
MINIREVIEW

Glucose-Fatty Acid Interaction in Skeletal Muscle and Adipose Tissue in Insulin Resistance

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Summary

Insulin resistance (IR) is the result of long-lasting positive energy balance and the imbalance between the uptake of energy rich substrates (glucose, lipids) and energy output. The defects in the metabolism of glucose in IR and type 2 diabetes are closely associated with the disturbances in the metabolism of lipids. In this review, we have summarized the evidence indicating that one of the important mechanisms underlying the development of IR is the impaired ability of skeletal muscle to oxidize fatty acids as a consequence of elevated glucose oxidation in the situation of hyperglycemia and hyperinsulinemia and the impaired ability to switch easily between glucose and fat oxidation in response to homeostatic signals. The decreased fat oxidation results into the accumulation of intermediates of fatty acid metabolism that are supposed to interfere with the insulin signaling cascade and in consequence negatively influence the glucose utilization. Pathologically elevated fatty acid concentration in serum is now accepted as an important risk factor leading to IR. Adipose tissue plays a crucial role in the regulation of fatty acid homeostasis. The adipose tissue may be the primary site where the early metabolic disturbances leading to the development of IR take place and the development of IR in other tissues follows. In this review we present recent evidence of mutual interaction between skeletal muscle and adipose tissue in the establishment of IR and type 2 diabetes.

Key words

Insulin resistance • Fatty acid • Adipose tissue • Skeletal muscle • Glucose-fatty acid cycle

Introduction

Insulin resistance is generally taken to mean a reduced ability of insulin to stimulate glucose transport and utilization. This metabolic abnormality, associated with dyslipidemia, hypertension and hyperinsulinemia, is characteristic for the “Insulin Resistance Syndrome” and often leads to the development of type 2 diabetes. The

mechanisms underlying the development of insulin resistance are undoubtedly numerous. There is growing body of evidence indicating that net positive energy balance and disordered fat storage and mobilization are central factors in the pathogenesis of this metabolic disorder (Lewis *et al.* 2002). Beside this, insulin resistance may be induced by other metabolic disorders, i.e. imbalance of several hormones like catecholamines

(Reaven 1996), glucocorticoids (Ward *et al.* 2003) or growth hormone (Rizza *et al.* 1982, Hansen *et al.* 1986). Mutation of some members of insulin signaling cascade (insulin receptor, insulin receptor-substrate) also leads to the manifestation of insulin resistance. Nevertheless, such mutation constitutes a very rare cause of type 2 diabetes (Kahn 1998).

Estimation of whole-body insulin sensitivity and action with euglycemic clamp technique is mainly a reflection of the glucose disposal by the muscles (60-70 %) (DeFronzo *et al.* 1992) while adipose tissue only accounts for about 10 % of whole-body insulin-stimulated glucose uptake (Smith 2002). This fact has led to the extrapolation that whole body insulin resistance not only occurs in muscles, but also starts there. Nevertheless, the high correlation between disturbances in normal metabolism of adipose tissue (obesity, lipodystrophy) and the manifestation of insulin resistance indicates the important role of adipose tissue in its etiology (Kahn and Flier 2000). Recent findings have shown that insulin resistance and impaired insulin action occur early in adipose tissue (in fact, long before glucose intolerance develops) and adipose tissue is now recognized as the primary site of its origin (Hotamisligil 2000). Over the last decade a large number of factors with diverse functions secreted by adipose tissue has been identified and adipose tissue is now considered to be an endocrine organ (Ahima and Flier 2000). These factors include a variety of proteins termed adipocytokines either with insulin sensitizing effect (adiponectin, leptin) or promoting insulin resistance (TNF α , resistin etc), that act in an autocrine, paracrine or endocrine fashion to control various metabolic functions (Pittas *et al.* 2004). The detailed listing is beyond the scope of this review (for review see Mattison and Jensen 2003, Fasshauer and Paschke 2003). Adipose tissue has traditionally been considered an energy storage organ that accumulates the excess of energy in the form of triglycerides and releases it in the form of FFA during energy shortage. FFA serve not only as highly energetic fuel but also as important signaling molecules and their availability is an important factor influencing glucose utilization in muscles.

These findings suggest that altered lipid metabolism might directly contribute to the onset of insulin resistance, particularly in the muscle and liver, leading to compensatory hyperinsulinemia which is believed to exacerbate the condition. In this review we will concentrate on the role of free fatty acids in the pathogenesis of insulin resistance.

The association of elevated FFA serum levels and the development of insulin resistance

Fatty acids are an important source of energy for many tissues, including skeletal muscle, which is the main site of the manifestation of insulin resistance. On the other hand, as surfactants they are toxic substances and even when bound to protein carrier their elevated concentration in the serum is considered to be one of the important risk factors for the development of insulin resistance and type 2 diabetes (Shafir and Raz 2003). The elevated free fatty acid (FFA) serum levels were demonstrated in the serum of subjects at high risk of type 2 diabetes (obesity, lipodystrophy, autonomous nervous system dysfunction) or in some experimental models of insulin resistance induced by diet manipulation (high-sucrose feeding). The acute elevation of fatty acid levels in healthy volunteers, which can be generated by intralipid/heparin infusion, can also result in whole body and muscle insulin resistance. According to very short half-life of FFA in the serum (about 2 min) it is possible to expect that FFA are quickly internalized and their increased intracellular availability may lead to changes in metabolism of glucose at the level of substrate competition, enzyme regulation, intracellular signaling and/or gene transcription that account for the insulin resistance seen in states such as metabolic syndrome and type 2 diabetes (Kraegen *et al.* 2001).

