

# Mitochondrial Function and Diabetes: Consequences for Skeletal and Cardiac Muscle Metabolism

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REVIEW ARTICLE

# Mitochondrial Function and Diabetes: Consequences for Skeletal and Cardiac Muscle Metabolism

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## Abstract

**Significance:** An early hallmark in the development of type 2 diabetes is the resistance to the effect of insulin in skeletal muscle and in the heart. Since mitochondrial function was found to be diminished in patients with type 2 diabetes, it was suggested that this defect might be involved in the etiology of insulin resistance. Although several hypotheses were suggested, yet unclear is the mechanistic link between these two phenomena. **Recent Advances:** Herein, we review the evidence for disturbances in mitochondrial function in skeletal muscle and the heart in the diabetic state. Also the mechanisms involved in improving mitochondrial function are considered and, whenever possible, human data is cited. **Critical Issues:** Reported evidence shows that interventions that improve skeletal muscle mitochondrial function also improve insulin sensitivity in humans. In the heart, available data from animal studies suggests that enhancement of mitochondrial function can reverse aging-induced changes in heart function, and can be protective against cardiomyopathy and heart failure. **Future Directions:** Mitochondria and their functions can be targeted with the aim of improving skeletal muscle insulin sensitivity and cardiac function. However, human clinical intervention studies are needed to fully substantiate the potential of mitochondria as a target to prevent cardiometabolic disease. *Antioxid. Redox Signal.* 24, 39–51.

## Mitochondria: Their Role in the Prevention and Treatment of Type 2 Diabetes Mellitus

**T**YPE 2 DIABETES MELLITUS is a chronic disease that is characterized by insulin resistance and elevated plasma glucose concentrations. During the postprandial phase, skeletal muscle is the major site of glucose uptake and a reduced insulin-stimulated glucose uptake into the muscle is suggested to play an important role in the development of whole body insulin resistance and type 2 diabetes. At a molecular level, skeletal muscle insulin resistance has been shown to be initiated by interference of fatty acid intermediates with insulin signaling (17, 38, 68), resulting in a reduced translocation of glucose transporter 4 (GLUT4) to the muscle cell membrane. In this respect, a reduced fat oxidative capacity and, more recently, a reduced overall mitochondrial metabolism have been postulated to underlie the develop-

ment of lipid-induced insulin resistance. In fact, mitochondrial dysfunction was suggested to be involved in the etiology of insulin resistance and type 2 diabetes (58). A decline in mitochondrial function has also been suggested to occur in the heart of type 2 diabetic patients, underlying certain forms of cardiomyopathy and heart failure (12, 65). Indeed, type 2 diabetic patients have a two-fold increased risk of developing heart failure, irrespective of hypertension and cardiovascular disease (21).

Most human data on the role of mitochondrial function in type 2 diabetes are available from the skeletal muscle. This organ can be easily accessible for obtaining biopsies, enabling *ex vivo* tissue analysis of mitochondrial metabolism. For cardiac muscle, this is more difficult and advances in this field strongly depend on noninvasive methodology. As an exception, recent clinical studies examined cardiac tissue that was sampled during heart surgery and examined *ex vivo* for

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mitochondrial respiration (3, 67, 73). Currently, our knowledge about human cardiac mitochondrial metabolism is expanding rapidly. Here, we will review the role of mitochondrial function in the muscle and heart, with an emphasis on human data.

#### Mitochondrial dysfunction in skeletal muscle

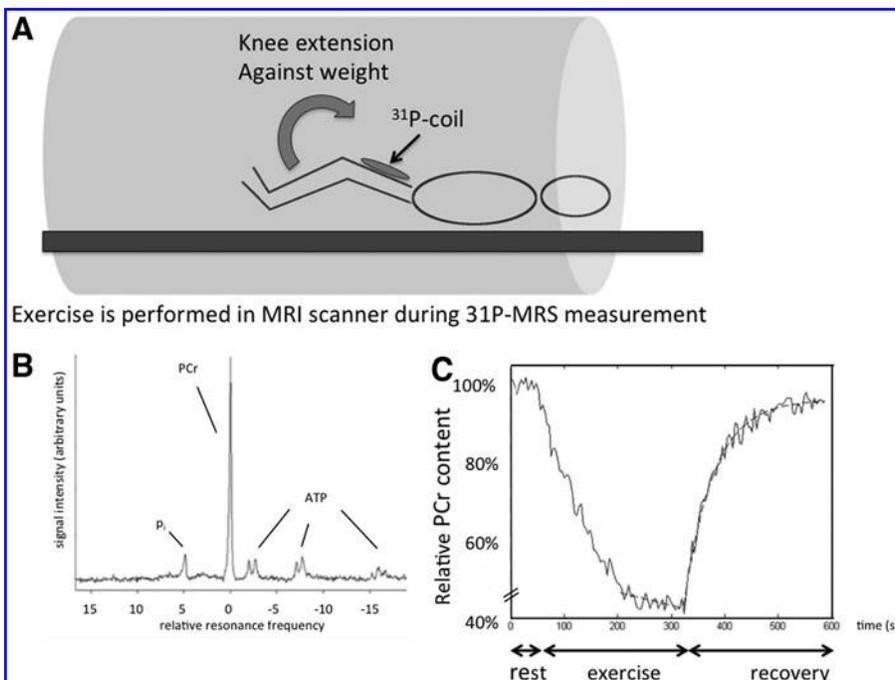
In the past decade of the previous century, it became recognized that type 2 diabetic patients are characterized by a reduced fat oxidative capacity. Early on in the present century, interest turned toward skeletal muscle mitochondria when a coordinated reduction of a large cluster of oxidative genes (OXPHOS) was reported in the skeletal muscle of patients with type 2 diabetes; a diminished expression of the coordinating transcription factor peroxisome proliferator coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) was also reported (64, 69, 78). Interestingly, these changes in mitochondrial markers were already visible in healthy subjects who were at an increased risk of developing diabetes, pointing out mitochondrial dysfunction as an early event in the development of diabetes.

