

# Fatty acid-induced mitochondrial uncoupling in adipocytes as a key protective factor against insulin resistance and beta cell dysfunction: a new concept in the pathogenesis of obesity-associated type 2 diabetes mellitus

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**Abstract** Type 2 diabetes is associated with excessive food intake and a sedentary lifestyle. Local inflammation of white adipose tissue induces cytokine-mediated insulin resistance of adipocytes. This results in enhanced lipolysis within these cells. The fatty acids that are released into the cytosol can be removed by mitochondrial  $\beta$ -oxidation. The flux through this pathway is normally limited by the rate of ADP supply, which in turn is determined by the metabolic activity of the adipocyte. It is expected that the latter does not adapt to an increased rate of lipolysis. We propose that elevated fatty acid concentrations in the cytosol of adipocytes induce mitochondrial uncoupling and thereby allow mitochondria to remove much larger amounts of fatty acids. By this, release of fatty acids out of adipocytes into the circulation is prevented. When the rate of fatty acid release into the cytosol exceeds the  $\beta$ -oxidation capacity, cytosolic fatty acid concentrations increase and induce mitochondrial toxicity. This

results in a decrease in  $\beta$ -oxidation capacity and the entry of fatty acids into the circulation. Unless these released fatty acids are removed by mitochondrial oxidation in active muscles, these fatty acids result in ectopic triacylglycerol deposits, induction of insulin resistance, beta cell damage and diabetes. Thiazolidinediones improve mitochondrial function within adipocytes and may in this way alleviate the burden imposed by the excessive fat accumulation associated with the metabolic syndrome. Thus, the number and activity of mitochondria within adipocytes contribute to the threshold at which fatty acids are released into the circulation, leading to insulin resistance and type 2 diabetes.

**Keywords** Adipocytes · Fatty acids · HAART · Mitochondria · Thiazolidinedione · Type 2 diabetes mellitus

## Abbreviations

FAD	flavin adenine dinucleotide
HAART	highly active antiretroviral therapy
MIDD	maternally inherited diabetes and deafness
mt	mitochondrial

## Introduction

Type 2 diabetes mellitus is generally associated with an enhanced energy intake and too little physical exercise. Also genetic factors determine the susceptibility to develop this disease. Whole-body insulin resistance and a state of low grade inflammation are early marker of the disease process [1]. Subsequently, hyperglycaemia develops due to

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an accelerated decline in beta cell function [2–4]. Type 2 diabetes mellitus is often accompanied by other co-morbidities such as hypertension and dyslipidaemia. Together, they constitute the metabolic syndrome [2].

Here we present the concept that removal of fatty acids within white adipocytes by fatty acid-induced uncoupled mitochondrial  $\beta$ -oxidation protects the organism against fatty acid leakage out of adipocytes, thereby preventing fatty acid-induced insulin resistance in liver and muscle and lipotoxicity in pancreatic beta cells. A consequence of this concept is that mitochondrial dysfunction in adipocytes, either inherited or acquired, makes the organism more prone to develop insulin resistance and type 2 diabetes.

