How Free Fatty Acids Inhibit Glucose Utilization in Human Skeletal Muscle

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Rat muscle studies suggest competition between free fatty acids (FFA) and glucose for oxidation, resulting in glucose-6-phosphate accumulation. However, FFA decrease glucose-6-phosphate in human skeletal muscle, indicating direct inhibition of glucose transport/phosphorylation. This mechanism could redirect glucose from muscle to brain during fasting and explain the insulin resistance associated with high-lipid diets and obesity.

In overnight-fasted humans, plasma free fatty acid (FFA) concentrations vary between 0.1 and 0.5 mmol/l, primarily due to different rates of lipolysis. Under certain physiological and pathophysiological conditions that are associated with impaired insulin action (insulin resistance; Table 1), plasma FFA concentrations may be markedly increased. Bierman et al. (1) first reported that plasma FFA are elevated in uncontrolled diabetes mellitus. Subsequent studies extended these findings also to nondiabetic but insulin-resistant humans, such as obese patients and first-degree relatives of type 2 diabetic patients (14). Plasma FFA concentrations correlate with the degree of hyperglycemia, skeletal muscle insulin resistance, and the risk of developing type 2 diabetes (14).

It is therefore not surprising that the potential relationship between lipids, particularly FFA, and skeletal muscle glucose metabolism has been the subject of numerous studies during the past several decades. Exactly 40 years ago, Randle et al. (13) postulated a mechanism describing the competition between FFA and glucose for mitochondrial oxidation that would lead to decreased glucose utilization in the presence of increased lipid concentrations. This hypothesis was based primarily on studies in isolated muscle preparations of rat diaphragms or hearts. Subsequent studies in humans mostly did not confirm these results, suggesting that this competition between FFA and glucose for oxidation is not operative under in vivo conditions. Only about a decade ago did development and application of new techniques such as nuclear magnetic resonance spectroscopy (NMRS) make it possible to test a series of hypotheses on metabolic pathways in humans in vivo (14, 20).

NMRS studies on skeletal muscle metabolism

Previously, intramuscular concentrations of metabolites could only be measured in tissue biopsies, and metabolite fluxes were assessed indirectly from arteriovenous balances across the leg or forearm and from isotopic dilution by using labeled metabolites. In vivo NMRS made it possible to monitor cellular concentrations of metabolites and metabolic fluxes in skeletal muscle of humans in vivo (14, 20). Intramuscular glycogen and triglyceride contents are measured with $^{13}$C NMRS and $^1$H NMRS, whereas $^{31}$P NMRS allows quantification of intramuscular concentrations of glucose-6-phosphate (G6P) and energy-rich phosphates such as ATP. Combining NMRS methods with isotopic dilution techniques now made it possible to monitor the time course of changes of intracellular metabolites noninvasively.

The glucose-fatty acid cycle (Randle hypothesis)

This hypothetical mechanism is based on the concept that FFA compete with glucose for mitochondrial oxidation (glucose-fatty acid cycle) (13). According to Randle (12), the essential components of this hypothesis are 1) that the relationship between glucose and FFA metabolism is reciprocal, 2) that in vivo and in vitro oxidation of FFA may inhibit catabolism of glucose in muscle, and 3) that effects of FFA oxidation are mediated by inhibition of phosphofructokinase-1 (PFK) and the pyruvate dehydrogenase (PDH) complex (Fig. 1A). Further components of this hypothesis are 4) that the essential mechanism is an increase in the mitochondrial concentration ratio of acetyl-coenzyme A (acyt-CoA) to CoA, which inhibits the PDH complex and leads to inhibition of PFK by citrate and of hexokinase (HK) by G6P and 5) that FFA oxidation inhibits the effect of insulin to accelerate sugar transport in rat heart.

According to this notion, oxidation of FFA first alters intramitochondrial metabolite concentrations, which then would inhibit the activities of glycolytic key enzymes. The diminished rate of glycolysis would increase G6P concentrations, which in turn would cause a decrease of glucose phosphorylation by allosteric inhibition of HK (Fig. 1A). From the observations that diabetes, starvation, FFA, and ketone bodies inhibit glucose uptake but cause marked intracellular accumulation of glucose in the presence of insulin, Randle (12) concluded that inhibition of glucose phosphorylation by elevated G6P is responsible for the impaired glucose uptake. Finally, this author recently postulated that studies in humans do not provide clear evidence for inhibition by FFA of insulin-stimulated glucose transport and that this effect is associated with repletion of glycogen stores (12).

