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Intramuscular triacylglycerol and insulin resistance: Guilty as charged or wrongly accused?

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Abstract

The term lipotoxicity elicits visions of steatotic liver, fat laden skeletal muscles and engorged lipid droplets that spawn a number of potentially harmful intermediates that can wreak havoc on signal transduction and organ function. Prominent among these so-called lipotoxic mediators are signaling molecules such as long chain acyl-CoAs, ceramides and diacyglycerols; each of which is thought to engage serine kinases that disrupt the insulin signaling cascade, thereby causing insulin resistance. Defects in skeletal muscle fat oxidation have been implicated as a driving factor contributing to systemic lipid imbalance, whereas exercise-induced enhancement of oxidative potential is considered protective. The past decade of diabetes research has focused heavily on the foregoing scenario, and indeed the model is grounded in strong experimental evidence, albeit largely correlative. This review centers on mechanisms that connect lipid surplus to insulin resistance in skeletal muscle, as well as those that underlie the antilipotoxic actions of exercise. Emphasis is placed on recent studies that challenge accepted paradigms.

Keywords

Skeletal muscle; Lipid; Fat oxidation; Mitochondria; Exercise; Obesity; Diabetes; Insulin action

1. Introduction

Modern technology has created a favorable climate for the “perfect metabolic storm”. Triggered by lifestyle habits that promote overnutrition and inactivity, Westernized societies are confronting an epidemic surge in the incidence of obesity and its attendant comorbidities. Foremost among these is type 2 diabetes mellitus, which is projected to reach a global incidence of 300 million cases by the year 2020. This grim epidemiological forecast has inspired a new area of biochemistry research, commonly referred to as lipotoxicity, in which scientists are seeking to unravel the molecular mechanisms that link increasing adiposity to glucose intolerance, tissue dysfunction and metabolic failure. The purpose of this review is to consider new insights into the metabolic events that connect lipid oversupply to insulin resistance in skeletal muscle, with particular focus on recent studies

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that challenge the popular notion that obesity and diabetes are disorders that stem from impaired fat oxidation.

2. Intramuscular lipids, insulin resistance and the athletes' paradox

During the past two decades there has been heavy emphasis on understanding the connection between intramuscular triacylglycerol (IMTG) content and insulin action. Interest in this topic grew from numerous reports describing a strong negative association between IMTG content and insulin sensitivity [28]. This relationship is evident in obese and diabetic humans as well as in several rodent and cell culture models of metabolic disease and/or chronic lipid exposure [3,40,41]. Intracellular triacyglycerols are packaged into lipid droplets, which are increasingly recognized as highly dynamic and functionally important organelles in all cell types [57]. In general, the triacylglycerol molecules themselves are viewed as relatively benign in nature; however, by virtue of their high turnover rates, these storage depots are presumed to spawn lipid signaling molecules such as long chain acyl-CoAs, diacylglycerol (DAG) and ceramides. Abnormal accumulation of these and other biologically active lipid metabolites is thought to engage stress-responsive serine kinases that impede insulin activation of its cell surface receptor, as well as downstream signaling molecules such as insulin receptor substrate 1 (IRS-1) and Protein Kinase B/Akt [33,63,69]. Taken together, these observations have fostered widespread support for the idea that ectopic intramuscular lipid accumulation is a principal contributor to obesity-associated insulin resistance.

Despite strong correlative evidence for the foregoing lipotoxicity theory, questions surrounding the pathophysiological relevance of IMTG accumulation began to surface with reports showing that lipid droplet accumulation in highly insulin-sensitive endurance athletes is similar to or even greater than that found in patients with type 2 diabetes [27,89,90]. This phenomenon, now well known as the “athletes' paradox”, suggested that lipid droplet accumulation *per se* is not sufficient to disrupt insulin action. With this discovery attention shifted toward mitochondrial function and disease-associated imbalances between lipid storage and catabolism. Investigators proposed that athletes tolerate high IMTG levels because trained muscles, enriched with mitochondria, have robust oxidative capacity and strong impetus for lipid degradation. Thus, enhanced fatty acid flux into mitochondria might prevent build up of toxic lipid species in other cellular compartments [23]. Conversely, the mitochondrial dysfunction theory of insulin resistance predicts that impaired or insufficient mitochondrial uptake and oxidation of fatty acids results in their alternative use as substrates for generating harmful lipid signaling molecules [69]. The notion that reduced β-oxidation represents a primary metabolic lesion in obesity gained support from several cross-sectional studies showing that isolated muscle strips, tissue homogenates or cultured myocytes from severely obese and/or diabetic humans exhibit diminished rates of fat oxidation when compared to specimens derived from healthy control subjects [9,41,46,87]. In addition, muscle insulin resistance often (but not always [86]) coexists with abnormalities in mitochondrial morphology, reduced NADH:O_2 oxidoreductase activity, diminished *in vivo* rates of ATP synthesis and TCA cycle flux, as well as low expression levels of genes involved in mitochondrial biogenesis and oxidative metabolism [47,49,59,68,78]. These traits are most often observed in association with aging,

severe obesity and type 2 diabetes but were also evident in young insulin-resistant offspring of parents with diabetes [60,62].

