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Mitochondrial Dysfunction in White Adipose Tissue

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Abstract

While mitochondria in brown adipose tissue and their role in non-shivering thermogenesis have been widely studied, we have only a limited understanding of the relevance of mitochondria in white adipose tissue for cellular homeostasis of the adipocyte, and their impact on systemic energy homeostasis. A better understanding of the regulatory role that white adipocyte mitochondria play on whole body physiology becomes increasingly important. White adipose mitochondrial biogenesis can effectively be induced pharmacologically using a number of agents, including PPAR γ agonists. Through their ability to influence key biochemical processes central to the adipocyte, such as fatty acid esterification and lipogenesis, as well as their impact on production and release of key adipokines, mitochondria exert a critical role on systemic insulin sensitivity.

Mitochondria and energy homeostasis

The essential roles of mitochondria in numerous aspects of metabolic regulation position them center stage in the control of global energy homeostasis. Mitochondrial metabolism is both the origin and target of multiple nutrient signals that orchestrate integrated physiological responses to maintain cellular insulin-sensitivity. In particular, glucose and lipid metabolism are largely dependent on mitochondria to metabolize these nutrients and generate cellular energy in the form of ATP [1]. All cells are affected by mitochondrial dysfunction. However, the primary tissues most negatively influenced by suboptimal mitochondrial performance are those tissues that most heavily rely on mitochondrial function, such as skeletal- and heart-muscle, liver and adipose tissue.

A metabolic imbalance of nutrient signal input, energy production and/or oxidative respiration, results in “mitochondrial dysfunction”; while we appreciate the association of changes of mitochondrial function with the pathogenesis of obesity-driven insulin resistance [2, 3], it is still widely debated whether it is a cause or a consequence of insulin resistance. In particular, energy-sensing cellular rheostats detect explicit signals of mitochondrial activity, such as the NAD⁺:NADH ratio, the AMP:ATP ratio or acetyl-CoA levels, such signals become dysregulated with the onset of obesity and type 2 diabetes (T2DM).

While a major role of the function of white adipose tissue (WAT) in regulating energy-intake, energy expenditure and insulin resistance has been established, the functional role of

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WAT mitochondria has received less attention. Over the last decade, several studies have highlighted the potential relevance of mitochondria in cellular physiology of the adipocyte in WAT and its impact on systemic metabolic regulation. We want to i) highlight the impact that mitochondrial activity has on WAT function, focusing explicitly on the white adipocyte; ii) discuss the means by which mitochondria in adipocytes become compromised and how such perturbations alter whole-body homeostasis; iii) elaborate on how the intracellular dynamics and key pathways within the adipocyte acclimatize to mitochondrial dysfunction and, iv) highlight promising therapeutic avenues that aim to improve adipocyte mitochondrial function.

Adequate mitochondrial function is essential for white adipocyte biology

WAT is now established as a major endocrine organ impacting directly or indirectly the physiological functions in almost all cell-types. Representing around 10% of total body-weight in lean adults, WAT can achieve >50% in obese subjects [4]. It is therefore not surprising that any obesity-induced changes in WAT mitochondria can substantially disrupt whole-body energy homeostasis.

The white adipocyte displays a unique structure, most frequently seen with a single, large lipid droplet associated with relatively low cytoplasmic volume and reduced mitochondrial density. Despite containing relatively low mitochondrial mass compared to overall size, the adipocyte interprets nutritional and hormonal cues in its micro-environment, then coordinates its mitochondrial response to either oxidize incoming fatty acids (FAs) and carbohydrate fuels through the tricarboxylic acid cycle (TCA) cycle and the respiratory chain, or store these fuels safely in the form of triglycerides until whole-body energy requirements signal for their release [5].

Mitochondria play an essential role for many different pathways in the adipocyte. A synchronized initiation of both adipogenesis and mitochondrial biogenesis indicate that mitochondria play a pertinent role in the differentiation and maturation of adipocytes [6]. A recent study by Tormos and colleagues confirmed that early events of enhanced mitochondrial metabolism, biogenesis and reactive oxygen species (ROS) production (specifically through complex III of the ETC) are critical to initiate and *promote* adipocyte differentiation in an mTORC1-dependent manner. Consistent with this idea, antioxidant treatment blocks adipocyte differentiation, whereas ROS, through exogenous hydrogen peroxide treatment of cells, restored the differentiation process, as judged by increased adipocyte lipid accumulation and induction of adipogenic genes [7]. An intriguing suggestion is that ROS, primarily in the form of H₂O₂, are essential to initiate the PPAR γ transcriptional machinery necessary to evoke adipocyte differentiation. Alternatively, ROS may play an important role in insulin signal transduction [8]. While extremely high levels of ROS unquestionably cause cellular damage, ROS production in moderation may, however, serve to maintain cellular homeostasis by creating a tolerable oxidative environment that permits and sustains pre-adipocyte differentiation *without* inflicting cellular damage. Future studies examining the precise function of ROS complementing the intricate process of adipocyte differentiation should prove illuminating, particularly in the context of a metabolically not well-balanced environment.

