Muscle lipid accumulation and protein kinase C activation in the insulin-resistant chronically glucose-infused rat

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Laybutt, D. Ross, Carsten Schmitz-Peiffer, Asish K. Saha, Neil B. Ruderman, Trevor J. Biden, and Edward W. Kraegen. Muscle lipid accumulation and protein kinase C activation in the insulin-resistant chronically glucose-infused rat. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E1070–E1076, 1999.—Chronic glucose infusion results in hyperinsulinemia and causes lipid accumulation and insulin resistance in rat muscle. To examine possible mechanisms for the insulin resistance, alterations in malonyl-CoA and long-chain acyl-CoA (LCA-CoA) concentration and the distribution of protein kinase C (PKC) isozymes, putative links between muscle lipids and insulin resistance, were determined. Cannulated rats were infused with glucose (40 mg·kg−1·min−1) for 1 or 4 days. This increased quadriceps muscle LCA-CoA content (sum of 6 species) by 1.3-fold at 1 day and 1.4-fold at 4 days vs. saline-infused controls (both P < 0.001 vs. control). The concentration of malonyl-CoA was also increased (1.7-fold at 1 day, P < 0.01, and 2.2-fold at 4 days, P < 0.001 vs. control), suggesting an even greater increase in cytosolic LCA-CoA. The ratio of membrane to cytosolic PKC-ε was increased twofold in the red gastrocnemius after both 1 and 4 days, suggesting chronic activation. No changes were observed for PKC-α, -δ, and -η. We conclude that LCA-CoAs accumulate in muscle during chronic glucose infusion, consistent with a malonyl-CoA-induced inhibition of fatty acid oxidation (reverse glucose-fatty acid cycle). Accumulation of LCA-CoAs could play a role in the generation of muscle insulin resistance by glucose oversupply, either directly or via chronic activation of PKC-ε.

hyperglycemia; hyperinsulinemia; malonyl-CoA; long-chain acyl-CoA

systemic glucose oversupply (“glucose toxicity”) has been shown to generate muscle insulin resistance in rats (9, 20) and humans (5, 32, 43). One possible mechanism entails an inhibition of muscle fatty acid oxidation in the presence of increased glucose metabolism and a consequent accumulation of muscle lipids. This mechanism, which acts as a “reverse glucose-fatty acid cycle,” is hypothesized to involve malonyl-CoA (31, 33, 36). Malonyl-CoA inhibits carnitine palmitoyltransferase I, the enzyme that transports long-chain fatty acid CoA (LCA-CoA esters) into mitochondria for oxidation; therefore, increases in its concentration could lead to the retention of LCA-CoA in the cytosol of the muscle cell. There is increasing evidence that these interactions are linked to muscle insulin resistance. Evidence that malonyl-CoA is elevated in insulin-resistant states has been recently reviewed (33). Second, although the causal links are yet to be clarified, there is evidence that muscle lipid accumulation is associated with insulin resistance. For example, an increase in muscle triglyceride content has been observed in rats made insulin resistant by fat feeding (41) and glucose infusion (20), as well as in insulin-resistant KKAy and ob/ob mice and fa/fa and Goto-Kakizaki (GK) rats (reviewed in Ref. 33). In humans also, increased muscle triglyceride content has been associated with reduced insulin sensitivity (28, 29). Although triglycerides, the storage form of lipid, are a marker of excess lipid accumulation in the muscle cell, it is perhaps more likely that insulin resistance is linked to increases in the cytosolic concentration of LCA-CoA already defined above (31, 35). In support of this notion, chronic increases in tissue LCA-CoA concentration have been demonstrated in the insulin-resistant high fat-fed rat (8, 27). We feel it is important to extend investigation to the case of glucose toxicity to examine the commonality or otherwise of metabolic interactions that may lead to insulin resistance. Whether LCA-CoA accumulation accompanies the insulin resistance induced by chronic glucose oversupply is not known and will be investigated here.