3. Mitochondrial dysfunction: Cause or consequence of metabolic disease?

Although correlative studies seem to implicate faulty mitochondria and impaired β-oxidation as predisposing risk factors for insulin resistance, still uncertain is whether diminished fat oxidation reflects an underlying cause or a late stage consequence of the disease process. The idea that a high oxidative capacity protects against skeletal muscle insulin resistance is attractive because it could explain the potent insulin sensitizing effects of habitual exercise. Recently however, this hypothesis has been challenged on the basis that it contradicts several fundamental principles of oxidative phosphorylation and energy balance [34]. First, the mitochondrial dysfunction theory of insulin resistance implies that mitochondrial content and/or capacity dictates resting rates of fat oxidation. However, this prediction goes against the widely accepted chemiosmotic model of respiration [75], which holds that absolute rates of fuel oxidation under aerobic conditions depend ultimately on ATP turnover (i.e., workload). A high work rate increases the intramitochondrial ADP/ATP ratio, which promotes proton influx from the inner mitochondrial space through ATP synthase. This in turn necessitates increased breakdown of carbon energy to generate reducing equivalents that fuel the electron transport system (ETS) and maintain mitochondrial membrane potential. Also noteworthy is that skeletal muscles harbor enormous mitochondrial reserve, enabling exponential increases in energy output during vigorous physical activity. Under resting conditions ATP demands are low and the ETS operates at only a small fraction of total respiratory capacity. Expanded mitochondrial mass therefore affords the advantage of enhanced fat oxidation during moderate to high intensity exercise but should not have much impact at rest. Why then should a marginal decline in oxidative potential cause lipotoxicity and insulin resistance in the typical sedentary individual?

Another central tenet of the mitochondrial dysfunction paradigm is that impaired fat oxidation gives rise to glucose intolerance, whereas high rates of fat oxidation promote glucose uptake and disposal. This concept conflicts with compelling evidence that fatty acids and glucose compete as metabolic substrates [74]. At a constant work rate, provision of fatty acids inhibits glycolytic flux and glucose oxidation because the lipid substrate provides at least some portion of the acetyl-CoA that feeds the TCA cycle. In the context of exercise, increased lipid delivery and catabolism allows for glucose/glycogen sparing, which then permits prolonged activity at a given submaximal intensity and/or a burst of anaerobic output in the later stages of an event [35]. Conversely, a generalized limitation in oxidative capacity or a specific impingement of β-oxidation necessitates a compensatory increase in glucose uptake and glycolytic ATP production. This is evident during an exercise challenge when comparing trained versus untrained states [35] and is sometimes observed under resting conditions in patients affected by inborn errors in fatty acid oxidation [55,91]. Thus, a large body of evidence predicts that a deficit in β- oxidation would increase, not decrease, glucose utilization.

4. Fat oxidation: Friend or foe?

To gain new insights into the consequences of lipid surplus a recent series of investigations employed a targeted, high throughput metabolic profiling approach to survey several two-state models of lipid exposure [3,49,50,65]. The methods used in these studies focused on quantifying discrete clusters of chemically related metabolites using mass spectrometry as the analytical platform. Approximately 150 intermediary metabolites in five chemical classes (acylcarnitines, organic acids, amino acids, long chain acyl-CoAs and free fatty acids) were measured in serum, urine and/or tissue extracts [6]. These analyses provided a more comprehensive snapshot of intermediary metabolism as compared to many previous studies. It is important to emphasize, however, that steady-state metabolite concentrations in tissues and blood represent the net balance between production, consumption, import and export. Additionally, many intermediates participate in multiple metabolic pathways. For these reasons, static measurements do not provide definitive information regarding substrate flux. Notably, interpretation of these data sets was informed by experiments in which substrate oxidation and mitochondrial function were assessed by several complementary methods, including whole body indirect calorimetry, *in vitro* flux analysis using radiolabeled tracers, and polarographic measurement of respiration.

