Free fatty acids and skeletal muscle insulin resistance

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Purpose of review

Acute exposure to fatty acids causes insulin resistance in muscle, and excess dietary lipid and obesity are also strongly associated with muscle insulin resistance. Relevant mechanisms, however, are still not fully elucidated. Here we examine the latest evidence as to why lipids might accumulate in muscle and the possible mechanisms for lipid-induced insulin resistance.

Recent findings

Muscle lipid metabolites such as long chain fatty acid coenzyme As, diacylglycerol and ceramides may impair insulin signalling directly. Crosstalk between inflammatory signalling pathways and insulin signalling pathways, mitochondrial dysfunction and oxidative stress have also been put forward as major contributors to the development or maintenance of lipid-induced insulin resistance in muscle. Several animal models with gene deletions in pathways of fatty acid synthesis and storage also show increased metabolic rate, reduced intramuscular lipid storage and improved insulin action when challenged with a high lipid load.

Summary

Studies in genetic and dietary obese animal models, genetically modified animals and humans with obesity or type 2 diabetes suggest plausible mechanisms for effects of fatty acids, lipid metabolites, inflammatory pathways and mitochondrial dysfunction on insulin action in muscle. Many of these mechanisms, however, have been demonstrated in situations in which lipid accumulation (obesity) already exists. Whether the initial events leading to muscle insulin resistance are direct effects of fatty acids in muscle or are secondary to lipid accumulation in adipose tissue or liver remains to be clarified.

Keywords

AMP kinase, ceramides, diacylglycerol, insulin resistance, muscle triglyceride, obesity

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Introduction

The hypothesis that insulin resistance is related to triglyceride accumulation in muscle and liver dates back to studies of 15–20 years ago showing that triglyceride accumulates in muscle of high fat-fed rats, coincident with insulin resistance [1,2]. Since then the relevance of muscle lipid accumulation to insulin resistance in humans has been demonstrated, and basic studies have indicated plausible mechanisms whereby lipid accumulation could generate insulin resistance [3,4,5•,6••]. The challenges now are not so much whether excess fatty acids can cause muscle (and liver) insulin resistance, but which of the putative mechanisms dominate in particular tissues and circumstances and whether there are different forms of lipid-induced insulin resistance (e.g. acute free fatty acid-induced versus longstanding obesity).

Intramyocellular triglyceride content and insulin resistance

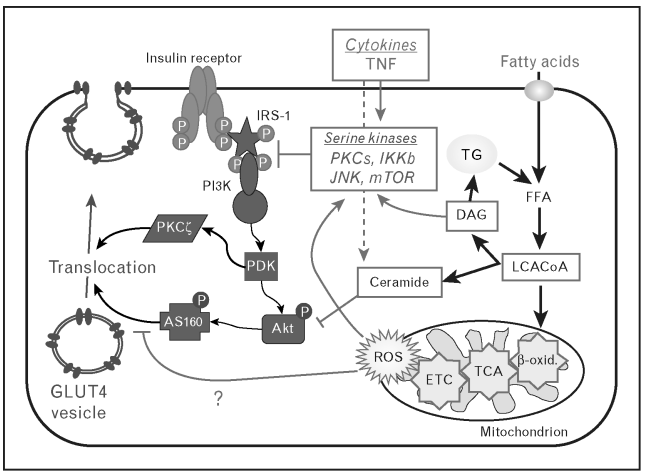
An association between intramyocellular triglyceride (IMTG) and insulin resistance is now well established in many animal models [3,4,5•,6••] and further evidence has appeared over the last 12 months. For example dexamethasone-treated [7] and endothelial nitric oxide synthase knockout [8] mice were shown to have impaired glucose tolerance or insulin resistance and increased muscle IMTG. Similarly overexpression of muscle acyl coenzyme A (CoA):diacylglycerol acyltransferase 2 (DGAT2), an enzyme which catalyses triglyceride synthesis, resulted in increased IMTG [and ceramide and long chain fatty acid CoAs (LCACoAs)] with insulin resistance in glycolytic muscle, a muscle type which normally accumulates less triglyceride than oxidative