Free fatty acids and glucose uptake in muscle

The first evidence that cardiac and skeletal muscles possess mechanisms that allow these tissues to shift readily back and forth between carbohydrate and fat as oxidative energy sources depending primarily on the availability of FFA was obtained by Randle *et al.* (1963). Based on their original experiments showing that the increased availability of FFAs decreased carbohydrate uptake and increase glucoso-6-phosphate in isolated perfused rat heart and hemidiaphragm Randle and colleagues formulated a theory that became known as glucose-fatty acid cycle or Randle cycle. The key points of this model were: increased availability of FFAs in the circulation produces an increase in intramuscular acetyl-CoA and citrate content, which results in the inhibition of pyruvate dehydrogenase and phosphofructokinase, and this in turn reduces glucose oxidation and glycolysis. Subsequent increase in intracellular glucose-6-phosphate concentration would inhibit hexokinase II activity, which

would result into an increase of intracellular glucose concentration and a decrease of muscle glucose uptake.

Originally, the glucose-fatty acid cycle was developed to explain the abnormalities in carbohydrate metabolism via higher fatty acid availability associated with diabetes mellitus, starvation and carbohydrate deprivation. Later, when it was found that insulin resistance is almost always associated with obesity and type 2 diabetes and these metabolic states are also characterized by elevated levels of free fatty acids (Schalch and Kipnis 1965), the concept of glucose-fatty acid cycle was extended to insulin-resistant states. The effort of many investigators was concentrated on the question whether increased fat oxidation could be responsible for the development of insulin resistance and type 2 diabetes.

The development of the method of euglycemic-hyperinsulinemic clamping brought the possibility to study how several hour infusion of intralipid/heparin in healthy volunteers leading to the elevation of systemic FFAs alters insulin-stimulated glucose metabolism. More recently the glucose fatty acid interactions were re-examined using whole body indirect calorimetry (to determine oxidative and non-oxidative glucose utilization) in combination with hyperinsulinemic clamping (to determine insulin sensitivity). Some of the groups showed that raising plasma FFA concentrations increased fat oxidation and inhibited both carbohydrate oxidation and glucose uptake, as would be predicted by the glucose fatty acid cycle (Thiebaud *et al.* 1982, Felley *et al.* 1989). In contrast, other groups found an inhibitory effect of fatty acids on glucose oxidation but not on glucose uptake (Bevilacqua *et al.* 1987, Bonadonna 1989). The explanation of this apparent contradiction was provided in the study of Boden *et al.* (1991) who reported that in healthy volunteers the fatty acid-mediated inhibition of insulin-stimulated glucose uptake develop only after 4 h of fat infusion. Thus insufficient time of lipid plus insulin infusion (2 h) was the most likely reason why the inhibitory effect of fatty acids on glucose uptake was not found in some studies (Boden 1997). Boden *et al.* (1991) also showed that lipid infusion produced insulin resistance in glucose disposal only several hours after it had already decreased glucose oxidation which suggested that the glucose fatty acid cycle might not be responsible for insulin resistance. In accordance with this are the results of Kelley *et al.* (1993) who found that lipid infusion produced a decrease of insulin-stimulated glucose oxidation, but there was a

greater decrease in glycogen synthesis. This result would not have been predicted by the mechanism used to explain the glucose-fatty acid cycle.

The NMR spectroscopy opened a new methodological approach. The group of Shulman (2000) used ^{13}C and ^{31}P NMR spectroscopy to measure muscle glycogen content and glucose-6-phosphate concentration in leg muscle of healthy subjects who were maintained under euglycemic hyperinsulinemic conditions with either low (0.2 mmol/l) or high (2 mmol/l) plasma fatty acid levels (Roden *et al.* 1996). Increasing of plasma fatty acid concentration to about 2 mmol/l for 5 h caused a reduction of approximately 50 % in insulin stimulated rates of leg muscle glycogen synthesis and the similar reduction of whole body glucose oxidation together with a decrease in intracellular glucoso-6-phosphate concentration compared to controls maintained at low plasma FFA levels. In contrast to the changes predicted by Randle cycle, which would expect that fat-induced insulin resistance would result in an increase of intramuscular glucoso-6-phosphate, Shulman and coworkers found that the drop in muscle glycogen synthesis was preceded by a fall in intramuscular glucoso-6-phosphate. These data suggest that increases in plasma fatty acid concentration initially induce insulin resistance by inhibiting glucose transport (indicated by glucoso-6-phosphate concentration) and that the reduction in muscle glycogen synthesis and glucose oxidation follows (Shulman 2000). The reduction in insulin-activated glucose transport and phosphorylation activity in normal subjects reached under experimental condition (i.e. by maintenance of healthy volunteers at high plasma fatty acid levels by intralipid/heparin infusion) is similar to that seen in obese individuals (Petersen *et al.* 1998), patients with type 2 diabetes (Rothman *et al.* 1992) and lean normoglycemic insulin-resistant offspring of type 2 diabetic individuals (Rothman *et al.* 1995).

Glucose-fatty acid cycle in reverse

Since the introduction of “glucose-fatty acid cycle” concept several investigators focused their attention on the reverse process, i.e. on the effect of glucose availability and oxidation on fatty acid oxidation. Several studies showed that when glucose availability increases, the fat oxidation decreases (Wolfe and Peters 1987, Wolfe *et al.* 1988). The experimental design employed in these experiments did not exclude a

possibility that this response is mediated, at least in part, by insulin-induced inhibition of lipolysis, resulting in the decreased fatty acid availability. However, Sidossis *et al.* (1996) showed that infusion of glucose plus insulin decreases plasma fatty acid oxidation and increases carbohydrate oxidation even when fatty acid availability is maintained constant by infusion of exogenous lipids. This finding suggests that accelerated carbohydrate oxidation directly inhibits fatty acid oxidation by mechanism other than inhibition of lipolysis.

