

Mitochondria are not static organelles, but exist largely as a reticular network. Mitochondria are constantly engaged in the process of fusion and fission, providing morphological plasticity to allow adjustments in response to the prevailing cellular stresses and metabolic requirements [65]. Mitochondrial fusion is mediated by the mammalian GTPases mitofusin 1 and mitofusin 2, as well as optic atrophy protein 1 (Opa1). Fusion occurs in a two-step process, which initially involves fusion of the outer membrane (mediated by mitofusins), followed by subsequent fusion of the inner membrane (driven by Opa1) [66,67]. Fission is regulated by another GTPase, dynamin-related protein 1 (Drp1), which resides in the cytosol and is recruited to the mitochondrial surface to engage other key components of the fission machinery (e.g. Fis 1) [68,69]. The fusion process is thought to allow two mitochondria to functionally complement each other through the exchange and repartitioning of their respective components (e.g. copies of the genome, metabolic enzymes and metabolites). Fission on the other hand is important both in the separation of the organelle into daughter cells during cell division and also in isolating and targeting damaged mitochondria for degradation. Collectively the balance of fusion and fission allows mitochondria to form a spectrum of shapes from small individual units to elongated interconnected networks.

In muscle cells, the mitochondrial network is arranged into two discrete, but interconnected pools – the subsarcolemmal (SS) pool near the cell surface, and the intermyofibrillar (IMF) pool in the interior of the cell between myofibres [70-72]. These two pools of mitochondria have been reported to display some differences in their metabolic characteristics, with SS mitochondria appearing to be more responsive to increase their oxidative capacity following an exercise stimuli than IMF mitochondria [57,73]. Despite the differences between mitochondrial pools, it has been proposed that the arrangement of mitochondria may 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 ATP synthesis in the IMF pool, where energy demands are highest during contraction [71].

3. Mitochondrial dysfunction in muscle and its association with insulin resistance

As detailed above, mitochondria represent complex organelles and perturbations in any aspect of mitochondrial regulation and function, could impact on metabolic homeostasis. The

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mitochondrial theory of insulin resistance has developed over the last 10-15 years and is based on the notion that defective mitochondrial metabolism will result in inadequate substrate oxidation, leading to a buildup of lipid metabolites and the subsequent development of insulin resistance. Support for this theory comes from many studies in humans and rodents, which have largely examined skeletal muscle and are reviewed below.

In the late 1990's and early part of last decade, several groups published studies showing that muscle from obese and insulin resistant subjects displayed reduced oxidative enzyme activity [74-76]. Some of these studies also examined lipid oxidation either in muscle homogenates, or by making RQ measurements across the leg, and it was shown that fatty acid oxidation was also decreased in obese, insulin resistant subjects compared to age-matched controls, potentially suggesting that defects in mitochondrial metabolism may be involved in the development of obesity and insulin resistance [74,75]. In 2002, Kelley's group showed that there was lower NADH:O_2 oxidoreductase activity and reduced mitochondrial size, as determined by electron microscopy, in muscle of obese subjects with insulin resistance and/or T2D compared to controls [77]. A year later, two influential microarray studies were published, reporting 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 [78,79]. These microarray studies were considered particularly important, as they documented a reduction in the master regulator of mitochondrial biogenesis, PGC-1α, and thus they provided a mechanism for the reduced oxidative gene expression. They were also important, as they showed that abnormal mitochondrial gene expression could be observed in insulin resistant relatives of patients with T2D and thus may be a pathogenic factor in the 'pre-diabetic' state. Overall the conclusion from these studies was that depressed PGC-1α levels, due to genetic predisposition, physical inactivity, or excessive caloric intake, could lead to a reduction in mitochondrial content, predisposing individuals to develop insulin resistance and T2D.

In the ensuing decade since these landmark studies were published, there has been intense research into this field and one issue that has arisen is how to best measure mitochondrial function/dysfunction. Numerous approaches have been employed, including measurements of parameters in frozen muscle samples (e.g. mRNA, protein content, oxidative enzyme activity and mtDNA), functional assessment of substrate oxidation in fresh samples (e.g. radiolabelled fatty acid oxidation, mitochondrial respiration measurements) and non-invasive magnetic resonance spectroscopy (MRS) with ^{31}P or ^{13}C to determine *in vivo* ATP synthesis rates, phosphocreatine resynthesis rates or TCA cycle activity as an index of mitochondrial function. All these assays provide some indication of mitochondrial function, however they may not always correlate with each other and this needs to be considered when interpreting studies in this area. Details of a number of key studies in this area are presented in the following sections.

In line with the microarray studies noted above, mRNA levels for a variety of mitochondrial genes have been shown to be reduced in muscle biopsies obtained from various insulin resistant populations, including lean insulin-resistant offspring of patients with T2D [80], obese subjects [81], patients with polycystic ovarian syndrome [82] and subjects with estab-

lished T2DM [83,84]. The level of mtDNA was also shown to be lower in both obese, insulin resistant subjects and obese subjects with T2D [85,86]. Heilbronn et al. [81] demonstrated reduced protein expression of respiratory chain subunits in obese insulin-resistant subjects and consistent with these findings, a recent proteomics study comparing lean, obese and T2D subjects, showed patterns of reduced mitochondrial proteins in the insulin-resistant subjects [87]. The activity of specific enzymes involved in oxidative pathways have been reported to be lower in various insulin-resistant populations [81,86,88,89] and additionally electron microscopy studies have reported reduced mitochondrial size and density in insulin-resistant muscle [77,80,86]. Interestingly, in the studies reporting mitochondrial deficiencies, there has been disparate results regarding which population of mitochondria may underlie the functional defects. Ritov et al. [86] reported that the number and functional activity of subsarcolemmal mitochondria was reduced in obesity and T2D, while a more recent study found similar subsarcolemmel mitochondrial content in lean controls, lean insulin-resistant non-diabetic subjects and insulin-resistant T2D subjects, however intermyofibrillar mitochondrial content was reduced in the latter two groups [90]. Differences in mitochondrial function may not only be present within different intramuscular populations, but also between different muscles across the body. Rabol and colleagues used high resolution respirometry to measure mitochondrial function in saponin-permeabilised fibres from m. deltoideus and m. vastus lateralis and observed reduced respiratory capacity only in the leg muscles of type 2 diabetic subjects compared to lean controls [91].

In addition to the above studies, several investigators have also measured *in vivo* mitochondrial function using MRS. Petersen et al. [92] studied lean, healthy elderly subjects using hyperinsulinemic-euglycemic clamps and MRS measurements and observed marked insulin resistance in skeletal muscle of the elderly subjects compared to weight-matched controls. This impairment in insulin action was associated with a 40% reduction in ATP synthesis capacity, and a pronounced accumulation of intramuscular fat. The same group published a paper the following year in which they studied lean insulin-resistant offspring of patients with T2D using the same methods. The insulin-resistant offspring displayed a 60% reduction in insulin-stimulated glucose uptake into muscle and this was again associated with increased intramyocellular lipid and reduced basal mitochondrial ATP synthesis capacity [93]. A subsequent study by Petersen et al. [94] also reported reduced-insulin-stimulated ATP synthesis in first-degree relatives of subjects with T2D and in later work it was shown that family history of T2D was associated with reduced TCA cycle flux [95]. In several other studies, patients with T2D have been shown to have reduced ATP synthesis capacity or phosphocreatine recovery rates, indicative of reduced mitochondrial function in these populations [96-99]. A further interesting case report using MRS showed that a MELAS patient with mtDNA mutations, displayed insulin resistance in muscle association with reduced baseline and insulin-stimulated ATP synthesis capacity [100].

A number of investigations have sought to determine if there is an intrinsic difference in the functional capacity per mitochondrion that may underlie the reductions in mitochondrial function reported with MRS. Some studies examining respiration or fatty acid oxidation in isolated mitochondria or permeabilised muscle fibres, have reported that the functional

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capacity per mitochondrion in insulin resistant and/or type 2 diabetic subjects is similar or only very mildly reduced [85,88,91,101-103] in insulin-resistant individuals, but when normalized to muscle mass, a substantial reduction is seen in insulin-resistant subjects [85,88,101]. These studies therefore only see marked differences when mitochondrial capacity is expressed per unit mass of skeletal muscle and thus indicate that *in vivo* mitochondrial defects observed with MRS may be more strongly related to reductions in mitochondrial number, than to substantial intrinsic mitochondrial defects. However, an elegant study by Phielix et al. [97] measured both *in vivo* mitochondrial function (with MRS) and *ex vivo* mitochondrial respiration in muscle from the same patients with T2D and they reported that in this population of subjects, the *in vivo* defects in mitochondrial function could be attributed to impairments in intrinsic mitochondrial substrate oxidation. Another study from this group also observed similar differences in intrinsic mitochondrial function in T2D patients compared to BMI-matched controls [96].

One limitation of the aforementioned studies is that they only provide static measurements of different populations at a given time and are unable to delineate whether the observed defects in mitochondrial metabolism are primary drivers of insulin resistance or arise as a consequence of decreases in insulin action. In this regard, intervention studies in rodents and humans have provided some experimental evidence that manipulations which result in declines in insulin action, are also associated with mitochondrial dysfunction. For example, infusion of fatty acids into humans for 6–48h to mimic the effects of chronic lipid overload 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 [104-106]. In healthy male subjects, high-fat feeding for 3 days was sufficient to reduce mRNA levels of PGC1α, PGC-1β and several other mitochondrial genes in skeletal muscle [107]. Similarly, genetic, or high-fat diet-induced obesity and insulin resistance in rodents has been reported by several groups to reduce mitochondrial gene expression, protein expression and mitochondrial respiration in skeletal muscle [107-111]. Providing additional evidence of a link between mitochondrial dysfunction and insulin resistance is the fact that antiretroviral therapy used to suppress human immunodeficiency virus infection causes insulin resistance in association with mtDNA copy number [112]. Collectively, the above studies illustrate that there are many instances where defects in mitochondrial metabolism and impairments in insulin action occur in conjunction with each other in skeletal muscle.

4. Mitochondrial dysfunction in tissues other than muscle

4.1. Liver

The liver plays a major role in regulating glucose homeostasis, producing glucose during the fasting state and storing glucose after the ingestion of a meal. Hepatic insulin resistance causes impaired glycogen synthesis and reduced suppression of endogenous glucose and is closely correlated with excess accumulation of lipid in liver. Chronic elevation of liver lipid content is referred to as non-alcoholic liver disease (NAFLD) and this condition progresses to non-alcoholic steatohepatitis (NASH) when inflammatory and fibrotic processes become involved.

