Lipid metabolism, exercise and insulin action

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Abstract

Skeletal muscle constitutes 40% of body mass and takes up 80% of a glucose load. Therefore, impaired glucose removal from the circulation, such as that which occurs in obesity and type 2 diabetes, is attributable in large part to the insulin resistance in muscle. Recent research has shown that fatty acids, derived from adipose tissue, can interfere with insulin signalling in muscle. Hence, insulin-stimulated GLUT4 translocation to the cell surface is impaired, and therefore, the rate of glucose removal from the circulation into muscle is delayed. The mechanisms provoking lipid-mediated insulin resistance are not completely understood. In sedentary individuals, excess intramyocellular accumulation of triacylglycerols is only modestly associated with insulin resistance. In contrast, endurance athletes, despite accumulating large amounts of intramyocellular triacylglycerols, are highly insulin sensitive. Thus it appears that lipid metabolites, other than triacylglycerols, interfere with insulin signalling. These metabolites, however, are not expected to accumulate

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in athletic muscles, as endurance training increases the capacity for fatty acid oxidation by muscle. These observations, and others in severely obese individuals and type 2 diabetes patients, suggest that impaired rates of fatty acid oxidation are associated with insulin resistance. In addition, in obesity and type 2 diabetes, the rates of fatty acid transport into muscle are also increased. Thus, excess intracellular lipid metabolite accumulation, which interferes with insulin signalling, can occur as a result of impaired rates of fatty acid oxidation and/or increased rates of fatty acid transport into muscle. Accumulation of excess intramyocellular lipid can be avoided by exercise, which improves the capacity for fatty acid oxidation.

**Introduction**

The prevalence of obesity and type 2 diabetes has increased dramatically in recent years. There is good reason to believe that changes in lifestyle (reduced physical activity and increased energy intake) have contributed to this problem in the latter half of the twentieth century. Indeed, a landmark study has shown that a modest change in lifestyle over 2.8 years [i.e. brisk walking 150 min/week and a restricted energy intake to induce a 7% or approx. 5.6 kg (12.3 lb) weight loss] is twice as effective, and far more economic, than daily drug (metformin) treatment in preventing the onset of type 2 diabetes [1]. However, the molecular mechanisms that are at the root of lifestyle-induced type 2 diabetes remain elusive.

**Causes of insulin resistance in skeletal muscle**

Glucose uptake into muscle occurs via a well-known facilitated diffusion process involving the glucose transporter GLUT4. Under basal conditions most of the GLUT4 protein is present within intramyocellular depot(s). When glucose levels in the circulation are increased, as is normally the case after a meal, insulin levels also rise. At the plasma membrane of the muscle cell, insulin binds to its receptor, which then initiates a signalling cascade that serves to induce the translocation of GLUT4 from its intracellular depot(s) to the cell surface, thereby increasing the rate of glucose transport into the muscle cell (for a review see [2]) (Figure 1). Muscle contraction can also recruit GLUT4 to the cell surface by activating a signalling system that is independent of the insulin signalling system. Insulin resistance occurs when inadequate quantities of GLUT4 are recruited to the cell surface by insulin, due to impairments in the insulin signalling cascade.

Type 2 diabetes patients, as well as a high proportion of obese individuals, exhibit insulin resistance. It is now widely recognized that skeletal muscle is one of the key sites in which insulin resistance is present. Under normal circumstances, about 80% of a glucose load is taken up by the skeletal muscles of the human body. Because skeletal muscle comprises about 40% of body weight, insulin resistance in this tissue accounts for a major proportion of
the whole body insulin resistance (i.e. delayed clearance of glucose from the circulation in response to insulin).