### Mitochondria and fatty acids

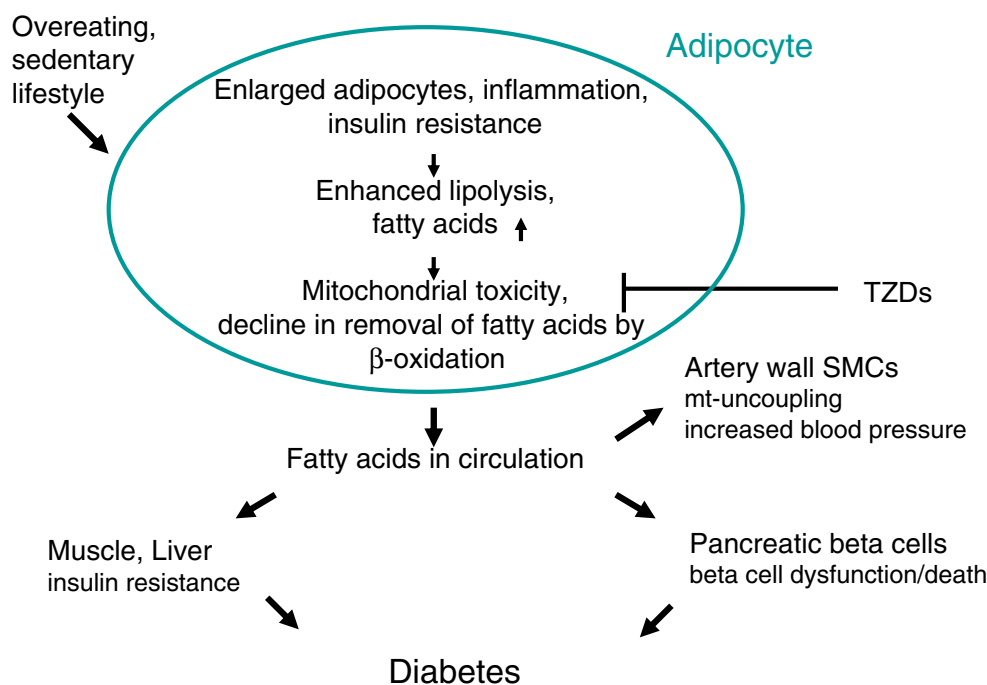
One of the main functions of mitochondria, in addition to producing ATP, is to remove fatty acids by  $\beta$ -oxidation. In this way, mitochondria are able to remove NEFA and to protect the organism against fatty acid-induced insulin resistance and pancreatic beta cell lipotoxicity.  $\beta$ -Oxidation can take place in all cell types relevant for glucose homeostasis, including muscle, liver and adipocytes. During this process, fatty acids are oxidised in the mitochondrial matrix by  $\text{NAD}^+$  and flavin adenine dinucleotide (FAD), yielding acetyl coenzyme A, which is further degraded to  $\text{CO}_2$  by the citric acid cycle. The resulting NADH and  $\text{FADH}_2$  need to be recycled into  $\text{NAD}^+$  and FAD by the respiratory chain so that additional fatty acid molecules can be oxidised. This requires a supply of ADP, which is converted into ATP by respiratory chain activity. The rate of conversion of ATP back into ADP, which is determined by the metabolic activity of the cell, determines the rate at which fatty acids can be removed by mitochondrial activity. If the capacity of the cell to oxidise fatty acids is to be enhanced, ATP needs to be reconverted into ADP at an increased rate. This occurs during exercise in contracting muscle. The resulting enhanced rate of ADP generation provides a major additional sink for the oxidation of fatty acids within the body. Only when mitochondria are in the uncoupled state can regeneration of  $\text{NAD}^+$  and FAD occur without the conversion of ATP into ADP. The released energy is then converted into heat. Uncoupling is a physiological adaptation process, normally regulated by specific proteins [5]. It is remarkable that, like uncoupling proteins, fatty acids are capable of inducing mitochondrial uncoupling, especially when their concentrations exceed the binding capacity of fatty acid binding proteins [6–8]. This implies that when unbound fatty acids are present in the cytosol of cells, mitochondria become less efficient at ATP production and generate more heat during the oxidation of NADH and  $\text{FADH}_2$  derived from food.

### Mitochondrial uncoupling in adipocytes by fatty acids may protect the organism against fatty acid-induced insulin resistance and lipotoxicity

White adipocytes contain large amounts of mitochondria in their tiny cytosolic compartment [9, 10]. There is no obvious reason why these cells need such a large capacity to produce ATP. We propose that, when uncoupled by fatty acids, these mitochondria prevent the release of fatty acids out of the adipocytes when the antilipolytic action of insulin is attenuated.