The results of Kelley and Mandarino (1990) who found, using leg balance technique, that glucose oxidation was increased in leg muscle of type 2 diabetic subjects studied postabsorptively under the conditions of fasting hyperglycemia were consistent with these findings. Furthermore, when hyperglycemia was reduced to normal levels by insulin infusion designed to suppress hepatic glucose output in subjects with type 2 diabetes, leg glucose oxidation decreased and fat oxidation increased. The increased glucose oxidation in skeletal muscle was found in obese insulin-resistant rats (Torgan *et al.* 1990, Cortez *et al.* 1991) and in skeletal muscle of insulin-resistant non-obese hereditary hypertriglyceridemic (HHTg) rats incubated *in vitro* in the presence of 1 mmol/l palmitate (Cahová *et al.* 2004a,b). The alterations in fatty acid metabolism in insulin-resistant muscle were demonstrated also during fasting. Blaak *et al.* (2000) demonstrated, using forearm balance technique and indirect calorimetry, that FFA uptake and oxidation in muscle are impaired in the forearm muscles of type 2 diabetic subjects during fasting and after isoproterenol stimulation. The failure to augment lipid oxidation during fasting conditions may be one of the mechanisms leading to the accumulation of lipid metabolism intermediates which may in turn contribute to the disturbances in glucose metabolism seen in insulin resistance.

Metabolic inflexibility of fatty acid utilization in insulin resistance

In lean, healthy individuals, skeletal muscle displays substantial metabolic flexibility, with the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions (Andres *et al.* 1956) to the suppression of lipid oxidation and increased glucose uptake, oxidation and storage under insulin-stimulated conditions (Kelley *et al.* 1990). Ukropcova *et al.* (2005) found that the capacity of skeletal muscle to oxidize fat under appropriate

conditions is related to leanness, aerobic fitness and insulin sensitivity. It has been proposed that the impaired ability to increase the dependence upon fat oxidation is related to the pathogenesis of insulin resistance in skeletal muscle (Kelley and Mandarino 2000, Storlien *et al.* 2004). In order to evaluate whether impaired muscle capacity to increase fat oxidation is related to the pathogenesis of insulin resistance, it is necessary to explain apparently contradictory observation described above that insulin resistance in muscle can be induced by elevated plasma fatty acid levels. On the other hand, there is also a convincing set of evidence that insulin-resistant skeletal muscle can manifest reduced efficiency of fat oxidation during fasting metabolism despite elevated levels of plasma FFA (Blaak *et al.* 2000). Kelley and Mandarino (2000) described the concept of “metabolic inflexibility” as one of the characteristic features of insulin-resistant skeletal muscle. Metabolically healthy skeletal muscle is characterized by the ability to switch easily between glucose and fat oxidation in response to homeostatic signals. The skeletal muscle of individuals with type 2 diabetes and obesity shows a great reduction of metabolic flexibility. This includes the lower reliance on fat and a greater reliance on glucose oxidation during fasting and lower stimulation of glucose oxidation and impaired suppression of fat oxidation by insulin (Kelley 2005).

The malonyl-CoA as a fuel sensing and signaling mechanism

One possible mechanism that explains the diminished oxidation of fatty acids during hyperglycemia and hyperinsulinemia is based on the role of malonyl-CoA as a link between fuel metabolism and signal transduction in skeletal muscle. The predominant function of malonyl-CoA in skeletal muscle is probably allosteric inhibition of the activity of carnitine palmitoyl transferase I (CPT I). CPT I is essential enzyme enabling the FFA entry into mitochondria and thus their oxidation. The intracellular concentration of malonyl-CoA in muscle is acutely (in minutes) regulated according to the metabolic state of the cell. In early studies McGarry *et al.* (1983) showed that the concentration of malonyl CoA in skeletal muscle is diminished by 80 % after 48 h starvation, in keeping with the increased need for fatty acid oxidation in the fasting state. Saha *et al.* (1995) showed that the concentration of malonyl-CoA increased several times in rat soleus muscle when incubated in the

presence of glucose and insulin within 20 min and within 6 h when the muscle was inactivated by denervation (i.e. in the situations when the need for fatty acid oxidation is decreased). The rapid response of malonyl-CoA concentration to changes in fuel supply or energy expenditure of the muscle cells has been referred to as the malonyl-CoA fuel sensing and signaling mechanism (Ruderman *et al.* 1999).

The existence of malonyl-CoA fuel sensing and signaling mechanism in humans is suggested by the observation of Bavenholm *et al.* (1998) that the concentration of malonyl-CoA increased in human leg muscle during an euglycemic hyperinsulinemic clamp and whole body fatty acid oxidation was markedly diminished in evaluated subjects. In the above mentioned study Sidossis *et al.* (1996) reported that decreases in fatty acid oxidation in humans undergoing concomitantly euglycemic-hyperinsulinemic clamp and intralipid infusion are accompanied by the decrease in the oxidation of long-chain fatty acylcarnitine in muscle suggesting inhibition of CPT I. The authors explain their findings by the increase of malonyl-CoA concentration in the situation of accelerated glucose metabolism.

A number of findings suggest an association between sustained elevations in the concentration of malonyl-CoA and insulin resistance in skeletal muscle. The high levels of malonyl-CoA were found in muscle of several animal models of insulin resistance (hyperglycemic and/or hyperinsulinemic) including the *fa/fa* rat (Saha *et al.* 1997), KKA^y mouse (Saha *et al.* 1994) and rats infused with glucose for 1-4 days (Laybutt *et al.* 1997). The increased levels of malonyl-CoA were also proved in rat muscle in which the insulin resistance was induced by denervation (Saha *et al.* 1994). On the other hand, the only increase of intracellular concentration of malonyl CoA is not sufficient explanation for the development of insulin resistance. The elevation of intracellular malonyl CoA was not proved in models of high-fat diet induced insulin resistance (Ruderman *et al.* 1999).