muscle [9]. Turning to human studies, the use of the noninvasive magnetic resonance spectroscopy (MRS) technique for assessing IMTG [10] has facilitated a number of studies over the last year. Improved insulin sensitivity achieved by low-calorie diets in patients with type 2 diabetes was accompanied by a reduction in IMTG in two recent studies [11,12]. Furthermore insulin resistance associated with ageing [13], growth hormone administration [14] and post-burn trauma [15] have all been associated with increased IMTG. Consistently, when peripheral insulin sensitivity was unaltered by various interventions there was also no change in IMTG [16–18], even though in one case circulating free fatty acid was reduced [16]. All these studies add to the numerous earlier studies pointing to the importance of IMTG as a correlate of muscle insulin resistance. There continue to be, however, other reports that suggest IMTG accumulation is not the whole story. For example some lean study participants have substantial muscle insulin resistance without increased IMTG [19••] and some high-BMI study participants have less IMTG than might be expected for their degree of insulin resistance [20]. Interestingly a recent study [21] suggested that IMTG is similar in obese individuals with type 2 diabetes (T2D) and BMI-matched nondiabetic controls, although the T2D individuals had impaired mitochondrial oxidative function and greater insulin resistance. To summarize what we perceive as current opinion, it is that IMTG is a useful marker of the level of cytosolic lipid accumulation. It is more likely, however, that the active lipid metabolites such as LCACoAs, diacylglycerols and ceramides are the culprits leading to insulin resistance (Fig. 1) [3,4,5•,6••] and, as considered below, there is often, but not always, a correspondence between levels of these metabolites and IMTG. Thus IMTG should not be regarded as a ubiquitous marker of insulin resistance. In addition there is also an issue regarding the precision of determining IMTG. It is possible that some of the variation in the literature is related to the inherent difficulty of this determination, in the light of possible adipocyte contamination in muscle biopsies, imprecision in MRS scanning and other issues. A recent study that compared three commonly used methodologies for determining muscle lipid content [22] is 'food for thought'.

Muscle lipid intermediates and not intramyocellular triglyceride are the 'culprit'

Important studies have appeared over the last 12 months to reinforce the view that it is the active lipid intermediates rather than IMTG that result in lipid-induced muscle insulin resistance. In the first Liu and colleagues [23••] demonstrated that direct upregulation of the triglyceride synthesis enzyme DGAT1 in muscle of mice resulted in increased IMTG but with improved muscle insulin sensitivity, reasonably interpreted as a consequence of reduced lipid intermediates. Consistent findings were also reported in humans; in a key study it was found that a single bout of exercise, which increased expression of DGAT1 and other lipogenic enzymes, resulted in increased muscle triglyceride but a partitioning away from other 'harmful' lipid metabolites, and reversal of fatty acid-induced insulin resistance [24••]. This latter study helped to explain the so-called 'athlete's paradox' whereby endurance athletes can have increased insulin sensitivity despite high muscle IMTG [25]. It is also consistent with recent data from cell systems [26,27]. An interesting corollary of this work is that it might well be that in some cases an increased IMTG may be indicative of a protective mechanism maintaining insulin sensitivity if it is associated with a reduction in lipid metabolite levels [23••,24••].

Figure 1 Potential mechanisms of fatty acid-induced insulin resistance in muscle



Exposure to excess fatty acids results in the accumulation of intramyocellular lipid species such as diacylglycerol (DAG) and ceramides. DAG is thought to activate serine kinases that can serine phosphorylate and reduce the signal transduction capacity of insulin receptor substrate 1 (IRS-1). Ceramides can interfere with insulin signalling at the level of Akt and are produced *de novo* from fatty acids or by release from sphingolipids in response to stress cytokines such as tumour necrosis factor (TNF). TNF and other cytokines associated with fat accumulation can also activate serine kinases directly via inflammatory signalling pathways. Excess fatty acid oxidation in the mitochondria, via the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC), can lead to an increase in fatty acid metabolites and reactive oxygen species (ROS) which can activate intracellular stress kinases but may possibly have effects on insulin signalling to GLUT4 translocation at unknown points downstream of Akt and AS160 function. TG, triglyceride.