In subsequent years, mitochondrial function in type 2 diabetic patients and healthy subjects was investigated with a wide range of methodologies, identifying aberrations in various aspects of appearance and performance of mitochondria in diabetes, which will be described next. Some (87, 92, 101), but not all studies (20) that used *in vivo* phosphorus magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) identified phosphocreatine (PCr) recovery after exercise to be delayed, suggesting a decreased *in vivo* mitochondrial capacity in type 2 diabetic patients. In these experiments, the time course of PCr is monitored by  $^{31}\text{P}$ -MRS *in vivo*, while subjects perform an exercise protocol inside the MR scanner. During exercise, PCr is consumed in skeletal muscle to form the adenosine triphosphate (ATP) needed for contraction. After cessation of exercise, PCr concentrations are quickly restored to resting values.

Using  $^{31}\text{P}$ -MRS methodology, the recovery of PCr can be examined in detail (Fig. 1). It was shown earlier that the resynthesis of PCr is almost exclusively fueled by aerobic metabolism (83) and therefore, the rate constant (or half-time) of PCr recovery can be used as an index of mitochondrial capacity (46). Indeed, parameters of PCr kinetics correlate with *ex vivo* markers of mitochondrial function and whole body oxygen uptake ( $\text{VO}_{2\text{max}}$ ) (52, 97).

Alternative  $^{31}\text{P}$ -MRS methods using saturation transfer methods examined ATP synthesis rates in muscle at rest and found decreased ATP synthesis rates in diabetic and prediabetic subjects, which was interpreted as hampered mitochondrial function (80). However, one should be aware that in this experimental setting, resting ATP synthesis is assessed, which is different from the investigation of maximal capacity of mitochondria (usually referred to as mitochondrial "function"). Saturation transfer data are even more difficult to interpret as not only oxidative but also especially glycolytic (nonmitochondrial) metabolism is examined by this method. As a result, the saturation transfer method overestimates the ATP synthesis rate many fold (45). Notwithstanding the limitations of saturation transfer methods, PCr recovery data correlated with ATP saturation transfer data, indicating that differences in unidirectional ATP synthesis rate as determined by saturation transfer may still give some indication of mitochondrial impairment (88).

Apart from noninvasive *in vivo* methodologies, an *ex vivo* analysis of mitochondrial respiration in muscle biopsies has been performed. In fresh muscle biopsies, oxygen consumption can be monitored in permeabilized muscle fibers or isolated mitochondria by high-resolution respirometry, *via* measurement of oxygen consumption on adenosine diphosphate (ADP) addition and substrates for oxidation. Using this methodology, basal as well as maximal ADP-stimulated respiration was reported to be decreased in type 2 diabetic patients (66, 82), and it persisted even after correction for mitochondrial density.



**FIG. 1. Determination of PCr kinetics by  $^{31}\text{P}$ -MRS.** (A) A patient is monitored with an MRI scanner and  $^{31}\text{P}$ -MRS while performing exercise; PCr kinetics is recorded in the active muscle. (B) A  $^{31}\text{P}$  spectrum of the vastus lateralis muscle. The resonance of inorganic phosphate ( $\text{P}_i$ ), PCr, and ATP is depicted. (C) Time course of PCr during exercise and recovery of a patient with type 2 diabetes (male, age = 67 years, BMI = 31 kg/m<sup>2</sup>). Spectra were measured every 4 s. A mono-exponential curve can be fitted to the curve of recovery of PCr after exercise to determine the half-time or rate constant. ATP, adenosine triphosphate; BMI, body mass index; MRS, magnetic resonance spectroscopy; PCr, phosphocreatine.

In addition to functional measurements, small mitochondria with altered morphology were reported in skeletal muscle of type 2 diabetic patients (27, 44) when investigated by electron microscopy, suggesting increased mitochondrial damage in diabetes. In search for explanatory mechanisms, increased levels of reactive oxygen species (ROS) were reported in skeletal muscle from type 2 diabetic subjects (4, 35). ROS may damage various organelles, including mitochondria. Hyperglycemia is known to increase ROS production (for review, see Ref. 28); therefore, hyperglycemia, inherent to diabetes, may also play a role in increasing mitochondrial ROS concentrations *in vivo*. Furthermore, damaged mitochondria, in turn, are known to produce higher levels of ROS (70), leading to a vicious cycle of mitochondrial damage (Fig. 2). In addition, the smaller and damaged mitochondria may also be a reflection of the impaired mechanisms of mitochondrial quality control (6).

Our knowledge about mitochondrial dynamics is currently rapidly increasing and processes involving mitochondrial fusion, fission, and mitophagy are investigated in detail (26a, 50a, 72, 86). It is suggested that along with age and metabolic disease, the cycle of mitochondrial fusion and fission is disturbed, resulting in a less stringent quality control, which decreases the ATP-generating capacity of mitochondria (reviewed in Ref. 60). Hallmark conditions of obesity and type 2 diabetes such as hyperglycemia and hyperlipidemia stimulate processes of mitochondrial fission in cell culture (103), resulting in fragmentation of the mitochondrial network, thereby hampering fusion–fission dynamics. In line with this, Mitofusin2 (Mfn2) was found to be reduced in skeletal muscle of type 2 diabetic patients (7) and a polymorphism in Mfn2