In addition, in differentiating preadipocytes, mitochondria must generate and sustain enough ATP to support highly energy-consuming lipogenic processes, while still maintaining normal cellular activity [9]. During nutrient uptake, mitochondria must provide acetyl-CoA derived from glucose metabolism as substrates for fatty acid (FA) synthesis. The conversion of the glucose metabolite pyruvate to acetyl-CoA occurs exclusively within the mitochondrial matrix. Furthermore, glycerol-3-phosphate, a precursor substrate for FA-

esterification, is produced by mitochondria and is required for the packaging of lipids in the form of triglycerides (TGs) into the lipid-droplet. In light of this, it has been proposed that “FFA recycling in the adipocyte” (a TG-to-FA cycle) is a crucial sequence of events that determine systemic FFA concentrations [10]. Many of these processes are substrate-driven. Given that mitochondrial β -oxidation rates are interconnected with glyceroneogenic pathways and FA-esterification processes [11], this highlights the potential to alleviate FA flux through the oxidative pathway by capturing and esterifying fatty acids into the local TG pool.

Mitochondrial dysfunction in adipocytes: contributors and consequences

Compromised mitochondrial function may arise from acute cellular or systemic disruption. A more precise definition of mitochondrial dysfunction is provided in **Text Box 1**. Key contributors of mitochondrial dysfunction include: excessive nutrient supply, which subsequently contributes to ROS formation and toxic lipid species production, genetic factors, endoplasmic reticulum (ER) stress (which is detailed in **Text Box 2**), aging and/or pro-inflammatory processes and altered mitochondrial fission that severely disrupt the dynamic mitochondrial network fission events; all single-handedly or collectively, contribute to insulin resistance. The contributions of acyl-carnitine accumulation and ceramide production are described in **Text Box 3**.

The onset of obesity and T2DM

A wealth of evidence ties the suboptimal function of mitochondria to obesity and T2DM. Indeed, during excessive nutritional overload, similar to skeletal muscle cells, adipocytes harbor low levels of ATP, concomitant with a build-up of NADH [6, 13]. The metabolic pendulum therefore shifts towards lipid storage, reduced mitochondrial biogenesis with enhanced glycolytic ATP synthesis. These alterations at the level of mitochondria further result in additional lipid accumulation and a potential for subsequent progression to insulin resistance.

At a cellular level, compromised mitochondrial function leads to glucose- and lipid-handling disorders and pathological TG accumulation (“steatosis”) in cell-types most vulnerable to lipotoxicity [14]. High levels of glucose and FFAs have been reported to directly stimulate mitochondrial dysfunction in 3T3-L1 adipocytes [15]. Moreover, studies have documented that mitochondrial loss in WAT correlates with the development of T2DM [16, 17]. Indeed, mitochondrial levels in white adipocytes derived from epididymal fat-pads of *ob/ob* mice are approximately 50% lower, compared to lean control mice [16]. Furthermore, Choo and colleagues demonstrated a reduction in mitochondrial number and function, a decrease in mitochondrial DNA (mtDNA) content and electron transport chain (ETC) enzymatic activity, in addition to an altered mitochondrial network; all contributing to a decline in oxidative phosphorylation (OXPHOS) and β -oxidation in WAT of diabetic mice [18]. Moreover, elegant microarray profiling studies have revealed that several mitochondrial genes critical for mitochondrial function and OXPHOS, in addition to PPAR α , ERR α and PGC1 α , are downregulated in obese, high-fat diet (HFD)-fed, insulin-resistant mice, and in *db/db* mice [3, 19]. This suggests that mitochondrial biogenesis is highly compromised in WAT of obese insulin-resistant mouse models. Similarly, in patients with insulin resistance, T2DM and severe obesity, the abundance of mitochondria and the expression of key genes pertinent to mitochondrial function are significantly reduced in WAT [20], in concert with decreased adipocyte oxygen-consumption rates and ATP production [21]. Finally, comparisons between identical twin pairs discordant for obesity have demonstrated markedly reduced mtDNA levels and decreased mitochondrial mass in the obese twins. WAT, despite identical mtDNA sequences [22]; this strongly emphasizes the importance of environmental factors, such as obesity, in the regulation of mitochondrial mass.

Lipid-induced ROS formation

The mitochondrial ETC is a major site of ROS production in adipocytes. Studies utilizing isolated mitochondria indicate that 0.2% to 2% of oxygen consumed by cells is incompletely metabolized; such that when an electron is accepted from the ETC, superoxide anions are generated as obligatory by-products [23], with complexes I and III representing the primary source of ROS [24]. In particular, the type of ROS species may be also be important and may vary from cell type to cell type. For instance, $O_2^{\cdot-}$ is considered the primary ROS species, with H_2O_2 a potent downstream secondary messenger [24]. More specifically, mitochondrial $O_2^{\cdot-}$ reacts with iron-containing proteins to generate molecules of H_2O_2 [25] to initiate potent downstream signals that trigger redox-dependent signaling in the cytosol. In light of this, several studies have highlighted the association between enhanced mitochondrial-derived H_2O_2 and insulin resistance, particularly in the context of excessive nutrient-intake that result in metabolic imbalance [26, 27].