Application of the foregoing tools showed that diet-induced and genetic forms of glucose intolerance were associated with high rates of incomplete fat oxidation [49,50], which occurs when carbon flux through the β-oxidation machinery outpaces entry of acetyl-CoA (the final product of β-oxidation) into the TCA cycle. This phenotype was initially uncovered by profiling of 37 independent acylcarnitine species ranging in size from 2 to 22 carbons [49]. The acylcarnitine metabolites are byproducts of substrate degradation formed from their respective acyl-CoA intermediates by a family of carnitine acyltransferases that reside principally (but not exclusively) in mitochondria. Most even chain species reflect incomplete fatty acid oxidation; odd chain species stem primarily from amino acid catabolism, whereas acetylcarnitine derives from acetyl-CoA, the universal degradation product of all metabolic substrates. Among the metabolites evaluated, the acylcarnitine profile emerged as the most striking. Several of the even chain, fatty acid-derived acylcarnitine intermediates were elevated in muscle of obese rodents, but decreased after a 2-week exercise intervention that restored glucose tolerance. Likewise, *in vitro* measurement of [^{14}C]oleate catabolism revealed disproportionally high rates of incomplete relative to complete fat oxidation in isolated muscle mitochondria from obese compared to lean rodents. By contrast, exercise training boosted mitochondrial capacity to completely oxidize lipid substrate [49]. A subsequent study showed that insulin resistant states were marked by increased whole body fat oxidation, impaired switching to carbohydrate substrate during the fasted to fed transition, and coincident reductions in muscle levels of several TCA cycle intermediates [50]. These perturbations were evident in multiple rodent models of glucose intolerance, both at the whole body level and in isolated mitochondria. In aggregate, these studies showed that the early stages of obesity and insulin resistance are accompanied by increased rather than reduced β-oxidation.

The aforementioned findings suggested that excessive fat supply, absent physical activity, results in a persistent mismatch between β-oxidation and TCA cycle activity. Similar

observations emerged from other laboratories that likewise reported increased oxidative capacity, β-oxidation enzyme activities and mitochondrial biogenesis in parallel with high fat feeding and the onset of both obesity and insulin resistance in rodents [26,30,51,85,96]. Moreover, a growing number of studies have reported a negative association between circulating and/or tissue acylcarnitines and glucose tolerance. For example, feeding rats a diet enriched with both fat and branched chain amino acids caused insulin resistance in association with elevated intramuscular levels of both even and odd chain acylcarnitine metabolites [64]. In another recent study, genetic ablation of Nur77, a transcriptional regulator of glucose utilization genes in skeletal muscle, increased susceptibility to diet-induced obesity and insulin resistance in parallel with elevated IMTG content and accumulation of even-chained acylcarnitine species [18]. Human studies have also reported increased plasma acylcarnitine levels in association with insulin resistance and diabetes [1,37,52,64].

Given that the vast majority of acylcarnitines are produced by mitochondria, their association with glucose intolerance led investigators to revisit an idea originally proposed by Sir Philip Randle; namely, that excessive fat oxidation triggers insulin resistance [74]. With the advent of genetic engineering modern science is now better equipped to test the Randle hypothesis using more sophisticated tools. Thus, the critical question of whether or not slowing fat oxidation would rescue insulin action was addressed using knockout mice lacking malonyl-CoA decarboxylase (MCD). This enzyme promotes β-oxidation by degrading malonyl-CoA, a natural inhibitor of carnitine palmitoyltransferase 1 (CPT1), which catalyzes the first committed step in the β-oxidation pathway. Loss of MCD results in partial CPT1 inhibition [50,88]. As a result, the *mcd*$^{-/-}$ mice had decreased acylcarnitine accumulation in muscle, enhanced rates of glucose oxidation, and resisted diet-induced glucose intolerance, despite high intramuscular levels of triacylglycerol and long chain acyl-CoAs [50,88]. One limitation of this study was that DAG and ceramides were not evaluated, thus it is possible that *mcd* deficiency led to an unanticipated reduction in these specific lipid species. Nonetheless, the antidiabetic phenotype of the *mcd*$^{-/-}$ mice suggests that increased fat oxidation is compulsory for the development of lipid-induced glucose intolerance. Studies in skeletal myocytes grown in culture produced similar results. For example, treatment with the CPT1 inhibitor, etomoxir, prevented lipid-induced insulin resistance in rat L6 myotubes [50]. Likewise, another study showed that lowering of MCD activity in primary human skeletal myocytes using small interfering RNAs invoked a clear shift from fatty acid to glucose oxidation and a corresponding increase in both basal and insulinstimulated glucose uptake [13]. These outcomes were associated with increased cell surface levels of the GLUT-4 glucose transporter, but surprisingly no enhancement in insulin signaling at the levels of IRS-1 tyrosine phosphorylation, PI3-kinase activity, or serine phosphorylation of Akt. Thus, MCD inactivation appeared to encourage glucose uptake and utilization through mechanisms independent of the canonical insulin signaling pathway. Still unknown is how the absence of *mcd* in the knockout mouse model impacts insulin signaling in specific target tissues. This is a key question that warrants additional investigation.