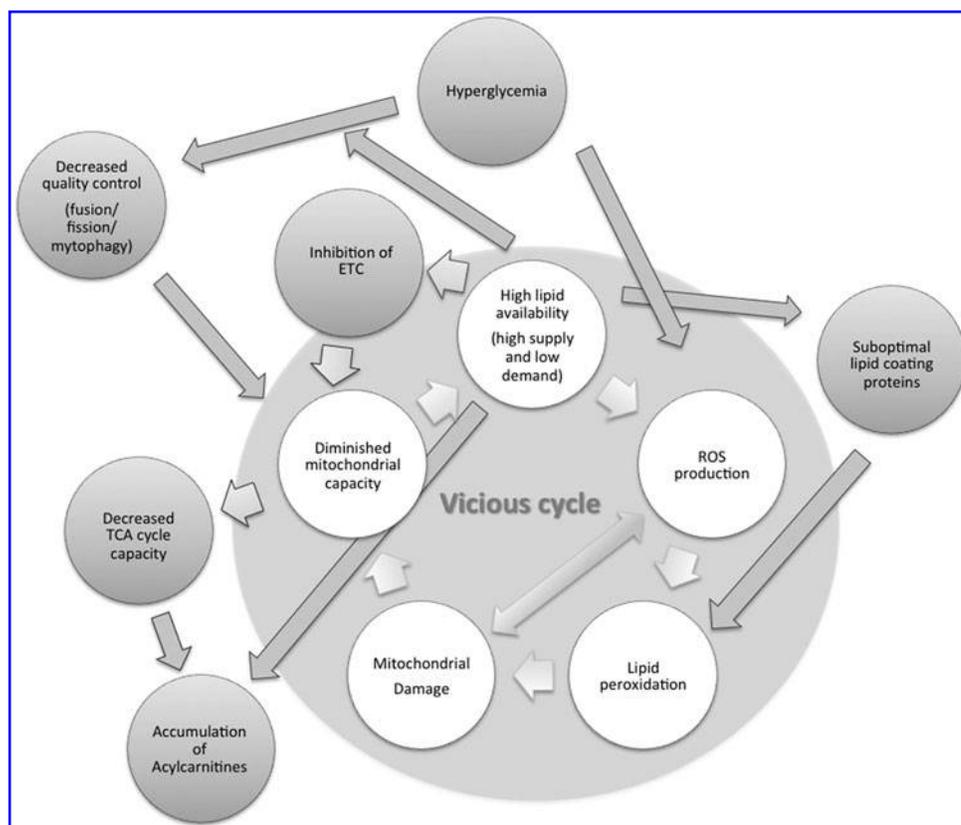
was reported to be associated with type 2 diabetes. These findings point out a possible role of disturbed mitochondrial dynamics in metabolic disease in the presence of substrate excess.

In summary, it is evident that the occurrence of type 2 diabetes is associated with a low mitochondrial capacity in the skeletal muscle. Mitochondrial dysfunction in skeletal muscle occurs at several levels, ranging from morphological perturbations to changes in gene- and protein expression, to impaired function according to *in vivo* and *ex vivo* data. However, as most data have been obtained from cross-sectional studies, and subject groups are not always carefully matched for age, body mass index, and physical activity, it is not completely clear as to what extent obesity, age, and physical inactivity contribute to the differences reported. Some studies indicate that even when controlling for these factors, a small but significant difference in mitochondrial function persists (92). Underlying mechanisms, such as ROS levels and increased fission, are currently under investigation (26a).

#### Mitochondrial dysfunction in cardiac muscle

It is well known that type 2 diabetic patients have an increased risk of developing heart failure (21). In overt heart failure, ATP production becomes insufficient to sustain normal contraction (37); thus, mitochondrial involvement is evident. However, early mitochondrial changes due to diabetes are less well established in humans (87a). The first evidence of disturbances in energy metabolism in cardiac muscle of diabetic patients stems from <sup>31</sup>P-MRS studies investigating cardiac PCr/ATP ratio *in vivo*

**FIG. 2. Schematic representation of the complex relationship between high lipid availability and mitochondrial dysfunction.** High lipid availability can cause diminished mitochondrial function *via* increased ROS levels and lipid peroxidation (89). Impaired mitochondrial function, in turn, can limit fat oxidative capacity, disturbing the balance between lipid availability and lipid use, further increasing lipid surplus in a vicious cycle. Alternatively, high lipid availability can directly affect electron transport in the respiratory chain (1). Combined with high levels of glucose, lipid surplus can hamper mitochondrial dynamics (103), interfering with normal mitochondrial quality control, thereby contributing to the deterioration of mitochondrial function. ROS, reactive oxygen species.



in “asymptomatic” patients (23, 87). The PCr/ATP ratio is considered an indicator of cardiac energy status, as PCr hydrolysis is the first rescue to maintain ATP concentration in the face of insufficient ATP synthesis (see phosphorus spectrum in Fig. 3). Therefore, mitochondrial dysfunction is expected to result in a decreased PCr/ATP ratio. Remarkably, mild diminishment of PCr/ATP ratio already occurs at a very early stage, when patients are asymptomatic with respect to cardiac disease (23, 87). This hints at a possible causal role of mitochondrial aberrations increasing the heart susceptibility to failure. Two independent studies reported decreased cardiac PCr/ATP ratio by 20%–35% in patients suffering from type 2 diabetes (23, 87).

A recent study showed increasing deterioration of mitochondrial metabolism in pigs during development of pace-induced heart failure, according to lessened conversion of hyperpolarized pyruvate to glutamate and CO<sub>2</sub> (94). “Hyperpolarization” of pyruvate enhances manifold the nuclear magnetic resonance sensitivity of this metabolite, making it possible to observe its processing further into downstream metabolites (e.g., glutamate and CO<sub>2</sub>), even if concentrations are low. Interestingly, the steady decrease in oxidative pyruvate metabolism that precedes the manifestation of heart failure was paralleled by a similar decrease in PCr/ATP levels, both of which were measured noninvasively by MRS. These data suggest that the PCr/ATP ratio may indeed be a valuable marker of cardiac mitochondrial function at the onset of heart failure.