Mitochondrially-generated ROS can further be viewed as an integrated physiological ‘distress’ signal in the adipocyte, with changes that occur according to nutrient, hormonal and/or oxygen fluxes. In the context of excess caloric-intake, increased lipid-uptake into the adipocyte yields increases in mitochondrial substrate load, subsequently increasing ETC activity and ROS production. ROS in turn, oxidize cellular lipids to yield lipid hydroperoxides that generate reactive lipid aldehydes; these modify intracellular proteins, DNA, RNA, carbohydrates, as well as other lipids [28]. In light of this, what do high ROS levels indicate to the adipocyte? Moreover, which type of ROS species is predominantly generated in the adipocyte under times of nutrient excess? ROS can, as a cellular metabolism sensor, control insulin-sensitivity of adipocytes and thus their endocrine functions. Indeed, enhanced ROS levels have been causally linked to an alteration in insulin sensitivity in numerous model systems [8, 15, 29, 30].

In vitro studies demonstrate that hyperglycemic conditions, non-esterified fatty acids (NEFAs) or TNF α treatment of 3T3L1-adipocytes results in ROS accumulation [31, 32], reduced mitochondrial biogenesis, decreased insulin-sensitivity [15, 29] and enhanced pro-inflammatory cytokine secretion [31]. Such effects result in a state of “oxidative-stress”, which further ensues a downregulation in PPAR γ and adiponectin [33]. In contrast, De Pauw and colleagues noted that mild uncoupling of 3T3-L1 adipocyte mitochondria, as triggered by FCCP or TNF α treatment, did increase ROS production, however did not alter mitochondrial biogenesis [34]. Rather the abundance of adipocyte pyruvate carboxylase, a major enzyme that participates in *de novo* fatty acid synthesis and glyceroneogenesis, was significantly downregulated; this was accompanied with a reduction in adipocyte triglyceride (TG) content [34]. This study presents a new and intriguing mechanism whereby treatments that uncouple mitochondria may serve to effectively limit TG accumulation in adipocytes, specifically by mitigating the pro-lipogenic properties of pyruvate carboxylase [34]. Future studies will have to examine more closely whether such effects in adipocytes are beneficial in minimizing obesity-induced lipotoxicity.

Interestingly, ROS themselves can directly inhibit oxygen-consumption in adipocytes, which results in enhanced lipid accumulation [30]. Normal rates of oxygen-consumption can be restored by the ROS scavengers pyruvate or N-acetylcysteine [30]. Insulin signaling studies in 3T3-L1 adipocytes show that insulin receptor substrates (IRS) -1 and -2 are carbonylated by reactive lipid species, resulting in reduced insulin-stimulated tyrosine phosphorylation and targeted degradation [35]. Adipocyte ROS have further been proposed to mimic the effects of insulin, through association of insulin action on glucose transport; suggesting that low levels of ROS generation can be an integral part to insulin-signaling [36]. Moderate ROS generation can also inhibit the proliferation of adipogenic progenitors [37] without induction of apoptosis [6], and potentially lead to the formation of large, dysfunctional

hypertrophic adipocytes. These events can be reversed upon antioxidant treatment that restores the dysregulation of adipokine balance to promoting adipogenesis [37].

Oxidative-stress has further been described clinically, as well as in WAT of many additional mouse models of obesity, such as the KKAY mice, diet-induced obesity (DIO) mice and *db/db* mice [28, 29, 33]. These metabolic settings can be further modified through overexpression of NADPH-oxidase and repression of antioxidant enzymes, such as superoxide dismutase 2 (SOD2), glutathione peroxidase (GPx) and catalase [33]. More specifically, Curtis and colleagues demonstrated that repression of the antioxidant enzyme glutathione-S-transferase (GSTA4) in WAT leads to increased protein damage (carbonylation), enhanced ROS production, and overall mitochondrial dysfunction [28]. Microarray analyses of human subcutaneous WAT (sWAT) revealed that GSTA4 expression is reduced in obese insulin-resistant individuals [28, 38]. Furthermore, both GSTA4-silenced 3T3-L1 adipocytes and WAT from GSTA4 knockout mice display a significant impairment in OXPHOS with increased ROS formation [28]. Collectively, we appreciate that substrate overload within the adipocyte increases ROS production. Yet, despite a persistently high burden of FFAs under even normal physiological conditions, the adipocyte displays tolerance to unusually high levels of ROS under basal conditions, without concomitant cell-damage [30]; in fact, the majority of other cell-types cannot sustain such a high level of ROS without undergoing apoptosis. The onset of obesity however, promotes a relatively *sluggish* behavior of the scavenging system within the adipocyte. This exacerbates the existing high basal levels of ROS, which leads to an inhibition of oxygen-consumption. Consequently, substrates cannot be oxidized efficiently and TG storage is favored.