Why do lipids accumulate in muscle?

There may be multiple metabolic causes of increased cytosolic lipid accumulation in muscle in insulin-resistant states, with an obvious question as to how much is due to increased uptake versus reduced utilization of fatty acids. It is likely that, in animal models of insulin resistance involving diets with a high fat content, a high muscle

uptake of fatty acids is the most significant factor. In fact we have found that in high fat-fed rats there is an adaptation to increase the clearance of fatty acids into muscle [28], presumably to make better use of what is the dominant energy substrate. A lot of recent research, however, has focused on the idea that lipid may accumulate in muscle because of a reduced oxidation of fatty acids. Strong data support a decreased number of mitochondria in muscle from obese individuals with T2D [29] and other studies show co-ordinated decreases in gene expression for a number of mitochondrial electron transport proteins [30,31]. Whether a mitochondrial defect can be induced by a lipid metabolic overload is currently a matter of debate. For example it has been reported that a high dietary fat intake can downregulate oxidative gene expression in muscle of humans and rodents [32], whereas more recent studies found that fat feeding increased fatty acid oxidation enzymes and mitochondrial fatty acid oxidative capacity [33•,34•]. Other reports do suggest changes in ATP production rates [35] and mitochondrial function after prolonged fat feeding [36••] but not at the early time points (2–5 weeks) when muscle insulin resistance is already clearly evident in fat-fed rodents [1,37]. Thus it seems likely that there is an initial upregulation of both fatty acid clearance into muscle, and the subsequent ability to oxidize the fatty acids in insulin-resistant rodents, although presumably the latter is insufficient to prevent cytosolic lipid accumulation in the presence of a high fatty acid supply to muscle. In addition it is possible that there are functional abnormalities in regulatory mechanisms that control muscle triglyceride turnover leading to or contributing to insulin resistance [6••]. Clearly intramuscular lipolysis is complex, with new data on humoral contributors CGRPα [38•] and ATGL [39•] emerging in 2007–2008. It is a case of 'watch this space'.

AMP-activated protein kinase and muscle lipid metabolism

The AMP-activated protein kinase (AMPK) pathway plays a key role in the sensing and regulation of tissue energy metabolism and its activation in muscle can, *inter alia*, lead to increased fat oxidation [40]. Pointing to its potential importance to human muscle metabolism is that a gain-of-function mutation in the γ subunit of AMPK leads to reduced IMTG and increased muscle glycogen levels [41]. AMPK activation results in decreased malonyl CoA, lessening inhibition of carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme that controls the transfer of cytosolic LCACoAs into mitochondria for oxidation. Small increases in muscle CPT-1 activity, at least in rodents [42], can direct lipids away from storage and into oxidation, stressing the importance of this regulatory step in opposing cytosolic lipid accumulation. AMPK activation also decreases ceramide synthesis, fatty acid-induced NFκB activation, and mTOR activity, all of which have been implicated in the generation of insulin resistance [43].

It is possible that a dysregulation of the AMPK pathway may itself be a contributing factor to the metabolic derangement associated with lipid accumulation and muscle insulin resistance. A number of animal models of insulin resistance in the literature suggest such a dysregulation [43] and recent additions include high fat feeding [44], glucose infusion [45] and ageing [46,47••]. In contrast to the animal data, however, human studies have yielded conflicting results. Supporting a possible dysregulation were reports that muscle AMPK activity was reduced in obese individuals [48,49] and in those with T2D [49], and that obese individuals and those with T2D had attenuated exercise activation of AMPK [50]. Other studies, however, suggest normal functioning of the AMPK pathway in human obesity and T2D [51–54]. At the moment reasons for these conflicting results are not clear, although it may be that reduced AMPK activity is associated with increased adiposity rather than with T2D *per se*. Thus comparisons between healthy individuals and those with T2D when BMI is matched may not show differences in AMPK activity [52–54]. Nevertheless this would not explain all findings [51]. It is hoped that some of these issues can be clarified in the near future.