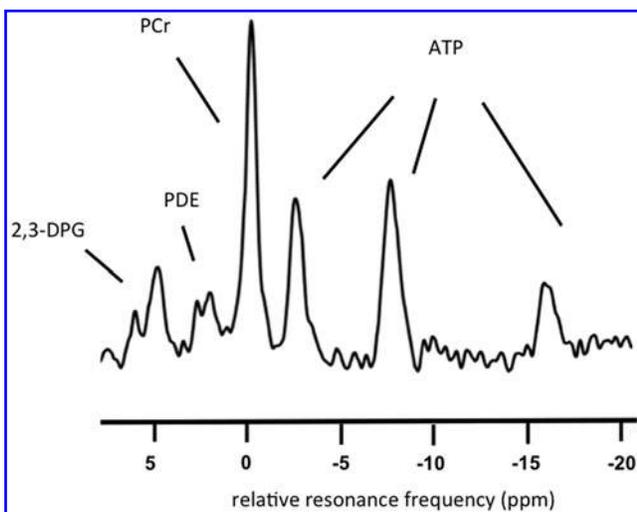
As in skeletal muscle, mitochondrial function of the heart can be affected by perturbations in many processes such as biogenesis, increased damage *via* ROS, and decreased quality control. A few recent studies (3, 67, 73) sampled atrial tissue in diabetic and control subjects during coronary artery bypass graft surgery. During such a surgery, a small atrial sample could be taken to investigate this in greater detail in humans (3, 67, 73). Two studies investigated *ex vivo* mitochondrial function in atrial tissue by high-resolution respirometry in diabetic patients and control subjects, and both found defects

in atrial myofibers from diabetic patients. While Anderson *et al.* (3) found no difference in state 3 respiration on pyruvate or succinate in diabetic patients, state 3 respiration was lower in permeabilized heart muscle fibers from diabetic subjects when supported by glutamate/malate. Since the latter substrate combination feeds complex I of the respiratory chain, these findings suggest a complex I abnormality in cardiac mitochondria from diabetic patients. The emission of H<sub>2</sub>O<sub>2</sub> was also higher in diabetic atrial tissue, either in the presence of increasing concentrations of succinate while inhibiting ATPase with oligomycin or during submaximal state 3 respiration. Furthermore, a more oxidative milieu was found in diabetic patients, as can be judged by a higher concentration of oxidized glutathione (GSSG) with respect to total glutathione (GSHt). Oxidative stress was further demonstrated by higher steady-state lipid peroxidation and nitrosation as assessed by immunoblot of proteins from atrial homogenates (hydroxynonenal [HNE] and 3-nitrotyrosine modified proteins).

While these results indicate impaired mitochondrial function, no evidence for decreased mitochondrial biogenesis was found, as PGC-1 $\alpha$  expression and peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) protein levels were unchanged (3). In the second, more recent study, Montaigne *et al.* (67) showed that several respiratory chain complex activities, namely from complex II and III, were lowered in atrial tissue from diabetic patients compared with nondiabetic patients. Furthermore, state 3 respiration supported by fatty acid-like, pyruvate, or succinate substrates was reduced in permeabilized fibers in cardiac tissue from diabetic patients. These data also point out an inefficient generation of ATP, as indicated by a poor coupling of oxidative phosphorylation (*i.e.*, low respiratory control ratio [RCR] on palmitate and pyruvate-supported respiration in diabetic tissue).

Furthermore, an increased atrial ROS production (as measured by electron paramagnetic resonance spectroscopy) and elevated activity of anti-oxidant enzymes (mitochondrial MnSOD and cytosolic catalase) were found (67). Again, also in this study, no defect in PGC-1 $\alpha$  expression was detected. Together with the finding that citrate synthase activity and mitochondrial density (as determined by electron microscopy) were similar in the cardiac tissue of diabetic patients and the control subjects, these data indicate normal mitochondrial biogenesis in the human diabetic heart (67). The disturbance in mitochondrial function might be relevant for contractile function, since diabetes was also associated with pronounced contractile dysfunction in these patients. The twitch force that developed *ex vivo* by atrial trabeculae from diabetic patients was significantly lower than in nondiabetic subjects. Although Ca<sup>2+</sup> handling was also affected in the hearts from diabetic patients and the Ca<sup>2+</sup> retention correlated with *ex vivo* twitch force, the latter was also correlating with parameters of mitochondrial function, suggesting a possible link between these two functional variables. It was proposed that hyperglycemia may be at the basis of the mitochondrial impairments, as parameters of mitochondrial function correlated negatively with HbA1C plasma concentrations (67).

Furthermore, mitochondria were smaller in atrial tissue from diabetic patients and the expression of fusion-related MFN1 protein was lower in tissue from diabetic patients and correlated negatively with HbA1C plasma concentrations. However, other dynamic-related proteins were unchanged (67).



**FIG. 3.** <sup>31</sup>P spectrum of the myocardium of a young healthy volunteer, acquired with one-dimensional MR spectroscopic imaging. The resonances of 2,3-DPG, PDE, PCr, and ATP are depicted. The PCr/ATP ratio can be calculated as a parameter of energy status. PDE, phosphodiester.

In summary, there is convincing evidence from *in vivo* and *ex vivo* studies that cardiac mitochondrial function is disturbed in the diabetic state. The underlying mechanism seems not to be related to altered PGC-1 $\alpha$  or PPAR expression and mitochondrial biogenesis, while mitochondria of diabetic patients show more uncoupling, higher ROS production, and changes in their fusion–fission dynamics. In this setting, the increased uncoupling may be at least partially due to the activation of uncoupling proteins (UCP2,3), which are known to be regulated by oxidative stress (14). This may be a protective mechanism, reducing oxidative stress at the cost of efficiency. In that respect, it has been shown that db/db mice are characterized by increased ROS and enhanced mitochondrial uncoupling (13). This notion is further supported by the finding that a polymorphism that results in lower transcription of UCP2 was associated with oxidative stress in humans (85).

### Functional Consequences of Mitochondrial Dysfunction for Insulin Resistance in Humans

Insulin sensitivity in skeletal muscle is an important determinant of whole body insulin action, as skeletal muscle is the major site of insulin-stimulated glucose uptake (22). Increased skeletal muscle lipid content (intramyocellular lipid [IMCL]) has been repeatedly found to be strongly related to insulin resistance (39, 50, 77) and elevated in type 2 diabetic patients as well as in subjects with an increased risk of developing diabetes later in life (39).

In endurance trained athletes, who are also characterized by high IMCL levels, this correlation between IMCL and insulin resistance is, however, absent. In that context, it was emphasized by us and others that the combination of high lipid availability and low oxidative capacity may be at the basis of insulin resistance. Seemingly, lipid infusion has a less pronounced effect in hampering insulin sensitivity in subjects with a high mitochondrial capacity. During lipid infusion, insulin sensitivity decreased by only 29% in endurance trained subjects as compared with 63% in untrained subjects (81), suggesting that a high mitochondrial function could protect from diabetes.