Compromised mitochondria alter the intracellular machinery of the adipocyte

During the development of obesity and T2DM, WAT expansion and adipocyte hypertrophy persists, to accommodate surplus nutrient-intake. Preadipocytes initiate adaptive responses to such metabolic challenges by adjusting the abundance and/or morphology of mitochondria and mtDNA content to ultimately remodel their highly organized mitochondrial network within the cell. However, how these adipocyte-specific pathways are altered and how the adipocyte adapts to substantial adjustments in mitochondrial demand is not clear.

Mitochondrial dysfunction impacts the fundamental intracellular dynamics in the mature adipocyte at multiple levels: i) the integration of adipogenesis with the rate of mitochondrial biogenesis during metabolic challenge is affected; ii) the adipocyte lipogenic and lipolytic pathways are altered in response to mitochondrial dysfunction and, iii) the production and release of adiponectin, a key adipokine involved in many aspects of cellular lipid homeostasis, is affected by mitochondrial changes. The latter is detailed in **Text Box 4**.

Preadipocyte differentiation and mitochondrial biogenesis

Adipocytes undergo two stages of maturation: differentiation and hypertrophy. During differentiation, newly recruited small adipocytes sustain peak levels of metabolic activity, increased substrate consumption and enhanced insulin sensitivity. Numerous *in vitro* studies consistently demonstrate that mitochondrial biogenesis is markedly increased during the preadipocyte differentiation, with a marked upregulation in mitochondrial protein levels [9, 16, 21, 45]. Elegant studies by Corvera and colleagues reveal that as preadipocytes embark upon the adipogenic process, there is an initial massive 20- to 30-fold proliferation of mitochondria within the cell, concomitant with an increase in β -oxidation [16, 45]. This is

orchestrated by a concerted action of PPAR γ , CREB, ERR α , C/EBP α , C/EBP β and PGC1 α [46, 47].

In light of this, others have reported that preadipocyte differentiation is accompanied with increases in the relative abundance of mtDNA copy number [48], concomitant with strong induction in components of mitochondrial genome replication and transcriptional machinery, such as the nuclear-encoded mitochondrial transcription factor A (TFAM) [49], in addition to constituents of deoxynucleotide metabolism required for mtDNA replication [50]. Interestingly, through small interference RNA-mediated knockdown of TFAM in 3T3-L1 adipocytes, Shi *et al.* further observed reduced ETC capacity and respiratory rates; this was accompanied with impairment in glucose transport. However, a paradoxical increase in insulin-stimulated Akt phosphorylation ensued as well, the underlying reasons for which were not clear [49].

Through use of mitochondrial respiratory inhibitors, or knockdown of genes involved in mitochondrial biogenesis, recent studies highlight that inhibition of respiration and OXPHOS severely impairs preadipocyte differentiation, leading to abnormal TG storage capacity of the mature adipocyte [9, 49, 51]; this coincides with reductions in PGC-1 β , PPAR γ , C/EBP α , SREBP-1c [9] and an upregulation of CREB [52] expression levels.

Mature white adipocytes can reduce their mitochondrial content, particularly with age, obesity and T2DM [18]. Indeed, older adipocytes that undergo hypertrophy can lose much of their functional metabolic potential. Obesity-induced alterations in mitochondrial biogenesis substantially deteriorate the preadipocyte differentiation program during WAT expansion [16, 18, 45]. Furthermore obesity-mediated ROS induction exerts detrimental inhibitory effects upon preadipocyte proliferation and differentiation [37]; thereby potentially curbing the development of *healthy* WAT during the development of obesity. Taken together, white preadipocyte differentiation and metabolic activities coincide with increases in mitochondrial biogenesis, while a reduction in mitochondrial mass is associated with adipocyte hypertrophy and loss of metabolic flexibility observed in obesity.

Lipogenesis, lipolysis and FA re-esterification

Mitochondria play a critical regulatory role in several major metabolic pathways that occur in the adipocyte, i.e. *de novo* lipogenesis, lipolysis and FA re-esterification. To sustain lipogenesis, mitochondria provide key intermediates for the synthesis of TGs through the actions of pyruvate carboxylase [53]. More specifically, an abundant and well-functioning pool of adipocyte mitochondria is required to generate acetyl- CoA and glycerol 3-phosphate for FA activation and synthesis, at a rate sufficient to sustain their esterification in TG; this is further enhanced by the activation of the glyceroneogenic pathway and mitochondrial anaplerosis [54].

