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Review

Mitochondrial involvement in skeletal muscle insulin resistance: A case of imbalanced bioenergetics

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ABSTRACT

Skeletal muscle insulin resistance in obesity associates with mitochondrial dysfunction, but the causality of this association is controversial. This review evaluates mitochondrial models of nutrient-induced muscle insulin resistance. It transpires that all models predict that insulin resistance arises as a result of imbalanced cellular bioenergetics. The nature and precise origin of the proposed insulin-numbing molecules differ between models but all species *only* accumulate when metabolic fuel supply outweighs energy demand. This observation suggests that mitochondrial deficiency in muscle insulin resistance is not merely owing to intrinsic functional defects, but could instead be an adaptation to nutrient-induced changes in energy expenditure. Such adaptive effects are likely because muscle ATP supply is fully driven by energy demand. This market-economic control of myocellular bioenergetics offers a mechanism by which insulin-signalling deficiency can cause *apparent* mitochondrial dysfunction, as insulin resistance lowers skeletal muscle anabolism and thus dampens ATP demand and, consequently, oxidative ATP synthesis.

1. Introduction

The concentration of blood glucose needs to be maintained within a relatively narrow range as hypoglycemia and hyperglycemia cause medical complications [1–3]. Despite fluctuating nutrient supply, healthy people achieve tight glycemic control through well-orchestrated biochemical interplay between organs. When circulating glucose rises, for example after a meal, pancreatic β cells increase glucose uptake and breakdown thus fueling mitochondrial respiration and boosting ATP synthesis through oxidative phosphorylation – the consequent glucose-induced rise in the cytoplasmic ATP/ADP ratio is a key signal that provokes β cells to secrete insulin [4]. In turn, the rise in circulating insulin instructs a range of different organs – including skeletal muscle, liver, white adipose tissue and the brain – to adjust their activity to the elevated nutrient supply and restore the glucose concentration to its original level. For instance, skeletal muscle takes up much glucose in response to insulin and, given its comparably large mass, is responsible for more than 70% of total body glucose disposal [5]. Insulin sensitivity of skeletal muscle is thus of critical importance for maintaining blood glucose homeostasis.

Although human insulin sensitivity varies naturally [6–8], persistent insulin resistance reflects a pathological state that associates firmly with disease. Monogenic deficiencies are for example responsible for severe insulin resistance syndromes [9,10], whilst environmental cues account for the loss of insulin sensitivity that links to diseases like type 2 diabetes [11]. Insulin resistance is a key feature of the metabolic syndrome, a cluster of disorders that collectively increase the risk of developing type 2 diabetes and cardiovascular disease [12]. Obesity is also a hallmark of this syndrome and indeed appears to be the most significant environmental risk for the development of insulin resistance [11,13].

The mechanism by which obesity causes skeletal muscle insulin resistance is incompletely understood, but bioenergetic failure has been implicated, which is not surprising as acquired obesity reflects an imbalanced whole body energy metabolism. However, the causal relation between mitochondrial dysfunction and insulin resistance is disputed fiercely. Irrespective of causality, the relative importance of mitochondrial functions that associate with loss of insulin sensitivity remains unclear. This review evaluates mitochondrial models of nutrient-induced insulin resistance of skeletal muscle, which all predict that pathology emerges when nutrient supply outweighs energy demand. Technical issues that may account for discrepancies between studies are described and it is emphasised that possible mitochondrial deficiency is best evaluated in context of cellular bioenergetic control.

2. Insulin sensitivity

2.1. Signalling

Insulin-sensitive cells express receptors that phosphorylate insulin receptor substrates (IRS1 in skeletal muscle) when activated by insulin triggering 2 major protein kinase cascades, the phosphatidylinositol

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3-kinase (PI3K) – protein kinase B (AKT) and the Ras-mitogen-activated protein kinase (MAPK) pathway [14] (Fig. 1). The insulin receptor (IR) and IRS isoforms are a 'critical node' in a complicated insulin signalling network that permits direct interaction with several other pathways, for example initiated by insulin growth factor-1 and cytokines [15]. Both IR and IRS1 are activated by tyrosine phosphorylation, and inhibited by protein tyrosine phosphatases (PTPs) and serine phosphorylation [15]. When activated in skeletal muscle, IRS1 recruits and activates PI3K that, in turn, allows activation of AKT2 [15]. As reviewed by others [16–18], the PI3K-AKT pathway is a mechanism that induces recruitment of glucose transporter protein (GLUT-4) to the plasma membrane and thus mediates the considerable insulin-stimulated muscle glucose uptake. The same pathway inactivates glycogen synthase kinase-3 [15], which promotes a net decrease of the extent to which glycogen synthase (amongst other enzymes) is phosphorylated, and thus accounts for the insulin stimulation of glycogenesis [19]. Additional muscle processes promoted by insulin include mitochondrial biogenesis [20], mitochondrial protein synthesis [21] as well as cell growth and differentiation [22]. The PI3K-AKT pathway mediates the additional anabolic effects of insulin through stimulation of the mammalian target of rapamycin [23], whilst the MAPK pathway cooperates to transmit the message of insulin to increase skeletal muscle growth and differentiation [14].

2.2. Bioenergetics

Glycogen storage is a well understood and corroborated anabolic fate of the glucose that is taken up by muscle in response to insulin [24]. Alternatively, the muscle-disposed glucose may have a catabolic destiny conserving energy as ATP [25–27] (Fig. 2). Insulin stimulation of muscle ATP synthesis has been attributed to increased mitochondrial oxidative capacity [25] as it coincides with enhanced mitochondrial protein synthesis [21,25] and with increased mRNA levels and activities of mitochondrial enzymes involved with substrate catabolism [25]. Consistently, insulin stimulates glucose oxidation [28], mitochondrial biogenesis [20] as well as coupling efficiency of oxidative phosphorylation [29]. It is important to note, however, that muscle bioenergetics are almost fully controlled by energy demand (*cf.* Section 6), such that ATP supply adapts rapidly to any change in ATP turnover [30]. Therefore, insulin effects on glucose oxidation may well be the indirect consequence of anabolic changes (Fig. 2) as the muscle processes that are stimulated by insulin all rely on endergonic mechanisms. Glucose uptake involves targeted exocytosis of intracellular compartments that sequester GLUT-4 protein [17] and thus relies on GTP-driven trafficking pathways [31]. Glycogenesis consumes both ATP and UTP, which is necessary to activate glucose molecules before glycogen synthase can add them to the extending glucan chains [32]. Protein synthesis and DNA synthesis are needed for promoting cell growth (including mitochondrial biogenesis) and differentiation, and represent major ATP consuming processes [33,34].

3. Insulin resistance

Obesity is characterized by elevated levels of circulating non-esterified fatty acids (NEFAs) and cytokines [35,36]. This excess of nutrients and inflammatory molecules is believed to link obesity causally with muscle insulin resistance [37], but exact mechanisms remain to be established. Cytokines activate inflammation- and stress-related signalling pathways that directly intersect with the insulin signalling network [15]. Cytokines may also influence insulin signalling by triggering unfolded protein response pathways under conditions that cause endoplasmic reticulum (ER) stress [38–40]. The role of ER stress in the pathology of insulin resistance in muscle, however, is currently unclear [38]. When faced with a chronic NEFA surplus, skeletal muscle cells produce many lipid species that link with decreased insulin sensitivity [41–49]. Species include triacylglycerol (TAG), diacylglycerol (DAG), ceramide and derived gangliosides, and acylcarnitines, which could in