A range of different parameters have been studied in rodents and humans with respect to liver mitochondrial metabolism. The collective findings indicate that the liver appears to be able to adapt to an excess of lipid by upregulating fatty acid oxidative capacity and TCA cycle activity, but this is not always coupled to a concomitant increase in electron transport chain activity, and as a consequence reactive oxygen species are produced (see [113] for an excellent review on the topic). There are also some *in vivo* MRS studies that have examined indices of mitochondrial metabolism in individuals with NAFLD and T2D, with the findings generally indicating mild abnormalities in mitochondrial function in these populations [114-116].

4.2. Adipose tissue

4.2.1. White adipose tissue

White adipose tissue (WAT) serves a principal role as the most important energy store in the body. However it has become increasingly clear over the last decade that WAT is also an active endocrine organ, releasing adipokines that influence whole-body energy homeostasis and insulin action. Mitochondrial content in WAT is low compared to other tissues, however the diversity of mitochondrial proteins in WAT has been shown to be greater than in muscle and heart [117]. Intact mitochondrial metabolism is critical for maintaining normal WAT functions, such as the appropriate synthesis and secretion of adipokines and cycling reactions involved in lipid synthesis [118].

WAT mitochondrial content has been reported to be reduced in insulin-resistant humans and rodents. In women with T2D, electron transport chain genes were shown to be downregulated in visceral WAT independently of obesity and perhaps as a consequence of TNFalpha-induced inflammation [119]. In obese humans, mtDNA copy number was reported to be lower than in control subjects and was directly correlated with basal and insulin-stimulated lipogenesis [120]. In rodent models of genetic or dietary-induced obesity and insulin resistance, there are reductions in mtDNA copy number, mitochondrial density and mitochondrial OXPHOS activity [121-123]. Administration of thiazolidinediones promotes mitochondrial biogenesis in WAT in animals and humans, in conjunction with improved whole-body insulin sensitivity [46,123], suggesting that specific changes in WAT mitochondrial metabolism in obesity and T2D, may be imparting whole-body metabolic consequences. Indeed, recent work has shown adipose-restricted alterations in mitochondrial activity can have profound effects on global glucose and lipid homeostasis [124,125].

4.2.2. Brown adipose tissue

Unlike WAT, the principal function of brown adipose tissue (BAT) is energy dissipation, rather than energy storage. BAT has a high mitochondrial density per gram of tissue, and the unique presence of uncoupling protein 1 (UCP1) allows brown adipocytes to couple the oxidation of lipids, not to ATP synthesis, but to heat generation via proton leak across the mitochondrial inner membrane. Interest in brown adipose tissue has recently soared on the back of 3 important papers published in 2009 that unequivocally demonstrated the presence of functional BAT in humans [126-128]. There is an inverse correlation between BAT activity (as

assessed by fluorodeoxyglucose PET) and obesity, suggesting that individuals with low BAT mitochondrial activity, may be prone to obesity and other metabolic diseases [126,127,129-131].

4.3. Heart

Like skeletal muscle, translocation of GLUT4 in response to insulin occurs in myocardium. This process is blunted in insulin-resistant humans and animals in association with other abnormalities in fuel metabolism ([132-134]. With respect to mitochondrial metabolism, genetic and diet-induced obesity and type 2 diabetes in rodents is associated impaired mitochondrial function [135-137]. MRS studies in individuals with T1DM, T2DM, obesity and/or NAFLD have also reported that that there is a decreased ratio of phosphocreatine:ATP in myocardium, potentially indicating derangements in mitochondrial substrate metabolism in these populations [138-142].

5. What factors lead to mitochondrial dysfunction in insulin resistance?

As noted in the previous section, mitochondrial dysfunction is frequently documented in insulin resistant states and there are many possible factors that may underlie this relationship.

5.1. Insulin resistance

Insulin is a potent anabolic hormone and it has been proposed that mitochondrial dysfunction may emerge secondary to insulin resistance. Insulin infusion in humans leads to increases in mitochondrial gene expression, higher oxidative enzyme activity and elevated ATP synthesis in muscle [143,144]. This response is attenuated in insulin-resistant T2D individuals, supporting a direct role for insulin resistance leading to mitochondrial dysfunction [143]. Further evidence for this notion comes from a study by Karakelides et al, who showed that acute insulin removal from subjects with type 1 diabetes, caused reductions in mitochondrial ATP production and in mitochondrial gene expression in skeletal muscle [145]. Additionally a recent study in patients with congenital defects in insulin signal transduction, reported that mitochondrial function (assessed by phosphocreatine recovery rates) in muscle was reduced in this population [146]. Finally a recent study that induced insulin resistance by prolonged fasting, also reported defects in mitochondrial function [147]. Overall these studies indicate that insulin can directly regulate mitochondrial biogenesis and metabolism, and therefore it is plausible that some of the mitochondrial defects observed in insulin-resistant subjects, could be a consequence of the insulin resistance itself.

5.2. Altered mitochondrial dynamics

Any perturbation in the dynamics of the mitochondrial network could potentially contribute to the pathogenesis of insulin resistance in skeletal muscle. The complex process of mitochondrial fission and fusion has been described above and alterations in key proteins mediating these dynamic events have been reported in insulin resistant and obese states. The expression

of mitofusin 2 (MFN2), which appears to have additional pleitropic effects in cells beyond the maintenance of the mitochondrial network [148-152], is reduced in the skeletal muscle of obese insulin-resistant humans, type 2 diabetic humans and diabetic Zucker rats [149,153] and correlates with the capacity for glucose oxidation [154]. Repression of MFN2 in L6E9 muscle cells and 10T/2 fibroblasts results in decreased glucose oxidation, cellular respiration, mitochondrial membrane potential, and causes fragmentation of the mitochondrial network [149] and liver-specific deletion of MFN2 results in glucose intolerance and impairments in insulin signaling [155]. Recent work has also shown that mice deficient in the mitochondrial protease OMA1, display obesity and altered metabolic homeostasis, due to altered processing of the inner membrane fusion protein OPA1 and disruptions in mitochondrial morphology and fuel metabolism [156]. It has also been reported that abnormalitieis in mitochondrial fission events may play a role lipid-induced insulin resistance. In C2C12 muscle cells, palmitic acid (but not other long-chain fatty acids) was shown to induce mitochondrial fragmentation in conjunction with insulin resistance and this effect could be blocked by genetic or pharmacological inhibition of Drp1 [157]. Analysis of tissues from *ob*/*ob* mice and high-fat fed mice in this study revealed increased Drp1 and Fis1 levels and pre-treatment of *ob*/*ob* mice with the Drp1 inhibitor Mdivi-1 resulted in a mild improvement in insulin action in these animals. Collectively these studies suggest that alterations in the equilibrium of mitochondrial fission and fusions events may play some role in the pathogenesis of insulin resistance.

5.3. Reduced physical activity

Physical inactivity has recently been reported to be as big a risk factor for non-communicable diseases as smoking, stressing the importance of exercise in metabolic health [158]. Exercise is one of the major stimuli for mitochondrial biogenesis and chronic inactivity results in decreases in mitochondrial number in muscle [159]. A number of studies have shown that obesity and other metabolic disorders are characterised by decreased physical activity levels and elevations in sedentary behaviour [160-162]. Interestingly the sedentary behaviours (e.g. sitting time) do not seem to be influenced by changes in weight and have been suggested to be biologically determined [161]. Given these differences, it is likely that some of the mitochondrial defects reported in overweight or obese insulin-resistant subjects may be explained, in part, by low levels of physical activity.

5.4. Genetic and epigenetic factors

There is evidence in the literature that the metabolic phenotype of skeletal muscle may be strongly influenced by genetic programming. For example, despite being cultured under similar conditions for several weeks, studies have shown that primary human skeletal muscle cells in culture display a similar metabolic phenotype (e.g. gene expression and lipid partitioning) to that of the donor subject from which they originated [163,164]. Mutations in nuclear-encoded genes involved in mitochondrial function (e.g. PGC-1α, NDUFB6) have been linked with insulin action and T2D, as have mtDNA deletions [165,166]. An emerging area of research is also the regulation of mitochondrial function by epigenetic factors. Barres et al showed that the promoter of PGC-1a is methylated at non-CpG sites and exposure of primary human

myotubes to hyperlipidemia or inflammatory stimuli, promoted PGC-1α hypermethylation. Intriguingly PGC-1α hypermethylation was observed in muscle of T2D patients in conjunction with reduced mitochondrial density [167]. PGC-1α hypermethylation has also been linked with insulin resistance in non-alcoholic fatty liver disease [168]. A number of other studies have also reported that methylation of other mitochondrial genes (e.g. NDUFB6 and ATP50) as well as TFAM, can be regulated by methylation and associated with insulin resistance. [166,168-170]. One recent study has also shown that methylation of mitochondrial DNA is also correlated with severity of NAFLD [171]. In addition to methylation, acetylation can also influence gene transcription and the potential importance of this epigenetic factor is highlighted by a recent study showing that pharmacological inhibition of HDAC1 in cells and obese animals could promote mitochondrial biogenesis and improve metabolic phenotype [172]. Overall the above studies indicate that specific chromatin modifications may influence mitochondrial function in insulin resistance and T2D.

5.5. Oxidative stress

Oxidative stress can be defined as a chronic imbalance between the production of reactive species and the protection against these species by antioxidant defenses, ultimately leading to macromolecular damage. Reactive oxygen species (ROS) are an unavoidable byproduct of metabolic reactions within cells and a major site for ROS production is the mitochondrion [173]. Studies from a number of different groups have shown that in genetic or diet-induced obese rodents, there is increase ROS production [4,5,174,175]. Importantly, most [4,5,175,176], but not all studies [177] show that insulin action is improved by genetic or pharmacological attenuatation of mitochondrial ROS production, indicating an especially important role for generation of reactive species in this organelle. Since mitochondria are particularly susceptible to oxidative attack [178,179], it is possible that overactive ROS generation in response to obesity or high dietary lipid supply, may lead to defects in mitochondrial function. Indeed, Bonnard et al. fed mice a long-term diet rich in fat and sugar and concluded that under their specific experimental conditions, oxidative stress was involved in the induction of mitochondrial dysfunction [108].