The decreased ability of insulin to stimulate glucose transport in muscle of individuals with insulin resistance could be caused by a block in insulin signal transduction or an inability to move the GLUT4 transporter to the membrane. To differentiate between these alternatives, several studies have investigated whether signals other than insulin could stimulate glucose transport in insulin resistant muscle; if so, then the insulin resistance must be caused by inadequate insulin signal transduction. Most such studies (for a review see [3]) demonstrate that glucose transport is stimulated normally by muscle contraction and hypoxia. There are also a number of studies that directly demonstrate that insulin signalling is depressed in insulin resistant muscle of obese and diabetic animals and humans. It has been demonstrated that insulin receptor autophosphorylation, IRS-1 (insulin receptor substrate 1) tyrosine phosphorylation,
as well as insulin activation of phosphatidylinositol 3-kinase and PKB/Akt (protein kinase B) were depressed in muscle of morbidly obese patients (for a review see [3]). These changes were accompanied by serine phosphorylation of the insulin receptor and IRS-1, both of which have been shown to depress the tyrosine kinase activity of the insulin receptor.

These changes in insulin signal transduction raise the question of which kinase phosphorylates the insulin receptor/IRS-1 and what activates that kinase in insulin resistant muscle. Several lines of evidence support the hypothesis that lipid accumulation in muscles of obese and diabetic individuals activates PKC (protein kinase C) which leads to serine phosphorylation and inactivation of IRS-1 and the insulin receptor. The β-isof orm of PKC is elevated in insulin resistant muscle of morbidly obese patients and lipid-treated human subjects. In obese animal models and in rats infused with lipid, the θ-isof orm of PKC is activated. These studies demonstrate an association between insulin resistance and PKC but do not demonstrate a cause and effect relationship. However, when human muscle is incubated with a PKC activator the muscle becomes less responsive to insulin and when insulin resistant human muscle is treated with a PKC inhibitor insulin sensitivity is restored. These studies support the role of PKC as an agent of insulin resistance. However, other factors, such as oxidative stress (accumulation of reactive oxygen species) and reduced fat oxidation may also play a role in causing insulin resistance.

**Fatty acids and skeletal muscle insulin resistance in obesity and type 2 diabetes**

It is now known that fatty acids contribute to insulin resistance in skeletal muscle. Whilst insulin resistance can be induced rapidly within about 4–5 h in humans, when fatty acids are infused [4], the mechanisms involved in this process are not fully understood. Traditionally, the well-known Randle (glucose–fatty acid) cycle has been widely used to explain the mechanism behind fatty acid-induced insulin resistance in skeletal muscle. However, this explanation has been questioned.

Recently, research has begun to identify other mechanisms by which fatty acids or their metabolites induce insulin resistance. These studies have begun to suggest that an increase in intramyocellular lipid accumulation and/or a reduction in fatty oxidation induce defects in the insulin signalling cascade that impairs the recruitment of GLUT4 to the cell surface (i.e. skeletal muscle insulin resistance). However, the mechanisms promoting the intramyocellular accumulation of triacylglycerols and fatty metabolites (fatty acyl-CoA, ceramides and diacylglycerol) are not clear. Such increases can be attributed to an increase in circulating fatty acid concentrations, increased rates of fatty acid transport into muscle and/or reductions in the rates of fatty acid oxidation. Below we examine how some of these processes are associated with insulin resistance in skeletal muscle. Understanding these processes provide direction
for therapeutic strategies, especially exercise intervention. This is a healthy, economic and non-pharmacological approach by which to combat insulin resistance in obesity and type 2 diabetes in modern industrialized societies.

**Fatty acids and intramyocellular lipids**

Fatty acids are stored primarily as triacylglycerols in subcutaneous and deep visceral adipose tissue. These depots are large (in a 70 kg male there is 9–15 kg adipose tissue, or 350–586 MJ) and represent approx. 95% of the total energy stores in humans. Physiologic signals (e.g. catecholamines, cortisol and reduced insulin concentrations) lead to the hydrolysis of triacylglycerol thereby releasing fatty acids into the circulation that are delivered to a number of tissues, including skeletal muscle. These blood-borne fatty acids are a primary source for skeletal muscle fatty acid oxidation, and precursors for the formation of intramyocellular fatty acyl-CoAs, diacylglycerols, ceramides and triacylglycerols.