Fatty acid metabolites and insulin action in muscle

As discussed in previous sections, there is growing body of evidence that the increased oxidation of fatty acids is not the primary cause of insulin resistance. On the contrary, the perturbations in lipid metabolism that result in accumulation of intracellular fatty acid

metabolites in muscle cell, either through increased delivery or decreased metabolism, induce insulin resistance. The first step in the metabolism of fatty acids in any tissue is the activation of the fatty acid to its long chain acyl coenzyme A (LC-AcCoA) derivative. Then LC-AcCoAs can be transported into the mitochondria *via* the action CPT I and oxidized in the beta-oxidation pathway. Alternatively, the LC-AcCoAs may be esterified to mono- and diglycerides and stored as triglycerides or incorporated into phospholipids in membranes (Faergeman and Knudsen 1997). The concentration of LC-AcCoAs in muscle is increased by fasting (Carroll *et al.* 1983), decreased by insulin (Oakes *et al.* 1997a,b) and is therefore an indicator of the extent of lipid metabolism in a tissue (Kraegen *et al.* 2001).

The common feature of various situations leading to the development of insulin resistance is the increased cytosolic concentration of LC-AcCoAs, which may represent a link between fatty acid metabolites and insulin action in muscle. LC-AcCoA levels are increased in muscle from fat-fed insulin-resistant rats and their concentration was not significantly reduced by 2 h insulin infusion during hyperinsulinemic euglycemic clamp (Oakes *et al.* 1997a). Another study (Oakes *et al.* 1997b) showed that insulin resistance was ameliorated by diet or exercise and that this effect was closely related to the ability of insulin to suppress the intracellular LC-AcCoA content. Insulin resistance generated by chronic infusion of glucose is accompanied by accumulation of LC-AcCoAs in muscle (Saha *et al.* 1995). The LC-AcCoA levels are elevated in muscle of rats fed high sucrose diet for two weeks (Cahová *et al.*, unpublished results). Based on these findings we can conclude that intracellular LC-AcCoA content increases as the result of various mechanisms all of them leading to the development of insulin resistance. The intracellular LC-AcCoA content may increase as the consequence of their increased availability in circulation (e.g. after high-fat diet) or as the consequence of an increased glucose supply followed by the rise of intracellular malonyl-CoA content and the inhibition of fatty acid entry into the mitochondria. It has been hypothesized that the elevated intracellular LC-AcCoA levels negatively influence the metabolism of glucose, but their precise role has not yet been fully elucidated. LC-AcCoA as a signaling molecule may influence a variety of cellular processes and may act *via* different mechanisms. Thompson and Cooney (2000) showed that LC-AcCoA inhibited the activity of hexokinase isolated from rat and human soleus muscle

in vitro. Hexokinase is the first enzyme of intracellular glucose metabolism and its inhibition may significantly reduce the glucose transport into the cell.

Another possible target of LC-AcCoA represents protein kinase C the role of which in the insulin signaling is now extensively studied. According to this hypothesis the increased content of intracellular fatty acid metabolites (like LC-AcCoA but also diacylglycerol or ceramides) activates a serine/threonine kinase cascade possibly initiated by protein kinase theta. This series of events leads to the phosphorylation of serine/threonine sites on insulin receptor substrates. Serine-phosphorylated forms of these proteins fail to activate PI 3-kinase, resulting in decreased activation of glucose transport and other downstream events. If this hypothesis is correct, any perturbations that lead to the accumulation of fatty acid metabolites might be expected to result in insulin resistance (Shulman 2000, Hegarty *et al.* 2003).

Chicco *et al.* (1999) investigated the mutual dependence of the development of insulin resistance and the content of intracellular LC-AcCoA and triglycerides during long-term (30 weeks) feeding of high sucrose diet in Wistar rats. The content of LC-AcCoA increased after the first three weeks of high sucrose feeding in gastrocnemius muscle, but this rise was not accompanied by any changes in the activity of pyruvate dehydrogenase or glycogen synthase measured in the same tissue. The glucose infusion rate measured during hyperinsulinemic-euglycemic clamp was at this time slightly, but significantly decreased. The increased amount of LC-AcCoA in the muscle cells was in this experiment rather an early indicator of the disturbances of lipid metabolism than the direct cause leading to the impairment of insulin sensitivity. Thompson and Cooney (2000) showed during the *in vitro* experiments that the glucose uptake in soleus muscle is decreased after four hours incubation in the presence of various fatty acids and that this decrease correlates with the intracellular LC-AcCoA content. In contrast, no correlation between LC-AcCoA content and the glycogen synthesis was found in this study. Therefore, it is likely that lipids regulate glycogen synthesis and glucose transport *via* different mechanisms. Similar results were also obtained by our group. The two weeks of high sucrose feeding led to a significant increase in the LC-AcCoA and triglyceride content in soleus muscle, but had no effect on glycogen synthesis *in vitro*. Interestingly, the glycogen synthesis was significantly decreased after the addition of palmitate into incubation medium (Cahová *et al.*,

unpublished results). In the study of Poynten *et al.* (2003) two weeks of administration of nicotinic acid induced insulin resistance in humans manifested as the lowering of glucose infusion rate during euglycemic hyperinsulinemic clamp. Induction of insulin resistance with nicotinic acid was related to elevated circulating fatty acids, decreased fat oxidation and decreased non-oxidative glucose disposal but not to increased intramuscular lipid content or LC-AcCoA content.

Interesting results challenging the "lipotoxic" model of skeletal muscle insulin resistance were reported by Perdomo *et al.* (2004). Their studies demonstrated that overexpression of CPT I in L6 myotubes increases the rate of fatty acid oxidation and enhances insulin stimulation of glucose disposal. When the cells were preincubated with palmitate, CPT I overexpression ameliorated the negative effects of fatty acids on insulin-stimulated glycogen synthesis, glucose uptake and phosphorylation of protein kinase B. These effects of CPT I overexpression were independent of changes in intracellular triacylglycerol, diacylglycerol, ceramide, or LC-AcCoA content. These data suggest that at this model the beneficial effect of β -oxidation on insulin sensitivity was mediated by a mechanism independent of lipid accumulation.

Taken together, these data indicate that the role of LC-AcCoA and other intermediates of lipid metabolism in the pathophysiology of insulin resistance have not yet been fully elucidated and further experimental work is needed. The potential interactions between glucose and lipid metabolism in skeletal muscle in insulin resistance are summarized in Figure 1.