Currently, there are different hypotheses on how mitochondrial dysfunction, in concert with a high lipid bioavailability (high IMCL), could hamper insulin sensitivity in the skeletal muscle. On the one hand, a predisposition for low mitochondrial function and therefore low fat oxidative capacity was suggested to be causing IMCL accumulation in sedentary subjects in the first place (80). In turn, intracellular lipid excess with respect to demand might be hampering insulin signaling *via* cytosolic accumulation of lipid intermediates such as diacylglycerol (DAG), ceramides of long-chain acyl-CoA or long-chain acyl-carnitine species (58). The consequences of high lipid bioavailability may be aggravated by suboptimal “packaging” of lipid droplets (IMCL) by lipid coating proteins (as recently reviewed in Ref. 11).

Alternatively to the suggestion of an initial defect in mitochondria, their reduced function may also be a consequence of high fatty acid abundance, causing mitochondrial damage *via* increased ROS levels and lipid peroxidation (89). Similarly, an imbalance between mitochondrial tricarboxylic acid (TCA) cycle activity and lipid availability was suggested to

lead to fatty acids being trapped in the form of long-chain acyl-carnitine hampering insulin sensitivity directly (2) or diminishing the availability of free carnitine, which was also associated with insulin resistance (55, 75).

Finally, a vicious cycle of weakened mitochondrial capacity and quality on the one hand, and excessive lipid substrate on the other hand, may be at the basis of cellular changes, leading to the development of insulin resistance (Fig. 2). Interestingly, diminished mitochondrial function was also shown to be associated with a loss of muscle mass (sarcopenia) (43). As skeletal muscle is responsible for the main part of insulin-induced glucose uptake, the loss of muscle mass, for example, during aging, further aggravates whole-body insulin resistance.

Although several mechanisms have been proposed to mediate insulin resistance during metabolic challenge, the exact mechanisms of how insulin sensitivity is diminished are yet unknown.

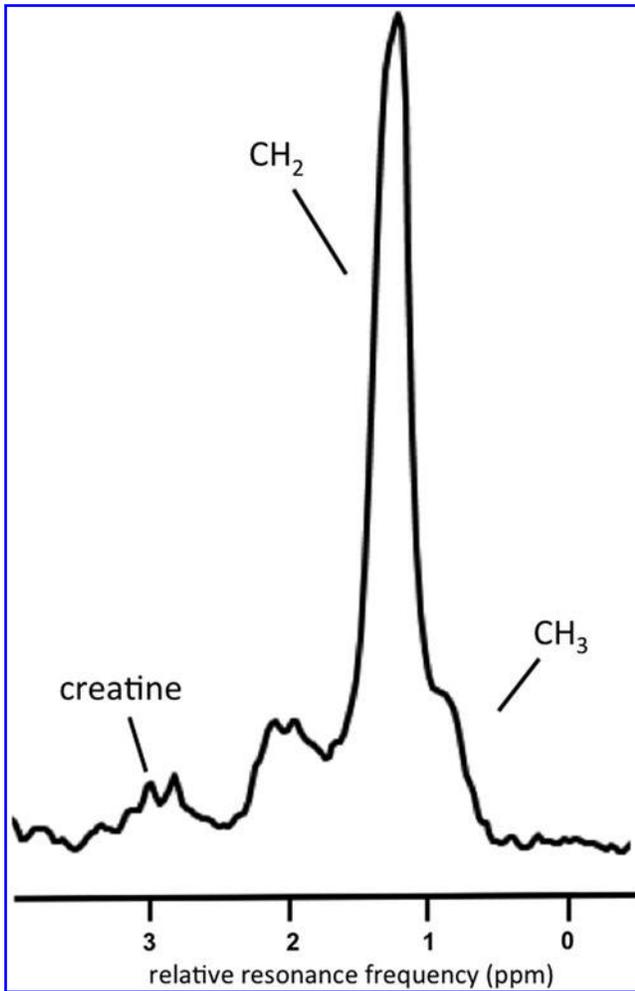
The debate on how mitochondrial fatty acid load and oxidative capacity are related to insulin resistance is also highly relevant for cardiac muscle. Insulin resistance in the heart is less crucial for whole-body glucose uptake, but it makes the heart inflexible with respect to substrate selection. Ultimately, this may increase the susceptibility to cardiac injury and apoptosis.

In the heart, an oversupply of lipids seems to be central in the initiation of unfavorable metabolic remodeling and insulin resistance as seen in diabetic cardiomyopathy. In overweight and diabetic humans, elevated cardiac fat content in the heart is observed (61), investigated with MRS (see lipid spectrum in Fig. 4). It has been proposed that elevated PPAR $\alpha$  activation, due to increased fatty acid availability, is involved (25). PPAR activation stimulates mitochondrial biogenesis and increases fatty acid uptake, leading to lipid accumulation in the heart of overweight and diabetic patients. Furthermore, fatty acid-induced PPAR $\alpha$  stimulation also augments the expression of pyruvate dehydrogenase kinase 4 (PDK4) that reduces carbohydrate metabolism by inhibiting the conversion of pyruvate to acetyl-CoA, thereby hampering the entry of the end product of glycolysis to the TCA cycle.

Although PDK4 activation would favor the oxidation of fatty acids, it also diminishes an insulin-stimulated switch to glucose oxidation, making the heart less metabolically flexible and insulin resistant. Furthermore, the accumulation of cardiac lipids is associated with the formation of lipid metabolites that were shown to interfere with insulin signaling *in vitro*, such as DAG and ceramides (17, 42). This would decrease metabolic flexibility in the heart and increase its dependence on fatty acids. Importantly, the high rates of fatty acid oxidation in the heart are associated with elevated oxygen consumption and high ROS production (57). In turn, ROS are believed to be able to damage mitochondria, lowering their function in diabetes (10, 99). In particular, cardiolipin, a phospholipid that stabilizes the inner mitochondrial membrane, was shown to be prone to oxidative damage (53). Therefore, mitochondrial quality and optimal functioning of the mitochondrial network seems to be challenged in the presence of high lipid availability and insulin resistance.