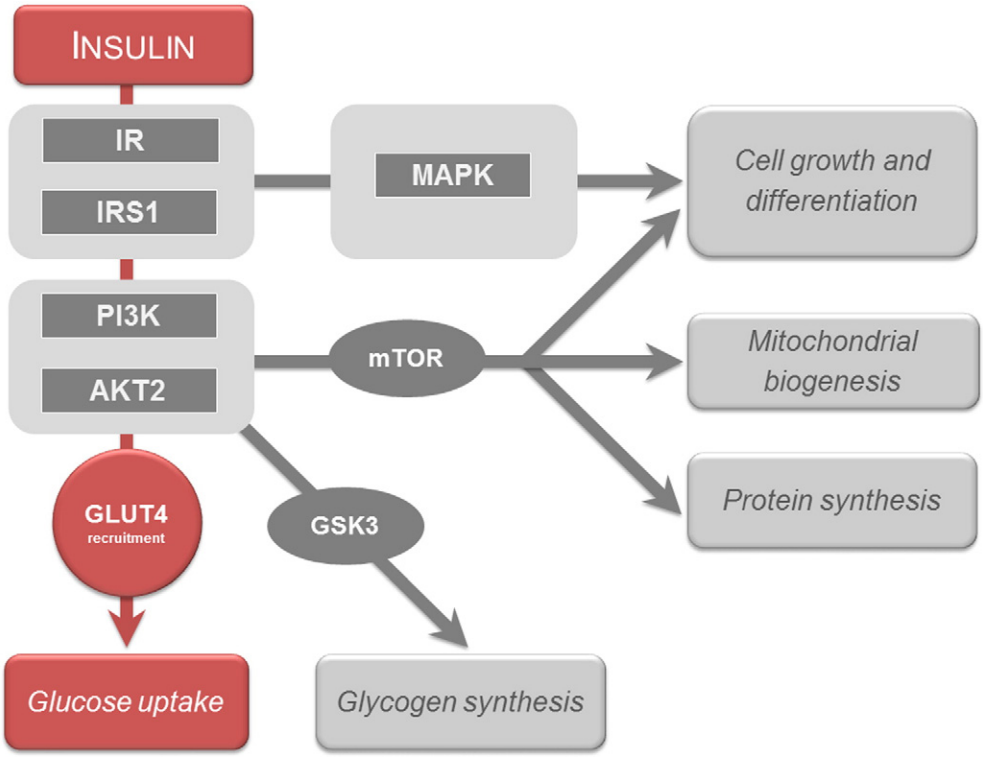

Fig. 1. Skeletal muscle insulin signalling. Insulin activation of its receptor (IR) and receptor substrate (IRS1) triggers both the PI3K–AKT and MAPK pathways that transmit insulin's message for skeletal muscle to engage with a series of anabolic processes. The main result is increased glucose uptake. GSK3, glycogen synthase kinase-3; mTOR, mammalian target of rapamycin.

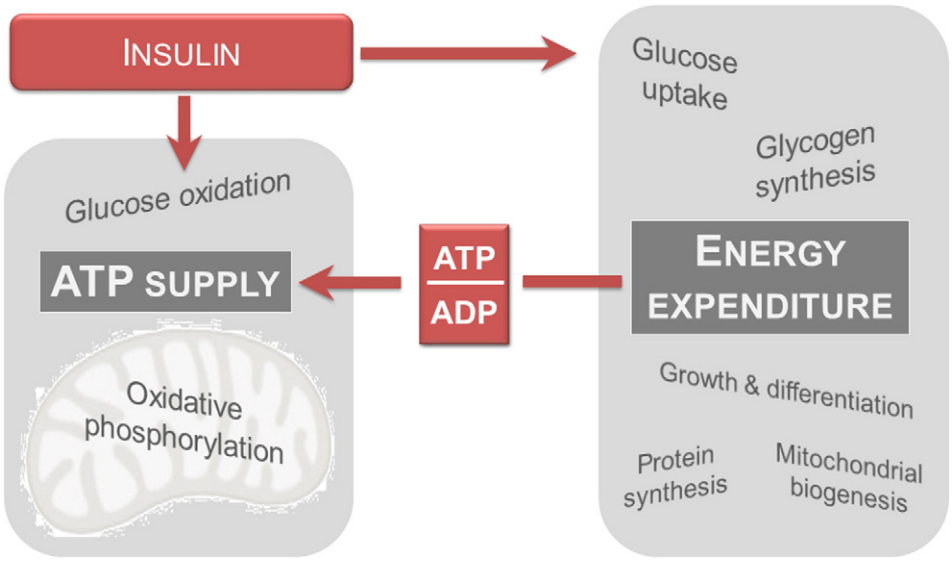

Fig. 2. Insulin effects on cellular bioenergetics. Glucose disposed in response to insulin can be broken down to produce ATP. This catabolic fate is supported by insulin stimulation of mitochondrial ATP synthesis, which may also indirectly result from increased energy demand of anabolic processes stimulated by insulin.

principle be considered maladaptive signals that arise from disordered lipid metabolism [50,51] when nutrient supply outweighs energy demand. Bioenergetic failure indeed features in most suggested explanations for the emergence of harmful lipids, but models differ on the precise involvement of mitochondrial activity (*cf.* Section 4). Moreover, imbalanced energy metabolism also disturbs cellular redox biology [52,53], which is reflected by generation of undesirable reactive oxygen species ([54] – ROS) that dampen insulin sensitivity. Reactive nitrogen species may also play a role [55].

3.1. Lipids

Strong association between intramyocellular TAG and skeletal muscle insulin sensitivity was established towards the end of last century [56–58] and has since been firmly established in rodent models of metabolic disease [59,60] and in obese and diabetic humans [61–63]. However, the occurrence of intramuscular TAG is not deleterious *per se*, as is evident from the so-called athlete's paradox that embodies the counterintuitive observation that muscle lipid content of highly trained

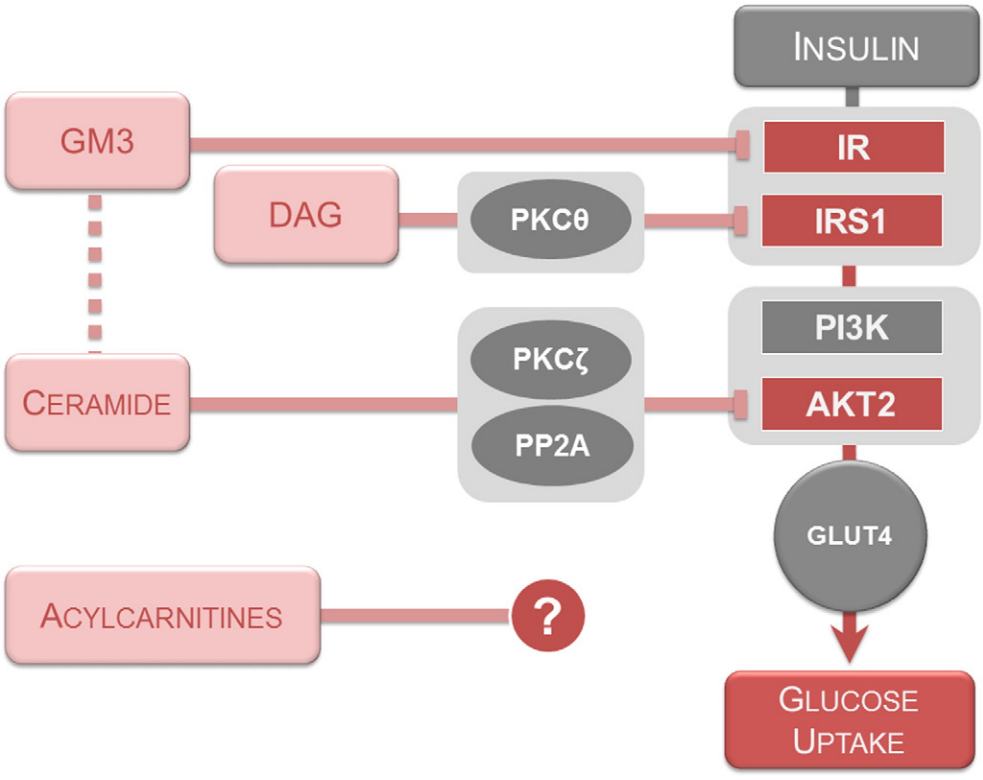

Fig. 3. Disturbance of skeletal muscle insulin signalling by lipids. Diacylglycerol (DAG) and ceramide activate atypical protein kinase C (PKC) isoforms that inhibit the insulin receptor substrate (IRS1) and protein kinase B (AKT2), respectively. Ceramide also achieves AKT2 inhibition through activation of protein phosphatase 2A (PP2A). GM3 is a ceramide-derived ganglioside that inhibits the insulin receptor (IR).

people correlates positively with their insulin sensitivity and oxidative capacity [64]. Indeed, endurance-trained athletes may accumulate as much, if not more, ectopic TAG than insulin-resistant type 2 diabetes patients [65]. TAG-containing lipid droplets are highly dynamic entities [66], however, and their high turnover is now generally held responsible for the production of deleterious lipid species that cause insulin resistance. In this respect, evidence has accrued for the notion that DAG and ceramide link to muscle insulin resistance [44, 46] (Fig. 3).