The role of adipose tissue in the development of insulin resistance

The skeletal muscle is the main tissue responsible for the insulin-stimulated glucose disposal and it was also considered to be the most important site where pathological mechanisms leading to the development of insulin resistance originate. Nevertheless, there is strong evidence from animal models indicating that this extrapolation is not fully justified. As reported by Abel *et al.* (2001) the animals with adipose-specific GLUT4 gene deletion developed whole body insulin resistance what implicated the important role of the adipose tissue for whole-body glucose disposal. Another interesting finding in the animal models is that muscle GLUT4 depletion is associated with a marked increase in

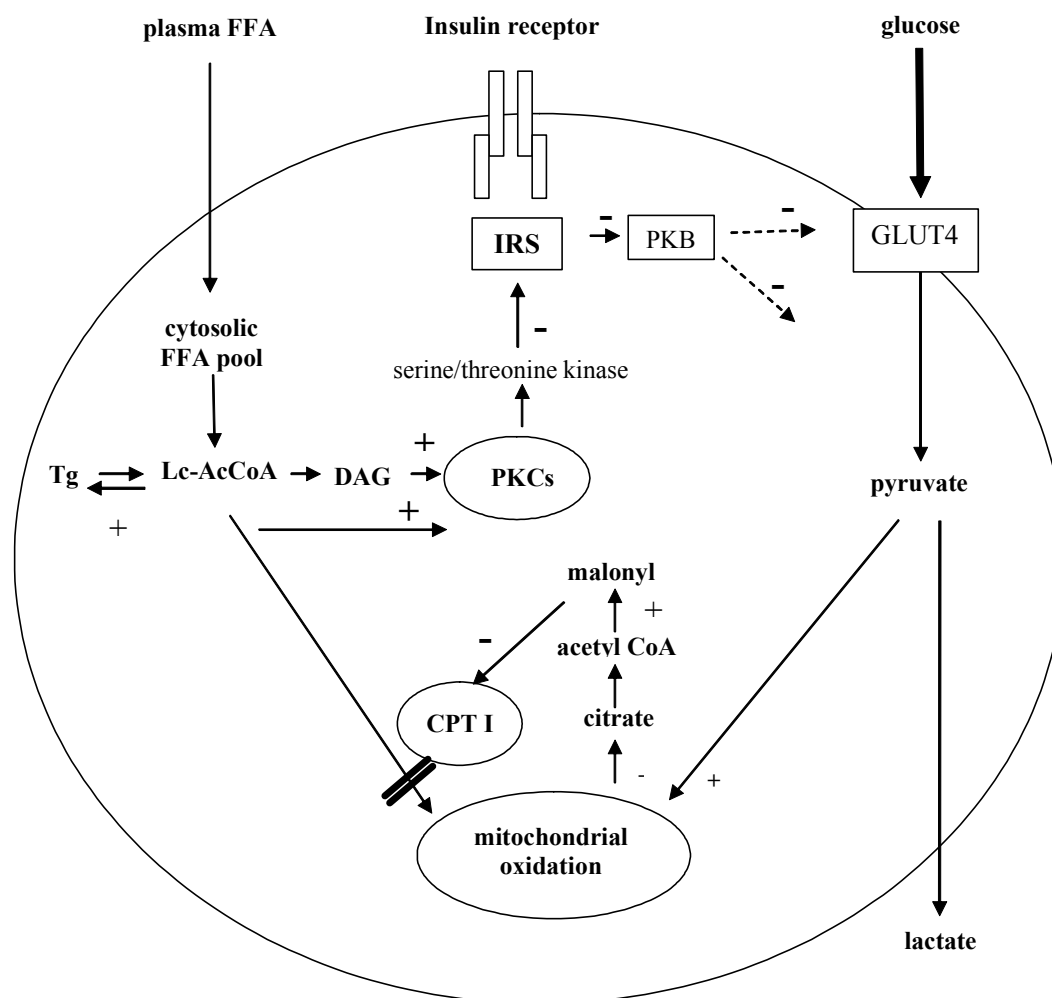


Fig. 1. Potential interactions between lipid and glucose metabolism in skeletal muscle in insulin resistance. When glucose for oxidation is present in excess of immediate needs of the cell, the mitochondrial and subsequently cytosolic concentration of citrate increases. Citrate serve as a source of cytosolic acetyl CoA which is converted by carboxylation to malonyl CoA. Malonyl CoA inhibits CPT I and prevents further entry of LC-AcCoA into mitochondria for oxidation what results in their accumulation in the cytosol. The lipid metabolism intermediates may activate PKC and further members of serine/threonine kinase cascade leading to phosphorylation of serine and threonine sites on insulin receptor and insulin receptor substrates. Serine phosphorylated forms of these proteins fail to activate downstream members of insulin signaling cascade, resulting in decreased activation of glucose transport and other downstream events (e.g. glycogen synthesis). + potential activators, - potential inhibitors.

glucose uptake by the fat and with the expanded adipose tissue mass (Kim *et al.* 2000). This cross-talk between tissues supports the possibility that insulin resistance may be initiated in one tissue and followed by a series of events in other tissues (Smith 2002).

The impaired tyrosine phosphorylation of IRS-1 associated with approx. 50 % reduction in PI-3 kinase activity was identified in skeletal muscle of diabetic and insulin-resistant subjects. The insulin-stimulated translocation of GLUT4 transporter is impaired in skeletal muscle of type 2 diabetic subjects (Zierath *et al.* 1996), but the total GLUT4 protein content and mRNA specific expression was not different from normal muscle cells (Garvey *et al.* 1992). As the consequence, insulin-

stimulated glucose transport is reduced in skeletal muscle from type 2 diabetic subjects. Interestingly, the recent *in vitro* experiments showed that this phenomenon is reversible in skeletal muscle. In the above mentioned work of Abel *et al.* (2001) muscles from insulin-resistant mouse restored normal insulin responsiveness after several hours of incubation in medium with normal glucose concentration (5 mmol/l). Similarly, Zierath *et al.* (1994) reported that preincubation of the human diabetic muscle strips for two hours at a physiological glucose concentration normalizes the insulin response and on the contrary, preincubation of the tissue biopsies from normal subjects for only 2 h at high glucose concentration impairs the effect of insulin.

