Lipid and insulin infusion-induced skeletal muscle insulin resistance is likely due to metabolic feedback and not changes in IRS-1, Akt, or AS160 phosphorylation

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Am J Physiol Endocrinol Metab 297: E67–E75, 2009. First published April 14, 2009; doi:10.1152/ajpendo.90945.2008.—Type 2 diabetes is characterized by hyperlipidemia, hyperinsulinemia, and insulin resistance. The aim of this study was to investigate whether acute hyperlipidemia-induced insulin resistance in the presence of hyperinsulinemia was due to defective insulin signaling. Hyperinsulinemia (300 mU/l) with hyperlipidemia or glycerol (control) was produced in cannulated male Wistar rats for 0.5, 1 h, 3 h, or 5 h. The glucose infusion rate required to maintain euglycemia was significantly reduced by 3 h with lipid infusion and was further reduced after 5 h of infusion, with no difference in plasma insulin levels, indicating development of insulin resistance. Consistent with this finding, in vivo skeletal muscle glucose uptake (31%, P < 0.05) and glycogen synthesis rate (38%, P < 0.02) were significantly reduced after 5 h compared with 3 h of lipid infusion. Despite the development of insulin resistance, there was no difference in the phosphorylation state of multiple insulin-signaling intermediates or muscle diacylglyceride and ceramide content over the same time course. However, there was an increase in cumulative exposure to long-chain acyl-CoA (70%) with lipid infusion. Interestingly, although muscle pyruvate dehydrogenase kinase 4 protein content was decreased in hyperinsulinemic lipid-infused rats, this decrease was blunted in muscle from hyperinsulinemic lipid-infused rats. Decreased pyruvate dehydrogenase complex activity was also observed in lipid- and insulin-infused animals (43%). Overall, these results suggest that acute reductions in muscle glucose metabolism in rats with hyperlipidemia and hyperinsulinemia are more likely a result of substrate competition than a significant early defect in insulin action or signaling.

lipotoxicity; hyperlipidemia; in vivo metabolism; long-chain acyl-CoA; pyruvate dehydrogenase kinase 4

ELEVATED CIRCULATING FATTY acids have been proposed to be a major contributing factor in the pathogenesis of skeletal muscle insulin resistance. A number of mechanisms involved in insulin resistance arising from lipid oversupply have been suggested; the majority of these mechanisms ultimately result in defective activation of the insulin-signaling pathway to reduce GLUT4 translocation to the plasma membrane (39). These proposed defects in insulin signaling are thought to occur predominantly via two loci: reduced activating tyrosine phosphorylation resulting from increased inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1) or reduced serine/threonine phosphorylation of Akt. A number of mediators have been proposed to inhibit IRS-1 activation: JNK activation (51), potentially by fatty acid signaling through Toll-like receptors-2 and -4 (37) or by accumulation of ceramide (37, 50); IKK-NF-κB (28); and diacylglyceride (DAG) activation of PKCs (56). These pathways have been suggested to increase inhibitory phosphorylation of IRS-1 on Ser307, which can decrease IRS-1 tyrosine phosphorylation and IRS-1-associated phosphatidylinositol 3-kinase activity (39). Degradation of IRS-1 protein levels has also been suggested as a process whereby insulin signaling is reduced in insulin-resistant states (57). Inhibition of Akt activation is thought to be due to increased activation of the protein phosphatase 2A (45) or PKCζ/λ (40) by accumulated ceramide.