DAG accumulates in isolated NEFA-exposed myotubes [67] and its level is increased in skeletal muscle of metabolically compromised rodent models [60,68,69] and of humans exhibiting lipid-induced muscle insulin resistance [70,71]. The mechanism by which DAG lowers insulin sensitivity of mice [72] and human [70,71] involves a novel protein kinase C isoform (PKCθ), whose activation dampens insulin signalling *via* serine phosphorylation of IRS1 ([73,74] – Fig. 3). Together, these studies provide compelling evidence to suggest that DAG is a causative mediator of obesity-related muscle insulin resistance. However, the link is not universal since the exquisitely insulin-sensitive athletes mentioned above exhibit higher intramuscular DAG levels than their sedentary equivalents, either of normal weight or obese [75]. Related, insulin resistance in obese people does not always coincide with higher DAG levels [76] and, reciprocally, high myocellular DAG does not necessarily imply loss of insulin sensitivity, as has recently been demonstrated in mice [77]. Differences in subcellular localization and composition/saturation of DAG species possibly explain the discrepancies between studies [78].

Skeletal muscle ceramide levels are elevated in insulin-resistant rodent models [79–82], obese humans [75,76,83,84], and in palmitate-exposed C2C12 myocytes [85]. Ceramide dampens insulin signalling by inhibiting AKT2 activity [81,85–91] (Fig. 3) by blocking its translocation to the plasma membrane *via* activation of atypical PKC isoform zeta (PKCζ) [89], and by promoting its dephosphorylation *via* protein phosphatase 2A (PP2A) activation [88,90]. In spite of this mechanistic support, the link between ceramide and muscle insulin resistance is not absolute as human skeletal muscle rendered insulin-insensitive by *acute* lipid infusion does not exhibit increased ceramide levels [71], whilst a positive link between ceramide and insulin sensitivity has indeed also been reported [92].

Gangliosides are ceramide-derived glycosphingolipids that can influence receptor-mediated signal transduction [49]. Inhibition of glycosphingolipid synthesis [93] or genetic ablation of ganglioside GM3 [94] for example enhances murine insulin sensitivity, a phenotype that links with increased tyrosine-phosphorylation of the insulin receptor ([94] – Fig. 3). Consistently, skeletal muscle insulin-resistance in obese rats links to low abundance of NEU3 sialidase, an enzyme responsible for GM3 degradation, and such degradation is repressed when L6 myocytes are exposed to fatty acids [95]. In contrast, however, transgenic mice overexpressing NEU3 sialidase exhibit lowered insulin sensitivity [96].

Increased but incomplete mitochondrial beta oxidation in various insulin-resistant skeletal muscle models coincides with a rise in acylcarnitines [97]. Such species indeed accumulate in human muscle [98] and plasma [99,100] when subjects are fed a high-fat diet, but signs of incomplete NEFA oxidation are not always detected [100]. It is possible that acylcarnitines are merely guilty by association and that the real culprits are the acyl-CoA esters from which they derive. In this respect, it is relevant that carnitine insufficiency associates with insulin resistance [101] and that carnitine supplementation improves insulin sensitivity [102,103]. Irrespective of their carrier, it is currently unclear how fatty acyls may provoke insulin resistance [47,51], but it should be noted that insulin resistance may emerge independently of changes in canonical insulin signalling [104] and that sirtuin-mediated protein deacylation is a mechanism with impact on the metabolic syndrome [105–107] that could conceivably afford fatty acyl moieties control over insulin resistance [108].

3.2. Reactive oxygen species

The relation between ROS and insulin sensitivity is a delicate one, as skeletal muscle does not respond optimally to insulin without ROS engagement [109], but becomes resistant to it when ROS levels are persistently high [53,54]. This duality underscores the importance of ROS in cell signalling [110] and highlights the need for tight regulation of their production and turnover, as chronic ROS surplus causes oxidative stress [111].

Mitochondrial hydrogen peroxide (H_2O_2) release in permeabilised skeletal muscle fibers from rats and humans is increased by a high-fat diet that renders these muscles insulin resistant [112]. Diet-induced loss of insulin sensitivity is prevented in rats when their antioxidant capacity is increased either pharmacologically with mitochondria-targeted antioxidants or genetically through the overexpression of mitochondrial catalase [112]. Similarly, increased antioxidant capacity in transgenic mice that globally overexpress peroxiredoxin 3, decreases H_2O_2 release from isolated skeletal muscle mitochondria, an effect that links with improved systemic glucose tolerance of these mice [113]. Mopping up mitochondrial superoxide with superoxide dismutase mimetics largely prevents development of palmitate-induced insulin resistance in cultured L6 myotubes, whilst antimycin A-stimulated mitochondrial superoxide lowers insulin sensitivity of such cells [114]. Consistently, mice overexpressing mitochondrial superoxide dismutase [114] or catalase [115], are partly protected against insulin resistance resulting from a high-fat diet and old age, respectively. Although some evidence is indirect (*cf.* Section 5.2.1), the observations suggest ROS *cause* insulin resistance, a mechanism first demonstrated in adipocytes and, systemically, in mice [116]. In addition to [112], a few more associations between obesity and markers of oxidative stress have been reported in human [117,118], and it is worth notice that lipid peroxide levels are increased in skeletal muscle of obese subjects [119].

ROS is an umbrella term that covers a mixed bunch of species with different chemical and biological properties. In this respect, H_2O_2 is the most likely ROS to act as a second messenger, largely owing to its ability to oxidise thiols [110] and thus to regulate the redox proteome [120]. Indeed, through oxidation of cysteine sulphydryl groups, H_2O_2 inhibits global phosphatase (PP2A) activity in skeletal muscle [121], which causes activation of a series of stress-sensitive kinases (including JNK, ERK and IKKβ) that in turn inhibit IRS1 *via* serine phosphorylation ([122] – Fig. 4). By similar modulation of 'cysteine switches', H_2O_2 inhibits protein tyrosine phosphatases such as PTP1B [123] and PTEN [53], which prevents deactivation of IRS1 and PI3K, respectively (Fig. 4), and is held responsible for physiological H_2O_2 stimulation of insulin signalling.

3.3. Reactive nitrogen species

Obesity and type 2 diabetes are characterized by decreased NO bioavailability in animals [124,125] and human [126,127]. NO deficiency is owing to decreased nitric oxide synthase (NOS) activity, and to reactivity with ROS that may effectively act as NO scavengers [55]. Endothelial NOS3 exhibits insulin-sensitizing effects promoting insulin and glucose delivery to muscle [128] and stimulating muscle fat oxidation [129]. Interestingly, vascular endothelial NO limitation may be overcome by dietary nitrate supplementation [130,131]. Dietary nitrate reverses metabolic defects of NOS3-deficient mice [130], and benefits glucose homeostasis in rodents [132,133]. Moreover, nitrite and NO stimulate insulin signalling in L6 muscle cells by increasing GLUT4 recruitment [132]. Mechanisms by which dietary nitrate affects skeletal muscle bioenergetics are reviewed elsewhere [134]. Muscle NO appears less beneficial than vascular NO [55] as muscle-inducible NOS2 promotes insulin resistance in mice [135] *via* S-nitrosation of the insulin receptor (IR), IRS1 and AKT [136,137].