To prove the operation of the glucose-fatty acid cycle in human skeletal muscle, it is mandatory to show 1) increased intramitochondrial acetyl-CoA/CoA ratios and reduced activ-
ity or flux through the PDH complex, 2) increased intramitochondrial citrate concentrations and reduced PFK-mediated flux, 3) increased intracellular G6P and reduced flux through the muscle isoenzyme of HK, HK-II, 4) impaired glycolysis and glucose oxidation, and 5) unchanged or even increased glycogen synthesis. Finally, these alterations should also be present under pathological conditions of increased FFA availability such as insulin resistance and type 2 diabetes (13).

Employing either oral lipid-rich meals or intravenous infusion of lipid emulsions, many studies in humans showed that a 1.5- to 26-fold rise in plasma FFA concentrations results in reduction of insulin-stimulated glucose disposal ranging from 0 to 55% and glucose oxidation between 11 and 100% (22). Nevertheless, in some studies FFA caused a greater decrease of nonoxidative than oxidative glucose metabolism (2, 8, 16). Moreover, despite an increased acetyl-CoA/CoA ratio (3), the PDH complex was inhibited only in some (2, 3, 8) but not all (19, 22) muscle biopsy studies in humans. Intracellular citrate concentrations were not affected by increased plasma FFA (3), and PFK was not altered by acipimox-induced reduction in plasma FFA (22). Likewise, HK activity was unchanged (7), as were intracellular G6P concentrations (2, 3). G6P was slightly increased only after 6 h of plasma FFA elevation in one study (2), which may be an artifact resulting from variable breakdown of glycogen to G6P during tissue handling (17).

In summary, studies in humans indicate that other mechanisms than the glucose fatty acid cycle must be operative to fully explain how increased plasma FFA concentrations cause reduction of whole body glucose disposal.

The unifying hypothesis

FFA effects on glucose transport/phosphorylation in human skeletal muscle. To test components of the glucose-fatty acid cycle, we applied $^{13}$C NMRS with [1-$^{13}$C]glucose to measure glycogen concentrations combined with $^{31}$P NMRS to measure G6P noninvasively in calf muscle of healthy humans (16). Intravenous lipid infusions were used to induce 10-fold elevation of plasma FFA concentrations, and insulin-stimulated conditions were created by the euglycemic-hyperinsulinemic clamp tests, which result in fasting plasma glucose and postprandial peripheral insulin concentrations. As a result of this approach, plasma FFA elevation decreases whole body glucose disposal by 46% and muscular glycogen synthesis by 50%. These results are in agreement with previous findings that a twofold elevation of plasma FFA decreases nonoxidative glucose metabolism and glycogen synthase activity in skeletal muscle biopsies (2). Surprisingly, the reduction in glucose disposal and glycogen synthesis started at 3 h of lipid/heparin infusion and was preceded by 60% inhibition of the insulin-stimulated rise in muscular G6P at as early as ~1.5 h. Glucose oxidation was 40% lower after 2 h of lipid infusion.

Simultaneous monitoring of the time courses of intracellular glycogen and G6P concentrations by combined $^{13}$C and $^{31}$P NMRS made it possible to identify the rate-controlling step of glycogen synthesis. Intracellular G6P is positioned between glucose transport/phosphorylation and glycogen synthase enzymes. According to metabolic flux control, G6P reflects the relative activities of these enzymes and the rate of glycolysis. If glycogen synthase activity is selectively impaired, slower removal of G6P would result in a rise of intracellular G6P. In contrast, G6P was markedly lower during plasma FFA elevation, suggesting that glucose transport and/or phosphorylation are the primary targets for reduction of glycogen synthesis under these conditions (Fig. 1B).