The majority of in vivo studies that have implicated defects in insulin signaling as a mechanism for insulin resistance have done so in models of hyperlipidemia, including high-fat feeding (12, 55, 58) and acute lipid infusion (11, 18, 20, 50). Additional work has shown that incubation of isolated muscles or cultured myotubes with high fatty acid [predominantly palmitate (C16)] concentrations leads to an impairment in insulin signaling and reduced insulin action (1, 19, 56). For the lipid infusion studies, numerous protocols have been used to investigate how lipid oversupply induces insulin resistance. The majority of these investigations have involved infusion of lipid (and heparin) for 0.5–24 h followed by commencement of insulin sensitivity measures such as hyperinsulinemic-euglycemic clamp (0.5 to 2 h) (3, 8, 16, 29, 30, 49, 50, 52). With such a degree of variation in the lipid infusion protocols, it perhaps is no surprise that a range of mechanisms, which are mentioned above, have been proposed to support the development of insulin resistance. Further complicating the identification of the mechanisms involved is some conjecture over the involvement of some of these mechanisms, including JNK signaling. Bhatt et al. (3) demonstrated that lipid infusion did not alter skeletal muscle JNK activity. However, they did not measure insulin signaling. Furthermore, a recent in vitro study reported that, in isolated soleus muscle, 6 h of palmitate incubation reduced acute insulin-stimulated glucose uptake and membrane GLUT4 protein, did not change Akt phosphorylation (Thr308 and Ser473), and reduced AS160 phosphorylation (1). In the majority of investigations of the mechanisms associated with lipid oversupply-induced insulin resistance in skeletal muscle, insulin was not elevated from the commencement of the lipid infusion.
infusion (3, 8, 11, 16, 18, 20, 29, 30, 49, 50, 52). This is important, inasmuch as hyperlipidemia is often associated with hyperinsulinemia in human subjects with type 2 diabetes (4).

Therefore, the aim of the present study was to determine whether the onset of insulin resistance in skeletal muscle caused by acute lipid and insulin infusion was associated with defective insulin signaling.

METHODS

Animals

All surgical and experimental procedures were approved by the Animal Ethics Committee (Garvan Institute/St. Vincent’s Hospital) and were in accordance with the National Health and Medical Research Council of Australia’s guidelines on animal experimentation.

Adult male Wistar rats (Animal Resources Centre, Perth, Australia) were communally housed at 22 ± 0.5°C with a controlled 12:12-h light-dark cycle (lights on from 0700 to 1900). They were fed ad libitum a standard rodent diet (Rat Maintenance Diet, Gordons Specialty Feeds, Sydney, Australia) containing 10% fat, 69% carbohydrate, and 21% (wt/wt) protein plus fiber, vitamins, and minerals. After 1 wk of acclimatization, rats were anesthetized with a combination of ketamine (Ketalar, 80 mg/kg) and xylazine (Ilium Xylazil, 20 mg/kg) injected intraperitoneally. Cannulas were implanted into the right and left jugular veins under aseptic conditions. Catheters were exteriorized at the back of the neck. Rats were housed individually after surgery and handled daily to minimize stress. Average body weight on the experimental day was 300–350 g.

Lipid Infusion

Animals were deprived of food for ≥5 h overnight and randomly assigned to the lipid-infused or control group before study. The lipid-infused group received 12% Intralipid (diluted with normal saline from Intralipid 20%; Baxter Healthcare, Sydney, Australia). The fatty acid composition of Intralipid is as follows: 1% myristic acid (14:0), 11% palmitic acid (16:0), 4% stearic acid (18:0), 20.8% oleic acid (18:1), 53% linoleic acid (18:2), 7% α-linoleic acid (18:3), and 3.2% other fatty acids. The rate of infusion was 2 ml/h, with a concomitant heparin infusion of 40 U/h to aid in the lipolysis of the triglyceride emulsion. To match the glycerol released from the triglyceride emulsion, control animals were infused with 3% glycerol. Both groups were infused for 0, 0.5, 1, 3, or 5 h. Both groups received insulin (Actrapid, Novo Nordisk, Copenhagen, Denmark) to achieve levels similar to those observed in the glucose infusion model (∼300 mU/l) (22); lipid-infused animals received 0.35 U·kg⁻¹·h⁻¹, and the control group received 0.6 U·kg⁻¹·h⁻¹. Glucose (50% solution) was infused via a peristaltic roller pump (model 101U/R, Watson-Marlow, Falmouth, UK). A blood sample was taken every 30 min, and the glucose infusion rate (GIR) was adjusted to maintain a whole blood glucose concentration of 4.5–5.5 mM. In one cohort, 33.8 μCi of 2-deoxy-D-[1,6,2-H³]glucose ([H³]2DG) and 22.5 μCi of [U-14C]glucose (Amersham Biosciences, Buckinghamshire, UK) were administered as an intravenous bolus 60 min before the animals were euthanized. Blood samples (200 μl) were taken at 2, 15, 30, 45, and 60 min after administration of the bolus for estimation of plasma tracer and glucose concentration. Then the animals were euthanized with an overdose of pentobarbital sodium (Nembutal, Abbott Laboratories, Sydney, Australia). Tissues were rapidly removed, freeze-clamped, and stored at −80°C for subsequent analyses. Rats had free access to water throughout the infusion.