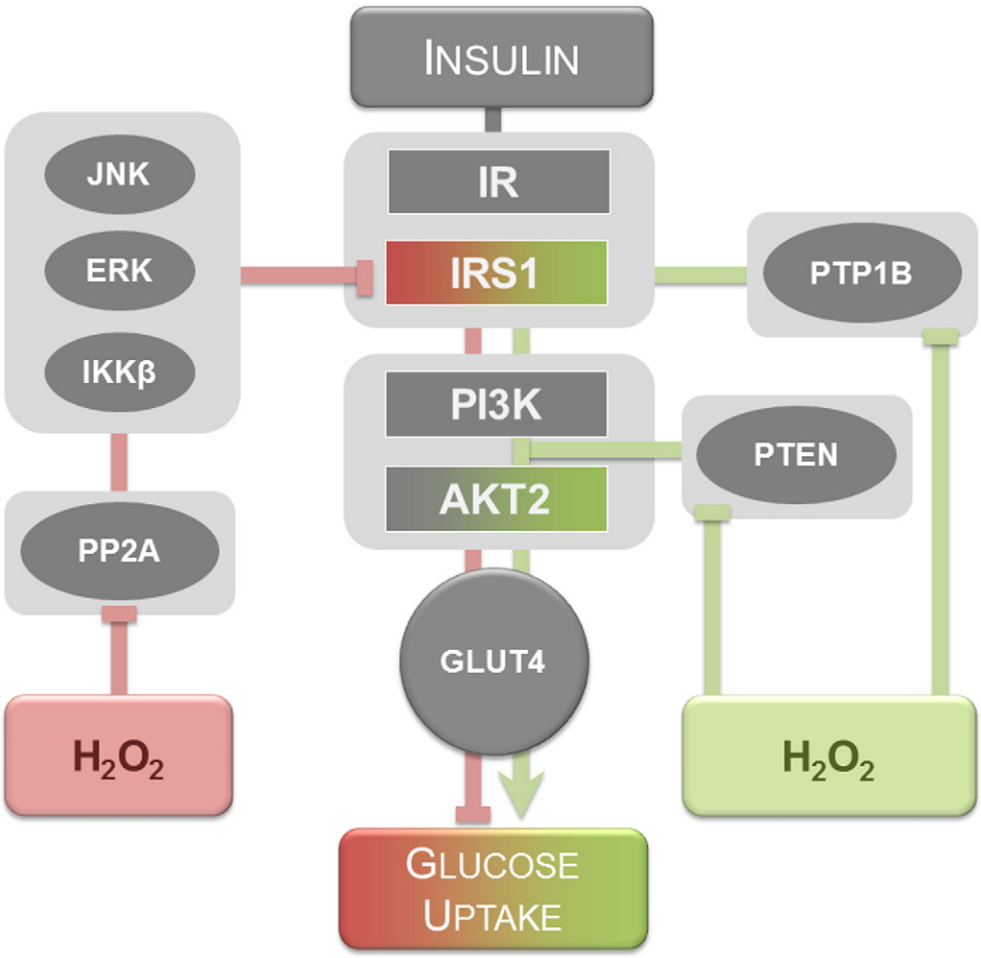

Fig. 4. Disturbance of skeletal muscle insulin signalling in obesity by reactive oxygen species. H_2O_2 is able to both promote and attenuate insulin-provoked glucose uptake (green and red lines, respectively). Inhibition of phosphatases such as the phosphatase and tension homologue (PTEN) and protein tyrosine phosphatase 1B (PTP1B) stimulates insulin signalling, whereas inhibition of PP2A dampens signalling secondary to activation of stress-sensitive kinases including c-Jun N-terminal kinase (JNK), extracellular signal regulated kinase (ERK) and inhibitory-κβ kinase β (IKKβ). See text for further detail.

4. Mitochondrial models of insulin resistance

Acquired obesity results from imbalanced systemic energy metabolism and it thus comes as no surprise that mitochondria are implicated heavily in the skeletal muscle insulin resistance that arises from chronic over-nutrition. Lipid intermediates and ROS have been suggested to accumulate in muscle as a result of nutrient mismanagement. Breakdown of major dietary nutrients converges in mitochondria where turnover of common carbon compounds produces reducing power that fuels oxidative phosphorylation (Fig. 5). When energy demand is low, mitochondrial carbon does not fuel ATP synthesis, but instead provides building blocks for anabolic processes such as lipid biosynthesis. Mitochondria are thus key to a well-balanced nutrient metabolism and are likely players in the emergence of myocellular lipids and ROS in obesity. The following models of muscle insulin resistance are based on this premise.

4.1. Oxidative capacity

Work by Shulman and colleagues challenged the 'Randle paradigm' [138,139] that NEFAs lower muscle insulin sensitivity by metabolic inhibition of glucose catabolism (*cf.* Section 4.3). Spectroscopy-based measurements revealed that muscle glucose disposal in type 2 diabetic patients is limited by glucose uptake, an observation that led to the hypothesis that insulin-stimulated GLUT4 recruitment is inhibited by lipid signalling molecules such as DAG and ceramide [140] (Fig. 3). The oxidative capacity model of muscle insulin resistance predicts that 'mitochondrial insufficiency' is responsible for the accumulation of these lipid anabolites, because it precludes β oxidation of the excess NEFAs that skeletal muscle faces in obesity [141,142] (Fig. 5). Originally based on links between human insulin resistance and decreased mitochondrial enzyme activity, fatty acid oxidation [143–146] and mitochondrial size [147], the prediction was corroborated by studies suggesting that rates of mitochondrial ATP synthesis and substrate oxidation – measured by molecular resonance spectroscopy (MRS, *cf.* Section 5.2.1) – are comparably low in elderly individuals with insulin-resistance [148], in lean, young, but insulin-resistant offspring of type 2 diabetic parents [26,149,150], and in non-obese patients with well-controlled type 2 diabetes [151]. Low mitochondrial respiration was also found in type 2 diabetic patients when measured *ex vivo* [152,153]. The oxidative capacity prediction is further supported by molecular studies showing that PGC-1α-regulated oxidative phosphorylation genes are downregulated, in a coordinated manner, in human muscle of diabetic and insulin-resistant subjects [154,155] and in muscle of fat-fed individuals [156]. Despite much additional experimental evidence (*cf.* [44,48]), the oxidative capacity model is not supported universally. For example, human capacity for fat oxidation is not always decreased in obese, insulin-resistant and type 2 diabetic subjects, as many studies have revealed opposite associations [157–165]. Consistently, rodent studies have revealed that intermittent increase of plasma fatty acids causes muscle insulin resistance in rats and stimulates mitochondrial biogenesis [166]. Furthermore, high-fat diet increases the mitochondrial β oxidation capacity of insulin-resistant mice [167]