WAT mitochondria also play a critical role in the regulation of adipocyte lipolysis and the re-esterification of 'excess'. FAs within the adipocyte. Lipolytic stimulation of adipocytes, through fasting, exercise, or treatment with β -3 adrenergic receptor agonists, such as catecholamines and isoproterenol, or IL-6 and TNF α , can induce hydrolysis of stored TGs to FFAs [55]; this process is accompanied by potent stimulation of mitochondrial β -oxidation processes [56]. Furthermore, isoproterenol and TNF α treatment dramatically increase the expression of several mitochondrial proteins that participate in OXPHOS, β -oxidation, the TCA cycle and the structural and functional integrity of mitochondria [56]. Surprisingly, proteins associated with oxidative-stress dissipation are downregulated in lipolytically stimulated adipocytes [56]. Finally, heightened ROS-induced protein damage in WAT has been associated with increased lipolysis [28] and impaired insulin signaling [35].

Therapeutic interventions that improve mitochondrial function

Interventions that improve adipocyte mitochondrial function may also improve whole-body insulin- sensitivity. What are the current therapeutic strategies and promising new avenues that aim to improve adipose tissue mitochondrial function and effectively treat obesity-induced T2DM?

Pharmacological interventions: thiazolidinediones (TZDs)

Pharmacological interventions, such as the use of TZDs, are powerful measures to increase mitochondrial mass. PPAR γ agonists (TZDs) used to be one of the primary anti-diabetic drugs utilized to treat patients with T2DM, as they increase insulin-sensitivity of WAT and skeletal-muscle. In particular, the clinically used TZDs rosiglitazone and pioglitazone are known to increase plasma adiponectin levels, and stimulate adipocyte differentiation [70], thereby enhancing hyperplastic growth. Interestingly, numerous *in vitro* and *in vivo* studies document that TZDs enhance mitochondrial biogenesis, content, function, morphology and palmitate-stimulated oxidative capacity in white adipocytes [16, 18, 21, 45]. More specifically, whole transcriptome studies utilizing white adipocytes from *ob/ob* mice have revealed that TZDs upregulate a specific set of mitochondria-associated genes, one of which is PGC1 α , an established master regulator of mitochondrial biogenesis [16, 45]. Taken together, the notion of improving mitochondrial function through use of anti-diabetic TZD agents is in support of the concept that manipulating mitochondrial function is associated with improvements in insulin-sensitivity and may indeed be feasible therapy for T2DM. In addition to TZDs, the prospect of ‘browning’. WAT is emerging as a novel and potentially feasible therapeutic avenue in the future. **Text Box 5** highlights the more recent developments in the area of browning of WAT.

Controlled and specifically targeted mitochondrial uncoupling is another attractive pharmacological approach, however rather difficult to achieve in practice. This idea of a limited degree of uncoupling, thereby expending higher levels of energy at baseline on the basis of uncoupling mitochondria remains very attractive therapeutically. The key is to find a compound that becomes decreasingly effective as the level of depolarization/uncoupling increases, hence avoiding an “overheating” response. There remains much room for discovery in this arena, particularly as it relates to activities of these compounds specifically in adipocytes.

Antioxidants and chemical uncouplers

Obesity-associated insulin resistance can be partially alleviated through mitochondrial antioxidant treatment or overexpression of mitochondrial scavengers. Indeed, clinical studies have demonstrated that antioxidant treatments, such as vitamin E, vitamin C, N-acetylcysteine, glutathione and coenzyme Q₁₀ can improve insulin-sensitivity in diabetic patients [81]. With regards to the adipocyte, R- α -lipoic acid (LA), an antioxidant previously shown to yield protective effects on mitochondria [82], was shown to enhance mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes [83]. The latter effects occur primarily through stimulation of oxygen-consumption and β -oxidation, with concomitant upregulation in PPAR γ , PPAR α , CPT1, PGC1 α , TFAM and NRF1 gene expression levels [83].

Other therapeutic strategies enhancing adipocyte mitochondrial function can be achieved through use of chemical uncouplers, to dissipate energy as heat [84] and reduce ATP synthesis by increasing proton-leak. Indeed in the 1930s, the chemical uncoupler 2,4-dinitrophenol (DNP) was utilized as a dietary drug; massive weight losses were documented, as much as 3 kg per week and a 40% average increase in basal metabolic rates [85].

However, such uncoupling effects must be tightly controlled, as a hyper-metabolic state can lead to fatal uncontrolled thermogenesis in many tissues, including WAT, with chronically elevated body-temperatures [86]; as such, the use of uncouplers was discontinued. In acute doses however, mild 4-(trifluoromethoxy)phenylhydrazine (FCCP, also called Carbonyl cyanide) -induced mitochondrial uncoupling has been shown to reduce lipid-droplet TG accumulation in 3T3-L1 adipocytes; such effects parallel enhance lipolysis and downregulation of transcription factors involved in adipogenesis and lipogenesis [87]. Taken together, therapeutic interventions using either antioxidant supplements or mild uncoupling agents may be beneficial to enhance mitochondrial function and adipocyte metabolism. Such agents will however need a much broader therapeutic range to avoid the potential to massively overheat critical tissues.