When calculated across the entire muscle mass of the human body, the total quantity of intramuscular triacylglycerol is very small constituting only 1–2% of the triacylglycerol depot that is present in the adipose tissue mass in the human body (e.g. in a 70 kg male with 28 kg muscle the total intramyocellular triacylglycerol pool is estimated to be approx. 0.2 kg or 7.8 MJ). Within muscle, triacylglycerols are present as small lipid droplets, located adjacent to the muscle mitochondria (Figure 2), and may therefore function as a readily available fuel for oxidative metabolism, particularly during exercise. Indeed, despite earlier work to the contrary, recent research using stable isotope methodology,
H-magnetic resonance spectroscopy and electron and/or immunofluorescence microscopy, have all shown that a substantial amount of energy is liberated from the hydrolysis and subsequent oxidation of intramyocellular triacylglycerol depots in type 1 muscle fibres with endurance exercise (for a review see [5]).

Intradymocellular triacylglycerols and insulin resistance

Despite their low concentrations within the muscle cell, intramyocellular lipids, when present in excess, appear to be very significant in causing interference with insulin signalling in the muscle cell. In this manner intramyocellular lipids interfere with the recruitment of the glucose transporter, GLUT4, to the cell surface, that then results in a lowered rate of insulin-stimulated glucose transport into the muscle cell (i.e. insulin resistance) (Figure 3).

The first suggestion that intramyocellular lipids were associated with
insulin resistance in muscle resulted from work showing that increases in intramyocellular triacylglycerol were positively, but modestly, associated with the severity of insulin resistance [6]. An association between intramyocellular triacylglycerol accumulation and insulin resistance has now been confirmed in many other studies, particular those in which high fat feeding trials have been used to induce skeletal muscle insulin resistance.

There are, however, exceptions to this intramyocellular triacylglycerol-insulin resistance linkage, which seemingly produces a paradox. First, type 1 (oxidative) skeletal muscle fibres have a 3-fold higher content of intramyocellular triacylglycerols than type 2 (glycolytic) muscle fibres; yet, type 1 muscle fibres are more insulin sensitive than type 2 muscle fibres. Secondly, endurance trained athletes have higher concentrations of intramyocellular triacylglycerols than sedentary and/or diabetic individuals; yet, because of their training, these athletes are far more insulin sensitive than sedentary individuals.

However, these examples may be much less paradoxical than it appears. The greater intramuscular triacylglycerol storage in type 1 fibres or in the trained athlete allows a greater contribution of the intramuscular triacylglycerol pool as a substrate source during exercise [5], as rates of fatty acid oxidation are high in type 1 fibres or in muscles of athletes because of endurance training. In contrast, in the obese and/or type 2 diabetes patient, elevated intramuscular triacylglycerol stores seem to be secondary to increased plasma fatty acid concentrations and increased skeletal muscle fatty acid uptake. In these individuals, in whom fatty acid oxidation may be impaired, this leads to accumulation of intramuscular triacylglycerol and fatty acid intermediates (fatty acyl-CoAs, diacylglycerols and ceramides). These intermediates, rather than intramuscular triacylglycerol themselves, impair insulin signalling [7–9]. Therefore, intramyocellular triacylglycerol content is probably only a surrogate marker for dysregulated fatty acid metabolism in muscle of obese individuals and type 2 diabetes patients, whilst in trained athletes intramyocellular triacylglycerol content is not related to impaired fatty acid metabolism.

**Fatty acid oxidation and insulin resistance**

Intramyocellular triacylglycerol concentrations are not always a good marker of insulin resistance. Therefore it has been suggested that skeletal muscle oxidative capacity may provide a better relationship to insulin sensitivity. This conclusion would be consistent with the observation that (i) in type 2 diabetes patients, skeletal muscle oxidative capacity is a better predictor of insulin sensitivity than either intramyocellular triacylglycerol concentration or long-chain fatty acyl-CoA content [10], and (ii) the increase in the muscles’ oxidative capacity and the increase in insulin sensitivity are highly correlated when individuals undergo a rigorous exercise training program (reviewed in [11]).