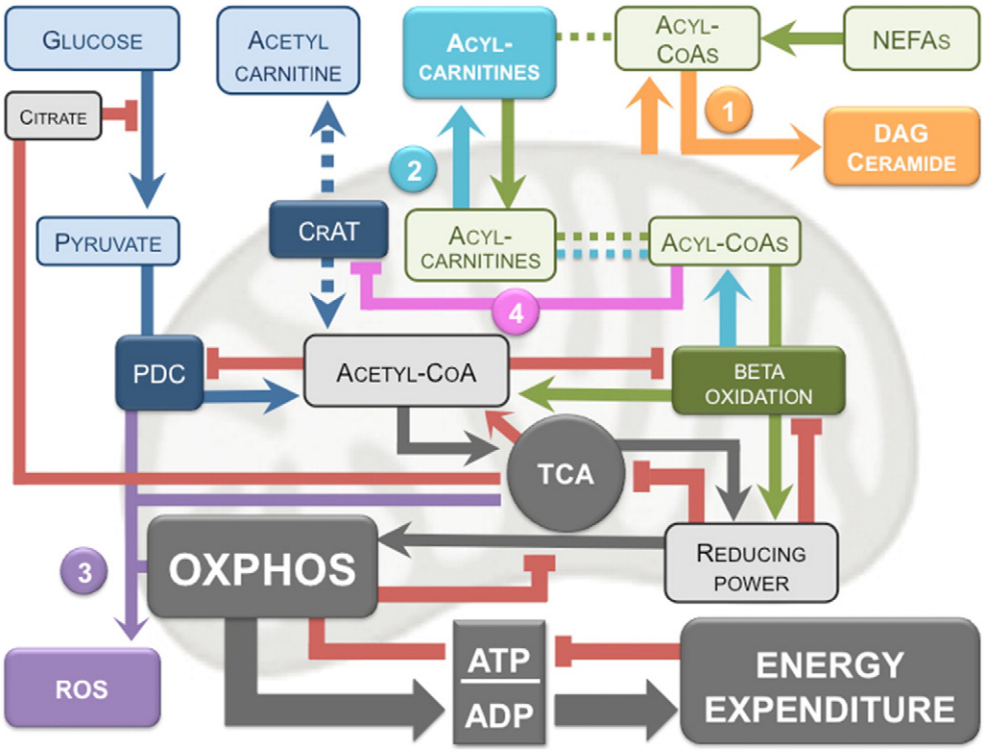

Fig. 5. Mitochondrial models of skeletal muscle insulin resistance. Glucose (dark-blue arrows) is broken down glycolytically to pyruvate in the cytoplasm, which is oxidised to acetyl-coenzyme A (CoA) by the pyruvate dehydrogenase complex (PDC) after being imported to mitochondria. Non-esterified fatty acids (NEFAs – green arrows) are activated to fatty-acyl-CoA esters in the cytoplasm. Acyl groups can be exchanged between CoA and carnitine carriers by various acyltransferases (dotted lines – CrAT = carnitine acyltransferase). Acylcarnitines are transportable across mitochondrial membranes. Mitochondrial acyl-CoA esters are broken down to acetyl-CoA *via* β oxidation, a process that also yields reducing equivalents that can be oxidised by the mitochondrial electron transfer chain. Turnover of acetyl-CoA (dark-grey arrows) *via* the tricarboxylic acid (TCA) cycle also generates such reducing power. The energy that is liberated from electron transfer is conserved as ATP *via* oxidative phosphorylation (OXPHOS). Glucose and NEFAs are only fully oxidised in this manner when muscle cells need energy. When nutrient supply outweighs energy expenditure, the system backs up (red lines): a relatively low ATP demand will increase the ATP/ADP ratio, reduce components of the electron transfer chain, boost the mitochondrial NADH/NAD^+ ratio, inhibit mitochondrial respiration, the TCA cycle and β oxidation, and provoke accumulation of acetyl-CoA and citrate, metabolites that inhibit PDC and phosphofructokinase, respectively. This reduced mitochondrial state promotes formation of molecules (DAG = diacylglycerol, ROS = reactive oxygen species) that act in various mitochondrial models of muscle insulin resistance: 1 = oxidative capacity (orange), 2 = lipid overload (blue), 3 = redox biology (purple), 4 = metabolic inflexibility (pink). See text for further detail.

and rats [168]. Any decline in muscle oxidative capacity does not tend to manifest itself before several months of high-fat feeding [169]. Recent studies into the relation between oxidative capacity and nutrient-induced muscle insulin resistance continue to yield discrepant results [170–173].

4.2. Lipid overload and redox biology

Muoio and colleagues first formulated the 'lipid overload' model having shown that obesity-related muscle insulin resistance associates firmly with intramuscular accumulation of fatty-acylcarnitine species [97,174]. Although it is unclear whether or not acylcarnitines provoke insulin resistance *per se* (*cf.* Section 3.1), these species are generated *via* acyltransferase-catalysed conversion of equivalent fatty-acyl-coenzyme-A (CoA) esters, which are perhaps the true culprits, that build up during incomplete NEFA breakdown (Fig. 5). The model predicts that incomplete breakdown occurs when mitochondrial lipid supply increases the rate of β oxidation rate more than the tricarboxylic acid (TCA) cycle and the mitochondrial electron transfer chain can handle or, more generally, when total nutrient supply outweighs energy demand [45]. In support for the suggested mismatch in obesity between mitochondrial β oxidation on the one hand and TCA cycle turnover and mitochondrial respiratory chain activity on the other, studies are cited [45] that show that β oxidation rate increases during early-stage nutrient-induced insulin resistance without change in mitochondrial respiratory capacity [168,175]. Further circumstantial support for the lipid overload model comes from links between acylcarnitine accumulation and insulin resistance in obese and type 2 diabetic human subjects [99,100,176–178] and in primary human skeletal muscle cells [179,180].

Chronic imbalance between the rate of β oxidation and the capacities of the TCA cycle and respiratory chain has also been suggested to increase mitochondrial ROS formation under conditions where fuel supply exceeds energy expenditure [53] (Fig. 5), which provides a mechanistic model as to how the H_2O_2 arises that dampens insulin signalling in obesity (Section 3.2). This 'redox biology' model of muscle insulin resistance [53] is conceptually similar to the 'lipid overload' model, and acylcarnitines and H_2O_2 may well accumulate concomitantly owing to the same bioenergetic imbalance. Importantly, these models both predict that increased β oxidation of NEFAs *attenuates* insulin sensitivity, which sets them apart from the 'oxidative capacity' model where insulin sensitivity is expected to *improve* as result of increased β oxidation [181]. It should be emphasised that effects on insulin sensitivity in this context will depend on the way in which increased lipid oxidation comes about. 'Pushing' lipid catabolism *via* pharmacological or genetic stimulation of mitochondrial β oxidation will not prevent the accumulation of insulin-desensitising lipid intermediates (Section 3.1) if insufficient demand for ATP precludes oxidation of the liberated reducing power. On the other hand, if lipid catabolism is 'pulled' by stimulating energy expenditure, then increased β oxidation will not have any pathological ramification. In other words, if β oxidation rate were amplified by increased ATP demand, for example secondary to physical exercise [182] or perhaps owing to decreased coupling efficiency of oxidative phosphorylation [183,184], then it is

unlikely that DAG, ceramide, acylcarnitines or ROS would accumulate. Insulin-numbing species implicated by the 'oxidative capacity', 'lipid overload' and the 'redox biology' models all *only* arise when cellular bioenergetic balance is disturbed (Fig. 5). It is thus possible that they contribute to insulin resistance in a concerted fashion.

The balance between nutrient supply and energy demand determines whether lipid oxidation associates with high or low ROS generation [53], which highlights that there is *no* unique relation between mitochondrial respiratory rate and ROS production [185]. Such a relation is frequently implied in redox biology literature where electron leak from the respiratory chain, which causes superoxide and H_2O_2 formation by incomplete reduction of oxygen, is often unhelpfully quantified as proportion of the *total* electron transfer rate through the chain. The driving force of ROS production is the reduction potential of the site that reduces oxygen incompletely [186], not the mitochondrial respiratory rate. Depending on how mitochondrial oxygen consumption is stimulated, ROS production may increase *or* decrease [185]. The redox biology model assigns a role for mitochondrial H_2O_2 as both beneficial and deleterious effector of insulin signalling under physiological and obese conditions, respectively (Fig. 4), and it is argued that the phenotype depends on the strength and persistence of the H_2O_2 signal [53]. It is equally conceivable that 'good' and 'bad' ROS are produced at different subcellular locations [54] or that the insulin signalling benefits are mediated by the 'non-radical' H_2O_2 [111], whilst insulin resistance emerges from 'radical' oxidative stress (*cf.* Section 5.2.2).

4.3. Metabolic inflexibility

Metabolic flexibility can be defined as the capacity of human skeletal muscle to switch from *lipid* uptake and oxidation in a fasted state to *glucose* uptake, oxidation and storage in a fed state when insulin levels are increased [145]. Such switching between metabolic fuels was first described by Randle and colleagues who discovered that skeletal muscle cells suppress glucose oxidation when supplied with NEFAs and facilitate fat oxidation instead [138]. This Randle 'cycle' [138,139] is achieved *via* acute metabolic regulation: increased mitochondrial β oxidation leads to accumulation of acetyl-CoA and citrate that lower glucose breakdown by respectively inhibiting the pyruvate dehydrogenase complex (PDC) and phosphofructokinase (Fig. 5). The consequent buildup of glucose-6-phosphate is accountable for inhibition of hexokinase, which, in turn, is suggested to limit glucose uptake [138,139]. Realizing that diabetes is characterized by increased circulating NEFA levels and decreased respiratory exchange ratios, it was proposed that the Randle cycle operates under conditions of nutrient excess and causes loss of muscle insulin sensitivity [187,188]. Interest in this metabolic dysregulation of insulin sensitivity faded a little when it was found that attenuated glucose uptake in obesity is caused by compromised GLUT4 recruitment instead of limited glucose phosphorylation [140]. Nonetheless, research on involvement of metabolic flexibility in the development of muscle insulin resistance has regained some momentum over recent years [108,189–191]. Muscle of obese subjects is characterized by a lack of metabolic flexibility, *i.e.*, it does not exhibit the expected sharp shift from lipid to glucose oxidation upon transition from a fasted to an insulin-stimulated state [145]. The apparent inflexibility is largely owing to the already comparably high reliance of obese individuals on glucose oxidation in the fasting state [143] and lack of further increased glucose oxidation during insulin infusion may not be surprising given the existing insulin resistance. However, the absolute rate of lipid oxidation in obese muscle does not change between fasting and insulin-stimulated conditions, which forms the basis for the 'metabolic inflexibility' model of muscle insulin resistance [145].