A possible link between LCA-CoA accumulation and a decrease in skeletal muscle insulin sensitivity involves the activation of protein kinase C (PKC) isozymes (7, 38, 40). Sustained alterations in PKC activity, or cellular location, which is a marker for activation, have been observed in models of muscle insulin resistance such as denervated muscle (16), fructose-fed rats (10), genetically insulin-resistant rats (3), and rats fed a high-fat diet (38). It has been suggested that PKC activation may interfere with normal insulin signaling at the level of the insulin receptor and insulin receptor substrate-1 (25, 30) and/or at the level of metabolic enzymes such as glycogen synthase (1).

The aim of this study was therefore to test this LCA-CoA, malonyl-CoA, PKC hypothesis in the glucose oversupply model of insulin resistance. The results describe the effects of 1- and 4-day glucose infusions, both of which cause insulin resistance, on the levels of LCA-CoA and malonyl-CoA and the distribution of various novel and conventional PKC isozymes.

Research Design and Methods

Animals. Surgical and experimental procedures performed in these studies were approved by the Animal Experimentation Ethics Committee (Garvan Institute of Medical Research) and were in accordance with the National Health and Medical Research Council (NH&MRC) guidelines. High-fat diet-fed animals were included in this study to confirm the results of our previous studies in this model (35). Animals were fed a Western-style diet for 3 days to ensure that they are in a state of insulin resistance, after which they were randomized to one of three groups; glucose-infused (3 mg/kg per min), saline-infused, or sham surgery (n = 10 for each group). In the glucose-infused group, glucose was infused at a constant rate of 3 mg/kg per min for 4 days. All animals were sacrificed at 1 or 4 days after the end of treatment (n = 10 for each group).

Measurement of Shoulder Gait. The shoulder gait was recorded using a computerized gait analysis system, as described in detail previously (33). The shoulders were recorded at 10 Hz and were analyzed using the software program developed by the Laboratory for Biomechanics and Sensory Motor Control. The shoulder activity was quantified by calculating the mean peak shoulder gait velocity 48 hours after the end of treatment (n = 10 for each group).

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Adult male Wistar rats weighing ~350 g were maintained in separate cages with controlled 12:12-h light-dark cycle (light on 0600) and fed standard laboratory chow (rat and mouse cubes, Norco Stockfeeds, South Lismore, New South Wales, Australia). The experiments were performed on samples from a subset of rats used in another study in which muscle and adipose tissue responses to insulin were assessed after glucose infusion (20). To facilitate chronic infusion, rats were fitted with cannulas located in the right jugular vein and the left carotid artery as previously described (19). The cannulas were exteriorized at the vertex of the head and were attached to a swiveling infusion device (20). To maintain patency of the carotid cannula before study, we infused 0.9% saline with a slow delivery (4 ml/day) syringe pump (model A, Razel Scientific Instruments, Stamford, CT).

Chronic infusion. Chronic infusions were commenced 3 days after surgery. The infusion protocol was adapted from previously described protocols (13, 21). Rats were infused either with 50% glucose in water (50% dextrose, Viaflex, Baxter, Sydney, Australia) at a glucose infusion rate of 40 mg·kg⁻¹·min⁻¹ (1.6 ml/h) for 4 days, with 0.9% saline for 3 days and then 50% glucose in water at the glucose infusion rate of 40 mg·kg⁻¹·min⁻¹ for 1 day or with 0.9% saline at the same infusion rate as in the glucose-infused rats (control). Chronic infusion was performed via the carotid cannula with a peristaltic roller pump (Watson-Marlow model 101U/R, Falmouth, UK). A blood sample (400 µl) was collected via the jugular cannula initially and after 1 and 4 days of chronic infusion to determine plasma glucose and insulin concentrations. Analysis of tissue concentrations of skeletal muscle LCA-CoA (red quadriceps and soleus) and malonyl-CoA (red quadriceps) was performed on samples obtained from rats in which infusions continued to the completion of the study. The rats were killed with an overdose of pentobarbitone sodium (Lethabarb, 150 mg/kg iv injection), and the tissues were rapidly removed, freeze-damped, and stored at −70°C for subsequent analysis.