5.6. Post-translational regulation of mitochondrial function

As noted above, there is an emerging appreciation for the fact that specific mitochondrial enzymes and pathways may be regulated by post-translational modifications. Several groups have shown that mitochondrial acetylation is increased in tissues of diet-induced obese mice [180,181]. SIRT3 is a key regulator of mitochondrial acetylation and the expression of this deacetylase enzyme is markedly reduced in a number of different experimental models of insulin resistance and diabetes [181-183]. SIRT3 KO mice display insulin resistance in muscle [182] and these mice also exhibit an accelerated development of the metabolic syndrome when challenged with long-term high fat diet, in association with pronounced hyperacetylation of liver mitochondria [181]. Interestingly, in addition to showing that SIRT3 KO mice have a compromised phenotype, Hirschey et al have also shown that a point mutation in SIRT3 that results in reduced activity of this protein, is associated with the development of metabolic

syndrome in humans [181]. The above studies suggest that altered acetylation of mitochondrial proteins may associate with insulin resistance and impaired mitochondrial function, and while further study in this field is required, there is some evidence that other mitochondrial PTMs may also be altered in insulin resistance and T2D [184,185].

6. Mitochondrial dysfunction is not always linked with insulin resistance

Despite the frequent association of mitochondrial dysfunction and insulin resistance, evidence of a cause-and-effect relationship between the two is still lacking. In fact, a substantial literature now exists in both humans and rodents directly challenging the notion that deficiencies in mitochondrial oxidative capacity are an obligate part of the link between lipid accumulation (obesity) and insulin resistance.

6.1. Human studies

Trenell and colleagues used MRS to determine basal and maximal ATP turnover in muscle of well-controlled T2D patients compared with physical activity-, age- and weight-matched control subjects and observed no difference between the two groups [186]. A similar finding was reported in a separate population where post-exercise phosphocreatine recovery indicated similar mitochondrial function between obese patients in either the early or advanced stages of T2D and normoglycemic controls matched for age, body composition and habitual physical activity levels [187]. A further study from the same group also recently reported similar *in vivo* mitochondrial function with MRS in prediabetic subjects compared with age, BMI and activity-matched controls, despite the presence of insulin resistance (by HOMA-IR and OGTT) [83]. In young lean men born with low birth-weight, mitochondrial function by MRS and mitochondrial gene expression are intact, despite these subjects displaying several pre-diabetic characteristics [188]. Two groups have also reported measurement of *in vitro* ATP production capacity and respiratory charactersitics in mitochondria isolated from obese subjects and fail to see any difference compared to controls [189,190]. Studies in different ethnic groups have also provided data contrary to the mitochondrial dysfunction theory of insulin resistance. Nair et al. showed that despite being more insulin resistant than age, sex and BMI-matched North American subjects, Asian Indians exhibit higher mtDNA content, elevated expression of genes involved in oxidative phosphorylation, increased oxidative enzyme activity and greater mitochondrial ATP production rates in muscle [191]. In this study the authors also went on to stratify the Asian Indian group into those with T2D and those without, and despite the diabetic individuals displaying impaired insulin sensitivity and increased muscle lipid levels, there was no difference in the various markers of mitochondrial oxidative capacity.

The studies above provide evidence that at least in those populations, mitochondrial dysfunction does not seem to be present in a number of insulin resistant groups. In line with these examples of a discordant relationship between these two variables, several human intervention studies have also shown that changes in insulin sensitivity can occur without concurrent improvements in mitochondrial function. For instance, dietary restriction in overweight and

obese subjects enhanced insulin sensitivity, without altering mtDNA, cardiolipin content or NADH-oxidase activity [192]. Improved insulin sensitivity was reported in insulin-resistant subjects with a family history of T2D following 7 days of treatment with the anti-lipolytic agent acipimox, yet mitochondrial gene expression in muscle actually declined in these subjects [193]. Treatment of diabetic patients with rosiglitazone improved insulin sensitivity, without altering *in vivo* mitochondrial function or markers of mitochondrial content [194,195]. Recently Samocha-Bonet also showed that 28 days of high-fat overfeeding was sufficient to induce insulin resistance in health humans, without any detectable defects in various markers of mitochondrial function [6]. Shorter-term overfeeding studies in low birth-weight subjects also revealed a disconnect between the induction of insulin resistance and the response of mitochondrial metabolism [196].

6.2. Rodent studies

To complement the studies in humans, a number of investigators have used gene-manipulated mice to more directly test whether specifically targeting mitochondrial metabolism, can induce changes in insulin sensitivity. Mitochondrial oxidative capacity was shown to be compromised in muscle-specific TFAM knockout mice, however these animals exhibited improved glucose clearance during a glucose tolerance test and maintained insulin-stimulated glucose uptake in muscle [197]. TFAM knockout in adipose tissue was recently shown to protect against diet-induced obesity and insulin resistance, despite causing abnormalities in mitochondrial function [125]. Similar findings were reported in mice with liver or muscle-specific deletion of apoptosis-inducing factor. These animals exhibited a gene expression pattern of mitochondrial oxidative phosphorylation deficiency similar to that observed in human insulin resistance [78,79], however they were lean and insulin-sensitive and did not manifest the usual deleterious effects of a high-fat diet [198]. A number of groups have also targeted other regulators of mitochondrial function in mice. Due to their key role in mitochondrial biogenesis, muscle-specific knockout of PGC-1α or loss-of-function mutation of PGC-1β produced the expected decline in markers of mitochondrial function yet insulin sensitivity in muscle was preserved or in fact slightly enhanced in these animals compared to wild-type counterparts [26,199]. Two separately generated lines of muscle-specific PGC-1α transgenic mice have shown predictable increases in many mitochondrial parameters, but these animals are insulin resistant, potentially due to excessive fatty acid delivery into muscle [200] or decreased GLUT4 expression [201]. In other examples of a dissociation between insulin resistance and mitochondrial dysfunction, it has been shown that Zucker diabetic fatty (ZDF) rats display normal *in vivo* muscle oxidative capacity and improved activity of enzymes involved in lipid oxidation during the progressions to insulin resistance and T2D [202], while *db*/*db* mice and *ob*/*ob* mice have been shown to exhibit higher mitochondrial oxidative capacity in liver compared to lean control animals [203,204]. Collectively the above studies clearly demonstrate that targeted manipulation of mitochondrial function, does not produce 'predictable' alterations in insulin action. A caveat to these studies is that in genetically manipulated mice, there is a complete lack or substantial increase in the content/function of a specific protein and thus caution must be exercise when interpreting the findings, as the phenotype (or lack thereof) may be

partially related to other compensatory adaptations (e.g. activation of AMP-activated protein kinase or increase in some other lipid metabolism pathway) induced by the alterations in specific genes [197,200].

Dietary studies are another robust approach to test whether mitochondrial defects can be causally linked with changes in insulin action. Feeding rats an iron-deficient diet causes a deficiency in mitochondrial electron transport chain enzymes, however this is not associated with the development of insulin resistance [205]. In 2007, our group showed that high-fat feeding in mice induced insulin resistance and was associated with increased expression of a PGC-1α and a number of mitochondrial proteins, elevated oxidative enzyme activity and higher fatty acid oxidation rates [206]. Furthermore we also demonstrated that similar changes in markers of mitochondrial metabolism were present in muscle from high-fat fed rats, *db*/*db* mice and *fa*/*fa* Zucker rats. These findings of enhanced mitochondrial oxidative capacity occurring in parallel with the induction of insulin resistance, suggested that diet-induced insulin resistance does not involve mitochondrial dysfunction. At a similar time Garcia-Roves also showed that high-fat feeding with daily heparin injections in rats resulted in mitochondrial biogenesis in skeletal muscle [207] and subsequent work by the same lab the following year confirmed that oversupply of dietary lipid produced insulin resistance, despite an increase in mitochondria in muscle [208]. Since the time of these publications, a number of other groups have shown that diets rich in fat increase mitochondrial biogenesis in muscle, despite the fact that the same diets robustly induce whole-body and muscle-specific insulin resistance [209-211]. One interpretation of these findings is that skeletal muscle is mounting an appropriate response to the increase in caloric load, by upregulating catabolic pathways, however this response is inadequate or mismatched with the elevation in nutrient intake, thus resulting in ectopic lipid accumulation in muscle and insulin resistance. Interestingly we have shown that the upregulation of mitochondrial oxidative capacity is far greater when medium chain fatty acids are substituted for long chain fatty acids in the diet and this is sufficient to prevent the accumulation of myocellular lipid and the development of insulin resistance in muscle [212]. Enhancing mitochondrial metabolism acutely by genetic means in muscle, also seems to be sufficient to boost mitochondrial fuel oxidation to the point where insulin resistance is ameliorated in muscle in fat-fed animals [25,213].

One interesting observation to come out of the dietary studies in rodents is that from Muoio's group. They reported that the increased mitochondrial fatty acid oxidation that occurs in response to high-fat feeding, results in the generation of acylcarnitines as incomplete oxidation products [214]. This acylcarnitine signature, which is thought to disturb the mitochondrial CoA equilibrium and indicate a state of mitochondrial stress, has also been observed in humans with obesity and T2D [215,216]. Whether the acylcarnitines can directly contribute to the development of insulin resistance is currently unclear, but recent work suggests that these metabolites serve as a marker for the inability of mitochondria to efficiently transition between fuel substrates [217].

In summary, while there are a large number of studies that document an association between mitochondrial dysfunction and insulin resistance in lean and obese subjects, there is now a

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significant literature showing that alterations in mitochondrial function in muscle and insulin action are not always correlated. There are a myriad of differences between studies that may explain these discrepancies, including the particular population of individuals studied and their ethnic background and physical fitness level, the muscle group examined, the dietary regime employed in rodent and human studies (e.g. duration of feeding, fat content and composition) and the particular assay/technique used to assess mitochondrial function.