Exercise and caloric-restriction

Several studies indicate that aerobic endurance exercise improves whole-body glucose homeostasis by stimulating skeletal-muscle mitochondrial respiratory capacity and mitochondrial biogenesis; the latter through increasing gene expression of PGC1, NRF1 and TFAM [88]. In light of this, recent studies have demonstrated that metabolic stimuli, such as exercise- or hypoxic conditions, induce skeletal muscle PGC1 α expression, which can further stimulate pro-angiogenic factors such as vascular endothelial growth factor (VEGF) in an ERR α -dependent manner; such events may elicit exercise-induced neovascularization [80, 89]. This elegantly connects the regulation of mitochondrial oxygen-consumption to the delivery of oxygen and nutrients through the vasculature. Such a concept may also be highly relevant for the white adipocyte, particularly in the context of obesity, when WAT is under-vascularized, fibrotic and harbors very limited numbers of mitochondria. Indeed, it was recently shown that local adipocyte-specific upregulation of VEGF- A improves vascularization in WAT, concomitant with massive upregulation of UCP1 and PGC1 α levels [90]. This suggests that VEGF in the microenvironment of the adipocyte can promote mitochondrial biogenesis in WAT and thus, may confer substantial beneficial effects to mitochondrial metabolism and whole-body glucose and lipid homeostasis. Future studies examining whether well-established skeletal- muscle stimuli that lead to the biogenesis of mitochondria, such as exercise or a PGC1 α overexpression, can coordinate a synchronized improvement in angiogenic and mitochondrial programs in white adipocytes, should prove intriguing.

In addition to exercise, caloric-restriction can also induce mitochondrial biogenesis, oxygen-consumption, ATP synthesis and expression of the NAD⁺-dependent PGC1 α -deacetylase, SIRT1; such effects have additional ROS-reducing benefits. Mitochondrial biogenesis and/ or improvements of mitochondrial function may therefore be major contributors for the beneficial effects of exercise and caloric-restriction.

Concluding remarks and future perspectives

Mitochondria are a central control point of many metabolic pathways. Under various physiological conditions, such as excess nutrient overload, a sedentary lifestyle, genetic factors and/or aging, adipocyte mitochondrial pathways can become defective, in part due to an induction of ROS-stimulated oxidative injury to the adipocyte. The notion that WAT, or individual adipocytes harboring dysfunctional mitochondrial components, have the potential to reprogram multiple metabolic pathways to eventually severely impact glucose and lipid homeostasis at the whole-body level, is a new and intriguing concept in physiology, and further expands the view of mitochondria beyond their cell autonomous functions.

The notion prevails that it is a desirable goal to stimulate fuel oxidation in white adipocytes and shift the balance toward less fuel storage and more fuel consumption. However, the

possibility of reducing mitochondrial oxidative phosphorylation processes *exclusively* in subcutaneous WAT (the fat pad prone to “healthy” adipose tissue expansion) has the potential to minimize obesity-related lipotoxicity in other tissues. This would of course come at the expense of acquiring excess body fat. Taken together, relatively unexplored and unappreciated white adipocyte mitochondrial pathways are likely components of obesity and the pathogenesis of insulin resistance, even though many of the details remain to be worked out. However, based on what we know now, white adipose tissue mitochondria are emerging as highly attractive organelles for therapeutic interventions with the potential to have an impact on systemic metabolism.

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Text Box 1**Mitochondrial dysfunction**

The term mitochondrial dysfunction is frequently applied to mitochondrial studies that describe associations of inappropriate substrate use with obesity and T2DM. However, the exact definition of mitochondrial function is complex, challenging to interpret given the diversity of studies employed. Classically, mitochondrial dysfunction is defined as the incapacity of mitochondria to generate and sustain sufficient ATP levels, through oxidative phosphorylation, in response to energy demands [12]. However, the term is more frequently utilized to define maladaptive physiological responses of mitochondria that undergo single or multiple metabolic perturbations. This involves irregularities in processes such as substrate catabolism, calcium buffering, iron transport, intracellular mitochondrial shape and location, apoptosis and ROS production. In terms of adipocyte biology, one or more of these manifestations may severely tip the lipid storage-to-lipid oxidization balance and contributes directly to the development of obesity and whole-body insulin resistance.

Text Box 2**ER stress and mitochondrial dysfunction**

Recent studies have provided direct evidence that compromised mitochondrial function and ER stress are significant pathogenic components in the development of insulin resistance and T2DM [39, 40]; suggesting an intimate functional relationship between mitochondria and the ER.