5. Mitochondrial dysfunction and insulin resistance: A matter of definition?

The finding that insulin resistance tracks with enhanced rather than impaired capacity for β-oxidation aligns with several recent studies that have challenged the mitochondrial dysfunction paradigm. For example, mice lacking the mitochondrial transcription factor A (TFAM) have reduced oxidative capacity but increased whole body glucose tolerance and enhanced insulin-stimulated glucose uptake in isolated muscles [95]. A similar phenotype was reported as a consequence of muscle-specific deletion of apoptosis inducing factor (AIF), a protein that plays a key role in regulating the ETS [70]. Deficiency of AIF resulted in subtle disruptions in mitochondrial gene expression and oxidative phosphorylation, resembling those reported in humans with type 2 diabetes [68,70]. However, in contrast to the diabetic subjects, AIF null mice maintained insulin sensitivity and resisted the diabetogenic effects of a high fat diet. Likewise, whole body or muscle-specific deletion of the redox regulatory protein, thioredoxin interaction protein (TXNIP), resulted in impaired oxidative muscle metabolism while also producing mice that were exquisitely insulin responsive, even after high fat feeding [38]. In both the AIF and TXNIP knockout mice protection against diet-induced glucose intolerance was associated with heightened insulin-mediated activation of Akt. Additionally, the AIF null mice had increased activity of 5′ AMP-activated protein kinase, an energy sensing enzyme that stimulates glucose uptake in response to a rise in the cellular AMP/ATP ratio [43].

Although emerging reports appear to argue against a role for mitochondrial dysfunction as a root cause of muscle insulin resistance, the controversy might come down to a matter of definition. On the one hand, mitochondrial dysfunction is classically defined as a disruption in respiratory capacity that limits aerobic generation of ATP. This definition was satisfied in both the TFAM and MCK-AIF knockout mouse models. Considering that a moderate to severe deficit in mitochondrial function increases reliance on glycolytic metabolism, it is not so surprising that these genetic maneuvers elicited adaptations that enhance glucose uptake and insulin sensitivity. On the other hand, a broader interpretation of mitochondrial dysfunction encompasses perturbations that do not necessarily compromise oxidative potential. Thus, disturbances in mitochondrial fuel selection, anaplerotic and cataplerotic carbon flux, redox balance and production of reactive oxygen species (ROS) can occur in the absence of measurable defects in respiratory capacity [4,12,65]. Conceivably, these aspects of mitochondrial function might report on local energetic tone as a means to coordinate insulin sensitivity with cellular demand for glucose substrate.

Could there be a mitochondrial-derived signal that links lipid overload to impaired insulin action? One hypothesis that has gained momentum centers on the premise that mitochondrial-derived ROS modulate insulin signaling [10,81]. Thus, persistently high rates of fat oxidation might foster a mitochondrial microenvironment that is conducive to ROS production. Fatty acids are a rich source of reducing equivalents, even when undergoing partial degradation. The first step of β-oxidation, which is catalyzed by a family of acyl-CoA dehydrogenases, produces reducing equivalents in the form of $FADH_2$. Electrons from this molecule can enter directly into the Q cycle of the ETS, and in doing so bypass regulation at complex I to build the proton gradient across the inner mitochondrial membrane. Likewise, electrons from glycolysis can enter the Q cycle via the glycerol-3- phosphate shuttle. Thus,

in theory, a surplus of both glucose and lipid fuel should raise mitochondrial membrane potential. In coupled mitochondria, protons descend this gradient via ATP synthase, thereby coupling respiration to ATP synthesis. The ETS utilizes oxygen as an electron acceptor and is thus the primary cellular source of ROS such as superoxide anion and hydrogen peroxide (H_2O_2). When energy delivery is high (overfeeding) but ATP consumption is low (physical inactivity), a high ATP/ADP ratio reduces proton flux through ATP synthase and slows electron transport through the ETS. These conditions increase mitochondrial membrane potential and the local ratio of NADH/NAD, which in turn imposes negative pressure on several TCA cycle enzymes. In addition, inefficient electron transfer through ETS complexes I and III has been shown to increase the half-life of the superoxide anion and thus generation of the cellular H_2O_2 [7].