7. Are mitochondria good therapeutic targets for the treatment of insulin resistance and T2D?

While the precise role of mitochondria as pathogenic drivers of insulin resistance and T2D remains controversial, it should be recognized that even if mitochondrial defects arise secondary to the development of insulin resistance or as a consequence of obesity, reductions in oxidative fuel metabolism are likely to accelerate ectopic lipid deposition in non-adipose tissues and thereby exacerbate the insulin-resistant state. Therefore it would appear that stimulating mitochondrial biogenesis and fuel metabolism, could have beneficial effects for treating metabolic diseases. Evidence in this regard is summarised below

7.1. Exercise

Exercise robustly stimulates mitochondrial biogenesis in muscle and has a multitude of health benefits, including improving insulin action. An 8 week cycling regime in sedentary elderly subjects increased muscle fatty acid oxidative capacity and in parallel improved insulin-mediated glucose disposal [218]. A ten week exercise training program produced similar improvements in insulin sensitivity and muscle oxidative enzyme activity in both first degree relatives of T2D patients compared with age-, sex- and BMI-matched controls [219]. Toledo reported that 4 months of exercise training in T2D patients increased skeletal muscle mitochondrial density, cardiolipin content and mitochondrial oxidation enzymes, in conjunction with a 60% improvement in insulin sensitivity [220]. Aerobic cycling training for 10 weeks in obese, T2D male subjects induced an ~20% increase in insulin sensitivity and a 40% increase in mitochondrial content, with these changes of a similar magnitude to that observed in matched control subjects [221]. Twelve weeks of combined progressive training was also sufficient to overcome the *in vivo* mitochondrial impairment observed in a group of T2D patients, with a concomitant improvement in insulin sensitivity also observed [96]

7.2. Caloric restriction and modulation of sirtuin enzymes

Caloric restriction has been shown to improve insulin sensitivity in humans [222,223] and to improve markers of mitochondrial function in muscle of humans and rodents [224,225]. Many of the beneficial effects of calorie restriction have been proposed to be through stimulation of the SIRT1 pathway. Indeed studies have shown that activators of SIRT1 (e.g. resveratrol and more potent specific activators) can enhance mitochondrial metabolism and improve insulin

action in rodent models of insulin resistance and T2D [226-229]. Recently it was also shown that 30 days of resveratrol supplementation improved some markers of mitochondrial function in muscle of obese subjects, in parallel with improved HOMA-IR and reduced hepatic lipid levels [230]. These effects seem to be limited to metabolically compromised subjects, as resveratrol did not improve markers of glycaemic control in non-obese women with normal glucose tolerance [231]. An alternative approach to using direct SIRT1 activators to mimic calorie restriction, is to increase the intracellular levels of NAD^+, the obligate co-factor for the sirtuin reaction. Indeed two recent studies using different NAD^+ precursors, nicotinamide mononucleotide or nicotinamide riboside, have both reported beneficial metabolic effects on metabolic homeostasis in animals models of insulin resistance and T2D [232,233].

7.3. Mitochondrial uncoupling

As noted above, energy dissipation or wasting can occur in a process known as mitochondrial uncoupling. While this occurs naturally through uncoupling proteins, there are also a pharmacological agents that can induce mitochondrial uncoupling, such as DNP (2,4-dintirophenol). These uncoupling agents are generally lipophilic weak acids, that cause mitochondrial uncoupling by transporting protons across the mitochondrial inner membrane into the matrix, deprotonating and then exiting as anions before repeating the catalytic cycle. Uncoupling agents have been successfully used in the past as obesity treatments. In the 1930's DNP was shown to be an effective weight-loss drug in humans, providing an important proof-of-concept that the stimulation of energy expenditure by uncoupling is not necessarily compensated for by an increase in caloric intake [234]. Despite its success as an anti-obesity therapy, DNP was withdrawn from the market in 1938 as it (like most uncouplers) has a narrow therapeutic window, with overdoses causing serious complications (even death) by compromising cellular energy homeostasis. Due to the current obesity epidemic, and illicit sales via the internet, it is alarming to see that DNP has made a comeback as a weight loss agent, with predictable lethal results [235,236]. Thus, while mitochondrial uncoupling with DNP does not appears to be a safe weight-loss therapy, other more recently described uncoupling agents may potentially have a safer profile for use in humans [237-239]. An additional approach may to be to upregulate physiological uncoupling in brown adipose tissue, via sympathomimetic agents or agonists for thyroid hormone or bile acid receptors.

7.4. Natural compounds

Natural compounds are another rich source of potential therapeutics for obesity and type diabetes, as there is often a long history of use of these compounds in the treatment of metabolic diseases states. One such compound is berberine, a natural plant alkaloid that has been used as a traditional medicine in many Asian countries. Berberine was shown to improve insulin sensitivity in a range of animal models [240] and there is also evidence of beneficial effects in humans [241]. Although enhancing mitochondrial function appears to be an effective treatment for insulin resistance, we showed that berberine acted through inhibition of Complex 1 of the electron transport chain [242]. This mild mitochondrial inhibition led to the activation of AMPK and subsequent metabolic benefits. Interestingly this pattern of mild inhibition of

mitochondrial metabolism has been reported in other insulin-sensitising medicinal plants [243] and is also a characteristic of metformin and thiazolidinediones, which are frontline anti-diabetic therapies [242,244,245].

8. Concluding remarks

The mitochondrial dysfunction theory of insulin resistance, proposes that defects in mitochondrial metabolism are key events involved in the pathogenesis of insulin resistance. At present, the available literature does not provide strong evidence for this relationship, and there is mounting evidence that mitochondrial defects observed in insulin-resistant individuals are likely acquired (e.g. due to low physical activity or caloric excess), or develop secondary to the insulin resistance itself. Furthermore, with respect to muscle, another important issue that needs to be considered is whether the ~30% reduction in mitochondrial function that has been observed in some insulin-resistant humans would limit the oxidation of fatty acids, leading to lipid accumulation as proposed [16]. At rest the rate of oxygen utilization in muscle is low; and the fact that muscle has enormous capacity to increase substrate oxidation over normal levels, means that there is substantial 'spare' capacity in this system to maintain fatty acid utilization under normal free-living conditions when energy requirements are low. Despite the unanswered questions about the precise role that mitochondria play in insulin resistance and T2D, therapies targeting this important organelle, should be explored for the the treatment of insulin resistance and its associated metabolic disorders.

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References

[1] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006; 7: 85-96.

[2] Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004; 306: 457-61.

[3] Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. Science 2006; 313: 1137-40.

[4] Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT, Price JW, 3rd, Kang L, Rabinovitch PS, Szeto HH, Houmard JA, Cortright RN, Wasserman DH, Neufer PD. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 2009;

[5] Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghzal GJ, Stocker R, Van Remmen H, Kraegen EW, Cooney GJ, Richardson AR, James DE. Insulin resistance is a cellular antioxidant defense mechanism. Proc Natl Acad Sci U S A 2009; 106: 17787-92.

[6] Samocha-Bonet D, Campbell LV, Mori TA, Croft KD, Greenfield JR, Turner N, Heilbronn LK. Overfeeding reduces insulin sensitivity and increases oxidative stress, without altering markers of mitochondrial content and function in humans. PLoS One 2012; 7: e36320.

[7] Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011; 29: 415-45.

[8] Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest 2008; 118: 2992-3002.

[9] Kraegen EW, Cooney GJ. Free fatty acids and skeletal muscle insulin resistance. Curr Opin Lipidol 2008; 19: 235-41.

[10] Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell 2012; 148: 852-71.

[11] Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH. Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 1997; 46: 983-8.

[12] 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: 2005-11.

[13] Adams JM, 2nd, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes 2004; 53: 25-31.

[14] 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: 1477-84.

[15] Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ, Dyck DJ. 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: 1144-6.

[16] Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. Science 2005; 307: 384-7.

[17] Scarpulla RC. Transcriptional paradigms in Mammalian mitochondrial biogenesis and function. Physiol Rev 2008; 88: 611-38.

[18] Mossmann D, Meisinger C, Vogtle FN. Processing of mitochondrial presequences. Biochim Biophys Acta 2012; 1819: 1098-106.

[19] Arany Z. PGC-1 coactivators and skeletal muscle adaptations in health and disease. Curr Opin Genet Dev 2008; 18: 426-34.

[20] Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998; 92: 829-39.

[21] Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 2002; 277: 1645-8.

[22] Andersson U, Scarpulla RC. Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. Mol Cell Biol 2001; 21: 3738-49.

[23] Arany Z, Lebrasseur N, Morris C, Smith E, Yang W, Ma Y, Chin S, Spiegelman BM. The transcriptional coactivator PGC-1beta drives the formation of oxidative type IIX fibers in skeletal muscle. Cell Metab 2007; 5: 35-46.

[24] Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 2002; 418: 797-801.

[25] Wright LE, Brandon AE, Hoy AJ, Forsberg GB, Lelliott CJ, Reznick J, Lofgren L, Oscarsson J, Stromstedt M, Cooney GJ, Turner N. Amelioration of lipid-induced insulin

resistance in rat skeletal muscle by overexpression of Pgc-1beta involves reductions in long-chain acyl-CoA levels and oxidative stress. Diabetologia 2011; 54: 1417-26.

[26] Handschin C, Choi CS, Chin S, Kim S, Kawamori D, Kurpad AJ, Neubauer N, Hu J, Mootha VK, Kim YB, Kulkarni RN, Shulman GI, Spiegelman BM. Abnormal glucose homeostasis in skeletal muscle-specific PGC-1alpha knockout mice reveals skeletal muscle-pancreatic beta cell crosstalk. J Clin Invest 2007; 117: 3463-74.

[27] Lelliott CJ, Medina-Gomez G, Petrovic N, Kis A, Feldmann HM, Bjursell M, Parker N, Curtis K, Campbell M, Hu P, Zhang D, Litwin SE, Zaha VG, Fountain KT, Boudina S, Jimenez-Linan M, Blount M, Lopez M, Meirhaeghe A, Bohlooly YM, Storlien L, Stromstedt M, Snaith M, Oresic M, Abel ED, Cannon B, Vidal-Puig A. Ablation of PGC-1beta results in defective mitochondrial activity, thermogenesis, hepatic function, and cardiac performance. PLoS Biol 2006; 4: e369.

[28] Lelliott CJ, Vidal-Puig A. PGC-1beta: a co-activator that sets the tone for both basal and stress-stimulated mitochondrial activity. Adv Exp Med Biol 2009; 646: 133-9.

[29] Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor gamma coactivator 1alpha expression in muscle. Proc Natl Acad Sci U S A 2003; 100: 7111-6.

[30] Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature 2005; 434: 113-8.

[31] Lerin C, Rodgers JT, Kalume DE, Kim SH, Pandey A, Puigserver P. GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1alpha. Cell Metab 2006; 3: 429-38.

[32] Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 2007; 104: 12017-22.

[33] Ruas JL, White JP, Rao RR, Kleiner S, Brannan KT, Harrison BC, Greene NP, Wu J, Estall JL, Irving BA, Lanza IR, Rasbach KA, Okutsu M, Nair KS, Yan Z, Leinwand LA, Spiegelman BM. A PGC-1alpha Isoform Induced by Resistance Training Regulates Skeletal Muscle Hypertrophy. Cell 2012; 151: 1319-31.

[34] Gleyzer N, Vercauteren K, Scarpulla RC. Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. Mol Cell Biol 2005; 25: 1354-66.

[35] Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci U S A 1994; 91: 1309-13.