Consistent with the suggestion that the capacity for fatty acid oxidation
may be related to insulin sensitivity, several groups have demonstrated that mitochondrial content and oxidative capacity are reduced in insulin resistant obese and diabetes patients (for a review see [3]). The recent report that mitochondrial density is lower in insulin-resistant offspring of type 2 diabetic patients suggests that reduced oxidative capacity may represent an early factor in the development of insulin resistance and type 2 diabetes [12].

Further support for the key role of impaired fatty acid oxidation in insulin resistance has come from studies in L6 muscle cells and studies using isolated muscles. In both systems palmitate induced insulin resistance by inhibiting the activation of the key insulin signalling kinase, Akt/PKB. This however was reversed when rates of fatty acid oxidation were increased either by transfecting L6 cells with CPT 1 (carnitine palmitoyltransferase 1) [13], or by stimulating fatty acid oxidation in isolated muscle by stimulating AMPK (AMP-activated protein kinase) with the exercise-mimetic agent AICAR (5-aminomimidazole-4-carboxamide-1-β-D-ribonucleoside) [14]. The rescue of insulin resistance was not accompanied by the reduction of intramuscular lipid metabolites, either in L6 cells or in isolated muscles. However, in isolated muscle the degree of insulin resistance and its rescue were highly correlated with the improved capacity for fatty acid oxidation [14].

In humans, skeletal muscle fatty acid oxidation is reduced in severely obese individuals (BMI = 54 kg/m²), but not in less severe obese individuals (BMI = 30–35 kg/m²) (for a review see [3]). In addition, morbidly obese patients have depressed fat oxidation measured at the whole body level, in muscle homogenates, and in muscle cells in culture, and these changes correlate with insulin resistance. However, when these patients lose weight after gastric bypass surgery, insulin sensitivity is restored but fatty acid oxidation remains depressed (for a review see [3]). Thus, alterations in fatty acid oxidation in the latter individuals are not obviously associated with impairment in insulin sensitivity. Therefore, other mechanisms may also be involved in fatty acid-induced insulin resistance in skeletal muscle.

**Increased fatty acid uptake and insulin resistance**

Intramuscular triacylglycerol and fatty acid intermediate accumulation may also be associated with an increase in circulating concentrations of fatty acids and mechanisms regulating their uptake. Insulin resistance and type 2 diabetes are often associated with increased fat intake and obesity. A greater fat mass increases the basal, whole-body lipolytic rate, as in the insulin resistant state adipose tissue lipolysis is less inhibited. This can result in higher circulating concentrations of fatty acids, which would result in a greater accumulation of intramuscular triacylglycerol and fatty acid intermediates. Lowering the circulating concentrations of fatty acids by inhibition of adipose tissue lipolysis, increases the oxidation of intramuscular triacylglycerol, and presumably also reduces intramuscular fatty acid intermediates, at rest
and during exercise, in overweight type 2 diabetes patients [5,15]. Reducing circulating fatty acids is an effective strategy to improve insulin sensitivity in type 2 diabetes patients [5,15,16].

**Increased fatty acid transport and insulin resistance**

Recently, it has been found that fatty acid uptake into the muscle cell occurs via a highly regulated protein mediated mechanism, involving a number of fatty acid transporters (for review see [17]). A key fatty acid transporter is FAT/CD36 (fatty acid translocase), the homologue of human CD36. Thus, an increased rate of entry of fatty acids into muscle could be yet another mechanism whereby excess fatty acids accumulate within the muscle cell.