Analytic Methods

Blood and plasma glucose levels were determined by an immobilized glucose oxidase method (model YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured by radioimmunoassay kit (Linco, St. Louis, MO). Plasma nonesterified fatty acid (NEFA) levels were determined spectrophotometrically using a commercially available kit (NEFA-C, WAKO Pure Chemical Industries, Osaka, Japan).

Plasma and tissue levels of [H³] and [14C]-labeled tracers were measured as described previously to calculate whole body and muscle glucose uptake; tissue glycogen concentration and [14C]glucose incorporation rates into glycogen were also calculated (23). Red quadriceps (RQ) triglycerides were extracted using the method of Folch et al. (10) and quantified using an enzymatic colorimetric method (GPO-PAP reagent, Roche Diagnostics). DAG, ceramide, and long-chain acyl-CoA (LCαCoAs) were extracted and quantified according to published methods (2, 41). Pyruvate dehydrogenase (PDH) complex (PDHC) activity was measured in whole tissue homogenates prepared in 5 vol of 50 mM potassium phosphate, 10 mM EGTA, 2 mM dithiothreitol, 2% BSA, 10 μg/ml aprotinin, 10 μM leupeptin, and 1 mM PMSF. PDHC activity was determined spectrophotometrically at 30°C as previously described (27). PDHC activity was expressed relative to citrate synthase activity, which was determined at 30°C, as described previously (35).

Skeletal muscle homogenates were subjected to protein extraction and immunoblotting, as previously described (22). Densitometry was performed using IPLab Gel software (Signal Analytics, Vienna, VA). Antibodies for anti-insulin receptor-β were obtained from BD Biosciences (San Jose, CA); anti-phospho-Thr183/Tyr185 insulin receptor from BioSource International (Camarillo, CA); anti-phospho-Tyr1162 IRS-1 from Sigma-Aldrich (St. Louis, MO); anti-Akt, anti-phospho-Ser773 Akt, anti-glycogen synthase kinase (GSK)-3β, anti-GSK3α, anti-phospho-Ser119 GSK3α/β, anti-IRS-1, anti-IC3, anti-phospho-Thr183/Tyr186 JNK, and anti-JNK from Cell Signaling Technology (Danvers, MA); anti-AS160 from Upstate Biotechnology (Lake Placid, NY); and anti-14-3-3 from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-Thr117 AS160 was a gift from Symansis (Auckland, New Zealand).

Statistical Analysis

Statistical analyses were performed using Prism5 (Graphpad Software, San Diego, CA). Differences among relevant groups were assessed using unpaired Student’s t-test or ANOVA with Tukey-Kramer post hoc tests as appropriate. P < 0.05 was considered significant. Values are means ± SE.

RESULTS

Plasma Parameters Before and After Chronic Infusion of Lipid

There was no difference in basal blood glucose levels between control and lipid-infused animals (4.13 ± 0.15 and 4.06 ± 0.09 mM, respectively). During the infusions, average blood glucose was 4.84 ± 0.07 and 5.33 ± 0.07 mM for the control and lipid-infused groups, respectively (Fig. 1A). Fasting plasma insulin was 45 ± 14 mU/l and increased to an average of 280 ± 16 mU/l for the control group; in the lipid-infused group, fasting plasma insulin was 31 ± 6 mU/l and increased to an average of 314 ± 26 mU/l (Fig. 1B). No difference in basal plasma NEFA levels was observed between control and lipid-infused groups: 0.69 ± 0.05 and 0.65 ± 0.04 mM, respectively. Average circulating NEFA levels were 0.11 ± 0.01 and 1.60 ± 0.06 mM in control and lipid-infused animals, respectively (Fig. 1C). Overall, the two groups were well matched for circulating glucose and insulin levels, whereas NEFA levels were significantly different.
Glucose Metabolism and Insulin Sensitivity