### Mitochondrial Function as a Target to Improve Insulin Sensitivity?

Although the mechanisms underlying the link between mitochondrial function, ectopic lipid storage, and insulin



**FIG. 4.**  $^1\text{H}$  spectrum of the interventricular septum of the heart of a healthy volunteer with elevated BMI (BMI=30), acquired with MR spectroscopy. The lipid resonances of the  $\text{CH}_2$  and  $\text{CH}_3$  group of fatty acids are depicted. Using the unsuppressed water resonance (not shown), a fat/water ratio can be calculated to quantify the cardiac fat fraction.

resistance are still not completely unraveled, accumulating evidence shows that improvement in mitochondrial function and quality in skeletal muscle have a positive effect on insulin sensitivity, while less is known about the human heart.

#### *Physical activity and exercise training to improve mitochondrial capacity*

A robust way to improve the ATP-generating capacity of the mitochondrial system is by physical activity training. It is well known that contraction in the skeletal muscle activates AMP-activated protein kinase (AMPK) and induces expression of PGC- $1\alpha$ , nuclear respiratory factor 1 (NRF-1), and NRF-2, resulting in increased transcription of mitochondrial proteins and stimulation of mitochondrial biogenesis (9, 59). Therefore, regular exercise consistently activates these pathways, which in the long term lead to a higher mitochondrial capacity, at least partly due to an increased mitochondrial density. Studies from our group and

others reported enhanced mitochondrial function *in vivo* in response to physical activity programs consisting of endurance and strength training ranging from 2 weeks to 1 year (26, 62, 63, 101). Exercise training also leads to an elevated content in cardiolipin, known to be necessary for the assembly of respiratory chain complexes in the inner mitochondrial membrane (100). Importantly, type 2 diabetic patients were just as responsive as healthy subjects to exercise-induced improvements in mitochondrial function (63).

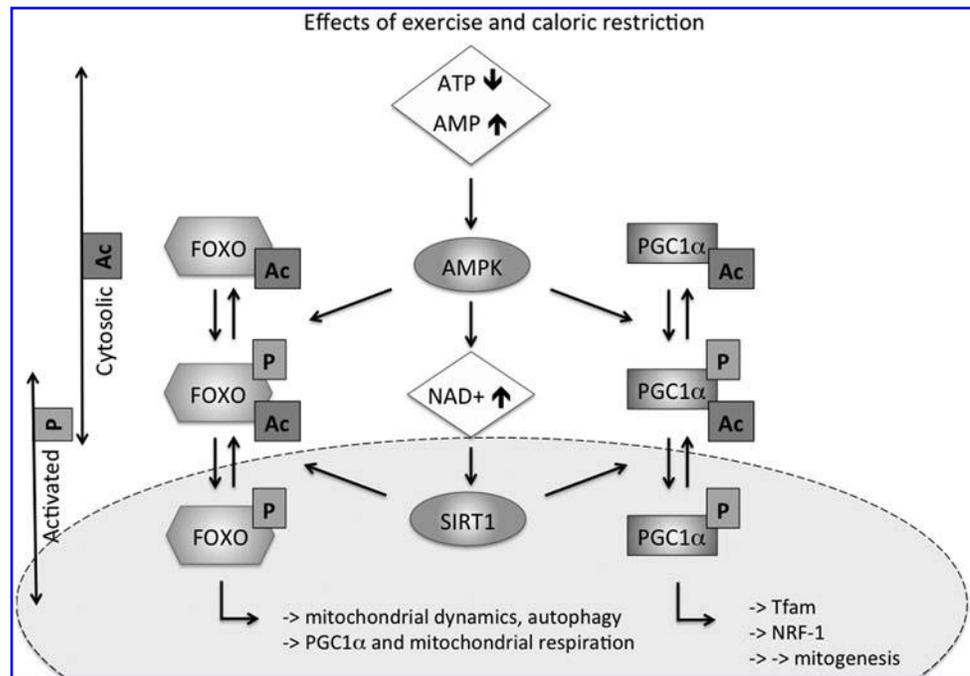
Training-induced improvements in *in vivo* mitochondrial function (as determined by  $^{31}\text{P}$ -MRS) were accompanied by increased mitochondrial oxidative capacity determined *ex vivo* using high-resolution respirometry (63). In fact, the reduced mitochondrial function observed in type 2 diabetic patients could be completely restored to control values in endurance training programs as short as 3 months. Enhanced mitochondrial function is also paralleled by decreased insulin resistance and augmented IMCL content.

These data show at least an association between improved mitochondrial function and insulin sensitivity in humans. It should be noted that the insulin-sensitizing effect of exercise training has long been recognized (34, 49), although the exact underlying mechanism leading to a more prominent insulin-induced translocation of GLUT4 in the trained state is still unclear. While it is remarkable that exercise-elicited enhancement of mitochondrial function is accompanied by improved insulin sensitivity, it should be acknowledged that exercise training causes various adaptations and it is unknown as to what extent the resulting improvement in mitochondrial function is responsible for the increased insulin sensitivity.

These adaptations are more difficult to investigate in the human heart, since cardiac tissue is not readily available; thus, for now, we have to rely on animal data or human  $^{31}\text{P}$ -MRS studies. In mice, daily swimming increased cardiac expression of transcription factors that orchestrate translation of mitochondrial proteins, such as PGC- $1\alpha$ , NRF-1, and mitochondrial transcription factor A (Tfam) (102). As in skeletal muscle, mitochondrial biogenesis in the heart is also stimulated by regular exercise, as reported in rodent models. In mice, exercise training increases mitochondrial volume and number (102). All these adaptations were endothelial nitric oxide synthase (eNOS) dependent, underscoring the relevant role of nitric oxide (NO) as a signaling molecule for training-induced cellular remodeling in the heart. In cardiomyocytes, eNOS becomes more active when phosphorylated by AMPK (18) and in the presence of high cellular calcium concentrations. Both cardiac AMPK activity and calcium concentrations are known to be enhanced by exercise (76), probably constituting important signaling routes leading to the improvement of mitochondrial function in the heart (Fig. 5).