Work by Muoio and colleagues [192] has provided new insight in the possible mechanism by which metabolic flexibility is lost in obesity, and has indeed revealed a novel mitochondrial player in the regulation of fuel selection. Carnitine acetyltransferase (CrAT) is located in the mitochondrial matrix where it converts acetyl-CoA and other short-chain fatty-acyl-CoAs to their equivalent, membrane-permeant carnityl esters. Acetyl-CoA export from mitochondria may alleviate PDC inhibition under conditions of high lipid availability, and emerging evidence indeed implicates CrAT deficiency in the development of muscle insulin resistance *via* a Randle-cycle-like mechanism (Fig. 5). For instance, muscle-specific CrAT knockout mice exhibit relatively high myocellular acetyl-CoA levels and low PDC activity, and have a diminished metabolic flexibility [192]. Dietary carnitine supplementation in human correlates positively with circulating acetylcarnitine levels, glucose tolerance, and metabolic flexibility [192,193]. Moreover, skeletal muscle CrAT activity associates with insulin sensitivity in human and rodent models [194,195] and is inhibited *in vitro* by palmitoyl-CoA [195]. Under physiological conditions, acetylcarnitine may well serve as a carbon sink that facilitates continued glucose breakdown in the insulin-stimulated state when energy demand is relatively low. When such demand increases, the buffered acetyl can be readily mobilized again. In this model, supra-physiological nutrient supply would cause CrAT deficiency and thus disturb the acetyl buffering system. Although quantification of (reversible) acetyl transfer between CoA and carnitine under physiological and pathological conditions will be necessary to test the model directly, it is of interest that mice depleted from skeletal muscle CrAT exhibit decreased exercise tolerance and that effective acetyl buffering is necessary for optimal human exercise performance [196]. The CrAT-enabled safe carbon sink would stop the TCA cycle and mitochondrial respiratory chain from being overloaded with glucose-derived acetyl-CoA when energy demand is low, and would thus prevent the accumulation of insulin-numbing molecules under such conditions (Fig. 5). The metabolic inflexibility model thus appears very similar indeed to the mitochondrial models of muscle insulin resistance discussed in Sections 4.1 and 4.2. In this respect, the metabolic gridlock that has been suggested to emerge from 'mitochondrial indecision' [108] will manifest itself whether or not fuel selection is regulated adequately. In other words, it seems unlikely that the TCA cycle should care about the origin of its acetyl-CoA (although compartmentalisation of mitochondrial acetyl-CoA is formally possible), and that oxidative phosphorylation should distinguish between the sources of its reducing equivalents. As long as there is energy demand, the system will make ATP irrespectively of the nutrients that fuel it, and equally indiscriminately, the system will back up when fuel supply outweighs ATP demand (Fig. 5). Several observations are relevant in this context. (i) Branched-chain amino acids may interact with NEFAs in the development of skeletal muscle insulin resistance [197] and their catabolism may contribute to the 'metabolic gridlock' proposed to account for metabolic inflexibility [108]. This combined nutritional effect is consistent with the idea that imbalance of fuel availability and energy demand in obesity lowers insulin sensitivity irrespective of the nature of the nutrients that are in excessive supply. (ii) Genetic activation [198] and indirect metabolic inhibition [192] of PDC both associate with skeletal muscle insulin resistance. In both cases insulin resistance is likely owing to acetyl-CoA, which accumulates as result of direct PDC activation or CrAT ablation respectively. In other words, the reason for acetyl-CoA accumulation is not important for its pathological consequences. (iii) Glycolytic inhibition of glucose breakdown does not necessarily exacerbate nutrient-induced insulin resistance [192], but may in fact ameliorate it [199], an apparent discrepancy that is explained by differences in *total* mitochondrial carbon accumulation between studies. (iv) AMP-activated protein kinase overrules Randle cycle mechanisms for selecting fuels [200] (*cf.* Section 6.1). Unregulated fuel selection thus appears not an issue when energy demand is relatively high.

5. The mitochondrial causality issue

5.1. Conflicting evidence

Causality of the association between mitochondrial dysfunction and skeletal muscle insulin resistance is a controversial issue. The evidence

in favour and against causal involvement of mitochondrial deficiency in disease development is split about equally, and 'pro' and 'contra' arguments are summarized in [201] and [202], respectively. Evidence in favour for a causal relation was provided in Section 4. The counterargument is partly based on the assertion that obese muscle should have sufficient mitochondrial respiratory reserve to deal with excessive nutrient supply. This legitimate assertion is based on many studies showing that fat oxidation capacity is relatively high in obese, insulin-resistant and type 2 diabetic humans (cited in Section 4.1), and is supported by the notion that type 2 diabetic patients increase their substrate oxidation rate some 40-fold in response to exercise [202]. However, 'reserve oxidative capacity' will be of little use if cellular energy demand is low (*cf.* Section 6.2). In addition to the rodent studies already cited [167,168], work on mouse models of mitochondrial dysfunction [203–205] is frequently used to argue against causal involvement of mitochondria in insulin resistance. Such models were generated by global genetic ablation of, respectively, the mitochondrial transcription factor Tfam [203], the mitochondrial apoptosis-inducing factor AIF [204] and the transcriptional coactivators PGC-1α and PGC-1β [205]. Interestingly, impaired mitochondrial activity is not linked with loss of insulin sensitivity, but these 'sledgehammer' approaches are somewhat confounded by likely non-mitochondrial effects, direct and adaptive, of the genetic knockout. For instance, genetic disruption of mitochondrial energy conservation renders the animal models fully dependent on anaerobic glucose metabolism for ATP production, which is not the ideal background against which to evaluate metabolic interactions between fuels [201]. Skeletal-muscle-specific knockout of carnitine palmitoyltransferase 1 more specifically prevents mitochondrial β oxidation of NEFAs [206]. Interestingly, muscle lipids accumulate in this mouse model without negative effect on insulin sensitivity [206]. Most compellingly, humans with inborn insulin signalling deficiency exhibit a decreased rate of phosphocreatine (PCr) recovery following exercise [207], which demonstrates that defects in muscle ATP synthesis *follow*, not precede, insulin resistance. Congenital lipodystrophy represents another inborn insulin resistance state and is also associated with a lowered PCr recovery rate [208]. The relation between mitochondrial function and skeletal muscle insulin sensitivity is evidently not a straightforward one, as is indeed suggested by the effect of short intensive exercise on muscle metabolism of healthy control individuals and offspring of mothers with diabetes: whilst ATP synthesis in isolated muscle mitochondria is increased by exercise in both groups, insulin sensitivity is only improved in the controls [209].

5.2. Measuring mitochondrial function

It should be evident that the mitochondrial causality issue remains far from being settled. Some factors that complicate the issue have a technical nature and include evolving opinion on how best to measure cellular insulin sensitivity (generally, signalling activity appears most reliably quantified by measuring functional endpoints [104]) and heterogeneity of insulin resistance models. Indeed, discrepancies between studies may arise from variation in the extent to which insulin resistance has progressed in the different models. For instance, mitochondrial capacity increases *early* during development of rat muscle insulin resistance, but only transiently, as capacity returns to the initial level when obesity and insulin resistance progress [210]. Similarly, the muscle mitochondrial oxidative capacity declines in mice put on a high-fat diet, but only after insulin resistance has been established firmly after months of high-fat feeding [169]. This loss of mitochondrial function has been attributed to the oxidative stress encountered in the insulin-resistant state [169]. Together with the possible reversibility of fat-induced insulin resistance [211], these observations underscore the importance of a precise definition of insulin resistance development when judging mitochondrial engagement with disease progression. Moreover, the mitochondrial causality debate is clouded a little by the at times vague definition of mitochondrial dysfunction, and by the difficulty of measuring such function reliably. Also relevant for the debate is the notion that the many functions of mitochondria are dictated by their dynamic morphology. Indeed, the role of mitochondrial dynamics in nutrient metabolism and the metabolic syndrome is becoming increasingly clear [212]. With respect to mitochondrial models of insulin resistance (Section 4), 2 key functions, oxidative phosphorylation and ROS production, warrant explicit discussion.