Whole body glucose metabolism. The GIR required to maintain euglycemia was stable throughout the 5-h infusion for the control group (Fig. 2A). In the lipid-infused group, GIR was stable for the first 2 h but was significantly reduced after 3, 4, and 5 h compared with 1 h of lipid infusion (Fig. 2A). The length of infusion had no significant effect on whole body glucose disappearance in the control or lipid-infused group (Fig. 2B). There was no significant effect on $R_Q$ between control and lipid-infused animals. Hepatic glucose output remained suppressed in the control group ($-0.34 \pm 2.24$ and $0.97 \pm 0.89 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at 3 and 5 h in the control group, respectively). However, there was a strong trend for an increase in hepatic glucose output in the 5-h lipid-infused group compared with the 5-h control-infused group ($4.75 \pm 0.24$ and $10.79 \pm 4.12 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at 3 and 5 h, respectively, in the lipid-infused group, $P = 0.07$).

Skeletal muscle insulin sensitivity and glucose metabolism. The ability of insulin to stimulate glucose uptake in muscle of control animals was similar after 3 and 5 h of infusion (Fig. 2C). Glucose uptake in RQ was significantly reduced after 5 h compared with 3 h of lipid infusion ($31\%$, $P = 0.008$; Fig. 2C). In the mixed-fiber tibialis cranialis and white quadriceps, there was also a significant reduction in glucose uptake after 5 h compared with 3 h of lipid infusion [33\% ($P = 0.005$) and 37\% ($P = 0.04$), respectively; data not shown]. Consistent with the reduction in glucose uptake, in vivo rates of glycogen synthesis in RQ muscle were significantly reduced in the 5-h lipid-infused group compared with the 3-h lipid-infused group (38\%, $P = 0.02$; Fig. 2D). This reduction in the rate of glycogen synthesis was not related to the glycogen content, which was similar to glycogen content in the control group (Fig. 2E).

Skeletal muscle insulin signaling. To determine whether changes in insulin signaling accompanied the development of insulin resistance in RQ, the activation state of several key components of the insulin-signaling pathway involved in glucose metabolism were examined. Phosphorylation of the insulin receptor (Tyr$^{1162/1163}$; Fig. 3A) was increased and remained elevated in control and lipid-infused animals. Additionally, phosphorylation of IRS-1 (Tyr$^{612}$) was significantly increased and sustained in control and lipid-infused animals (Fig. 3B). Phosphorylation of Akt (Ser$^{473}$; Fig. 3C) and its downstream targets involved in glucose metabolism, AS160 (Thr$^{642}$; Fig. 3D), GSK3β (Ser$^{21}$; Fig. 3E), and GSK3α (Ser$^{9}$; Fig. 3F), was increased and maintained with prolonged control and lipid infusion. There was no change in total protein expression of any of these signaling components. Densitometry measurements demonstrated no significant reduction in the phosphorylation state for most intermediates between control and lipid-infused animals at 3 or 5 h, despite the significant impairment in glucose uptake during this period of lipid infusion (Fig. 2C).

Skeletal muscle inflammatory signaling. Inflammatory signaling has been proposed as a mechanism that can impair insulin signaling (47). There was no change in the total protein content of IκBα, which was used as a surrogate marker of IKK-NF-κB signaling activation, in the lipid-infused or control group (Fig. 4A). Phosphorylation of JNK was also not altered with prolonged control or lipid infusion (Fig. 4B). The absence of changes in inflammatory signaling is consistent with the lack of alterations in insulin signaling in this model of hyperinsulinemia and hyperlipidemia.

Skeletal muscle lipid content. There was a significant increase in triglyceride content after 5 h of lipid infusion compared with 3 h of lipid infusion and 5 h of control infusion ($P < 0.01$; Fig. 5A). There was no significant difference in DAG or ceramide content between lipid and control infusion during the course of the infusion (Fig. 5, B and C). These findings are consistent with the fact that there was also no defect in key insulin-signaling intermediates (Fig. 3).