[36] Baar K, Song Z, Semenkovich CF, Jones TE, Han DH, Nolte LA, Ojuka EO, Chen M, Holloszy JO. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. FASEB J 2003; 17: 1666-73.

[37] Huo L, Scarpulla RC. Mitochondrial DNA instability and peri-implantation lethality associated with targeted disruption of nuclear respiratory factor 1 in mice. Mol Cell Biol 2001; 21: 644-54.

[38] Bruni F, Polosa PL, Gadaleta MN, Cantatore P, Roberti M. Nuclear respiratory factor 2 induces the expression of many but not all human proteins acting in mitochondrial DNA transcription and replication. J Biol Chem 2010; 285: 3939-48.

[39] Ristevski S, O'Leary DA, Thornell AP, Owen MJ, Kola I, Hertzog PJ. The ETS transcription factor GABPalpha is essential for early embryogenesis. Mol Cell Biol 2004; 24: 5844-9.

[40] Dufour CR, Wilson BJ, Huss JM, Kelly DP, Alaynick WA, Downes M, Evans RM, Blanchette M, Giguere V. Genome-wide orchestration of cardiac functions by the orphan nuclear receptors ERRalpha and gamma. Cell Metab 2007; 5: 345-56.

[41] Liesa M, Borda-d'Agua B, Medina-Gomez G, Lelliott CJ, Paz JC, Rojo M, Palacin M, Vidal-Puig A, Zorzano A. Mitochondrial fusion is increased by the nuclear coactivator PGC-1beta. PLoS ONE 2008; 3: e3613.

[42] Luo J, Sladek R, Carrier J, Bader JA, Richard D, Giguere V. Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. Mol Cell Biol 2003; 23: 7947-56.

[43] Alaynick WA, Kondo RP, Xie W, He W, Dufour CR, Downes M, Jonker JW, Giles W, Naviaux RK, Giguere V, Evans RM. ERRgamma directs and maintains the transition to oxidative metabolism in the postnatal heart. Cell Metab 2007; 6: 13-24.

[44] Luo J, Sladek R, Bader JA, Matthyssen A, Rossant J, Giguere V. Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-beta. Nature 1997; 388: 778-82.

[45] Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spiegelman BM, Staels B, Wahli W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol Rev 2006; 58: 726-41.

[46] Bogacka I, Xie H, Bray GA, Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. Diabetes 2005; 54: 1392-9.

[47] Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, Watanabe Y, Uchiyama Y, Sumi K, Iguchi H, Ito S, Doi T, Hamakubo T, Naito M, Auwerx J, Yanagisawa M, Kodama T, Sakai J. Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxida-

tion in skeletal muscle and attenuates metabolic syndrome. Proc Natl Acad Sci U S A 2003; 100: 15924-9.

[48] Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature 2007; 450: 736-40.

[49] Blattler SM, Cunningham JT, Verdeguer F, Chim H, Haas W, Liu H, Romanino K, Ruegg MA, Gygi SP, Shi Y, Puigserver P. Yin Yang 1 deficiency in skeletal muscle protects against rapamycin-induced diabetic-like symptoms through activation of insulin/IGF signaling. Cell Metab 2012; 15: 505-17.

[50] Ding WX, Yin XM. Mitophagy: mechanisms, pathophysiological roles, and analysis. Biol Chem 2012; 393: 547-64.

[51] Lemasters JJ, Qian T, He L, Kim JS, Elmore SP, Cascio WE, Brenner DA. Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. Antioxid Redox Signal 2002; 4: 769-81.

[52] Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 2008; 27: 433-46.

[53] Chen M, Sandoval H, Wang J. Selective mitochondrial autophagy during erythroid maturation. Autophagy 2008; 4: 926-8.

[54] Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, Wang J. Essential role for Nix in autophagic maturation of erythroid cells. Nature 2008; 454: 232-5.

[55] Sato M, Sato K. Degradation of paternal mitochondria by fertilization-triggered autophagy in C. elegans embryos. Science 2011; 334: 1141-4.

[56] Santos D, Cardoso SM. Mitochondrial dynamics and neuronal fate in Parkinson's disease. Mitochondrion 2012; 12: 428-37.

[57] Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, Cortright RN. Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. Am J Physiol Cell Physiol 2005; 288: C1074-82.

[58] Glancy B, Balaban RS. Protein composition and function of red and white skeletal muscle mitochondria. Am J Physiol Cell Physiol 2011; 300: C1280-90.

[59] Zhao X, Leon IR, Bak S, Mogensen M, Wrzesinski K, Hojlund K, Jensen ON. Phosphoproteome analysis of functional mitochondria isolated from resting human muscle reveals extensive phosphorylation of inner membrane protein complexes and enzymes. Mol Cell Proteomics 2011; 10: M110 000299.

[60] Kelly OM, McNamara YM, Manzke LH, Meegan MJ, Porter RK. The preservation of in vivo phosphorylated and activated uncoupling protein 3 (UCP3) in isolated skele-

tal muscle mitochondria following administration of 3,4-methylenedioxymethamphetamine (MDMA aka ecstasy) to rats/mice. Mitochondrion 2012; 12: 110-9.

[61] Anderson KA, Hirschey MD. Mitochondrial protein acetylation regulates metabolism. Essays Biochem 2012; 52: 23-35.

[62] Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, Kim J, Woo J, Kim JH, Choi BH, He B, Chen W, Zhang S, Cerione RA, Auwerx J, Hao Q, Lin H. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. Science 2011; 334: 806-9.

[63] Mailloux RJ, Seifert EL, Bouillaud F, Aguer C, Collins S, Harper ME. Glutathionylation acts as a control switch for uncoupling proteins UCP2 and UCP3. J Biol Chem 2011; 286: 21865-75.

[64] Doulias PT, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H. Nitric oxide regulates mitochondrial Fatty Acid metabolism through reversible protein s-nitrosylation. Sci Signal 2013; 6: rs1.

[65] Ferree A, Shirihai O. Mitochondrial dynamics: the intersection of form and function. Adv Exp Med Biol 2012; 748: 13-40.

[66] Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC. Structural basis of mitochondrial tethering by mitofusin complexes. Science 2004; 305: 858-62.

[67] Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. Mol Biol Cell 2009; 20: 3525-32.

[68] Yoon Y, Krueger EW, Oswald BJ, McNiven MA. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. Mol Cell Biol 2003; 23: 5409-20.

[69] Smirnova E, Griparic L, Shurland DL, van der Bliek AM. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol Biol Cell 2001; 12: 2245-56.

[70] Picard M, White K, Turnbull DM. Mitochondrial morphology, topology, and membrane interactions in skeletal muscle: a quantitative three-dimensional electron microscopy study. J Appl Physiol 2013; 114: 161-71.

[71] Skulachev VP. Mitochondrial filaments and clusters as intracellular power-transmitting cables. Trends Biochem Sci 2001; 26: 23-9.

[72] Westermann B. Molecular machinery of mitochondrial fusion and fission. J Biol Chem 2008; 283: 13501-5.

[73] Krieger DA, Tate CA, McMillin-Wood J, Booth FW. Populations of rat skeletal muscle mitochondria after exercise and immobilization. J Appl Physiol 1980; 48: 23-8.

[74] 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: E1130-41.

[75] 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: E1039-44.

[76] 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: 2051-60.

[77] Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002; 51: 2944-50.

[78] Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34: 267-73.

[79] Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ. 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 2003; 100: 8466-71.

[80] Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, Neschen S, White MF, Bilz S, Sono S, Pypaert M, Shulman GI. 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: 3587-93.

[81] 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: 1467-73.

[82] Skov V, Glintborg D, Knudsen S, Jensen T, Kruse TA, Tan Q, Brusgaard K, Beck-Nielsen H, Hojlund K. 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: 2349-55.

[83] van Tienen FH, Praet SF, de Feyter HM, van den Broek NM, Lindsey PJ, Schoonderwoerd KG, de Coo IF, Nicolay K, Prompers JJ, Smeets HJ, van Loon LJ. Physical activity is the key determinant of skeletal muscle mitochondrial function in type 2 diabetes. J Clin Endocrinol Metab 2012; 97: 3261-9.

[84] Wang M, Wang XC, Zhang ZY, Mou B, Hu RM. Impaired mitochondrial oxidative phosphorylation in multiple insulin-sensitive tissues of humans with type 2 diabetes mellitus. J Int Med Res 2010; 38: 769-81.

[85] 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: 790-6.

[86] 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.

[87] Hwang H, Bowen BP, Lefort N, Flynn CR, De Filippis EA, Roberts C, Smoke CC, Meyer C, Hojlund K, Yi Z, Mandarino LJ. Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. Diabetes 2010; 59: 33-42.

[88] Holloway GP, Thrush AB, Heigenhauser GJ, Tandon NN, Dyck DJ, Bonen A, Spriet LL. Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. Am J Physiol Endocrinol Metab 2007; 292: E1782-9.

[89] Ritov VB, Menshikova EV, Azuma K, Wood R, Toledo FG, Goodpaster BH, Ruderman NB, Kelley DE. Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Endocrinol Metab 2010; 298: E49-58.

[90] Chomentowski P, Coen PM, Radikova Z, Goodpaster BH, Toledo FG. Skeletal muscle mitochondria in insulin resistance: differences in intermyofibrillar versus subsarcolemmal subpopulations and relationship to metabolic flexibility. J Clin Endocrinol Metab 2011; 96: 494-503.

[91] Rabol R, Larsen S, Hojberg PM, Almdal T, Boushel R, Haugaard SB, Andersen JL, Madsbad S, Dela F. Regional anatomic differences in skeletal muscle mitochondrial respiration in type 2 diabetes and obesity. J Clin Endocrinol Metab 2010; 95: 857-63.

[92] Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003; 300: 1140-2.

[93] 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: 664-71.

[94] 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.

[95] Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, Shulman GI. Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients. Diabetes 2007; 56: 1376-81.

[96] Meex RC, Schrauwen-Hinderling VB, Moonen-Kornips E, Schaart G, Mensink M, Phielix E, van de Weijer T, Sels JP, Schrauwen P, Hesselink MK. Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes 2010; 59: 572-9.

[97] Phielix E, Schrauwen-Hinderling VB, Mensink M, Lenaers E, Meex R, Hoeks J, Kooi ME, Moonen-Kornips E, Sels JP, Hesselink MK, Schrauwen P. Lower intrinsic ADP-stimulated mitochondrial respiration underlies in vivo mitochondrial dysfunction in muscle of male type 2 diabetic patients. Diabetes 2008; 57: 2943-9.

[98] Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Jeneson JA, Backes WH, van Echteld CJ, van Engelshoven JM, Mensink M, Schrauwen P. 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: 113-20.