Nevertheless, this study could not rule out that the glucose-

<table>
<thead>
<tr>
<th>Condition</th>
<th>Plasma Free Fatty Acids, mmol/l</th>
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<tbody>
<tr>
<td>Fasting overnight, 10–12 h</td>
<td>~0.1–0.5</td>
</tr>
<tr>
<td>Low-carbohydrate/high-fat diet</td>
<td>~0.8</td>
</tr>
<tr>
<td>Exercise, ~2 h, trained subjects</td>
<td>~1.0</td>
</tr>
<tr>
<td>Exercise, ~2.5 h, moderately trained subjects</td>
<td>~1.6</td>
</tr>
<tr>
<td>Starvation, 69 h</td>
<td>~1.8</td>
</tr>
<tr>
<td>Epinephrine, intravenous</td>
<td>~1.8</td>
</tr>
<tr>
<td>Lean insulin-resistant offspring of type 2 diabetic parents</td>
<td>~0.6</td>
</tr>
<tr>
<td>Obese nondiabetic humans</td>
<td>~0.6–0.8</td>
</tr>
<tr>
<td>Overt type 2 diabetes mellitus</td>
<td>~0.7–0.9</td>
</tr>
<tr>
<td>Nonalcoholic fatty liver disease</td>
<td>~0.7</td>
</tr>
<tr>
<td>Lipoprotein lipase defect</td>
<td>~1.0</td>
</tr>
<tr>
<td>Oral fat loading in familial combined hyperlipidemia</td>
<td>~1.2</td>
</tr>
<tr>
<td>Type A insulin resistance</td>
<td>~3.3</td>
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fatty acid cycle is operative at lower, more physiological plasma FFA concentrations and that muscular G6P is initially increased. Thus we used $^3$P NMRS with increased time resolution and found that the insulin-stimulated rise in G6P is blunted by ~25% as early as 45 min after plasma FFA elevation in healthy humans (15). Furthermore, plasma FFA within the physiological range (Table 1) concentration-dependently inhibit both insulin-stimulated whole body glucose disposal and the increase in G6P (Fig. 2).

But these findings still cannot exclude that the glucose-fatty acid cycle is operative at lower, more physiological plasma FFA concentrations and that muscular G6P is initially increased. Thus we used $^3$P NMRS with increased time resolution and found that the insulin-stimulated rise in G6P is blunted by ~25% as early as 45 min after plasma FFA elevation in healthy humans (15). Furthermore, plasma FFA within the physiological range (Table 1) concentration-dependently inhibit both insulin-stimulated whole body glucose disposal and the increase in G6P (Fig. 2).

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FIGURE 1. Interaction of free fatty acids (FFA) with glucose (GLU) in skeletal muscle. FFA are taken up by fatty acid transporter protein-1 (FATP-1) and activated to fatty acyl-coenzyme A (FA-CoA), whereas GLU is taken up by glucose transporter-4 (GLUT-4) and phosphorylated by hexokinase-ll (HK). A: glucose-fatty acid cycle (Randle hypothesis). FA-CoA oxidation would increase the ratios of acetyl-CoA/CoA and of NADH/NAD$^+$, which inhibit the pyruvate dehydrogenase (PDH) complex. Increased citrate should further inhibit phosphofructokinase (PFK). These changes would slow down oxidation of GLU and pyruvate (PYR) and increase glucose-6-phosphate (G6P), which would probably stimulate glycogen (GLY) storage, inhibit hexokinase (HK), and decrease GLU transport. B: FFA interaction with glucose uptake. FA-CoA primarily activates isofoms of protein kinase C (PKC) and NF-$\kappa$B, which inhibits insulin signal transduction, resulting in decreased glucose transport/phosphorylation and glycogen synthesis. IRS, insulin receptor substrate; DAG, diacylglycerol; PI3K, phosphoinositide 3-kinase.

FIGURE 2. Concentration dependence of the inhibitory effects of FFA on insulin-stimulated whole body glucose disposal and increase in intracellular G6P concentrations in skeletal muscle of humans in vivo. Numbers represent the percent decrease from maximum values obtained at the lowest plasma FFA concentrations of ~0.1 mmol/l, which are due to insulin-induced inhibition of whole body lipolysis.
ily inhibit glucose transport in human skeletal muscle (Fig. 1B).

Together, these studies do not confirm the Randle hypothesis, which predicts a rise in G6P, but indicate that glucose transport becomes rate controlling even at physiologically increased plasma FFA. Moreover, the observed effects are identical to the metabolic changes typically found in skeletal muscle of insulin-resistant and type 2 diabetic individuals (14, 20).