To assess chronic alterations in the cellular localization of specific PKC isozymes under the same conditions as the determination of insulin resistance during glucose infusion (20), skeletal muscle (red gastrocnemius) was collected from additional saline-infused and 1- and 4-day glucose-infused rats in which, over a 2-h period, the chronic infusions were progressively reduced in six 20-min steps to equalize plasma glucose and insulin levels among the groups. The equalization of plasma glucose and insulin enabled the assessment of chronic alterations in PKC, avoiding any possible influence of different prevailing plasma glucose (17) and/or plasma insulin levels (3, 42) at the time of measurement of PKC translocation.

Tissue LCA-CoA measurement. LCA-CoAs were extracted and purified from skeletal muscle for detection by reverse-phase HPLC as previously described (15). Individual species were quantified corresponding to the following peaks: palmitoyl (16:0), palmitylcoyl (16:1), stearoyl (18:0), oleoyl (18:1), linoleoyl (18:2), and linolenoyl (18:3) CoA. The results are reported as the sum of these six identified LCA-CoA species.

Malonyl-CoA measurement. Malonyl-CoA levels in skeletal muscle were determined radioenzymatically as previously described (36).

Immunoblot analysis of PKC content. Skeletal muscle samples were demembranated, extracted, and processed to obtain cytosolic and solubilized membrane fractions as described previously (38). Equivalent amounts of the two fractions were subjected to SDS-PAGE. Proteins were electroblotted onto nitrocellulose membranes, which were probed with rabbit anti-peptide antibodies specific for PKC-α, -ε, and -δ isoforms (Santa Cruz Biotechnology, Santa Cruz, CA) or PKC-δ (GBP-BRL, Life Technologies, Mulgrave, Australia), and then were incubated with horseradish peroxidase-labeled goat anti-rabbit antibody (BioRad, Sydney, Australia; Ref. 38). Detection and quantitation of the horseradish peroxidase-conjugated proteins were performed as described elsewhere (39).

Analytic methods. Blood and plasma glucose levels were determined by an immobilized glucose oxidase method (YSI 23 AM glucose analyzer, Yellow Springs Instruments, Yellow Springs, OH) in 25-µl samples. Plasma insulin levels were measured in a double-antibody radioimmunoassay with a polyclonal antiserum and purified rat insulin as standard. Plasma nonesterified fatty acids (NEFA) were measured with an acyl-CoA oxidase-based colorimetric kit (WAKO NEFA-C, WAKO Pure Chemical Industries, Osaka, Japan).

Statistical analysis. Statistical analyses were performed with a statistics package (Statview, Abacus Concepts, Berkeley, CA). Differences among relevant groups were assessed by an unpaired Student’s t-test. A priori individual comparisons were made of 1- and 4-day glucose-infused groups vs. saline-infused controls and 1- vs. 4-day glucose-infused groups. Group sizes were 4–5 rats for LCA-CoA levels and 5–6 rats for other measurements.

RESULTS

Lipid accumulation and alterations in cellular PKC localization were examined in muscle obtained from 1- and 4-day glucose-infused rats and saline-infused rats, which served as controls. The results are interpreted here in light of the previous demonstration that insulin resistance is induced in muscle to equivalent degrees after 1 or 4 days of glucose infusion (20).

Plasma glucose and insulin concentrations. Mean plasma glucose and insulin concentrations during chronic glucose or saline infusions are shown in Table 1. After 1 day of glucose infusion, rats were hyperglycemic with a twofold elevation in plasma glucose compared with saline-infused rats (P < 0.001). They were also hyperinsulinemic with a 9.5-fold elevation in plasma insulin levels compared with controls (P < 0.001). Rats from the 4-day glucose-infused group were also hyperinsulinemic with a sixfold elevation in plasma insulin levels compared with saline-infused rats (P < 0.01). However, their plasma glucose concentrations had declined to levels not significantly different from those of the saline-infused group (Table 1), indicating increased systemic glucose clearance in the 4- vs. 1-day