Adipokines have been identified as endogenous regulators of the AMPK–malonyl CoA network and hence it is possible that cytosolic lipid retention and muscle insulin resistance are related to resistance to adipokine regulation [55••]. Two contenders are leptin and adiponectin, but other adipokines such as the lipocalins [56–58] appear to also have an important involvement in energy metabolic regulation, albeit so far ill-defined regarding muscle. Leptin can for example act to reduce elevated triglyceride content in muscle and liver in lipodystrophic insulin-resistant subjects (who have low leptin levels), and plasma levels are increased in obesity, suggesting central or peripheral leptin resistance develops. There is in addition direct in-vitro evidence for leptin resistance in skeletal muscle [55••]. Studies have also indicated that there may be resistance to adiponectin-induced AMPK activation and fatty acid oxidation in insulin-resistant high fat-fed rats [59•] and in muscle cells from obese individuals and obese individuals with T2D [60]. A problem remains, however, as to the physiological importance of adiponectin action in muscle, complicated by the multiple circulating forms of adiponectin, and its actions may arguably have greater metabolic importance centrally or in the liver [61•].

Which lipid intermediates cause muscle insulin resistance?

The major candidates among lipid metabolites for inducing muscle insulin resistance are LCACoAs, diacyglycerol

and ceramides [62••] (Fig. 1). Several additional studies have appeared over the last year linking ceramides to muscle insulin resistance, the majority view being that ceramides principally act downstream of IRS1 in the insulin signalling pathway to inhibit phosphorylation and activation of PKB/Akt [62••]. Increased muscle ceramides have been reported in men at risk of developing T2D [63] and several in-vitro studies on muscle cells provided strong support for the importance of ceramides in insulin resistance [64–66]. Putative links to insulin signalling, particularly involving Akt, have been further investigated [67]. Key interventional studies have been performed in rodents, pointing to causal links of ceramide synthesis to insulin resistance; thus pharmacological inhibitors of ceramide synthesis [68••], or of a ceramide metabolite glucosylceramide [69], have substantially ameliorated insulin resistance. While ceramide inhibitors can substantially improve glucocorticoid, saturated fat and obesity-related insulin resistance, they appear ineffective against insulin resistance induced by unsaturated fats [68••], not surprising as ceramides are the result of metabolizing saturated fatty acids.

The other strong contender to mechanistically link lipid intermediates to impaired insulin signalling are diacylglycerols (DAGs) principally activating DAG-sensitive PKCs, such as the novel PKCs θ and ε [3,4]. Studies such as those in the muscle UCP3 overexpressing mouse model [70•] provide strong evidence for the importance of the DAG/PKC pathway influencing the early steps of insulin signalling, and support earlier implications from the PKCθ knockout mouse [71]. In our own work we found that DAGs were elevated with the onset of insulin resistance in glucose-infused rats, although ceramides were not measured in this study [45]. Others find lowering of both ceramides and DAGs when insulin sensitivity is enhanced, as with effects of metformin or exercise in the Zucker diabetic fatty rat [72]. Lastly the potential importance of DAG was strengthened by an innovative study from Zierath's group [73••] in which muscle DAG levels were specifically elevated in mice by inhibiting diacylglycerol kinase, a regulator of DAG breakdown. The result included increased susceptibility to hyperglycemia-induced insulin resistance, impaired insulin signalling and glucose transport, together with development of mild obesity.

While debate will continue on the relative importance of the various cytosolic lipid intermediates in muscle insulin resistance, in 2007 an additional important study appeared [74••] suggesting that it is a mitochondrial rather than a cytosolic lipid overload, accompanied by incomplete fatty acid oxidation, that is particularly deleterious to muscle insulin action. At first glance the mitochondrial overload theory [75••] is seemingly at odds with other recent findings suggesting increased mitochondrial transfer of LCACoAs enhances insulin sensitivity [27,42,76,77]. It will be interesting to see how these alternate scenarios play out over the next year or so.