5.2.1. Oxidative phosphorylation

Cellular bioenergetic changes during development of muscle insulin resistance may be inferred from altered transcriptomic, proteomic and metabolomic signatures [154,155,213–215], and also from changes in mitochondrial density and biogenesis [216,217]. Although invaluable, such circumstantial evidence requires functional bioenergetic measurements to confirm the topological effects are indeed reflected by changes in activity. However, it is very challenging to measure real-time mitochondrial ATP synthesis in a reliable way, particularly in physiologically relevant models. For instance, the mitochondrial ATP synthesis capacity of *resting* human skeletal muscle has been inferred from unidirectional flux between inorganic phosphate (P_i) and ATP as measured *in vivo* by ^{31}P MRS magnetization transfer [26,148–151]. The interpretation of such transfer data is difficult for two reasons: (i) in resting muscle, the flux between P_i and ATP is dominated by a glycolytically mediated exchange, and (ii) resting ATP synthesis does not reflect mitochondrial respiratory capacity calculated from *ex vivo* or *in vivo* oxygen uptake measurements [218–221]. Based on thorough meta-analysis of published literature, Kemp and Brindle conclude that ^{31}P MRS magnetization transfer studies do not tell anything about mitochondrial function [221]. This conclusion clearly weakens the case in favour of causal involvement of mitochondrial dysfunction in development of muscle insulin resistance, as this case is largely built on magnetization transfer studies. ^{31}P MRS measurements of PCr recovery following exercise, on the other hand, do indeed reflect the mitochondrial capacity of making ATP [221], but have so far yielded conflicting evidence [222–227]. As argued in Section 5.1, PCr recovery deficiencies in human skeletal muscle with *inborn* insulin signalling defects suggest mitochondrial dysfunction is a result, not cause, of insulin resistance [207,208].

5.2.2. Mitochondrial ROS

The case for causal involvement of ROS in obesity-related skeletal muscle insulin resistance has been made convincingly [53,54]. Although such involvement implicates mitochondria as likely causal players in disease pathology, it remains unclear if harmful ROS indeed originate in mitochondria and, if so, which of the 11 ROS-producing sites that have been described to date [185] are responsible for their production. In this respect, it is worth note that PDC has recently been discovered as a mitochondrial site that generates H_2O_2 under conditions of nutrient excess [228]. Under physiological conditions, PDC forms a redox circuit with the nicotinamide nucleotide transhydrogenase to keep ROS levels low during pyruvate oxidation [193]. Interestingly, metabolic modelling links PDC to insulin resistance phenotypes [229] and the enzyme is part of the 'metabolic inflexibility' model of nutrient-induced muscle insulin resistance [108] (*cf.* Section 4.3).

Generally, it is unclear for many of the ROS-generating sites whether they produce superoxide, H_2O_2, or both [185]. It is important to establish the exact source and nature of the ROS that are produced under physiological and pathological conditions in skeletal muscle, as it will help distinguish between mechanisms that could explain beneficial and deleterious ROS effects on insulin signalling [54]. Current consensus [53,54] has it that the oxidative stress causing insulin resistance is *non-radical* [111], *i.e.*, that negative effects on insulin signalling are mediated through H_2O_2-modulation of 'cysteine switches' on relevant tyrosine kinases and phosphatases (*cf.* Section 3.2). However, radical ROS damage [111] is a likely contributor to pathology given the accumulation of lipid peroxidation products in human skeletal muscle of insulin resistant

obese people [119]. Lipid peroxidation is initiated by the highly reactive hydroxyl radical (HO•) that arises through reaction between H_2O_2 and free iron. This Fenton chemistry may be driven by superoxide that is responsible for the required reduction of free iron, a species that indeed accumulates in muscle of obese humans [230]. Mitochondria are not the only source of ROS in skeletal muscle cells, as superoxide can also be produced in the plasma membrane by NADPH oxidases [231], in peroxisomes during α and β oxidation of NEFAs [232,233] and in the ER as result of enzyme-catalysed sulphydryl oxidation during protein folding [234,235]. Indeed, debate on the relative importance of the various ROS sources for the regulation of insulin signalling is ongoing [53,54,236]. The need for establishing the exact sources of skeletal muscle ROS is underscored by the growing appreciation that ROS origin dictates whether their physiological effects are beneficial or detrimental [237,238]. Intuitively, the importance of location is obvious as ROS are short-lived and highly reactive by definition. In this respect, it is worth mention that mitochondria are mobile organelles [239,240] that, in principle, could find their way to any place within the cell. Precise understanding of ROS nature and origin is evidently very important if translational potential of possible modulation of redox biology in treatment or prevention of muscle insulin resistance is to be realized. Such understanding is currently hampered by the difficulty of measuring 'native' rates [241] of ROS production in physiologically meaningful systems. Recently developed superoxide suppressors [242,243] and targeted ROS probes [244] will be invaluable tools in this respect.

6. Energy demand

The mitochondrial causality debate is not only ongoing because of technical issues (Section 5.2), but also because it remains insufficiently appreciated that myocellular bioenergetics are fully controlled by energy demand. This control structure is evident from the observation that acute inhibition of ATP turnover in skeletal muscle cells causes an immediate decrease of mitochondrial ATP synthesis [34]. Moreover, muscle adapts to more persistent energy stress by increasing its mitochondrial capacity [245,246], a response that is regulated by the AMP-activated protein kinase (AMPK). The demand-driven flux of ATP is reminiscent of a market economy where supply of goods is dictated by consumer (not producer) needs. Importantly, by controlling ATP flux this way, skeletal muscle cells can satisfy wide-ranging energetic demands whilst keeping their phosphorylation potential – which links ATP supply with ATP demand – under tight homeostatic control and far removed from its thermodynamic equilibrium [247]. Indeed, the oxidative phosphorylation rate in skeletal muscle may fluctuate several orders of magnitude without discernable effect on the ATP/ADP ratio. The notion that skeletal muscle ATP synthesis is demand-driven has major ramifications for interpreting the association between mitochondrial dysfunction and nutrient-induced loss of insulin sensitivity. Muoio and Neufer indeed argue persuasively that many paradoxical observations in the literature are readily reconciled when the discrepant energetic needs of the studied experimental models are considered [45]. As discussed by Kemp and Brindle (*cf.* Section 5.2.1), quantification of 'mitochondrial' ATP synthesis by MRS-based measurement of unidirectional flux between P_i and ATP in *resting* muscle has no straightforward relation with mitochondrial oxidative capacity [221], for the simple reason that such resting flux is set by sub-maximal energy demand. Similarly, determination of skeletal muscle glycolytic capacity is easily confounded by restricted ATP demand [248]. In this section, the importance of energy demand for maintaining skeletal muscle insulin sensitivity is highlighted by pointing out how AMPK relates to the current clinical management of the metabolic syndrome, and by exploring the possibility that mitochondrial 'dysfunction' may arise during development of muscle insulin resistance as a consequence of compromised ATP turnover.