[99] Szendroedi J, Schmid AI, Chmelik M, Toth C, Brehm A, Krssak M, Nowotny P, Wolzt M, Waldhausl W, Roden M. Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. PLoS Med 2007; 4: e154.

[100] Szendroedi J, Schmid AI, Meyerspeer M, Cervin C, Kacerovsky M, Smekal G, Graser-Lang S, Groop L, Roden M. Impaired mitochondrial function and insulin resistance of skeletal muscle in mitochondrial diabetes. Diabetes Care 2009; 32: 677-9.

[101] 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: 2277-85.

[102] Larsen S, Stride N, Hey-Mogensen M, Hansen CN, Andersen JL, Madsbad S, Worm D, Helge JW, Dela F. Increased mitochondrial substrate sensitivity in skeletal muscle of patients with type 2 diabetes. Diabetologia 2011; 54: 1427-36.

[103] Mogensen M, Sahlin K, Fernstrom M, Glintborg D, Vind BF, Beck-Nielsen H, Hojlund K. Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes 2007; 56: 1592-9.

[104] 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: 136-40.

[105] Hoeks J, Hesselink MK, Russell AP, Mensink M, Saris WH, Mensink RP, Schrauwen P. Peroxisome proliferator-activated receptor-gamma coactivator-1 and insulin resistance: acute effect of fatty acids. Diabetologia 2006; 49: 2419-26.

[106] Richardson DK, Kashyap S, Bajaj M, Cusi K, Mandarino SJ, Finlayson J, DeFronzo RA, Jenkinson CP, Mandarino LJ. 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: 10290-7.

[107] Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, Smith SR. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 2005; 54: 1926-33.

[108] Bonnard C, Durand A, Peyrol S, Chanseaume E, Chauvin MA, Morio B, Vidal H, Rieusset J. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. J Clin Invest 2008; 118: 789-800.

[109] Boudina S, Sena S, Sloan C, Tebbi A, Han YH, O'Neill BT, Cooksey RC, Jones D, Holland WL, McClain DA, Abel ED. Early mitochondrial adaptations in skeletal muscle to diet-induced obesity are strain dependent and determine oxidative stress and energy expenditure but not insulin sensitivity. Endocrinology 2012; 153: 2677-88.

[110] Gomes AP, Duarte FV, Nunes P, Hubbard BP, Teodoro JS, Varela AT, Jones JG, Sinclair DA, Palmeira CM, Rolo AP. Berberine protects against high fat diet-induced dysfunction in muscle mitochondria by inducing SIRT1-dependent mitochondrial biogenesis. Biochim Biophys Acta 2012; 1822: 185-95.

[111] Lionetti L, Mollica MP, Crescenzo R, D'Andrea E, Ferraro M, Bianco F, Liverini G, Iossa S. Skeletal muscle subsarcolemmal mitochondrial dysfunction in high-fat fed rats exhibiting impaired glucose homeostasis. Int J Obes (Lond) 2007; 31: 1596-604.

[112] Fleischman A, Johnsen S, Systrom DM, Hrovat M, Farrar CT, Frontera W, Fitch K, Thomas BJ, Torriani M, Cote HC, Grinspoon SK. 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: E1666-73.

[113] Begriche K, Massart J, Robin MA, Bonnet F, Fromenty B. Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology 2013;

[114] Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA 1999; 282: 1659-64.

[115] Szendroedi J, Chmelik M, Schmid AI, Nowotny P, Brehm A, Krssak M, Moser E, Roden M. Abnormal hepatic energy homeostasis in type 2 diabetes. Hepatology 2009; 50: 1079-86.

[116] Krssak M, Hofer H, Wrba F, Meyerspeer M, Brehm A, Lohninger A, Steindl-Munda P, Moser E, Ferenci P, Roden M. Non-invasive assessment of hepatic fat accumulation in chronic hepatitis C by 1H magnetic resonance spectroscopy. Eur J Radiol 2010; 74: e60-6.

[117] Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha

VK. A mitochondrial protein compendium elucidates complex I disease biology. Cell 2008; 134: 112-23.

[118] Kusminski CM, Scherer PE. Mitochondrial dysfunction in white adipose tissue. Trends Endocrinol Metab 2012; 23: 435-43.

[119] Dahlman I, Forsgren M, Sjogren A, Nordstrom EA, Kaaman M, Naslund E, Attersand A, Arner P. Downregulation of electron transport chain genes in visceral adipose tissue in type 2 diabetes independent of obesity and possibly involving tumor necrosis factor-alpha. Diabetes 2006; 55: 1792-9.

[120] Kaaman M, Sparks LM, van Harmelen V, Smith SR, Sjolin E, Dahlman I, Arner P. Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. Diabetologia 2007; 50: 2526-33.

[121] Choo HJ, Kim JH, Kwon OB, Lee CS, Mun JY, Han SS, Yoon YS, Yoon G, Choi KM, Ko YG. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. Diabetologia 2006; 49: 784-91.

[122] Rong JX, Qiu Y, Hansen MK, Zhu L, Zhang V, Xie M, Okamoto Y, Mattie MD, Higashiyama H, Asano S, Strum JC, Ryan TE. Adipose mitochondrial biogenesis is suppressed in db/db and high-fat diet-fed mice and improved by rosiglitazone. Diabetes 2007; 56: 1751-60.

[123] Wilson-Fritch L, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP, Corvera S. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. J Clin Invest 2004; 114: 1281-9.

[124] Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, Askew GR, Simcox JA, McClain DA, Li C, Scherer PE. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. Nat Med 2012; 18: 1539-49.

[125] Vernochet C, Mourier A, Bezy O, Macotela Y, Boucher J, Rardin MJ, An D, Lee KY, Ilkayeva OR, Zingaretti CM, Emanuelli B, Smyth G, Cinti S, Newgard CB, Gibson BW, Larsson NG, Kahn CR. Adipose-Specific Deletion of TFAM Increases Mitochondrial Oxidation and Protects Mice against Obesity and Insulin Resistance. Cell Metab 2012; 16: 765-76.

[126] Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009; 360: 1509-17.

[127] van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009; 360: 1500-8.

[128] Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P. Functional brown adipose tissue in healthy adults. N Engl J Med 2009; 360: 1518-25.

[129] Ouellet V, Routhier-Labadie A, Bellemare W, Lakhal-Chaieb L, Turcotte E, Carpentier AC, Richard D. Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. J Clin Endocrinol Metab 2011; 96: 192-9.

[130] Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 2009; 58: 1526-31.

[131] Pfannenberg C, Werner MK, Ripkens S, Stef I, Deckert A, Schmadl M, Reimold M, Haring HU, Claussen CD, Stefan N. Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. Diabetes 2010; 59: 1789-93.

[132] Cook SA, Varela-Carver A, Mongillo M, Kleinert C, Khan MT, Leccisotti L, Strickland N, Matsui T, Das S, Rosenzweig A, Punjabi P, Camici PG. Abnormal myocardial insulin signalling in type 2 diabetes and left-ventricular dysfunction. Eur Heart J 2010; 31: 100-11.

[133] Peterson LR, Saeed IM, McGill JB, Herrero P, Schechtman KB, Gunawardena R, Recklein CL, Coggan AR, DeMoss AJ, Dence CS, Gropler RJ. Sex and type 2 diabetes: obesity-independent effects on left ventricular substrate metabolism and relaxation in humans. Obesity (Silver Spring) 2012; 20: 802-10.

[134] Wright JJ, Kim J, Buchanan J, Boudina S, Sena S, Bakirtzi K, Ilkun O, Theobald HA, Cooksey RC, Kandror KV, Abel ED. Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding. Cardiovasc Res 2009; 82: 351-60.

[135] Boudina S, Sena S, O'Neill BT, Tathireddy P, Young ME, Abel ED. Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial energetics in obesity. Circulation 2005; 112: 2686-95.

[136] Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, Aziz S, Johnson JI, Bugger H, Zaha VG, Abel ED. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. Diabetes 2007; 56: 2457-66.

[137] Zhang Y, Yuan M, Bradley KM, Dong F, Anversa P, Ren J. Insulin-like growth factor 1 alleviates high-fat diet-induced myocardial contractile dysfunction: role of insulin signaling and mitochondrial function. Hypertension 2012; 59: 680-93.

[138] Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. J Am Coll Cardiol 2009; 54: 1891-8.

[139] Perseghin G, Lattuada G, De Cobelli F, Esposito A, Belloni E, Ntali G, Ragogna F, Canu T, Scifo P, Del Maschio A, Luzi L. Increased mediastinal fat and impaired left ventricular energy metabolism in young men with newly found fatty liver. Hepatology 2008; 47: 51-8.

[140] Perseghin G, Ntali G, De Cobelli F, Lattuada G, Esposito A, Belloni E, Canu T, Costantino F, Ragogna F, Scifo P, Del Maschio A, Luzi L. Abnormal left ventricular energy metabolism in obese men with preserved systolic and diastolic functions is associated with insulin resistance. Diabetes Care 2007; 30: 1520-6.

[141] Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S, Clarke K. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003; 107: 3040-6.

[142] Metzler B, Schocke MF, Steinboeck P, Wolf C, Judmaier W, Lechleitner M, Lukas P, Pachinger O. Decreased high-energy phosphate ratios in the myocardium of men with diabetes mellitus type I. J Cardiovasc Magn Reson 2002; 4: 493-502.

[143] Asmann YW, Stump CS, Short KR, Coenen-Schimke JM, Guo Z, Bigelow ML, Nair KS. 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: 3309-19.

[144] 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 U S A 2003; 100: 7996-8001.

[145] Karakelides H, Asmann YW, Bigelow ML, Short KR, Dhatariya K, Coenen-Schimke J, Kahl J, Mukhopadhyay D, Nair KS. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. Diabetes 2007; 56: 2683-9.

[146] Sleigh A, Stears A, Thackray K, Watson L, Gambineri A, Nag S, Campi VI, Schoenmakers N, Brage S, Carpenter TA, Murgatroyd PR, O'Rahilly S, Kemp GJ, Savage DB. Mitochondrial oxidative phosphorylation is impaired in patients with congenital lipodystrophy. J Clin Endocrinol Metab 2012; 97: E438-42.

[147] Hoeks J, van Herpen NA, Mensink M, Moonen-Kornips E, van Beurden D, Hesselink MK, Schrauwen P. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. Diabetes 2010; 59: 2117-25.

[148] de Brito OM, Scorrano L. Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. Antioxid Redox Signal 2008; 10: 621-33.