Cytokines and inflammatory pathways

In the last few years a substantial amount of evidence has been published to support a role for cytokines and the activation of inflammatory signalling pathways in obesity and lipid-induced insulin resistance [78•]. Obesity is associated with higher plasma and tissue levels of a number of major cytokines (tumour necrosis factor, interleukin-6, MCP-1, PAI-1 to name a few). There is some controversy, however, about the extent to which dietary insulin resistance in muscle is the direct effect of local activation of inflammatory pathways. Recent studies report no activation of the IKK-β/NFκB pathway in muscle of animals fed a high-fat diet [79] and that overexpression of components of the IKK-β/NFκB pathway in muscle does not result in insulin resistance [80•]. Although other studies do report the activation of inflammatory pathways in muscle of dietary models of insulin resistance [81], the period of dietary intervention used to produce obesity and insulin resistance is usually 6–16 weeks whereas muscle insulin resistance can normally be detected after 2–3 weeks of high-fat feeding when there is little evidence of an increase in circulating cytokines or increased cytokine expression in adipose tissue [82]. It appears that cytokine production from macrophages within adipose tissue or other tissues (liver, vascular tissue and skeletal muscle) could cause activation of IKK-β and JNK in muscle in an endocrine, paracrine or autocrine fashion [83]. What is not clear, however, is whether the initiation of lipid-induced insulin resistance in muscle requires activation of inflammatory signalling pathways or whether these pathways are involved more in the maintenance or exacerbation of muscle insulin resistance once the obese, low-grade inflammation state has been established.

Insulin signalling and insulin resistance: need for reassessment

It is our opinion that it is now vital to determine what really is important for impairing muscle insulin-stimulated glucose uptake *in vivo*, particularly in T2D, and there are certainly some gaps in our knowledge. Current dogma would have it that muscle lipid intermediates primarily interfere with insulin signalling at either the IRS1 step (DAG/PKCs) or downstream at the PKB/Akt step (ceramides). Whether this causes defective muscle glucose metabolism, however, is uncertain. In common with the PKCs, IRS1 can be phosphorylated at multiple sites by a host of other Ser/Thr kinases: JNK, ERK, mTOR, and IKK, resulting in reduced insulin-stimulated tyrosine phosphorylation and in some cases proteolytic degradation. Effects may be very nonlinear, however,

with maximal insulin-stimulated glucose transport in adipocytes being achieved by activating only 10 and 20% of the total IRS1 and Akt, respectively [84]. A similar situation may also be the case in muscle. Adding to current uncertainties, recent studies have reported that insulin-stimulated Akt activation is in fact not impaired in muscle from obese individuals with insulin resistance, from glucose-intolerant first-degree relatives of patients with T2D and from patients with T2D [85,86]. Furthermore in rats made insulin resistant by 5 h of hyperglycaemia/hyperinsulinaemia [87], or in isolated soleus muscle made insulin resistant by palmitate incubation [88], there is no defect in insulin-stimulated Akt phosphorylation [87]. Lastly reduction of IRS1 levels in muscle by 60% by direct in-vivo genetic manipulation does not result in impaired insulin action [89••]. Hence we believe that the major mechanisms by which lipids and other factors cause insulin resistance may well be downstream of Akt or within a parallel pathway that remains to be defined. As a minimum we need to keep an open mind to this possibility.

Conclusion

To better define the molecular basis of insulin resistance, it will be necessary to establish the initial onset events that are triggered in response to a variety of insulin resistance insults so that we can begin to understand how these converge upon the insulin action pathway. It is currently unclear whether there is one common convergence point that accounts for all forms of insulin resistance and, if so, how this defect culminates in T2D. Much work is going on to dissect and understand the processes which are now on the 'short list' in linking muscle lipid accumulation to defective insulin signalling, but new prospects are opening up and we should keep an open mind as to which of these mechanisms will eventually turn out to be the most important in human insulin-resistant states such as obesity and T2D.

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