Insulin rapidly suppressed LCACoA content in the control and lipid-infused groups, and it remained suppressed for the duration of the control infusion (Fig. 5D). However, LCACoA content showed a trend toward an increase after 3 h of lipid infusion compared with control infusion. The accumulated exposure to LCACoA over the course of the infusion (measured as area under the curve) was increased by 70\% with lipid infusion compared with control infusion (Fig. 5, inset). LCACoAs have been suggested to inhibit hexokinase (HK) activity and, thereby, influence glucose uptake (46). As an indirect marker of HK activity, there was a 34\% reduction in the ratio of $[^{3}H]2DG$-6-phosphate to $[^{3}H]2DG$ after 5 h of lipid infusion compared with the control group (5.70 ± 1.55 vs. 3.77 ± 0.84). This represents a potential mechanism by which the rate of glucose uptake and glycogen synthesis was reduced in
lipid-infused animals (Fig. 2, C and D), independently of an insulin-signaling defect (Fig. 3).

**PDK content and PDHC activity.** PDHC regulates the entry of glucose carbons into the mitochondrial tricarboxylic acid (TCA) cycle for complete oxidation to CO₂. The activity of PDH is regulated by PDK, which phosphorylates PDH to inhibit its activity. Protein content of PDK4 was significantly reduced from basal levels (2.07 ± 0.23 arbitrary units) after 3 h of control or lipid infusion (42% (P = 0.01) and 30% (P = 0.04), respectively; Fig. 6A). PDK4 was further reduced after 5 h of control infusion (28%, P = 0.04). However, PDK4 was unchanged after 5 h of lipid infusion and, at this time point, was significantly elevated compared with 5 h of control infusion (59%, P = 0.01; Fig. 6A). This indicates that lipid infusion may reduce the ability of insulin to reduce PDK4 protein.

To determine whether the changes in PDK4 protein content altered activity of its downstream target, PDHC activity was investigated. PDHC activity was increased compared with basal level after 3 and 5 h of control infusion [1.2-fold (P = 0.02) and 1.7-fold (P = 0.005), respectively; Fig. 6B]. After 5 h of lipid infusion, PDHC activity was reduced (43%, P = 0.02) compared with 5 h of control infusion, which was consistent with the higher level of PDK4 protein.

**DISCUSSION**

In this study, we investigated whether oversupply of lipid in the presence of high insulin resulted in skeletal muscle insulin resistance due to an insulin-signaling defect. Despite skeletal muscle insulin resistance, we observed no attenuation of protein phosphorylation of intermediates of the insulin-signaling cascade, including the insulin receptor IRS-1, Akt, and its downstream targets AS160 and GSK3β. The insulin resistance in this model of hyperlipidemia and hyperinsulinemia was potentially due to changes in PDHC activity as a result of altered PDK4 content and prolonged exposure to elevated LCACoA. Overall, our results suggest that metabolic feedback, rather than significant alterations in insulin signaling, may mediate the onset of insulin resistance in this model.

The use of acute lipid infusion in rodents as a model of elevated circulating free fatty acids has provided some insight into the potential mechanism(s) by which insulin resistance may be generated by chronic energy imbalance. At the whole body level, lipid infusion reduces the GIR required to maintain euglycemia and, at the tissue level, reduces peripheral glucose disposal and insulin-mediated suppression of hepatic glucose production during insulin stimulation (5, 7, 29, 53). In skeletal muscle, insulin resistance is associated with reduced glucose uptake, glycolysis, and glycogen synthesis (5, 29). Furthermore, defects in activation of components of the insulin-signaling pathway have been linked with skeletal muscle in-