The ER plays a central role in calcium buffering, lipid synthesis, protein folding and maturation. Several factors that perturb ER function, such as increased demand or decreased capacity to fold proteins within the ER, result in accumulation of unfolded proteins; this triggers downstream signaling pathways, which are generically referred to as the unfolded protein response [39, 40]. This state of “ER stress” induces a succession of adaptive compensatory mechanisms, which include inhibition of protein translation, increased ER chaperone expression, ER-associated degradation and cell injury and subsequent apoptosis [39, 40]. Furthermore, the increased accumulation of unfolded proteins generates high levels of ROS as well, due to multiple thiol-disulfide exchanges mediated by ER oxidoreductases. As a “professional” secretory cell with high level production of many secretory proteins, the adipocyte is definitely prone to this phenomenon.

Adipocyte cellular homeostasis likely depends upon the functional relationship between mitochondria and the ER; crosstalk that may become disrupted during metabolic imbalance. Interestingly, the outer mitochondrial membrane (OMM) and the ER are connected by specific tethers; this facilitates ER proteins to associate directly with proteins and lipids of the OMM [39]. Certainly when the ER undergoes heightened stress, it is capable of relaying calcium signals to mitochondria that result in both ATP production and/or cellular apoptosis, thus inducing some degree of mitochondrial dysfunction. *Vice versa*, the ER requires ATP to function adequately. Recent studies have further demonstrated that elevated cytosolic free Ca^{2+} concentrations, due to mitochondrial dysfunction, elicit the ER stress response [40]. Collectively, bidirectional interactions exist between the mitochondrion and the ER; such that dysfunction on either side can severely disrupt metabolic homeostasis on the other end, ultimately leading to aberrant insulin signaling.

Text Box 3**Acyl-carnitine accumulation and ceramide formation**

Interestingly in obese states, skeletal-muscle mitochondria are surrounded by high lipid loads, which force an increase in oxidative respiration [13, 41]; in fact, elevated intramuscular TG storage is well recognized as a common feature of obese insulin-resistance. As such, high rates of β -oxidation outpace the metabolic fluxes through the TCA cycle, leading to a build-up of incompletely oxidized acyl-carnitine intermediates. Such disconnect between β -oxidation and TCA cycle activity promotes mitochondrial lipid-induced oxidative-stress and muscle insulin resistance [42].

Toxic lipid species, such as ceramide (a sphingolipid derived from the condensation of palmitoyl-CoA and serine) have been implicated as candidate mediators of insulin resistance [43, 44]. Diabetogenic agents, such as NEFAs, TNF α and glucocorticoids, all enhance ceramide levels, either through stimulation of its palmitoyl-CoA precursor, or increasing serine palmitoyltransferase activity, the key enzyme that catalyzes the first rate-limiting step in *de novo* ceramide biosynthesis [44]. Since dysfunctional adipocytes may serve as primary sites of excess ceramide synthesis, these sphingolipids may play a prominent role in the context of the suboptimal local mitochondrial environment [44]. Ceramides can further partition into mitochondrial membranes and can have pore-forming abilities; this may lead to a partial uncoupling of the mitochondrial membrane potential. However, the relevance of this mechanism in general and for the adipocyte specifically, still needs to be established more thoroughly.

Text Box 4

Adiponectin

Adipocytes secrete “adipokines”; cytokines and hormones that communicate systemically with other cell-types to maintain whole-body energy homeostasis [57, 58]. One adipose-derived hormone is adiponectin [59]; a mediator of improved insulin-sensitivity through its anti-hyperglycemic, anti-atherogenic and anti-inflammatory properties [43, 60–62]. Obese individuals exhibit low plasma levels of adiponectin [61], likely due to pathological alterations associated with hypertrophic adipocytes, which selectively suppress adiponectin synthesis or promote retention inside the adipocyte [63].

Adiponectin plays a vital role in mitochondrial processes; and *vice versa*, mitochondrial function is essential for the production and release of adiponectin from adipocytes. Indeed, stimulation of mitochondrial biogenesis in adipocytes, via adenoviral-mediated overexpression of the mitochondrial transcription factor nuclear respiratory factor-1 (NRF-1) [64], or treatment with endothelial nitric oxide synthase (eNOS), enhances adiponectin synthesis [64, 65]. Conversely, treatments impairing mitochondrial function or reducing mitochondrial biogenesis, such as TFAM- or eNOS-specific siRNAs, reduce adiponectin synthesis [64, 65]. Particularly, defective mitochondria exhibit enhanced ER stress and reduced adiponectin transcription; occurring through activation of c-Jun NH₂-terminal kinase and activating transcription factor 3 [64]. Similarly in obese *db/db* mice, adiponectin expression and mitochondrial content are reduced in WAT; this is reverted through TZD treatment [64].

Adiponectin-overexpressing mice display enhanced insulin-sensitivity and increased adipocyte mitochondrial density, concomitant with transcriptional upregulation of WAT lipid-storage and OXPHOS components [60]; the latter stands true even when transgenic mice are crossed into an *ob/ob* background [66]. Interestingly, the mitochondrial inhibitors oligomycin and antimycin A, decrease adiponectin expression through enhancing ROS production [33] and expression of the dominant-negative C/EBP isoform, CHOP10; potentially through inhibition of adiponectin message via interference with the C/EBP-binding region of the adiponectin gene promoter [67].