[149] Bach D, Pich S, Soriano FX, Vega N, Baumgartner B, Oriola J, Daugaard JR, Lloberas J, Camps M, Zierath JR, Rabasa-Lhoret R, Wallberg-Henriksson H, Laville M, Palacin M, Vidal H, Rivera F, Brand M, Zorzano A. Mitofusin-2 determines mitochondrial

network architecture and mitochondrial metabolism. A novel regulatory mechanism altered in obesity. J Biol Chem 2003; 278: 17190-7.

[150] Pich S, Bach D, Briones P, Liesa M, Camps M, Testar X, Palacin M, Zorzano A. The Charcot-Marie-Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. Hum Mol Genet 2005; 14: 1405-15.

[151] 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: 25060-70.

[152] Chen KH, Guo X, Ma D, Guo Y, Li Q, Yang D, Li P, Qiu X, Wen S, Xiao RP, Tang J. Dysregulation of HSG triggers vascular proliferative disorders. Nat Cell Biol 2004; 6: 872-83.

[153] Hernandez-Alvarez MI, Thabit H, Burns N, Shah S, Brema I, Hatunic M, Finucane F, Liesa M, Chiellini C, Naon D, Zorzano A, Nolan JJ. Subjects with early-onset type 2 diabetes show defective activation of the skeletal muscle PGC-1{alpha}/Mitofusin-2 regulatory pathway in response to physical activity. Diabetes Care 2010; 33: 645-51.

[154] 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: 2108-14.

[155] Sebastian D, Hernandez-Alvarez MI, Segales J, Sorianello E, Munoz JP, Sala D, Waget A, Liesa M, Paz JC, Gopalacharyulu P, Oresic M, Pich S, Burcelin R, Palacin M, Zorzano A. Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis. Proc Natl Acad Sci U S A 2012; 109: 5523-8.

[156] Quiros PM, Ramsay AJ, Sala D, Fernandez-Vizarra E, Rodriguez F, Peinado JR, Fernandez-Garcia MS, Vega JA, Enriquez JA, Zorzano A, Lopez-Otin C. Loss of mitochondrial protease OMA1 alters processing of the GTPase OPA1 and causes obesity and defective thermogenesis in mice. EMBO J 2012; 31: 2117-33.

[157] Jheng HF, Tsai PJ, Guo SM, Kuo LH, Chang CS, Su IJ, Chang CR, Tsai YS. Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. Mol Cell Biol 2012; 32: 309-19.

[158] Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, Lancet Physical Activity Series Working G. Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet 2012; 380: 219-29.

[159] Hoppeler H, Fluck M. Plasticity of skeletal muscle mitochondria: structure and function. Med Sci Sports Exerc 2003; 35: 95-104.

[160] 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: 2655-67.

[161] Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH, Jensen MD, Clark MM. Interindividual variation in posture allocation: possible role in human obesity. Science 2005; 307: 584-6.

[162] 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: 548-54.

[163] Ukropcova B, McNeil M, Sereda O, de Jonge L, Xie H, Bray GA, Smith SR. Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. J Clin Invest 2005; 115: 1934-41.

[164] Hulver MW, Berggren JR, Carper MJ, Miyazaki M, Ntambi JM, Hoffman EP, Thyfault JP, Stevens R, Dohm GL, Houmard JA, Muoio DM. Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. Cell Metab 2005; 2: 251-61.

[165] Barroso I, Luan J, Sandhu MS, Franks PW, Crowley V, Schafer AJ, O'Rahilly S, Wareham NJ. Meta-analysis of the Gly482Ser variant in PPARGC1A in type 2 diabetes and related phenotypes. Diabetologia 2006; 49: 501-5.

[166] Ling C, Poulsen P, Simonsson S, Ronn T, Holmkvist J, Almgren P, Hagert P, Nilsson E, Mabey AG, Nilsson P, Vaag A, Groop L. Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. J Clin Invest 2007; 117: 3427-35.

[167] Barres R, Osler ME, Yan J, Rune A, Fritz T, Caidahl K, Krook A, Zierath JR. Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 2009; 10: 189-98.

[168] Sookoian S, Rosselli MS, Gemma C, Burgueno AL, Fernandez Gianotti T, Castano GO, Pirola CJ. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor gamma coactivator 1alpha promoter. Hepatology 2010; 52: 1992-2000.

[169] Gemma C, Sookoian S, Dieuzeide G, Garcia SI, Gianotti TF, Gonzalez CD, Pirola CJ. Methylation of TFAM gene promoter in peripheral white blood cells is associated with insulin resistance in adolescents. Mol Genet Metab 2010; 100: 83-7.

[170] Ronn T, Poulsen P, Tuomi T, Isomaa B, Groop L, Vaag A, Ling C. Genetic variation in ATP5O is associated with skeletal muscle ATP50 mRNA expression and glucose uptake in young twins. PLoS One 2009; 4: e4793.

[171] Pirola CJ, Fernandez Gianotti T, Burgueno AL, Rey-Funes M, Loidl CF, Mallardi P, San Martino J, Castano GO, Sookoian S. Epigenetic modification of liver mitochon-

drial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut 2012;

[172] Galmozzi A, Mitro N, Ferrari A, Gers E, Gilardi F, Godio C, Cermenati G, Gualerzi A, Donetti E, Rotili D, Valente S, Guerrini U, Caruso D, Mai A, Saez E, De Fabiani E, Crestani M. Inhibition of Class I Histone Deacetylases Unveils a Mitochondrial Signature and Enhances Oxidative Metabolism in Skeletal Muscle and Adipose Tissue. Diabetes 2012;

[173] Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc) 2005; 70: 200-14.

[174] Hey-Mogensen M, Jeppesen J, Madsen K, Kiens B, Franch J. Obesity augments the age-induced increase in mitochondrial capacity for H2O2 release in Zucker fatty rats. Acta Physiol (Oxf) 2012; 204: 354-61.

[175] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006; 440: 944-8.

[176] Boden MJ, Brandon AE, Tid-Ang JD, Preston E, Wilks D, Stuart E, Cleasby ME, Turner N, Cooney GJ, Kraegen EW. Overexpression of manganese superoxide dismutase ameliorates high-fat diet-induced insulin resistance in rat skeletal muscle. Am J Physiol Endocrinol Metab 2012; 303: E798-805.

[177] Paglialunga S, van Bree B, Bosma M, Valdecantos MP, Amengual-Cladera E, Jorgensen JA, van Beurden D, den Hartog GJ, Ouwens DM, Briede JJ, Schrauwen P, Hoeks J. Targeting of mitochondrial reactive oxygen species production does not avert lipid-induced insulin resistance in muscle tissue from mice. Diabetologia 2012; 55: 2759-68.

[178] 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.

[179] Lesnefsky EJ, Hoppel CL. Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat. Biochim Biophys Acta 2008; 1777: 1020-7.

[180] Kendrick AA, Choudhury M, Rahman SM, McCurdy CE, Friederich M, Van Hove JL, Watson PA, Birdsey N, Bao J, Gius D, Sack MN, Jing E, Kahn CR, Friedman JE, Jonscher KR. Fatty liver is associated with reduced SIRT3 activity and mitochondrial protein hyperacetylation. Biochem J 2011; 433: 505-14.

[181] Hirschey MD, Shimazu T, Jing E, Grueter CA, Collins AM, Aouizerat B, Stancakova A, Goetzman E, Lam MM, Schwer B, Stevens RD, Muehlbauer MJ, Kakar S, Bass NM, Kuusisto J, Laakso M, Alt FW, Newgard CB, Farese RV, Jr., Kahn CR, Verdin E. SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. Mol Cell 2011; 44: 177-90.

[182] Jing E, Emanuelli B, Hirschey MD, Boucher J, Lee KY, Lombard D, Verdin EM, Kahn CR. Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signaling via

altered mitochondrial oxidation and reactive oxygen species production. Proc Natl Acad Sci U S A 2011; 108: 14608-13.

[183] Yechoor VK, Patti ME, Saccone R, Kahn CR. Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. Proc Natl Acad Sci U S A 2002; 99: 10587-92.

[184] Hojlund K, Yi Z, Lefort N, Langlais P, Bowen B, Levin K, Beck-Nielsen H, Mandarino LJ. Human ATP synthase beta is phosphorylated at multiple sites and shows abnormal phosphorylation at specific sites in insulin-resistant muscle. Diabetologia 2010; 53: 541-51.

[185] Hojlund K, Wrzesinski K, Larsen PM, Fey SJ, Roepstorff P, Handberg A, Dela F, Vinten J, McCormack JG, Reynet C, Beck-Nielsen H. Proteome analysis reveals phosphorylation of ATP synthase beta -subunit in human skeletal muscle and proteins with potential roles in type 2 diabetes. J Biol Chem 2003; 278: 10436-42.

[186] 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: 1644-9.

[187] 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: 643-53.

[188] Brons C, Jensen CB, Storgaard H, Alibegovic A, Jacobsen S, Nilsson E, Astrup A, Quistorff B, Vaag A. Mitochondrial function in skeletal muscle is normal and unrelated to insulin action in young men born with low birth weight. J Clin Endocrinol Metab 2008; 93: 3885-92.

[189] Karakelides H, Irving BA, Short KR, O'Brien P, Nair KS. Age, obesity, and sex effects on insulin sensitivity and skeletal muscle mitochondrial function. Diabetes 2010; 59: 89-97.

[190] Lefort N, Glancy B, Bowen B, Willis WT, Bailowitz Z, De Filippis EA, Brophy C, Meyer C, Hojlund K, Yi Z, Mandarino LJ. Increased reactive oxygen species production and lower abundance of complex I subunits and carnitine palmitoyltransferase 1B protein despite normal mitochondrial respiration in insulin-resistant human skeletal muscle. Diabetes 2010; 59: 2444-52.

[191] Nair KS, Bigelow ML, Asmann YW, Chow LS, Coenen-Schimke JM, Klaus KA, Guo ZK, Sreekumar R, Irving BA. Asian Indians have enhanced skeletal muscle mitochondrial capacity to produce ATP in association with severe insulin resistance. Diabetes 2008; 57: 1166-75.

[192] Toledo FG, Menshikova EV, Azuma K, Radikova Z, Kelley CA, Ritov VB, Kelley DE. Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite in-

creases in insulin action and decreases in intramyocellular lipid content. Diabetes 2008; 57: 987-94.

[193] 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: 743-52.

[194] Pagel-Langenickel I, Schwartz DR, Arena RA, Minerbi DC, Johnson DT, Waclawiw MA, Cannon RO, Balaban RS, Tripodi DJ, Sack MM. 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;

[195] 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: 2917-21.