The situation is quite different in adipose tissue. Adipocytes from type 2 diabetic subjects also have a marked reduction in the insulin-stimulated IRS-1 phosphorylation, which is mainly due to 70 % reduction in IRS-1 protein expression (Rondinone *et al.* 1997). Both PI-3 kinase activation and the downstream activation of PKB/Akt are significantly impaired in adipocytes from type 2 diabetics (Carvalho *et al.* 2000). In contrast to muscle cells, preincubation of the human fat cells for 16 h in physiological (5.6 mmol/l) or high (16.8 and 25 mmol/l) glucose concentration does not impair the acute insulin stimulatory effect on glucose uptake nor does preincubation of diabetic adipocytes at a physiological glucose concentration restore the acute insulin response after 6 h (Smith 2002). In contrast to skeletal muscle a marked reduction of both GLUT4 mRNA and protein was found in adipocytes (Rondinone *et al.* 1997).

All these data indicate that there are basic differences in insulin signaling and protein and gene expression between two major insulin-sensitive tissues. It can be speculated that the adipose tissue may initiate and/or be the initial target organ for insulin where insulin resistance develops. This could then lead to a series of events (such as increased FFA or various adipocytokine production) whereby insulin resistance is induced or augmented in muscle and liver (Smith 2002).

The role of adipose tissue in the regulation of FFA serum concentration

Adipose tissue is a major reservoir of energy stored as triglycerides which can be quickly released in the form of fatty acids. The net effect of lipolysis (i.e. production of fatty acids) depends on the whole complex of processes on the lipid droplet surface. The hydrolysis of stored triglycerides is regulated primarily by hormones like catecholamines *via* cAMP-dependent protein kinases. Nevertheless, recent results indicate the existence of additional regulatory mechanisms. Perilipins coat the lipid droplets of adipocytes and are thought to have an important role in the regulation of triglyceride hydrolysis. Perilipins are believed to inhibit the actions of lipases on intracellular lipids perhaps by acting as barriers to lipase access. Upon catecholamine stimulation, perilipins are hyperphosphorylated by protein kinase A and this phosphorylation has been suggested to facilitate lipase access to the lipid droplets (Londos *et al.* 2005). The perilipin knockout mouse exhibits reduced adipose tissue

mass, elevated basal lipolysis and impaired catecholamine-stimulated lipolysis. These animals also show increased tendency to develop glucose intolerance and peripheral insulin resistance (Tansey *et al.* 2001). Another important factor determining the intensity of lipolysis is the effectivity of removing of released fatty acids from the reactive center of hormone-sensitive lipase that depends predominantly on the capacity of fatty acid transporter proteins. The amount of fatty acids finally released into the circulation is determined by the reesterification of released fatty acids, i.e. on the availability of glucose.

Another important function of adipose tissue is its role in buffering the fatty acid fluxes both in postprandial period and during fasting. If this buffering function is impaired, then other tissues like liver and muscle are exposed to excessive fluxes of lipid fuels which may lead to the impairment of normal regulation mechanisms (Frayn 1998). Unlike the situation in any other tissue, fatty acid movement across the adipocyte membrane is bidirectional.

The role of adipose tissue in postprandial phase

In the postprandial state hormone sensitive lipase (HSL) is suppressed and the pathway of fatty acid esterification is stimulated, the FFAs released by lipoprotein lipase (LPL) from VLDL and chylomicrons are “trapped” in the form of triglycerides in adipose tissue. From this point of view, adipose tissue is a dynamic tissue with a very active pattern of metabolism. It plays a crucial role in buffering the flux of fatty acids in the circulation in the postprandial period (Frayn 2002). The rate of fatty acid uptake and esterification is highly controlled. It is stimulated by insulin, as was demonstrated both *in vivo* (Frayn *et al.* 1994) and *in vitro* (Dyck *et al.* 2001). In recent years another regulator of this pathway has been identified – acylation-stimulating protein (ASP) secreted by adipocytes into circulation. It is produced by two-step process involving three proteins of the alternate complement system C3, factor B and adipsin, all of which are synthesized and secreted by adipocytes (Cianflone *et al.* 2003). Its production is stimulated by the presence of chylomicrons (Maslowska *et al.* 1997). As was recently shown ASP production in adipose tissue *in vivo* is temporally correlated with the uptake of fatty acids (Saleh *et al.* 1998). This protein may be involved in the entrapment of fatty acids released by

Table 1. The effect of wortmannin on adrenaline- and methoxamine-stimulated lipolysis and glucose utilization in adipose tissue of high sucrose diet fed rats *in vitro*.

	Lipolysis		Glucose utilization	
	Glycerol ($\mu\text{mol/g w.w.}$)	FFA ($\mu\text{mol/g w.w.}$)	Lactate production $\mu\text{mol/g v.v.}$	Incorporation into lipids ($\mu\text{mol/g w.w.}$)
<i>Basal</i>	3.7 ± 0.3	1.6 ± 0.07	7.3 ± 0.5	1.5 ± 0.2
<i>Basal + wortmannin</i>	3.2 ± 0.5	$2.4 \pm 0.18^*$	$3.1 \pm 0.3^*$	1.0 ± 0.1
<i>Adrenaline</i>	$11.8 \pm 2.2^\#$	$9.1 \pm 1.5^\#$	6.1 ± 0.7	$3.3 \pm 0.2^\#$
<i>Adrenaline + wortmannin</i>	12.2 ± 1.8	$18.6 \pm 2.6^*$	$4.8 \pm 0.3^*$	$1.8 \pm 0.15^*$
<i>Methoxamine</i>	2.9 ± 0.6		6.3 ± 1.1	1.9 ± 0.1
<i>Methoxamine + wortmannin</i>	3.1 ± 0.5		$4.0 \pm 0.4^*$	$1.1 \pm 0.05^*$

Data are means \pm S.E.M., $n = 8-10$. * Significant effect ($p < 0.05$) of wortmannin, # significantly different ($p < 0.05$) from basal values.