6.1. AMP-activated protein kinase

AMPK is a serine/threonine protein kinase that has been highly conserved through evolution and is generally considered the 'master regulator' of energy metabolism in a wide range of tissues [249,250]. AMPK is activated under conditions of energetic stress (*e.g.* exercise) where high ATP demand increases the level of AMP. Broadly speaking, activated AMPK stimulates energy-liberating mechanisms and dampens ATP-consuming processes thus restoring cellular energy balance [251]. In skeletal muscle, AMPK for example promotes NEFA oxidation [252,253], glucose uptake [254,255] and protein degradation [256], whereas it attenuates lipogenesis [257,258], glycogenesis [259,260] and protein synthesis [261]. Simultaneous stimulation of NEFA oxidation and glucose uptake is at odds with the Randle cycle (*cf.* Section 4.3) and AMPK activation indeed overrules metabolic fuel selection mechanisms [200]. Notably, glucose uptake is virtually insulin-independent during exercise [262]. Moreover, chronic AMPK activation triggers mitochondrial biogenesis [245] and increases mitochondrial protein content [246]. Most evidence for the AMPK regulation of these physiological processes has been gathered from rodent work, but fuel homeostasis *via* AMPK regulation may well benefit human skeletal muscle insulin sensitivity [250]. Indeed, AMPK is seen as target for clinical management of muscle insulin resistance [263], although pharmacological AMPK stimulation without increasing energy expenditure fails to lower murine adiposity [264]. Importantly, however, it transpires that the insulin-sensitizing drugs metformin [265,266] and the thiazolidinediones [267,268] (TZDs), exert their beneficial metabolic effects at least in part by increasing AMPK activity. Mechanisms by which these antidiabetic drugs lead to AMPK activation have not been established conclusively [267]. Metformin inhibits mitochondrial respiratory complex I [269], which is expected to compromise oxidative phosphorylation and thus to lower cellular ATP levels [266,270]. Paradoxically, activated AMPK will boost NEFA oxidation, a process that will *not* operate without involvement of complex I, contrary to what has been suggested elsewhere [250,271]. It is interesting to note in this context that TZDs inhibit pyruvate uptake into mitochondria [272], an effect that could perhaps stimulate β oxidation, although it would prevent complete catabolism of the AMPK-increased glucose. Reciprocally, it has been shown that increased glucose utilization following chronic inhibition of NEFA oxidation [206,273] and consequent improvement insulin sensitivity [274] are associated with AMPK activation and increased exercise capacity, respectively.

6.2. ATP turnover

Opponents of the oxidative capacity model of skeletal muscle insulin resistance argue that resting skeletal muscle should have sufficient reserve respiratory capacity to deal with the excessive nutrient supply that prevails in obesity [202]. Such reserve will be inconsequential, however, if the demand for it is lacking. In other words, the *effective* oxidative capacity is largely determined by energy expenditure, and it is therefore conceivable that mitochondrial respiratory 'dysfunction' associated with elevated NEFA levels reflects decreased ATP turnover (Fig. 6). Indeed, insulin-numbing exposure of cultured rat and primary human myoblasts to palmitate coincides with a significantly decreased rate of *de novo* protein synthesis [34]. This anabolic process is a major ATP consumer in most cells and its inhibition by palmitate in skeletal muscle cells provokes mitochondria to reserve less ATP for making new protein. In response to palmitate, mitochondria also lower ATP supply that is used in human myoblasts for making DNA and RNA, and for maintaining appropriate sodium and potassium gradients across the plasma membrane. More generally, various saturated and unsaturated NEFAs dampen the overall absolute rate of mitochondrial ATP supply, which may well be a consequence of lower ATP demand [34]. This finding demonstrates that depressed mitochondrial respiration does not necessarily imply intrinsic defects, but could merely be an

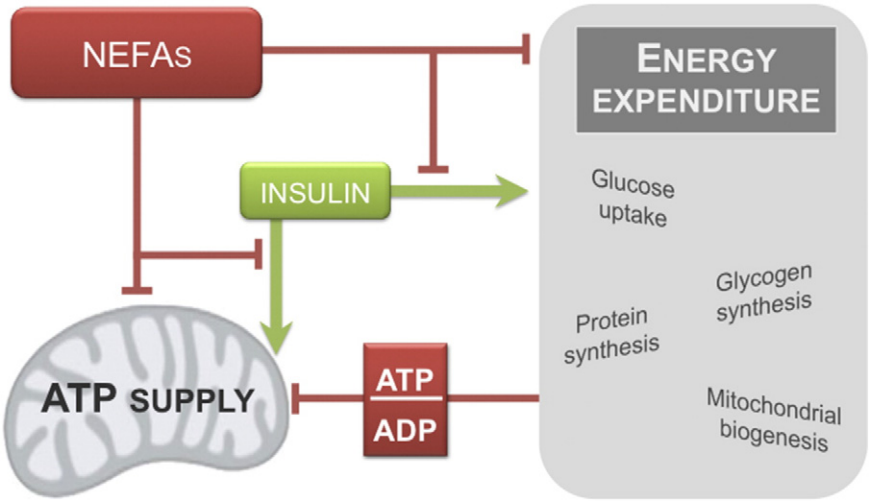

Fig. 6. Mitochondrial dysfunction in obese skeletal muscle. Oversupply of non-esterified fatty acids (NEFAs) may impair the oxidative phosphorylation machinery directly. In addition, mitochondrial activity may decrease in response to nutrient-induced attenuation of energy expenditure.

adaptation to altered energy demand. Lipotoxicity may in principle compromise many endergonic cellular functions [275] and possible harmful effects of palmitate on ER-mediated protein folding [276], a process that consumes much ATP, are worth mention in this respect. On the other hand, lipotoxicity may well trigger cellular stress responses that *increase* ATP turnover, and mitochondrial respiratory adaptation will likely reflect the net outcome of diverse NEFA effects on ATP-consuming processes. Indeed, indirect effects of nutrient excess on the effective oxidative capacity mediated *via* altered energy expenditure could also provide a mechanism for the apparent mitochondrial dysfunction that follows from inborn insulin signalling deficiency [207]. Congenital defects in the insulin receptor are expected to dampen the anabolic response of skeletal muscle to insulin (Figs. 2 and 6). The consequent attenuated energy demand may well be responsible for the apparent decrease of mitochondrial ATP synthesis capacity. From a clinical perspective, it is conceivable that the variable propensity of obese people to develop metabolic disease – almost three-quarters of people with a body mass index of more than 40 kg/m^2 do not suffer from diabetes [277] – partly relates to differences in the sensitivity of muscle energy expenditure to nutrient overload amongst individuals.

7. Conclusion

Mitochondrial dysfunction is associated with skeletal muscle insulin resistance and studies on human subjects with congenital insulin signalling defects demonstrate unequivocally that mitochondrial defects can result from *ab initio* insulin resistance. Evidence for a possible causal role of mitochondrial dysfunction in development of insulin resistance is less direct. Proposed mechanisms by which imbalanced bioenergetics can produce deleterious lipids and ROS are intuitively attractive, but support for the conceptually similar models remains circumstantial. The reported dampening effects of DAG, ceramide and hydrogen peroxide on insulin signalling are compelling, but links between mitochondrial oxidative capacity, lipid intermediates and insulin resistance are far from universal, and it is also not conclusively clear if harmful ROS indeed originate in mitochondria. Improved measurement of (i) lipid composition and subcellular location, (ii) real-time oxidative phosphorylation activity, (iii) native rates by which different ROS sources generate superoxide, hydrogen peroxide and, indirectly, hydroxyl radicals, and indeed of (iv) insulin sensitivity itself, which may deteriorate without changes in canonical signalling pathways, should enlighten the causality debate. As it stands, it seems plausible that changes in mitochondrial function that occur relatively late during development of muscle insulin resistance will exacerbate pathology. It is equally conceivable that mitochondrial insufficiencies or deficiencies coincide with harmful effects of nutrients and cytokines on other cellular targets, and that the onset of insulin resistance is triggered by multifarious *independent defects*. A challenge of this causal scenario would be to unravel the possible interplay between the initial effects, and to establish their relative importance. If mitochondria are indeed *not* involved during early disease pathology, then it remains to be demonstrated how insulin resistance causes the reported functional mitochondrial defects. Addressing this issue, mitochondrial function will be best evaluated in context of cellular bioenergetic control, as loss of insulin sensitivity likely remodels ATP-consuming processes, and mitochondria will indeed adapt to such remodeling of energy demand. It may thus be fairly obvious to conclude that mitochondrial involvement in obesity-related insulin resistance of skeletal muscle is 'a case of imbalanced bioenergetics', but it is currently far less trivial to judge which side of the balance is tipped to upset the peace.

Transparency document

The Transparency document associated with this article can be found, in online version.

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