**FFA effects on insulin signal transduction in human skeletal muscle.** The interaction of lipids with insulin signaling has been studied extensively in isolated tissues of rodents and cell cultures (8), but only a few studies examined the effects of plasma FFA elevation on elements of the insulin signal transduction cascade in vivo in human skeletal muscle. Lipid-infusion protocols combined with repetitive biopsies of the vastus lateralis muscle revealed that the FFA-induced effects in insulin-stimulated glucose metabolism are in fact associated with alteration of insulin signaling (5). Plasma FFA elevation almost completely abolished the increase in insulin receptor substrate (IRS-1)-associated phosphoinositide 3-kinase (PI3K) (5) (Fig. 1B). Moreover, IRS-1 tyrosine phosphorylation is also impaired, whereas phosphorylation of other elements such as the central molecule of insulin signal transduction, protein kinase B (PKB/Akt), seems to be intact under high-FFA conditions (10).

Recently, evidence was provided for the link between FFA and insulin signaling in human skeletal muscle. After 6 h of lipid infusion, reduction in whole body glucose disposal by 43% was accompanied by a threefold increase in diacylglycerol, fourfold increase in protein kinase C (PKC) activity with recruitment of the β and δ isoforms of PKC to the cell membrane, as well as a 70% decrease of NF-κB-α, an inhibitor of NF-κB (6) (Fig. 1B). Under similar conditions of lipid infusion or following a high-fat diet, rodents exhibit identical alterations in insulin signal transduction, which may be mediated through the increase of FA-CoA and/or rather of the ε (8) and θ isoforms (20) of PKC. Both PKC isoforms and NF-κB could contribute to insulin resistance by regulating phosphorylation of serine and threonine residues of IRS proteins. NF-κB comprises dimeric transcription factors, which are also involved in inflammation processes. Its subcellular localization is controlled by the inhibitory protein IκB, which binds to NF-κB and inhibits its uptake into cell nuclei.

Alternatively, accumulation of FA-CoA can also result from increased breakdown of intramyocellular triglycerides, which serve as an endogenous pool of FFA and correlate with insulin resistance as assessed from insulin-stimulated whole body glucose disposal (14).

Together, increased availability of FFA due to either circulating plasma FFA or triglycerides or from intramyocellular triglycerides could lead to elevated FA-CoA and translocation of PKC isoforms to the muscle cell membrane (Fig. 1B). This activation of PKC may stimulate the IκB-α/NF-κB system or may directly phosphorylate serine residues of the insulin receptor and its substrates. This will in turn inhibit the activity of IRS-associated PI3K, which is mainly responsible for the insulin induced recruitment of glucose transporter GLUT-4 to the cell membrane and thereby ultimately inhibit glucose transport (14) (Fig. 1B).

These mechanisms primarily describe the scenario of acute (short-term) elevation of plasma FFA concentrations. Under these conditions, FFA, independent of the source (either lipid/heparin infusion, which predominately increases unsaturated fatty acids from exogenous triglycerides, or the post-nicotinic acid rebound phenomenon, which increases FFA due to exaggerated breakdown of endogenous lipids) similarly impair insulin-stimulated glucose uptake. On the other hand, chronic dietary-induced increases in circulating FFA and membrane fatty acids, in particular in sarclemma, depend on the amount and quality of the ingested fat. Insulin resistance of glucose uptake seems to relate directly to elongation and desaturation of fatty acids in the outer layers of the sarcolemma in human skeletal muscle (4). Moreover, a rise in plasma FFA for 24 h or more could also inhibit glucose uptake by increasing the expression of fatty acid transporter protein-1 and decreasing the expression of GLUT-4 in skeletal muscle (23). In addition, in adipose tissue FFA bind to peroxisome proliferator-activated receptor-γ (PPAR-γ), which regulates lipolysis and release of adipocytokines. Thus FFA could also exert indirect effects in skeletal muscle and thereby chronically modulate insulin-stimulated glucose uptake (11).

**Conclusions**

Studies in humans clearly demonstrated that FFA directly inhibit glucose transport and phosphorylation in skeletal muscle, which is mainly responsible for glucose disposal under insulin-stimulated conditions and for impaired glucose uptake in insulin-resistant states such as obesity and type 2 diabetes mellitus. These findings also indicate an important role of nutrition, particularly increased consumption of lipids and FFA, for the pathogenesis of insulin resistance.

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