Table 1. Final plasma parameters of saline-infused control, 1-day glucose-infused, and 4-day glucose-infused rats while infusion was maintained

<table>
<thead>
<tr>
<th>Plasma Parameter</th>
<th>Saline</th>
<th>1-Day Glucose</th>
<th>4-Day Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>6.9 ± 0.2</td>
<td>14.1 ± 1.6†</td>
<td>7.5 ± 0.3§</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>25 ± 5</td>
<td>237 ± 28†</td>
<td>153 ± 21‡</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>0.45 ± 0.05</td>
<td>0.21 ± 0.03*</td>
<td>0.22 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5–6 rats/experimental condition. NEFA, nonesterified fatty acid. †P < 0.01 and §P < 0.001 vs. saline-infused controls. ‡P < 0.05 and ¶P < 0.001 for 4-day vs. 1-day glucose-infused rats.
infused groups. Although plasma glucose levels are lower in the final stages of 4-day infusion compared with 1-day infusion, the rate of systemic glucose disappearance to tissues should be similar or greater (after urine loss is considered) at the 1- and 4-day time points and should approximately equal the intravenous glucose infusion rate if hepatic glucose output is suppressed. NEFA levels were suppressed by chronic glucose infusion, consistent with induced hyperinsulinemia (Table 1).

LCA-CoA. The effects of glucose infusion on total (whole cell) LCA-CoA levels in red quadriceps muscle are shown in Fig. 1. Total levels were determined as the sum of all measured individual species (palmitoyl, palmitoleoyl, stearoyl, oleoyl, linoleoyl, and linolenoyl) of LCA-CoA. Consistent with the possibility that LCA-CoA may influence the development of insulin resistance, the concentration of LCA-CoA was increased 1.3-fold at 1 day and 1.4-fold at 4 days (both P < 0.001 vs. control) of glucose infusion. Similar increases were also observed for LCA-CoA in soleus muscle (data not shown), i.e., 1.8-fold at 1 day (P < 0.03) and 1.7-fold at 4 days (P < 0.02 vs. control) of glucose infusion.

Malonyl-CoA. The concentrations of malonyl-CoA in red quadriceps from rats chronically infused with glucose or saline are shown in Fig. 2. Malonyl-CoA levels were increased by 1.7-fold after 1 day (P < 0.01) and by 2.2-fold after 4 days (P < 0.001) of glucose infusion.

PKC. Chronic alterations in the cellular localization of PKC were assessed after complete cessation of saline and glucose infusions. Under these conditions, plasma glucose and insulin levels were similar in each group (plasma glucose: 6.6 ± 0.3, 6.8 ± 0.2, and 7.0 ± 0.2 mM; plasma insulin: 28 ± 3, 36 ± 13, and 28 ± 6 mU/l in saline, 1-day, and 4-day glucose-infused rats, respectively).

Immunoblots were carried out on cytosolic and membrane fractions prepared from red gastrocnemius muscle to determine the subcellular distribution of PKC-α, -δ, -ε, and -θ. Immunoblots obtained with antibodies specific for each of these isozymes are shown in Fig. 3. As previously observed (38, 39), the PKCs were predominantly cytosolic in muscle from control animals (87, 89, 73, and 89% of total for PKC-α, -δ, -ε, and -θ, respectively). The sum of the cytosolic and membrane-associated fraction is a measure of total PKC expres-
sion for each isozyme. The translocation of PKC isozymes from cytosolic to membrane fractions is often taken as an indication of PKC activation (38, 44). This was assessed by the ratio of membrane-associated to cytosolic PKC content (Table 2).

Alterations were observed in the localization of PKC-ε in muscle of the glucose-infused rat model of insulin resistance. PKC-ε located in the membrane fraction was increased after both 1 day (P < 0.01) and 4 days (P < 0.04) of glucose infusion compared with the control group. This was accompanied by decreased levels of PKC-ε in the cytosolic fraction, and as a result the ratio of membrane to cytosolic PKC-ε was increased twofold after both 1 day (P < 0.01) and 4 days (P < 0.03) of glucose infusion (Table 2), indicating chronic activation of the kinase. The proportion of total PKC-ε in the cytosol was reduced by 22% at 1 day (P < 0.001) and 20% at 4 days (P < 0.02) vs. controls. The reduction in PKC-ε in the cytosol was offset by the increase in membrane-associated enzyme, such that total levels of immunoreactive PKC-ε were not significantly different after glucose infusion (data not shown).