[196] Brons C, Jacobsen S, Hiscock N, White A, Nilsson E, Dunger D, Astrup A, Quistorff B, Vaag A. Effects of high-fat overfeeding on mitochondrial function, glucose and fat metabolism, and adipokine levels in low-birth-weight subjects. Am J Physiol Endocrinol Metab 2012; 302: E43-51.

[197] Wredenberg A, Freyer C, Sandstrom ME, Katz A, Wibom R, Westerblad H, Larsson NG. Respiratory chain dysfunction in skeletal muscle does not cause insulin resistance. Biochem Biophys Res Commun 2006; 350: 202-7.

[198] Pospisilik JA, Knauf C, Joza N, Benit P, Orthofer M, Cani PD, Ebersberger I, Nakashima T, Sarao R, Neely G, Esterbauer H, Kozlov A, Kahn CR, Kroemer G, Rustin P, Burcelin R, Penninger JM. Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. Cell 2007; 131: 476-91.

[199] Vianna CR, Huntgeburth M, Coppari R, Choi CS, Lin J, Krauss S, Barbatelli G, Tzameli I, Kim YB, Cinti S, Shulman GI, Spiegelman BM, Lowell BB. Hypomorphic mutation of PGC-1beta causes mitochondrial dysfunction and liver insulin resistance. Cell Metab 2006; 4: 453-64.

[200] Choi CS, Befroy DE, Codella R, Kim S, Reznick RM, Hwang YJ, Liu ZX, Lee HY, Distefano A, Samuel VT, Zhang D, Cline GW, Handschin C, Lin J, Petersen KF, Spiegelman BM, Shulman GI. Paradoxical effects of increased expression of PGC-1alpha on muscle mitochondrial function and insulin-stimulated muscle glucose metabolism. Proc Natl Acad Sci U S A 2008; 105: 19926-31.

[201] 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: 31385-90.

[202] De Feyter HM, Lenaers E, Houten SM, Schrauwen P, Hesselink MK, Wanders RJ, Nicolay K, Prompers JJ. Increased intramyocellular lipid content but normal skeletal

muscle mitochondrial oxidative capacity throughout the pathogenesis of type 2 diabetes. Faseb J 2008; 22: 3947-55.

[203] Holmstrom MH, Iglesias-Gutierrez E, Zierath JR, Garcia-Roves PM. Tissue-specific control of mitochondrial respiration in obesity-related insulin resistance and diabetes. Am J Physiol Endocrinol Metab 2012; 302: E731-9.

[204] Brady LJ, Brady PS, Romsos DR, Hoppel CL. Elevated hepatic mitochondrial and peroxisomal oxidative capacities in fed and starved adult obese (ob/ob) mice. Biochem J 1985; 231: 439-44.

[205] Han DH, Hancock CR, Jung SR, Higashida K, Kim SH, Holloszy JO. Deficiency of the mitochondrial electron transport chain in muscle does not cause insulin resistance. PLoS One 2011; 6: e19739.

[206] Turner N, Bruce CR, Beale SM, Hoehn KL, So T, Rolph MS, Cooney GJ. 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: 2085-92.

[207] Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, Holloszy JO. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. Proc Natl Acad Sci U S A 2007; 104: 10709-13.

[208] Hancock CR, Han DH, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proc Natl Acad Sci U S A 2008;

[209] de Wilde J, Mohren R, van den Berg S, Boekschoten M, Dijk KW, de Groot P, Muller M, Mariman E, Smit E. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. Physiol Genomics 2008; 32: 360-9.

[210] Sadler NC, Angel TE, Lewis MP, Pederson LM, Chauvigne-Hines LM, Wiedner SD, Zink EM, Smith RD, Wright AT. Activity-based protein profiling reveals mitochondrial oxidative enzyme impairment and restoration in diet-induced obese mice. PLoS One 2012; 7: e47996.

[211] Stephenson EJ, Camera DM, Jenkins TA, Kosari S, Lee JS, Hawley JA, Stepto NK. Skeletal muscle respiratory capacity is enhanced in rats consuming an obesogenic Western diet. Am J Physiol Endocrinol Metab 2012; 302: E1541-9.

[212] Turner N, Hariharan K, TidAng J, Frangioudakis G, Beale SM, Wright LE, Zeng XY, Leslie SJ, Li JY, Kraegen EW, Cooney GJ, Ye JM. Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent: potent tissue-specific effects of medium-chain fatty acids. Diabetes 2009; 58: 2547-54.

[213] Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, Cooney GJ, Febbraio MA, Kraegen EW. 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.

[214] Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, Muoio DM. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008; 7: 45-56.

[215] Mihalik SJ, Goodpaster BH, Kelley DE, Chace DH, Vockley J, Toledo FG, DeLany JP. Increased levels of plasma acylcarnitines in obesity and type 2 diabetes and identification of a marker of glucolipotoxicity. Obesity (Silver Spring) 2010; 18: 1695-700.

[216] Adams SH, Hoppel CL, Lok KH, Zhao L, Wong SW, Minkler PE, Hwang DH, Newman JW, Garvey WT. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. J Nutr 2009; 139: 1073-81.

[217] Muoio DM, Noland RC, Kovalik JP, Seiler SE, Davies MN, DeBalsi KL, Ilkayeva OR, Stevens RD, Kheterpal I, Zhang J, Covington JD, Bajpeyi S, Ravussin E, Kraus W, Koves TR, Mynatt RL. Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. Cell Metab 2012; 15: 764-77.

[218] 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: 737-9.

[219] Ostergard T, Andersen JL, Nyholm B, Lund S, Nair KS, Saltin B, Schmitz O. 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.

[220] Toledo FG, Menshikova EV, Ritov VB, Azuma K, Radikova Z, DeLany J, Kelley DE. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. Diabetes 2007; 56: 2142-7.

[221] Nielsen J, Mogensen M, Vind BF, Sahlin K, Hojlund K, Schroder HD, Ortenblad N. Increased subsarcolemmal lipids in type 2 diabetes: effect of training on localization of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. Am J Physiol Endocrinol Metab 2010; 298: E706-13.

[222] Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A, Ravussin E. 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: 1337-44.

[223] Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Holloszy JO. Improvements in glucose tolerance and insulin action induced by in-

creasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr 2006; 84: 1033-42.

[224] Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, Smith SR, Ravussin E. Calorie restriction increases muscle mitochondrial biogenesis in healthy humans. PLoS Med 2007; 4: e76.

[225] Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO. Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 2005; 310: 314-7.

[226] Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Mataki C, Elliott PJ, Auwerx J. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab 2008; 8: 347-58.

[227] Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006; 127: 1109-22.

[228] Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature 2007; 450: 712-6.

[229] Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006; 444: 337-42.

[230] Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, Moonen-Kornips E, Hesselink MK, Kunz I, Schrauwen-Hinderling VB, Blaak EE, Auwerx J, Schrauwen P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab 2011; 14: 612-22.

[231] Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S, Schechtman KB, Gu C, Kunz I, Rossi Fanelli F, Patterson BW, Klein S. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. Cell Metab 2012; 16: 658-64.

[232] Canto C, Houtkooper RH, Pirinen E, Youn DY, Oosterveer MH, Cen Y, Fernandez-Marcos PJ, Yamamoto H, Andreux PA, Cettour-Rose P, Gademann K, Rinsch C, Schoonjans K, Sauve AA, Auwerx J. The NAD(+) precursor nicotinamide riboside en-

hances oxidative metabolism and protects against high-fat diet-induced obesity. Cell Metab 2012; 15: 838-47.

[233] Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 2011; 14: 528-36.

[234] Tainter ML, Stockton AB, Cutting WC. Use of dinitrophenol in obesity and related conditions. Final report. JAMA 1935; 105: 332-336.

[235] Miranda EJ, McIntyre IM, Parker DR, Gary RD, Logan BK. Two deaths attributed to the use of 2,4-dinitrophenol. J Anal Toxicol 2006; 30: 219-22.

[236] Tewari A, Ali T, O'Donnell J, Butt MS. Weight loss and 2,4-dinitrophenol poisoning. Br J Anaesth 2009; 102: 566-7.

[237] Lou PH, Hansen BS, Olsen PH, Tullin S, Murphy MP, Brand MD. Mitochondrial uncouplers with an extraordinary dynamic range. Biochem J 2007; 407: 129-40.

[238] Severin FF, Severina, II, Antonenko YN, Rokitskaya TI, Cherepanov DA, Mokhova EN, Vyssokikh MY, Pustovidko AV, Markova OV, Yaguzhinsky LS, Korshunova GA, Sumbatyan NV, Skulachev MV, Skulachev VP. Penetrating cation/fatty acid anion pair as a mitochondria-targeted protonophore. Proc Natl Acad Sci U S A 2010; 107: 663-8.

[239] Antonenko YN, Avetisyan AV, Cherepanov DA, Knorre DA, Korshunova GA, Markova OV, Ojovan SM, Perevoshchikova IV, Pustovidko AV, Rokitskaya TI, Severina, II, Simonyan RA, Smirnova EA, Sobko AA, Sumbatyan NV, Severin FF, Skulachev VP. Derivatives of rhodamine 19 as mild mitochondria-targeted cationic uncouplers. J Biol Chem 2011; 286: 17831-40.

[240] Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y, Ye JM, Lee CH, Oh WK, Kim CT, Hohnen-Behrens C, Gosby A, Kraegen EW, James DE, Kim JB. Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states. Diabetes 2006; 55: 2256-64.

[241] Dong H, Wang N, Zhao L, Lu F. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. Evid Based Complement Alternat Med 2012; 2012: 591654.

[242] Turner N, Li JY, Gosby A, To SW, Cheng Z, Miyoshi H, Taketo MM, Cooney GJ, Kraegen EW, James DE, Hu LH, Li J, Ye JM. 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: 1414-8.

[243] Eid HM, Martineau LC, Saleem A, Muhammad A, Vallerand D, Benhaddou-Andaloussi A, Nistor L, Afshar A, Arnason JT, Haddad PS. Stimulation of AMP-activated protein kinase and enhancement of basal glucose uptake in muscle cells by quercetin

and quercetin glycosides, active principles of the antidiabetic medicinal plant Vaccinium vitis-idaea. Mol Nutr Food Res 2010; 54: 991-1003.

[244] Brunmair B, Staniek K, Gras F, Scharf N, Althaym A, Clara R, Roden M, Gnaiger E, Nohl H, Waldhausl W, Furnsinn C. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? Diabetes 2004; 53: 1052-9.

[245] 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: 607-14.