LPL and their transport to the surface of adipocytes.

The necessary condition determining the effective buffering function of adipose tissue is rapid alterations in adipose tissue metabolism. The adipose tissue in lean individuals can switch from a negative to a positive FFA balance during the transition from fasting to the postprandial state. In insulin-resistant obese individuals the adipose tissue FFA balance remains negative postprandially (Frayn *et al.* 1996).

This observation is also supported by the results of our group comparing the response of isolated adipose tissue to previous glucose load *in vivo*. While the adipose tissue of normal rats increased the glucose uptake within 30 min after glucose load and transformed glucose into fatty acids through *de novo* fatty acid synthesis, the adipose tissue of non-obese HHTg rats did not respond to increased supply of glucose at all (Cahová *et al.* 2004a,b). There are many findings that several functions of adipose tissue necessary for effective buffering are impaired in insulin resistance. The impaired ability of insulin to suppress HSL has been repeatedly reported (Chen *et al.* 1987, Groop *et al.* 1989). Similarly, the ability of insulin to up-regulate LPL activity and hence triacylglycerol clearance is impaired in obesity, a frequent risk factor for type 2 diabetes (Ong and Kern 1989, Coppack *et al.* 1992). *In vitro*, the defect of the ability of adipose tissue to increase fatty acid uptake in coordination with their increasing concentration was found in non-obese animal model of insulin resistance – HHTg rats (Cahová *et al.* 2004a,b). The inefficient trapping of fatty acids in adipose tissue in the postprandial period leads to the inappropriately elevated FFA concentrations in the postprandial period accompanied by various pathological

consequences resulting in the insulin resistance.

The role of adipose tissue in fasting

In the fasting, the main function of adipose tissue is to supply energy in the form of FFAs which are predominantly released from intracellular triglycerides by HSL. There is a large outward flow of FFAs from the cells to the circulation. Nevertheless, a continuous cycle of fatty acid release by hydrolysis of endogenous triglycerides and their reesterification occurs in the adipose tissue. The intensity of both processes depends on the acute metabolic needs of the organism. Adrenaline, besides stimulation of HSL, also stimulates glucose uptake into adipocytes. The positive effect of adrenaline on glucose uptake in adipose tissue was known since early sixties (Mosinger 1968), but the exact biological significance and mechanism were poorly understood. From this point of view there is a profound difference between the effect of adrenaline on skeletal muscle and adipose tissue as in muscle adrenaline strictly inhibits the glucose entry into the cell. The results of our group indicate that the glucose uptake and reesterification of released fatty acids after adrenaline stimulation is impaired at least in non-obese model of insulin resistance, hereditary hypertriglyceridemic rats. The difference is accentuated after short-term high sucrose feeding. While in controls adrenaline increased glucose incorporation into glycerol of triglyceride molecule by 70 % and FFA reesterification by 200 %, it had no effect in HHTg rats. In consequence, the greater portion of FFAs released by lipolysis is exported from adipose tissue into the medium compared with controls due to the lower glucose