Under the conditions of assessment, there were no changes observed in cytosolic, membrane-associated, or total levels of PKC-α, -β, or -δ (data not shown) after either 1 or 4 days of glucose infusion. Consequently, the ratios of membrane to cytosolic PKC-α, -β, and -δ were not different among the groups, suggesting that these kinases were not chronically activated during glucose infusion (Table 2).

**DISCUSSION**

The effects of glucose infusion on muscle lipid metabolites and the cellular localization of specific PKC isoforms were investigated. The main findings of this study are that prolonged (1 or 4 day) glucose infusion leads, in skeletal muscle of rats, to increased levels of 1) LCA-CoA and 2) malonyl-CoA and 3) to an increase in PKC-ε in the membrane-associated fraction, suggesting sustained activation of this enzyme. We have previously shown that prolonged glucose infusion leads to muscle triglyceride accumulation and insulin resistance (20). The present findings provide a possible mechanistic link whereby glucose infusion might lead to these events. They suggest that glucose infusion causes increased levels of malonyl-CoA, which by inhibiting the transfer of LCA-CoA into mitochondria, could contribute to its accumulation in the cytosol. Such increases in LCA-CoA or a related lipid product might activate one or more PKC isoforms (3, 16, 38), which, in turn, may induce insulin resistance (7, 38, 40). Additionally, from a technical viewpoint, LCA-CoA determination in muscle tissue samples is, in comparison with triglyceride determination, much less susceptible to artifactual contamination from adipose cell infiltration (11). Therefore, our results strengthen the possibility that lipid accumulation is a possible mediator of, or contributor to, the insulin resistance induced by glucose infusion.

In the proposed scheme, malonyl-CoA is seen to act as a mediating signal between tissue glucose supply and LCA-CoA levels through regulation of mitochondrial LCA-CoA transport. An alternative role is not readily apparent because malonyl-CoA cannot be further synthesized to fatty acids in muscle (6). In addition, a close inverse correlation has been demonstrated between malonyl-CoA levels and the rate of fatty acid oxidation in perfused heart (34) and isolated cardiac myocytes (4), which are similarly nonlipogenic. Thus, even in the presence of a low plasma FFA level, the accumulation of lipid in muscle of glucose-infused rats could be initiated by an insulin- and glucose-induced increase in malonyl-CoA. Because an increase in malonyl-CoA should inhibit LCA-CoA transport into mitochondria, this suggests that the LCA-CoA concentration in the cytosol was increased even more than indicated by the whole cell measurement. We would propose that the increase in cytosolic LCA-CoAs, together with expected increases in glycerol-phosphate, and the activities of enzymes such as glycerol-phosphate acyltransferase and phosphatidic acid phosphatase under hyperinsulinemic conditions, account for the observed increase in triglyceride synthesis (20).

Other factors may also contribute to an increase in cytosolic LCA-CoA in muscle of glucose-infused rats. One of these is augmented lipid synthesis in the liver, with a resultant increased export of very low-density lipoproteins to peripheral tissues. Another is the increase in muscle triglyceride mass itself, which would increase intramuscular LCA-CoA by mass action if its fractional turnover was maintained. It is significant that muscle LCA-CoA levels increased in glucose-infused groups despite a decrease in plasma NEFA levels (Table 1), suggesting alteration in intracellular LCA-CoA generation or metabolism. Regardless of the origin, this is the first demonstration that glucose-infused rats developed insulin resistance in vivo via a mechanism potentially involving LCA-CoA accumulation in skeletal muscle. The data also support the hypothesis that a disturbance in lipid metabolism is an important factor in the development of insulin resistance and its associated clinical disorders in humans (24).