Evidence is accumulating that inflammation of adipose tissue is an early step in the pathogenesis of type 2 diabetes. The combination of excessive food intake with a sedentary lifestyle results in the formation of large adipocytes overloaded with triacylglycerols. Through as yet unknown mechanisms the adipose tissue becomes inflamed and infiltrated by leucocytes. This leads to the release of inflammatory cytokines such as  $\text{TNF-}\alpha$  [11–14], a potent inducer of insulin resistance in adipocytes which also induces lipolysis. As a result, fatty acids are released out of the large triacylglycerol pool [15]. We propose that, initially, a part of these fatty acids are removed inside adipocytes by partially uncoupled mitochondrial  $\beta$ -oxidation. In doing so, these mitochondria create a threshold for fatty acid release into the circulation, which would otherwise trigger the development of whole-body insulin resistance and pancreatic beta cell lipotoxicity, the latter expected to occur when the body cannot utilise these released fatty acids as fuel, for example, in response to high food intake and little physical exercise. Our proposed mechanism is outlined in Fig. 1. When low amounts of fatty acids are released out of the triacylglycerol pool in insulin-resistant adipocytes, these fatty acids can be removed by coupled  $\beta$ -oxidation. The rate of fatty acid removal by this pathway is determined by the rate of ADP regeneration within adipocytes, which is likely to be a more or less constant factor, merely determined by cellular metabolism and not affected by variations in the lipolytic rate. We propose that, when the rate of fatty acid release exceeds the rate of fatty acid removal by coupled  $\beta$ -oxidation, the increase in cytosolic concentrations of fatty acids not bound to binding proteins (unbound) induces a partial uncoupling of the mitochondria. Uncoupling by fatty acids has been shown in multiple *in vitro* experiments [5–8, 16]. Evidence of uncoupling *in vivo* is provided by a study in which exposure of heart muscle to fatty acids resulted in a decrease in ATP production and a concomitant increase in oxygen consumption [17]. When uncoupled, the mitochondria are able to remove much larger quantities of fatty acids through generation of heat. The degree of uncoupling increases in with further elevations in fatty acid concentration, thus generating more heat. This represents a dynamic adaptation of the efficiency of the mitochondrion, which is





**Fig. 2** Proposed sequence of events leading to the development of hyperglycaemia during the metabolic syndrome. When adipocytes become overloaded with triacylglycerol, low-grade inflammation develops and inflammatory cytokines such as TNF- $\alpha$  induce insulin resistance in the adipocytes. This results in an elevated state of lipolysis. When fatty acids are inadequately removed within adipocytes because of mitochondrial dysfunction (for example, induced by fatty acids or HAART therapy, fatty acids appear in the circulation,

where they induce insulin resistance of muscle and liver and malfunction of pancreatic beta cells. The elevated circulating fatty acid concentrations may also uncouple mitochondria in artery wall smooth muscle cells, thereby elevating the risk of hypertension [46]. Thiazolidinediones (TZDs) ameliorate the disease process in two ways: (1) by creating more mitochondria in adipose cells [23, 36, 37], thereby enhancing the capacity for oxidation of fatty acids; and (2) by enhanced re-esterification of fatty acids [39, 40]

showed an abnormal morphology [24]. These data support the concept of mitochondrial dysfunction in adipose tissue in states of obesity and type 2 diabetes.

Furthermore, intake of high-fat food by healthy persons rapidly increases the basal metabolic rate. This increase is blunted in obese individuals, whereas obese individuals exhibit an increased thermogenesis. These observations are suggestive for a rapid effect of ingested fatty acids on thermogenesis by mitochondrial uncoupling. The data also suggest that, in obese individuals, mitochondria are already uncoupled or damaged and less responsive to further fatty acid-induced uncoupling [25].

In vitro studies have shown that fatty acid-induced uncoupling of the mitochondrial respiratory chain depends on the chemical nature of these fatty acids. Unsaturated fatty acids, such as oleic acid, are better uncouplers than saturated fatty acids [8]. This could imply that the inclusion of saturated fatty acids in the diet makes mitochondria less prone to uncoupling, resulting in the earlier release of fatty acids, i.e. at a lower fatty acid level.

A further implication of our model is that a decline in mitochondrial function in adipocytes reduces the ability of these cells to store triacylglycerol. Inadvertently released

fatty acids out of the triacylglycerol pool enter the circulation and are redistributed in other tissues. This occurs, for example, in individuals starting on highly active anti-retroviral therapy (HAART) [26–29]. The nucleoside analogues included in HAART inhibit mtDNA polymerase and induce a ~30–50% reduction in mtDNA content in adipocytes and other tissues. HAART is associated with the redistribution of peripheral fat to the central and other compartments and with an elevated risk of developing the metabolic syndrome and type 2 diabetes. We see this clinical phenotype as a result of release of fatty acids by peripheral adipocytes, which in turn is due to a decrease in mitochondrial capacity to remove fatty acids. The fatty acids, after entering the circulation, induce insulin resistance and lipotoxicity to pancreatic beta cells and become stored as ectopic triacylglycerol deposits.