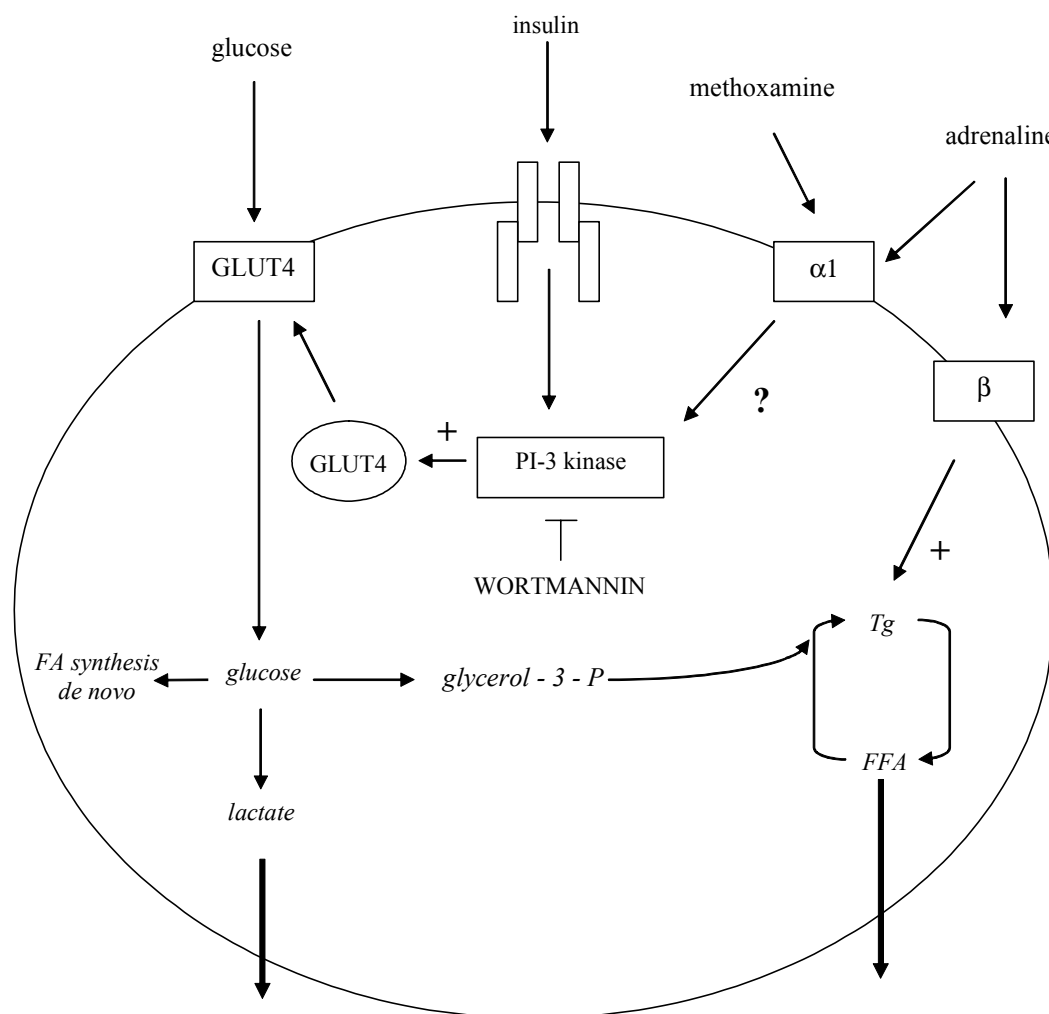


Fig. 2. The proposed mechanisms of adrenaline-stimulated glucose transport into adipocytes. The stimulation of β -adrenergic receptors activates the hydrolysis of intracellular triglycerides (Tg) and FFA production. Part of the released FFA is exported from adipose tissue but part of them, because of the existence continuous Tg/FFA cycle, is reesterified in the tissue. In consequence, the intracellular need of glucose for FFA reesterification increases. This process is dependent on PI-3 kinase as wortmannin significantly decreased the esterification of glucose and increased the FFA release from the tissue, probably as a result of lower glucose availability. The specific stimulation of α_1 -adrenoreceptors by methoxamine does not increase the lipolysis but tend to increase the glucose utilization for fatty acid synthesis *de novo*. Adrenaline activates both types of receptors concomitantly and the glucose transported into cell after the α_1 receptor activation may provide some surplus glucose for reesterification of FFA derived from adrenaline-stimulated lipolysis.

availability for FFA esterification in the situation of adrenaline-stimulated lipolysis.

Faintrenie and Geloën (1998) reported that selective stimulation of both α (by phenylephrine) and β adrenergic receptors (by propranolol) increased glucose uptake into adipocytes. α and β adrenoceptors are coupled with different intracellular effectors systems and therefore it is probable that stimulating effect of adrenaline on glucose utilization is mediated by more than one mechanism. Cheng *et al.* (2000), who employed the specific α_1 -adrenoceptor agonist, identified the specific role of these receptors in glucose uptake. They found that transport of glucose after specific stimulation

of α_1 -adrenoceptors is associated with GLUT4 translocation to the plasma membrane but the mechanism of this process remains to be identified.

Our experiment in normal rats showed that adrenaline, beside its main function, which is the stimulation of lipolysis and increase of NEFA production, concomitantly activates the glucose utilization what leads to the rendering a part of the released fatty acids in adipose tissue by their reesterification. Our experiments employing concomitantly the adrenaline and PI-3 kinase inhibitor wortmannin showed that the inhibition of glucose entry into adipocytes led to the significant increase of adrenaline-stimulated FFA release and

provided evidence supporting the important role of reesterification for final FFA release from adipose tissue in fasting animals (Table 1, Cahová, unpublished results).

Adrenaline realizes its effect on glucose utilization probably preferentially *via* β -adrenoceptor stimulation which leads to the increased NEFA production and consequently to the increase of intracellular need for glucose for their esterification. Nevertheless, the stimulation of α_1 -adrenoceptors may also contribute to the increased glucose supply to the cell. A possible mechanism is proposed in Figure 2.

Conclusions

The important role of fatty acids in the development of insulin resistance is now generally accepted. Nevertheless, the exact mechanism how fatty acids contribute to the establishment of insulin-resistant phenotype of skeletal muscle is still not completely elucidated. There is now growing evidence that the original theory (Randle or glucose-fatty acid cycle) supposing that the decreased glucose uptake in muscle is a result of increased fatty acid oxidation and suppressed glucose oxidation, cannot completely explain all experimental data. It seems that fatty acid oxidation in type 2 diabetes is decreased and the oxidation of glucose is elevated under the conditions of hyperglycemia and hyperinsulinemia. The increased oxidation of glucose leads to the rise of intracellular malonyl CoA content which is a potent inhibitor of CPT I and thus prevents the entry of fatty acids to mitochondrial oxidation. The theory of "lipotoxicity" explains the effect of fatty acids on muscle cells *via* the accumulation of lipid metabolism intermediates (LC-AcCoA, DAG, ceramides) in the

cytoplasm. These compounds interfere with insulin signaling cascade what results in a decrease of glucose transport into tissues. The impaired uptake of glucose by tissues increases plasma glucose concentration leading to further insulin secretion and hyperinsulinemia. It starts the vicious cycle when the normal regulation of the energy substrate metabolism is disturbed and the changes at the level of substrate competition, enzyme regulation, intracellular signaling and/or gene transcription develop. One of the manifestations of this situation is "metabolic inflexibility", which is defined as an impaired ability to switch easily between glucose and fat oxidation according to the metabolic needs of the organism.

In spite of the fact that the insulin resistance manifests itself mostly in skeletal muscle, there is a lot of evidence indicating the important role of adipose tissue in the pathophysiology of insulin resistance. The experiments, in which specific manipulation in adipose tissue (e.g. fat-specific GLUT4 knock-out) induced whole body insulin resistance, indicate that insulin resistance may be initiated in one tissue and development of insulin resistance in other tissues follows. Among other mechanisms, the adipose tissue may contribute to the development of insulin resistance by impaired buffering of fatty acid fluxes not only in postprandial period but also during fasting. In consequence the non-adipose tissues are exposed to excessive fluxes of lipid fuels, which may lead to the impairment of normal regulation mechanisms in these tissues.

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