Recent progress in ROS biology indicates that these agents are not merely arbitrary toxins but also act as bona fide participants in signal transduction. Further noteworthy is that oxidant stress is known to activate several of the serine kinases and transcription factors that have been linked to impaired insulin signaling, including c-jun amino-terminal kinases (JNKs), IkB kinase catalytic subunit β (IKK-β), NF-kappa B transcription factor (NF-kB) and protein kinase C (PKC; reviewed in [10,17]). Treatment of genetically obese/diabetic rodents with antioxidant nutrients, such as alpha-lipoic acid and *N*-acetylcysteine, has been shown to improve insulin action and glucose homeostasis [8,31,36]. Likewise, experiments in cultured 3T3-L1 adipocytes showed that several distinct genetic and nutritional maneuvers that reduced oxidative stress blocked insulin resistance caused by dexamethasone and TNFα [36]. In these earlier studies the role of muscle mitochondria as targets or mediators of the antioxidant interventions was not evaluated. However, more recent reports offered direct and compelling evidence that aberrant ROS production can provoke muscle insulin resistance [4,32]. In both rodents and humans insulin resistant states were associated with increased mitochondrial H_2O_2 emission potential and decreased skeletal muscle levels of glutathione, a commonly used biomarker of oxidative/reductive stress due to its important role in detoxifying H_2O_2 [4]. Moreover, genetic or pharmacological manipulations that lowered mitochondrial ROS production preserved insulin action in lipid-treated myotubes [32] and mice fed a high fat diet [4,32]. Results from another study further suggested that oxidative stress contributes to mitochondrial dysfunction in mice fed a Western type diet [11]. Taken together, these findings imply that mitochondrial dysfunction and insulin resistance have a common connection to redox imbalance. ROS signaling and redox sensing rely heavily on the interdependent glutathione and thioredoxin reducing systems. Both use the reducing power of NADPH to mitigate oxidative stress and to modulate reversible oxidation/reduction of protein thiols/disulfides. These socalled “sulfur switches” are gaining increasing recognition for their regulatory roles in cell signaling, mitochondrial function and metabolic control [14,44,45]. Also noteworthy is that these redox circuits intersect and regulate insulin signaling molecules such as PTEN, SHIP2 and PTP1B [14,38,44,45]. Despite several intriguing molecular connections between lipid-induced redox stress and muscle insulin resistance, this area of investigation has remained relatively underexplored.

6. Carnitine as an antilipotoxic therapy

The observation that tissue acylcarnitines accumulate in several instances of insulin resistance temps speculation that these metabolites might act as “lipotoxic” culprits. However, this suggestion is at odds with evidence that production of carnitine conjugates helps to avert mitochondrial dysfunction, owing at least in part to regeneration of free CoA [73,97]. Presuming that acylcarnitine production and efflux actually benefits mitochondrial function, perhaps prolonged exposure to lipid stress disrupts this defense mechanism by compromising carnitine availability. In accord with this prediction a recent study uncovered a strong signature of carnitine diminution in multiple rodent models of obesity and diabetes [65]. Whole body carnitine levels were decreased in insulin resistant states such as advanced age, genetic diabetes and diet-induced obesity. The obesity-related decline in free carnitine was associated with increased whole body fat oxidation, muscle accumulation of long chain acylcarnitines, a corresponding decline in short chain acylcarnitine species, and impaired substrate switching from fatty acid to pyruvate when measured in isolated mitochondria. Most notably, this and another study showed that carnitine therapy improved glucose tolerance and insulin responsiveness in rodent models of aging, genetic diabetes and high fat feeding [65,71], while also reversing obesity-related derangements in mitochondrial fuel selection [65]. In these studies the antidiabetic effects of carnitine were associated with a shift in fuel preference toward glucose oxidation, a surprising result given the prominent role of this nutrient in permitting mitochondrial import and oxidation of fatty acids. Importantly however, carnitine also enables mitochondrial export of excess carbon fuel. Fitting with the latter function, carnitine-supplemented rodents had robust increases in tissue efflux and urinary excretion of acetylcarnitine. This metabolite derives from acetyl-CoA via the action of carnitine acetyltransferase (CrAT), a mitochondrial enzyme that converts short chain CoA species to their acylcarnitine counterparts while also regenerating free CoA [21]. Accordingly, CrAT is presumed to play a key role in regulating the activities of mitochondrial enzymes that respond to the acetyl-CoA/CoA ratio, such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [73]. Investigators speculated that chronic lipid stress might lead to a specific compromise in the intramitochondrial pool of free carnitine, which in turn antagonizes CrAT activity, mitochondrial function, glucose/pyruvate oxidation and insulin action [65].