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Fig. 2. Effect of lipid infusion on whole body indexes of insulin sensitivity. A: glucose infusion rate (GIR) required to maintain euglycemia in rats infused with lipid or glycerol and insulin for 5 h. *P < 0.05 vs. 60 min of lipid infusion (by ANOVA). #P < 0.05 vs. control infusion (by ANOVA). B–D: rate of glucose disappearance (Rd), red quadriceps glucose uptake, and glycogen synthesis rate in animals after 3 and 5 h of glycerol or lipid infusion in the presence of hyperinsulinemia. *P < 0.05 vs. 3-h lipid infusion (by ANOVA). E: glycogen content in animals infused with lipid or glycerol and insulin for 5 h. Values are means ± SE (n = 5–7 rats per group). *P < 0.05 vs. Basal.
Sulin resistance. These defects include reduced tyrosine phosphorylation of IRS-1, which is thought to be due to increased inhibitory serine phosphorylation, reduced phosphatidylinositol 3-kinase activity, reduced Akt1 (but not Akt2 or Akt3) activation, decreased PKC activity, and lower glycogen synthase activity and phosphorylation (11, 17, 29, 30, 50). Accumulation of active lipid intermediates, such as DAG, ceramide, and LCACoA, has been linked with insulin resistance (5, 8, 20, 53, 56). The accumulation of these lipid intermediates has been suggested to activate stress pathways, including PKC (56), JNK (50), NF-κB, and the p38-MAPK pathways (3), to inhibit insulin signaling. Furthermore, a number of studies have demonstrated that depletion of lipid pools can prevent the generation of insulin resistance or restore insulin action (7, 20, 50, 53). In these studies, lipid infusions or in vitro fatty acid incubations have been performed before measurement of insulin sensitivity. In contrast to these previous studies, in the present experiment, lipid and insulin were administered concurrently from the commencement of the infusion to mimic the situation after a high-fat meal, when fatty acids and insulin are elevated. This protocol did not result in any reduction in the phosphorylation of key insulin-signaling intermediates involved in glucose metabolism. In a model similar to that employed in the present study, Ye et al. (54) reported that 6 h of lipid infusion with concurrent insulin infusion resulted in reduced Akt phosphorylation (Ser473). However, a major factor that may explain this discrepancy is...
the fact that the circulating lipid levels used by Ye et al. were much higher (~4 mM). Similarly, Yu et al. (56) reported that 5 h of lipid infusion increased insulin-stimulated serine phosphorylation (1.6-fold) and reduced tyrosine phosphorylation of IRS-1 (~30%). The observations reported in this study were from animals with circulating NEFA levels of ~8 mM, which is well above the physiological range (36). Our findings suggest that, in contrast to studies that “preload” the animal (and muscle) with lipid and then investigate insulin action, the maintenance of a constant insulin stimulus in the presence of physiological circulating lipid levels appears to prevent the attenuation of insulin signaling.

To determine potential mechanisms involved in the onset of the insulin resistance in this acute model of hyperlipidemia and hyperinsulinemia, we investigated a role for purported metabolic feedback mechanisms. The lipid intermediate LCACoA has been suggested to inhibit HK activity and, thereby, reduce glucose uptake (46). Accumulation of LCACoAs in association with insulin resistance has been demonstrated in high-fat-fed rats (9, 38) and 1- and 4-day glucose-infused rats (33). In similar studies, insulin resistance generated by lipid infusion in the presence of insulin was associated with an increase in LCACoA in rats (5) and humans (48). We observed a higher level of LCACoA during the lipid infusion, suggesting that the onset of insulin resistance generated in vivo by hyperlipidemia

Fig. 5. Effects of lipid infusion on skeletal muscle lipid intermediates: triglyceride (A), diacylglyceride (B), ceramide (C), and long chain acyl-CoA (LCACoA, D) content and accumulated exposure (insets). Lipid intermediates were measured in red quadriceps from control and lipid-infused animals in the presence of insulin. Values are means ± SE (n = 5–7 rats per group). *P < 0.05 vs. Basal; #P < 0.05 vs. 5-h control infusion.