In insulin resistant skeletal muscle in humans and animals, rates of fatty acid transport into muscle are markedly increased [18–20]. Such increased rates of fatty acid transport are already evident at an early age before type 2 diabetes is evident in diabetic fatty Zucker rats [20]. In obese humans, the increased rates of fatty acid transport into muscle are highly correlated with an increase in triacylglycerol accumulation, whereas rates of fatty acid oxidation are not increased [18]. In these studies, a permanent relocation of FAT/CD36 to the...
plasma membrane, but not an increase in FAT/CD36 expression, facilitates the increased rate of fatty acid transport into the insulin resistant skeletal muscles of obese and type 2 diabetic individuals [18]. This means that for a given level of circulating fatty acids, more will be taken up into the muscle when the plasma membrane FAT/CD36 content has been increased. This has led to a model which suggests that intramyocellular lipids accumulate in response to a greater rate of FAT/CD36-mediated fatty acid influx into the muscle cell, in the absence of any changes in fatty acid oxidation (Figure 4B). This is further supported by the inverse correlation between the plasma membrane content of the fatty acid transporter FAT/CD36 and the glucose transporter GLUT4 \( (r = -0.91) \) [20], during the transition from insulin resistance to severe type 2 diabetes in diabetic fatty Zucker rats. Thus in insulin resistant muscles, the subcellular locations of FAT/CD36 and GLUT4 are juxtaposed, with GLUT4 being retained in its intracellular depots whilst FAT/CD36 is permanently relocated to the plasma membrane. Clearly, the increased rate of fatty acid transport into the muscle cell is yet another mechanism that is associated with insulin resistance in this tissue.

It is also possible that changes in fatty acid transport across the plasma membrane are associated with changes in fatty acid oxidation, as unexpectedly FAT/CD36 is also present in the mitochondrion in rat [21] and human muscle [22,23], where it appears to interact with CPT 1. Blocking FAT/CD36 at the mitochondrion reduced fatty acid oxidation by 90%. During exercise in rats [21] and humans [23] muscle mitochondrial FAT/CD36 is increased via its translocation from an unknown intracellular depot. However, the significance of mitochondrial FAT/CD36 in insulin resistance and type 2 diabetes is not yet understood.

**Conclusion**

There is little doubt that lipids contribute to the onset of insulin resistance in muscle. Research is needed at several levels. In particular, we need to develop an understanding of the molecular mechanisms that contribute to the intramyocellular accumulation of lipid metabolites and their interference with insulin signalling. In addition, there is already sufficient knowledge to pursue important research at the applied level. Specifically, without knowing the molecular basis of action of lipid metabolites, effective exercise and dietary regimens need to be investigated, i.e. ones that limit intramyocellular lipid accumulation, either by limiting the availability of fatty acids in the diet and/or by effectively oxidizing them in muscle, as occurs when an exercise programme is maintained. These healthy, low cost, non-pharmacological strategies can provide an opportunity to prevent or even reverse insulin resistance.
Summary

• Intramyocellular triacylglycerol depots are modestly correlated with insulin resistance, but are unlikely to be the direct cause of insulin resistance.

• Accumulation of lipid metabolites (fatty acyl-CoAs, diacylglycerols and ceramides) within the muscle contribute to insulin resistance by interfering with the insulin activation of the signalling pathway involved in recruiting GLUT4 to the cell surface.

• It has been hypothesized that the fatty acid metabolites (fatty acyl-CoAs, diacylglycerols and ceramides) activate protein kinase C, which then leads to serine phosphorylation and inactivation of IRS-1. The latter is regarded as a molecular key event leading to insulin resistance as it prevents tyrosine phosphorylation and activation of IRS-1.

• Reduced rates of fatty acid oxidation by muscle may be a better indicator of insulin resistance than intramyocellular lipid accumulation.

• Reduced rates of fatty acid oxidation have been related to insulin resistance in severely obese individuals (BMI > 35 kg/m²), since this probably leads to the accumulation of intramyocellular lipid metabolites.

• Increased rates of fatty acid transport into muscle, due to the increased presence of a fatty acid transport protein (FAT/CD36) at the cell surface, is another mechanism whereby muscle can accumulate excess intramyocellular lipids.

• The imbalance between plasma fatty acids, skeletal muscle fatty acid uptake, storage and oxidation collectively contribute to the development of skeletal muscle insulin resistance. Therefore, limiting fatty acid accumulation in muscle, as occurs with regular exercise, is an effective strategy to maintain insulin sensitivity.

References


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