Interestingly, L-carnitine is an FDA-approved therapy for inborn errors in metabolism [56]. Patients afflicted with these disorders present with nonketotic hypoglycemia, muscle weakness, pronounced elevations in long chain acylcarnitines and systemic carnitine depletion [91]. Whereas numerous clinical studies have examined carnitine therapy as a strategy for treating inborn metabolic errors, renal dysfunction, heart failure and cognitive dysfunction (reviewed in [94,97]), few have investigated its use as an antidiabetic agent. Results from a limited number of small scale studies suggest that carnitine supplementation might confer insulin sensitizing actions in glucose intolerant or diabetic humans [16,58,72]. The precise mechanisms underlying the benefits of supplemental L-carnitine remain unclear, although mitochondrial energy metabolism and redox state have been suggested as probable targets [2,5,67,97]. Thus, by buffering intramitochondrial imbalances between acyl-CoA load and TCA cycle activity, this nutrient might play a critical role in combating the

damaging effects of nutrient surplus. Larger scale clinical trials are necessary to further explore the antidiabetic potential of carnitine therapy in humans.

7. Exercise as an antilipotoxic strategy

The salutary impact of physical activity on insulin sensitivity, and metabolic health in general, has been well recognized for decades. As the prevalence of the metabolic syndrome continues to rise, so too have efforts to understand the molecular mechanisms linking exercise to desirable health outcomes, with the lofty goal of progressing toward exercise mimetic drugs. Because exercise elicits an exquisitely complex set of physiological responses, the health benefits of routine activity are likely to stem from an equally complex set of mechanisms involving multiple organ systems. It is also likely that at least some of this benefit is secondary to redistribution of body fat and adjustments in whole body lipid balance. However, progress beyond this general presumption to more tangible "antilipotoxic" mechanisms has proven challenging. Much attention has centered on mitochondrial biogenesis and concomitant enhancement of oxidative capacity, both of which represent hallmark adaptations that occur in response to aerobic exercise training and coincide with insulin sensitization [35]. Routine physical activity also causes a fiber type shift, favoring conversion from white to red myofibers [35]. Cross-sectional studies have found an inverse relationship between the proportion of type I (red) fibers and risk of developing metabolic disease [82]. Yet unknown is whether this fiber conversion has a direct impact on disease risk or if it is simply a reflection of physical activity. Nonetheless, these findings have fueled widespread speculation that red skeletal muscles, enriched with mitochondria, confer protection against metabolic dysfunction. Clearly, exercise-induced mitochondrial adaptations and fiber type switching contribute to improved fitness and athletic performance; however, their presumed roles in combating obesity and diabetes are still based on circumstantial evidence.

The past decade has produced major advances in our understanding of several master transcriptional regulators of mitochondrial biogenesis and β-oxidation [61]. Scientists now have an opportunity to exploit this knowledge, along with the power of mouse genetics, to determine whether or not heightened oxidative potential protects against metabolic disease, independent of contractile activity. Two relevant muscle-specific mouse models have been characterized in which the myosin creatine kinase (MCK) promoter was used to drive overexpression of either peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor that promotes expression of β-oxidation genes, or PPARγ coactivator-1α (PGC-1α), a promiscuous transcriptional co-activator that stimulates mitochondrial biogenesis and transcription of several oxidative enzymes [19,24]. Both of these transgenic mouse models had increased expression of multiple mitochondrial proteins, leading to enhanced capacity for fat oxidation, but contrary to the predicted outcome they were more susceptible to whole body and skeletal muscle insulin resistance. In the MCK-PPARα mice, administration of oxfenicine, a pharmacological inhibitor of β-oxidation, improved glucose tolerance, suggesting excessive fat oxidation contributed to the diabetic phenotype [24]. The MCK-PGC1α transgene caused a 2.4-fold increase in mitochondrial content of type II fibers without affecting type I fibers [19]. The resulting boost in oxidative power enabled superior exercise performance relative to wild-type mice [15] but did not change fat mass, total body

energy expenditure or weight gain during a high fat diet. Reminiscent of the athletes' paradox, MCK-PGC1α mice had increased intramuscular levels of TAG, DAG and lysophosphatic acid when fed the fat-enriched diet [19]. Investigators surmised that the elevated DAGs were responsible for the insulin resistant phenotype of the mice. Equally plausible however, the combined increases in IMTG and mitochondrial content may have encouraged preferential oxidation of lipid substrate, thereby antagonizing the drive for glucose uptake and utilization as predicted by the Randle hypothesis. It is also possible that both mechanisms operate in concert. Further investigation is now necessary to pinpoint the precise diabetogenic lesions in these two transgenic models.