Adiponectin also exerts pleiotropic insulin-sensitizing effects on skeletal-muscle and liver. Specifically, adiponectin induces PGC1 α -mediated mitochondrial biogenesis and β -oxidation, through AMPK [68]. Liver adiponectin-knockout (Adpn-KO) mice display a pre-existing condition of hepatic-steatosis and mitochondrial impairment [69]. Conversely, adenoviral-mediated replenishment of adiponectin to Adpn-KO mice depletes lipid accumulation, restores oxidative capacity and minimizes lipid peroxidation [69]; highlighting potent hepatoprotective properties of adiponectin. Collectively, adiponectin-mediated alterations in WAT mitochondria may alter insulin action in skeletal-muscle and liver; promoting crosstalk through an adipose-hepatic-muscular axis. Finally, FA-derivatives may serve as endogenous ligands of PPAR γ , hence activate mitochondrial programs; interventions that alter pools of available intracellular lipids, such as TZDs, exercise or caloric-restriction, or simply lack of the cytoplasmic FA-binding protein aP2, may enhance mitochondrial biogenesis.

Text box 5**BAT and browning WAT: a therapeutic intervention to improving mitochondrial function?**

Brown adipose tissue (BAT), in contrast to WAT, participates in energy dissipation rather than energy storage. While a decline in the respiratory capacity of BAT impairs thermogenesis and thus has been linked to DIO [71], BAT has traditionally been regarded to have very minor physiological relevance in humans beyond early childhood. However, recent demonstrations using ^{18}F -fluorodeoxyglucose positron emission tomography identified significant deposits of BAT in adult humans, deposits that are more prominent in women than men, and declining activity with increasing adiposity [72]. These findings raise the possibility of therapeutic manipulation of BAT thermogenesis to promote weight-loss and insulin-sensitivity [73], while preventing lipid accumulation through UCP1-mediated mitochondrial uncoupling [74]. The discovery of active adult BAT therefore opens up an intriguing avenue in obesity research.

Another intriguing aspect of WAT mitochondrial research is that, given the high metabolic activities of BAT, attempts to pharmacologically induce a switch from WAT to a BAT-like phenotype through enhancement of mitochondrial biogenesis, is a highly promising concept, in the context of the prevailing obesity epidemic. Mechanistically, mitochondrial biogenesis is driven in part through induction of PGC1 α and PGC1 β [75], both of which serve as co-activators of nuclear transcription factors, such as NRF1, PPAR γ and PPAR α [76] and thus, are intimately involved in the induction of mitochondrial replication, lipid accumulation and β -oxidation, respectively.

The presence of brown-like adipocytes within WAT depots has indeed been correlated with improved metabolic phenotypes [77]. Another factor pertinent to the mitochondrial biogenesis process in adipose tissues is PRD-BF-1-RIZ1 homologous domain containing protein 16 (PRDM16), a transcriptional co-activator of PGC1 α and PGC1 β that upregulates genes involved in mitochondrial biogenesis, uncoupling and OXPHOS [78]. Interestingly, TZDs induce white-to-brown adipose tissue conversion through stabilization of PRDM16 protein [79], thus highlighting PRDM16 as a key “browning” determination factor. Finally, recent studies identified a novel PGC1 α -dependent myokine that promotes BAT-like development of WAT [80] and highlights efficient skeletal-muscle-to-WAT crosstalk. Specifically, muscle-derived PGC1 α , through stimulation of FNDC5, was shown to induce secretion of a novel hormone termed irisin [80]. Irisin can act directly on white adipocytes to promote their “browning” in an UCP1-mediated manner [80]. Collectively, these studies beckon the question of could the transformation of substantial amounts of BAT from WAT, or an enhancement in the metabolic activity of WAT serve as an antidote to obesity? Indeed, WAT research is certainly heading in the BAT direction.

Figure 1. Proposed Pathways of Mitochondrial Dysfunction in the White Adipocyte
 Metabolic challenges such as excess nutrient-intake that promote high FFAs concentrations and hyperglycemia, in addition to aging and ceramide formation, all increase mitochondrial ROS production to cause adipocyte mitochondrial dysfunction. Consequently, compromised mitochondrial function reduces mitochondrial biogenesis and mitochondrial DNA (mtDNA) content and decreases the rate of β -oxidation. As such, major adipocyte pathways are altered, such as adipogenesis, lipolysis, fatty acid esterification and adipocyte-derived adiponectin production; collectively, this promotes changes in insulin sensitivity. Improvements of mitochondrial function through therapeutic strategies, such as TZDs or

antioxidant treatment, exercise and/or calorie restriction can restore insulin action, which leads to normalize cellular homeostasis.