Another example of a mitochondrial dysfunction is represented by patients carrying a 3243A>G mutation in mtDNA. This mutation results in an attenuated mitochondrial function. Most of these patients develop the maternally inherited diabetes and deafness (MIDD) syndrome. Remarkably, these patients usually have a BMI of <25 kg/m<sup>2</sup>. Furthermore, they exhibit ectopic triacylglycerol deposits in

multiple tissues [30–33]. This clinical picture suggests the attenuated storage of triacylglycerol in adipose tissue. Around mid-life, these patients show a decrease in insulin secretion, which may result from lipotoxicity to pancreatic beta cells.

Congenital lipodystrophy is another clinical example showing that an inadequate storage of triacylglycerol in adipocytes contributes to ectopic triacylglycerol deposits and the development of severe whole-body insulin resistance and diabetes [34].

In contrast, improved mitochondrial function specifically in adipocytes is seen in individuals taking thiazolidinediones. These drugs are widely used to ameliorate whole-body insulin resistance in patients suffering from type 2 diabetes mellitus and the metabolic syndrome. These drugs bind to peroxisome proliferator-activated receptor- $\gamma$  receptors which are highly expressed in adipocytes and in cells from the immune system, but low in liver and muscle [35]. These drugs increase mitochondrial copy number and mitochondrial gene expression specifically in adipose tissue [23, 36, 37]. Clinically, these drugs improve storage of triacylglycerol in peripheral adipocytes and ameliorate fatty acid-related insulin resistance. Patients taking these drugs tend to store more triacylglycerol in their adipocytes, leading to weight gain [38]. Together, these clinical data suggest that correct mitochondrial function is needed for adequate storage of fatty acids as triacylglycerol in adipocytes. By this means the organism is protected against the fatty acid-induced development of insulin resistance and lipotoxicity to the pancreas.

As thiazolidinediones also induce the expression of genes involved in the neogenesis of glycerol, these drugs also enhance the re-esterification of fatty acids [36, 39, 40]. This also contributes to the removal of fatty acids from the circulation and protects adipocyte mitochondria against fatty acid-induced damage. Because part of the glycerol in adipocytes is synthesised through pathways that are dependent upon mitochondrial function [36], this suggests that changes in mitochondrial function also may affect the rate of glycerol production and thereby the rate of fatty acid esterification.

To be able to perform, uncoupled  $\beta$ -oxidation mitochondria require the presence of sufficient amounts of oxygen. It has been suggested that a state of hypoxia occurs in expanding adipose tissue during the development of obesity and that this state may contribute to the development of the metabolic syndrome [41, 42]. Our model predicts that hypoxia in adipocytes would lead to a reduced rate of fatty acid removal inside these cells and to more fatty acid release into the circulation.

The sensitivity of an individual to the development of type 2 diabetes as a result of lifestyle is determined by genetic factors. Multiple genetic variants that modulate the risk of an individual for the development of diabetes have been identified, including genetic variants related to mito-

chondrial function. A high penetrance mutation in mtDNA has been found to be associated with the MIDD syndrome. This mutation predominantly affects the activity of complex I of the respiratory chain [43]. In the same biochemical pathway a mutation in the *LARS2* gene, which encodes mitochondrial leucyl-tRNA synthetase 2, also modulates the risk for diabetes [44]. Furthermore, genetically determined changes in expression levels of components of the mitochondrial respiratory chain have been found to be associated with an increased risk of developing type 2 diabetes mellitus [45]. These genetic factors are likely to result in a decreased capacity of the mitochondria to remove fatty acids through uncoupled  $\beta$ -oxidation. Thereby, a genetic predisposition can lower the threshold for fatty acid release by adipocytes into the circulation and as a result enhance the risk for type 2 diabetes.

Our model of the pathogenesis of type 2 diabetes mellitus requires experimental verification of several points, and it certainly does not exclude the involvement of additional factors such as the coregulation of whole-body insulin action and insulin secretion through adipokines and the involvement of uncoupling proteins in setting the threshold for fatty acid-induced uncoupling of mitochondria. However, we see the way fatty acids interact with mitochondria in the cytosol of adipocytes as a major initiating event in the disease process leading to the metabolic syndrome and type 2 diabetes mellitus.

**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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