Very little data are available in the human heart; however, there are some indications that mitochondrial function is also improved by exercise training. Left ventricular PCr/ATP ratio was found to be elevated in life-long physically active men when compared with sedentary individuals (79), and a correlation between cardiac PCr/ATP and physical fitness was reported in healthy young men (48). However, effects on cardiac energy metabolism were less clear in patients with heart failure who were subjected to an 8-week mild training protocol. The training intervention improved functional

**FIG. 5. Scheme of molecular mechanisms that mediate the beneficial effects of CR and exercise on mitochondrial function.** CR and exercise activate AMPK and SIRT1 that result in phosphorylation (30, 40) and deacetylation (15, 84) of FOXO and PGC-1 $\alpha$ . PGC-1 $\alpha$  and FOXO activation stimulate the transcription of nuclear genes related to mitochondrial function. AMPK, AMP-activated protein kinase; CR, calorie restriction; PGC-1 $\alpha$ , peroxisome proliferator coactivator 1- $\alpha$ .



parameters such as left ventricular ejection fraction, but it did not affect the PCr/ATP ratio (33).

It is worth noting that cardiac lipid content, as determined by noninvasive  $^1\text{H-MRS}$ , was decreased after training in overweight/obese subjects. This would be in line with improved mitochondrial function, leading to normalization of cardiac lipid content (91). However, quite strikingly, a reduction in cardiac lipid content with training could only be detected in healthy subjects and did not occur in patients with type 2 diabetes (93). However, the explanation of this differential response to exercise is unclear.

Likewise, mitochondrial quality control in the heart may be beneficially influenced by physical activity, improving mitochondrial respiratory capacity. Induction of autophagy has been reported after endurance training in mice (32). Mfn2, an indicator of mitochondrial fusion activity, was increased in the cardiac muscle of mice after daily swimming (102). Diminished cardiac mitochondrial function in post-infarction rats, characterized by adverse mitochondrial network dynamics (reduced fusion and increased fission), was normalized after aerobic interval training. RCR and P/O were elevated after training and complex I, III, and IV activities were enhanced concomitantly with normalization of markers of mitochondrial fusion (mfn2 and OPA1) and fission (dynamin-related protein 1, DRP1) (41).

In summary, together with higher mitochondrial density, optimization of mitochondrial dynamics may also underlie the training-induced improvement in mitochondrial function, although more data are needed to understand how physical activity affects these processes (26a). Furthermore, more human studies will be necessary to investigate how exercise training may impact cardiac energy metabolism and whether exercise-induced improvement of cardiac function depends on enhanced cardiac mitochondrial function (31a).

#### Calorie Restriction and Calorie Restriction Mimetics to Improve Mitochondrial Function

The generally accepted health benefits of physical activity have urged researchers to find alternatives to exercise with the aim of modulating, and potentially improving, mitochondrial function. From cellular studies, it is well known that energy scarcity (fasting, calorie restriction [CR]) leads to PGC-1 $\alpha$  and forkhead box O protein (FOXO) activation, increasing the transcription of mitochondrial genes in various cell types (5, 71). PGC-1 $\alpha$  and FOXO need to be phosphorylated by AMPK (30, 40) and acetylated by sirtuin1 (SIRT1) (15, 84) to become active (Fig. 5). Furthermore, there are indications that CR increases autophagy (8) (by deacetylation of autophagy protein 5 (Atg5) and Atg7, via SIRT activation) (104), which may reflect a more stringent control of mitochondrial quality. Mitochondrial efficiency is increased with CR, preserving ATP generation at a lower oxygen cost while producing less ROS (56). Indeed, *in vitro* results show that the number of low-potential mitochondria is increased on CR (56).

These findings were mostly confirmed in both skeletal muscle and the heart in rodents *ex vivo*, with very pronounced reductions in ROS generation. Still, there is some discussion about the postulated stimulation of mitochondrial biogenesis by CR according to recent studies showing contradictory results (reviewed in Ref. 29). Nevertheless, human data are still very limited, even for skeletal muscle. One study showed that CR can indeed improve mitochondrial function also in humans. Six months of 25% CR in young overweight subjects improved oxidative capacity and increased the expression of PGC-1 $\alpha$ , TFAM, and SIRT1, as well as of mtDNA content in skeletal muscle tissue (19). On the other hand, weight reduction studies have not been consistent in reporting improved mitochondrial function (24).

In a very small study with overweight male subjects,  $\text{VO}_{2\text{max}}$  increased after 25% CR for 7 weeks together with

AMPK and SIRT activation in peripheral blood mononuclear cells. Interestingly, muscle cells that were cultured in serum from these calorie-restricted subjects showed an increase in both AMPK and SIRT activities and mitochondrial biogenesis (47). In another human study, long-term CR influenced transcriptional activity of the insulin-like growth factor/insulin signaling, mitochondrial biogenesis, and inflammation pathways, as evaluated by gene expression profiling (51).

For the heart, we have to rely largely on rodent studies. In mice, CR was shown to induce mitochondrial biogenesis by upregulating PGC-1 $\alpha$ , NRF-1, and TFAM (74). In addition, MFN1 and MFN2 were induced, indicating changes in mitochondrial dynamics (74). In rats, CR decreased the amount of acetylated mitochondrial proteins in the heart and, although not different with respect to the basal state, mitochondrial function was preserved on ischemia/reperfusion in the restricted group only, and this was accompanied by lower H<sub>2</sub>O<sub>2</sub> emission (96). Interestingly, the CR-mediated adaptation also produced functional consequences, since CR ameliorates aging-related diastolic dysfunction in rats (95).

Research on the health benefits of CR has also stimulated the search for compounds that could mimic its effects. In this context, sirtuins activation is a desired effect. One of the compounds that has received much attention lately is resveratrol (90, 96a). Resveratrol (3, 5, 4' trihydroxystilbene) is a polyphenol naturally present in several plants, and it is identified as a small-molecule activator of sirtuin 1 (SIRT1) (36). Resveratrol is believed to have CR-like effects, making it a promising candidate for treatment and prevention of metabolic diseases (16, 98, 105).