Fig. 6. Effect of lipid infusion on skeletal muscle pyruvate dehydrogenase kinase (PDK4) content (A) and pyruvate dehydrogenase complex (PDHC) activity (B). Activities were measured in the basal state and after 3 and 5 h of glycerol or lipid infusion in the presence of hyperinsulinemia in red quadriceps. Values are means ± SE (n = 5–7 rats per group). *P < 0.05 vs. basal; #P < 0.05 vs. 3 h control; ‡P < 0.05 vs. 5 h control (by 1-way ANOVA).
and hyperinsulinemia may be related to inhibition of HK activity by LCACoAs. Furthermore, the reduced ratio of \(^{[3}H\)2DG-6-phosphate to \(^{[3}H\)2DG suggests a reduction in HK activity with lipid infusion compared with control infusion. The ratio of \(^{[3}H\)2DG-6-phosphate to \(^{[3}H\)2DG has been used as an indirect marker of HK activity in vitro (32). However, the use of this measure as an estimate of in vivo HK activity is limited, in that it is not able to discriminate whether nonphosphorylated 2DG is intracellular, interstitial, or from the residual plasma within the tissue sample. Although a reduction in HK activity by LCACoAs is a plausible mechanism, it cannot be verified by ex vivo measurements of HK activity from lipid-infused tissues, because changes in enzyme activity are unlikely to be maintained once the allosteric regulator (e.g., LCACoA) has been diluted by homogenization. We cannot rule out a change in GLUT4 at the cell surface as a result of a mechanism not involving the canonical signaling pathways, nor can we rule out an intrinsic change in GLUT4 activity that may contribute to the decreased glucose uptake.

An alternative metabolic mechanism that may have contributed to the development of insulin resistance in this model of lipid oversupply is mitochondrial substrate competition. One of the first mechanisms proposed for skeletal muscle insulin resistance caused by oversupply of lipid was the Randle cycle (42). Simply stated, substrates (i.e., lipid and glucose) compete for oxidation in the mitochondria, and the resulting increase in circulating free fatty acids. This higher level of PDK4 protein content, most likely due to increased mRNA expression, may account for the reduced activity of PDHC, which could result in decreased glucose flux through the oxidative pathways. Taken together, it is possible that acute oversupply of lipid, in the presence of high insulin, caused a reduction in PDH activity (via PDK4), which may contribute to the reduction in insulin-stimulated glucose uptake.

The precise mechanism linking increased LCACoA content and reduced PDHC activity to reduced glucose uptake remains to be completely resolved. From the observations made in the model used in the present study, one may speculate that the reduced PDHC activity would result in reduced flux through the oxidative pathway of glucose metabolism. This may result in allosteric regulation of key glycolytic enzymes by intermediates, including HK by glucose-6-phosphate as well as LCACoA. This possible reduction of HK activity leads to reduced glucose uptake. At the molecular level, one of the primary sites at which fatty acids attenuate glucose metabolism is thought to be PDK regulation of PDHC. PDK has been shown to phosphorylate PDHC on multiple sites to regulate its activity (31). A number of studies have implicated PDK4 as a contributor to dysfunctional glucose metabolism in models such as high-fat-fed rats (21) and humans (6), Zucker diabetic fatty rats (44), and obese humans (43). Consistent with these findings, PDK4-knockout mice are protected from chronic (18 wk) high-fat-induced glucose intolerance and hyperglycemia (24). Similar to the present study, coinfusion of lipid and insulin into rats has been reported to blunt the reduction in PDK4, but not PDK2, protein content and mRNA expression compared with saline-insulin-infused controls (34). In young lean humans, coinfusion of lipid and insulin blunted the increase in PDHC activity and elevated PDK4 mRNA compared with saline-insulin-infused controls (48). Furthermore, in these subjects, fatty acid oxidation was increased, glucose oxidation was reduced, and acylcarnitine (β-oxidation intermediate) and LCACoA content were elevated. Interestingly, there was no difference in Akt phosphorylation in the lipid-infused group in this study. Furthermore, as shown by nuclear magnetic resonance, lipid infusion reduces glucose oxidation (PDHC-to-TCA flux ratio) in rodent skeletal muscle (16, 25). In the present study, PDHC activity in the control animals was significantly increased most likely as a result of reduced PDK4 protein content. However, this reduced PDK4 content was blunted by the presence of high circulating free fatty acids. This higher level of PDK4 protein content, most likely due to increased mRNA expression, may account for the reduced activity of PDHC, which could result in decreased glucose flux through the oxidative pathways. Taken together, it is possible that acute oversupply of lipid, in the presence of high insulin, caused a reduction in PDH activity (via PDK4), which may contribute to the reduction in insulin-stimulated glucose uptake.

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