

Mitochondria, lipotoxicity and skeletal muscle metabolism : implications for type 2 diabetes mellitus

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SUMMARY

Summary

The prevalence of obesity and its associated comorbidities is increasing dramatically worldwide. Obesity is considered a major risk factor for other chronic diseases such as type 2 diabetes mellitus (T2DM). One of the recognized characteristics in the early onset of T2DM is insulin resistance. Although the exact underlying mechanism of insulin resistance is not completely understood, lipid accumulation in skeletal muscle is strongly associated with T2DM. In obesity, caloric intake in excess of total body energy expenditure could result into augmented lipid accumulation, not only in adipose tissues but also in non-adipose tissues such as skeletal muscle. Excess lipid accumulation in skeletal muscle can result in toxic effects on its function and is therefore also referred to as lipotoxicity. Since skeletal muscle is responsible for the majority of the postprandial insulin-stimulated glucose uptake, skeletal muscle can be considered the most important site of insulin resistance. Because mitochondria are the primary cellular site for fatty acid oxidation and utilization, strategies to manipulate aspects of skeletal muscle mitochondrial function could potentially modulate lipid handling and therefore insulin sensitivity. Besides generating ATP, mitochondria inevitably produce reactive oxygen species (ROS). Elevated levels of ROS could result into oxidative damage in skeletal muscle, which in turn has been suggested to lead to disturbed mitochondrial function, insulin signaling, and eventually progression of insulin resistance. Blunting mitochondrial ROS production could be an approach to alleviate insulin resistance by lowering muscle oxidative stress. Thus, modulation of skeletal muscle mitochondrial oxidative capacity and/or oxidative stress could be potential strategies to tackle insulin resistance and T2DM.

In this context, a novel mitochondria-specific antioxidant; the Skulachev ion (SkQ, plastoquinonyl decyltriphenyl-phosphonium), has been reported to effectively reduce oxidative damage. The study in **chapter 2** confirmed that SkQ treatment can reduce oxidative stress in skeletal muscle *in vitro* and under high fat conditions *in vivo*. However, despite this relief of oxidative stress, SkQ treatment did not ameliorate lipid-induced insulin resistance. These findings illustrate that diet-induced oxidative stress is not necessarily a prerequisite for the development of skeletal muscle insulin resistance.

Another way to lower mitochondrial ROS production is via mitochondrial uncoupling; a process in which the mitochondrial proton gradient is dissociated from ATP production. In addition, mitochondrial uncoupling could enhance metabolic rate and insulin action. However, it is unknown whether mitochondrial uncoupling could be beneficial in the treatment of T2DM. By using the chemical uncoupler 2,4- dinitrophenol (DNP), we investigated in **chapter 3** whether chemical induced mitochondrial uncoupling could enhance muscle substrate oxidation and glucose homeostasis under diabetic conditions. Although we

used the maximal dose of DNP that was tolerated by the animals, no effects on muscle metabolism, lipid accumulation, or glucose tolerance were found. Therefore, based on these data, DNP could not be validated as an appropriate therapy for T2DM.

Various dietary strategies have also been described to beneficially affect insulin sensitivity, by lowering oxidative stress and/or by enhancing muscle metabolism. As such, in type 2 diabetic patients the consumption of soy has been reported to have advantageous effects as it lowers fasting insulin levels. Genistein, the most abundant and active phytoestrogen in soy, has been reported to have antioxidant activities and to improve skeletal muscle oxidative metabolism, thereby stimulating fatty acid handling and glucose uptake. In **chapter 4** it was evaluated whether genistein supplementation could antagonize the progression of the hyperinsulinemic normoglycemic state (pre-diabetes) toward full-blown T2DM in Zucker Diabetic Fatty rats. However, feeding genistein to these rats did not lead to improvements in whole-body glucose tolerance, skeletal muscle oxidative stress, or mitochondrial function. These data show that dietary supplementation of pure genistein does not alleviate symptoms associated with T2DM progression in rats.

In adipose tissue and liver, the recently identified mitochondrial protein mitoNEET has been suggested to be a powerful regulator of mitochondrial oxidative capacity, and thereby, of lipid accumulation. However, the function of mitoNEET in skeletal muscle has not been examined before. In **chapter 5** we overexpressed mitoNEET specifically in skeletal muscle of rats via *in vivo* gene electroporation and examined the effects on mitochondrial oxidative capacity and intramyocellular lipid accumulation. However, skeletal muscle specific overexpression of the mitochondrial protein mitoNEET did not result in changes in mitochondrial oxidative capacity and -density, whole-muscle substrate metabolism, or lipid content. In addition, mitoNEET protein expression levels in skeletal muscle were comparable between healthy and diabetic rats. Therefore, no indications were found for a relevant physiological role in skeletal muscle of mitoNEET in the transition from pre-diabetes toward advanced T2DM. These data argue against an important role for mitoNEET in glucose homeostasis in skeletal muscle.

Finally, UCP3 is also suggested to play a role in mitochondrial oxidative capacity and in the regulation of mitochondrial ROS production. Although the exact physiological role of UCP3 has not been established, several studies show a tight regulation of UCP3 expression by free fatty acid levels, supporting a regulating role of UCP3 in fatty acid handling. **Chapter 6** describes the role of UCP3 in maintaining mitochondrial function in a state of a maximally disturbed balance between lipid availability and oxidative capacity. For this purpose, wildtype and UCP3-ablated mice were subjected to a high-fat diet combined with a fat oxidation inhibitor (etomoxir). As anticipated, the etomoxir-intervention resulted in increased lipid accumulation in skeletal muscle and a reduction in fat oxidative capacity. However, no

SUMMARY

UCP3-related effects on skeletal muscle mitochondrial function or lipotoxicity were established. This reveals that even under severe *in vivo* lipid-challenged conditions UCP3 does not play a major role in the preservation of skeletal muscle energy metabolism. In the heart, however, it was observed that UCP3 may be crucial in protection against the development of arrhythmias and hence sudden cardiac death. The role of UCP3 in the heart is relatively unexplored and further investigation is required to establish its function in cardiac tissue.

In conclusion, several aspects of mitochondrial function have been addressed in relation to glucose homeostasis. The results of the studies described in this thesis indicate that mitochondrial ROS production does not contribute to the pathogenesis of insulin resistance and T2DM *per se*. Still, mitochondrial dysfunction may contribute to the development of insulin resistance and T2DM. Additional research is warranted to gain more insight in the role of mitochondrial oxidative capacity in the maintenance of insulin sensitivity.