Although current evidence is far from conclusive, at least in some cases exercise-independent increases in mitochondrial mass and fat oxidation failed to enhance glucose tolerance. These findings suggest that the antidiabetic effects of an active lifestyle might depend more on the bioenergetic expense of physical work than adaptive remodeling of the muscle. Indeed, it is well recognized that even an acute bout of exercise can lead to complete or near-complete reversal of impaired insulin-stimulated glucose uptake into skeletal muscle of obese humans and rodents [84]. Muscle contraction is known to stimulate translocation of the Glut4 glucose transporter via an insulin-independent mechanism (reviewed elsewhere [79]), but the insulin sensitizing properties of contractile activity are evident up to 24 h post-exercise, indicating that additional mechanisms come into play [84]. This was first described in studies showing that treadmill running caused a 2-fold increase in insulin-stimulated glucose uptake when assessed during the post-exercise period using a rat hindlimb perfusion model [76], and then later reproduced in human studies of one-legged exercise [77] and animals models of electrically stimulated muscle contraction [22].

Because the acute effects of exercise can be isolated to skeletal muscle, this experimental paradigm provides an attractive model for evaluating interactions between contractile activity and local insulin action. However, few studies have directly examined contraction-induced reversal of insulin resistance in accord with global changes in muscle lipid metabolites. Oakes et al. reported that a single bout of swim exercise reversed diet-induced insulin resistance in rodents in association with reduced intramuscular levels of malonyl-CoA and long chain acyl-CoAs, whereas IMTG were unchanged [66]. Similarly, a more recent study in humans found a strong correlation between lowering of malonyl-CoA and improved insulin sensitivity, both measured 4 h after one-legged exercise [25]. In this investigation the experimental protocol resulted in decreased glycogen levels but did not affect IMTG levels. A more comprehensive assessment of lipid intermediates was reported by Thyfault et al. [83], who applied the hindlimb perfusion model to studies of lean and obese Zucker rats. Metabolite concentrations were profiled in specimens harvested 10 min after contractile activity to capture the lipid milieu at the time of insulin administration. Analysis of mitochondrial-derived acylcarnitines showed that the contraction regimen elicited marked increases in β-oxidation and mitochondrial carbon flux, in parallel with reduced malonyl-CoA levels. Both the endogenous supply and mitochondrial use of fatty acid substrate appeared to be greater in muscle from the obese versus lean rats, despite similar rates of glycogen depletion. In line with this scenario, contraction caused a robust reduction in IMTG content of the obese muscles; however, diacylglycerol, monoacylglycerol and long chain acyl-CoAs were either unchanged or increased. Thus,

these findings revealed a strong association between improved insulin action and enhanced mitochondrial activity, but without corresponding reductions in leading candidate mediators of insulin resistance. Notably, one outcome of acute contraction universally observed in these and other studies (cited below) was the lowering of muscle glycogen. Accordingly, enhancement of insulin action in these models might be more closely connected to glycogen metabolism than lipid flux [20].