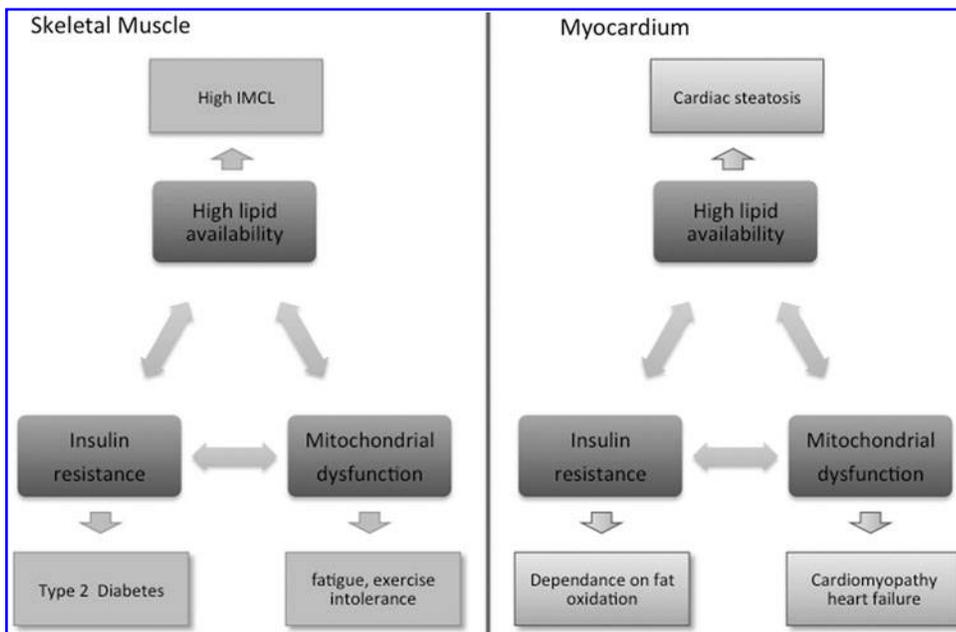
In humans, we showed that supplementing overweight healthy men with resveratrol improved skeletal muscle mitochondrial efficiency according to *ex vivo* high-resolution respirometry and the maximal mitochondrial respiration attained in the presence of complex I and complex II substrates. Furthermore, gene enrichment analysis revealed that resver-

atrol activates mitochondrial pathways related to ATP synthesis and oxidative phosphorylation. In accordance with earlier rodent data, resveratrol supplementation increased citrate synthase and AMPK activities while inducing increased SIRT1 protein levels in the skeletal muscle (98).

Regarding cardiac function, many beneficial effects of resveratrol on the rodent heart have been described; unfortunately, very little is known about its effects on the human myocardium. In aged rats, a comparatively similar increase in fractional shortening was determined after resveratrol treatment or endurance training. However, only resveratrol was able to protect against apoptosis (54). Resveratrol prevented adverse cardiac remodeling on pressure overload in mice. Likewise, markers of oxidative stress, cardiac hypertrophy, inflammation, fibrosis, hypoxia, and apoptosis, all of which were increased in response to pressure overload, were significantly reduced in the mice group treated with resveratrol (31). However, the occurrence of these salutary effects remains to be investigated in humans.

Clearly, more human data is needed on the impact of nutritional and/or pharmacological activation of mitochondrial metabolism on skeletal muscle insulin sensitivity and cardiac function. Currently, some registered drugs are known to be ligands of mitochondrial transcription factors and promote mitochondrial biogenesis, such as, for example, fibrates or glitazones that bind to PPAR $\alpha$  or PPAR $\gamma$ , respectively. Currently, any of the currently used drugs are as efficient as a healthy lifestyle, and more specific mitochondrial compounds need to be tested. In spite of the progress achieved in understanding mitochondrial function and how it can be boosted, current knowledge indicates that the most effective and safest way to stimulate mitochondrial function is *via* a moderate caloric intake and physical activity.

In conclusion, improving mitochondrial function has beneficial effects on insulin sensitivity, although the mechanisms are not yet fully elucidated. Since the skeletal muscle is the main organ responsible for insulin-stimulated glucose



**FIG. 6. High lipid availability, mitochondrial dysfunction, and insulin resistance interrelationship, and their consequences in cardiac and skeletal muscle.** Mitochondrial dysfunction is associated with tissue dysfunction in both organs, resulting in insulin resistance, exercise intolerance, and cardiomyopathy. Insulin resistance in the skeletal muscle is an early hallmark of type 2 diabetes development. In the heart, insulin resistance increases the dependence on fat oxidation. High lipid availability and accumulation of intracellular fat are the most likely involved in the development of insulin resistance and mitochondrial dysfunction.

uptake, the improvement of mitochondrial function is a promising target to improve whole body insulin sensitivity in type 2 diabetic patients (Fig. 6). This is even more important, as mitochondrial impairment has been shown to lead to muscle mass reduction.

In the heart, the available data from animal studies suggest that improvement of mitochondrial function can reverse aging-induced changes (Fig. 6). Interventions that improve mitochondrial function are protective against cardiomyopathy and ischemia/reperfusion damage. More studies are needed to confirm these findings in humans.

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#### Abbreviations Used

ADP = adenosine diphosphate  
 AMPK = AMP-activated protein kinase  
 ATP = adenosine triphosphate  
 BMI = body mass index  
 CR = calorie restriction  
 DAG = diacylglycerol  
 eNOS = endothelial nitric oxide synthase  
 GLUT4 = glucose transporter 4  
 HNE = hydroxynonenal  
 IMCL = intramyocellular lipids  
 MRS = magnetic resonance spectroscopy  
 NO = nitric oxide  
 NRF 1,2 = nuclear respiratory factor 1,2  
 PCr = phosphocreatine  
 PDK4 = pyruvate dehydrogenase kinase 4  
 PGC-1 $\alpha$  = peroxisome proliferator coactivator 1- $\alpha$   
 PPAR = peroxisome proliferator-activated receptor  
 RCR = respiratory control ratio  
 ROS = reactive oxygen species  
 TCA = tricarboxylic acid  
 UCP = uncoupling protein

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