Another intriguing idea that has been put forth suggests that muscle contraction protects against lipotoxicity by encouraging long chain acyl-CoA partitioning into IMTG [53,54,80]. Both acute and chronic exercise induce muscle expression of glycerolipid biosynthetic enzymes, which is coupled to increased IMTG synthesis and content [42,80]. These observations help to explain the high lipid levels in trained athletes. A recent study in humans showed that the insulin sensitizing properties of a single exercise session corresponded with elevated IMTG content and lower muscle levels of DAG, ceramide and muscle glycogen, all measured the day after exercise and following a lipid perfusion [80]. The study implies that sequestration of fatty acid into IMTG guards against less desirable fates. Similar outcomes have been observed in transgenic mice with muscle-specific overexpression of diacyglycerol acyltransferase (DGAT1), an enzyme that catalyzes that final committed step in IMTG synthesis [54]. In these mice, increased DGAT activity in muscle resulted in higher IMTG levels, decreased DAG and ceramide levels, and resistance to diet-induced glucose intolerance. Interestingly however, DGAT1 overexpression was also accompanied by a complex set of mitochondrial abnormalities, including altered morphology, a 45% reduction in mtDNA and increased mRNA expression of metabolic genes such as CPT1 and uncoupling protein 3 [53]. It is yet unclear whether this form of mitochondrial dysfunction influenced skeletal muscle substrate selection. A similar link between IMTG turnover and mitochondrial function has been described in mouse models with targeted deletion of adipose tissue triglyceride lipase (ATGL), a ubiquitously expressed lipolytic enzyme [29,92,93,98]. Deficiency of ATGL in whole body knockout mice results in severe metabolic disruptions, cardiac dysfunction and premature death [29], which occurs in association with increased tissue lipid droplet accumulation, decreased DAG and ceramide levels, and inhibition of both fatty acid oxidation and mRNA expression of oxidative genes. As a result, these mice exhibit increased glucose use, depleted liver and muscle glycogen levels, and enhanced whole body and skeletal muscle insulin sensitivity [29,39,48]. Taken together with the phenotype of the MCK-DGAT1 mice, these results build on evidence of a fascinating physical and functional dynamic between lipid droplets, mitochondrial biology and insulin action [57].

8. Summary and concluding remarks

Despite intense investigation, our current understanding of lipotoxicity and the molecular underpinnings of lipid-induced insulin resistance in skeletal muscle remains elusive. Research in this field has focused heavily on potential links between oxidative insufficiencies, IMTG accumulation and resulting perturbations in lipid signaling molecules. By contrast, this review featured new studies suggesting additional mechanisms that involve various forms of mitochondrial stress; including excessive β-oxidation, carnitine depletion, aberrant ROS production and redox imbalance. Mounting evidence argues against the notion

that skeletal muscle insulin resistance is caused by inherent or acquired deficiencies in oxidative metabolism. Instead, the early stages of lipid overload and glucose intolerance reflect a pronounced shift in substrate selection, in favor of fat oxidation. Whether or not this switch in metabolic currency plays a central role in triggering mitochondrial stress, insulin resistance and/or eventual mitochondrial failure, is yet an open question that warrants further study.

Relevant to this topic, there has been much speculation that the insulin sensitizing benefits of habitual exercise come secondary to skeletal muscle adaptations that enhance oxidative capacity. However, recent studies in genetically modified mouse models challenge this idea. Likewise, increased fat oxidation and coincident lowering of damaging lipid species are generally presumed to contribute to the antilipotoxic and antidiabetic actions of acute muscle contraction. Clearly, muscle contraction increases mitochondrial flux and degradation of carbon substrates, including but not limited to fatty acids. However, the seminal question of whether or not the insulin sensitizing properties of contractile activity require fatty acid degradation and corresponding reductions in lipid signaling molecules has yet to be answered experimentally.

Much of the foregoing discussion centers on findings from transgenic or knockout mouse models. The relevance of these studies to human physiology is an important consideration. In many cases, genetically modified mouse models involve complete loss of function or dramatic overexpression of the targeted protein, resulting in a cascade of metabolic insults and/or adaptations that might not apply to the clinical problem under investigation. Accordingly, results from these studies must be interpreted with caution. Still, the animal models permit proof-of-concept experiments that are difficult, or even impossible, to accomplish in humans. Potential relevance to human physiology and the etiology of human disease is strengthened when several distinct models lead to similar outcomes and interpretations. It is also important to emphasize that new insights pertaining to biological mechanisms of insulin resistance do not necessarily translate into attractive antidiabetic drug targets. For example, in the event that fat oxidation contributes to glucose intolerance, potential adverse consequences of inhibiting β-oxidation are likely to dampen enthusiasm for this specific therapeutic strategy. In a similar light however, emergent findings prompt concerns about the development of exercise mimetic drugs. Thus, pharmacological agents that promote fat oxidation and/or mitochondrial biogenesis, without a corresponding increase in energy expenditure, might do more harm than good when administered to inactive, glucose intolerant patients.

In summary, recent developments have raised new uncertainties surrounding the interplay between lipotoxicity, mitochondrial function and skeletal muscle insulin resistance. The intent of this paper was to highlight emerging controversies and inspire reexamination of popular paradigms. At least one general consensus in this field is that the recent surge in the prevalence of insulin resistance stems largely from inactivity and caloric excess, regardless of the energy source. The current debate regarding mechanisms of metabolic failure reflects the pathophysiological complexities of the disease and underscores the difficult challenge of developing safe and effective therapeutics. Unfortunately, the therapy that has thus far

proven most effective is the one that is least appealing to many patients (i.e., diet and exercise).

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