The results do not exclude the possibility that other mechanisms also contribute to the insulin resistance caused by glucose infusion; for example, increased glucose flux through the hexosamine biosynthesis pathway, a normally minor pathway of glucose metabolism,

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**Table 2.** Comparison of PKC (isozyme) localization as expressed by ratio of membrane-associated to cytosolic PKC content in skeletal muscle from previously saline- and glucose-infused rats

<table>
<thead>
<tr>
<th>PKC Isozyme</th>
<th>Saline</th>
<th>1-Day Glucose</th>
<th>4-Day Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>ε</td>
<td>0.38 ± 0.04</td>
<td>0.77 ± 0.08†</td>
<td>0.76 ± 0.14*</td>
</tr>
<tr>
<td>θ</td>
<td>0.12 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>δ</td>
<td>0.01 ± 0.11</td>
<td>0.10 ± 0.10</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats/experimental condition. Red gastrocnemius was the skeletal muscle used. *P < 0.03 and †P < 0.01 vs. saline-infused controls.
has been shown to cause impaired glucose transport in rat adipocytes (12, 23). Similarly, glucosamine accumulation may be a factor in the etiology of insulin resistance induced in skeletal muscle in vivo by glucose oversupply. Interestingly, increases in plasma FFA, which presumably lead to increases in intramuscular LCA-CoA, have been shown to increase the generation of glucosamine end products through activation of the hexosamine pathway in rats undergoing a euglycemic-hyperinsulinemic clamp (14). In addition, activation of the hexosamine pathway has been associated with enhanced PKC activity (12). The interaction of the hexosamine pathway and the lipid-PKC mechanism proposed here to cause insulin resistance requires further study, although the fact that plasma FFA levels decreased during glucose infusion makes an interaction of the hexosamine pathway and plasma FFA-induced enhancement of PKC seem unlikely.

Our findings are consistent with the notion that lipid accumulation leads to chronic activation of PKC-ε in muscle of glucose-infused rats. The increase in cytosolic LCA-CoA and a related lipid product, such as diacylglycerol, which is elevated in other models of insulin resistance (3, 10, 16, 38), may activate PKC-ε and subsequently generate insulin resistance. Activation of this isozyme, one of the major PKCs in skeletal muscle (37, 42), is common to insulin-resistant muscle of high-fat-fed (38), obese-Zucker (fa/fa), obese-aged, and GK rats (3). In the genetic models, the activation of PKC-ε is accompanied by alterations in several other PKC isozymes and in the fat-fed rats by alterations in PKC-δ (38). In the current study, the cellular distribution of PKC isozymes was assessed after plasma glucose and insulin equalization, suggesting that these factors do not actually play a role in causing the changes observed in PKC-ε. Altered distribution of the other novel PKCs present in skeletal muscle, PKC-δ and -6, and of PKC-α, the major conventional isozyme in muscle, was not observed here. This may be due either to differential compartmentalization within muscle cells of PKCs and the lipid pools affected by glucose infusion or a greater sensitivity of PKC-ε to changes in the particular lipid species affected, as discussed previously (39).

The findings presented here do not eliminate a possible involvement of PKC-δ in impaired glucose disposal during acute insulin stimulation. As we have previously postulated (38), insulin-stimulated lipid metabolism may potentiate the accumulation of PKC-δ-activating species, which could lead to an inhibitory effect of the kinase on glucose uptake. It is possible that the particular lipid species responsible for the cytosolic downregulation of PKC-δ and -6 in fat-fed rats (38) is not elevated by glucose infusion and that the chronic activation of PKC-δ and -6 is not necessary for the generation of insulin resistance. An alternative explanation, however, is that activation of PKC-δ and -6 occurs during glucose infusion but is rapidly reversed upon equalization of plasma glucose and insulin concentrations before PKC determinations. The absence of downregulation in the case of chronic glucose infusion may be due to the persistent hyperinsulinemia (except for the final 2 h of study), which increases mRNA levels of several PKC isozymes in skeletal muscle, compensating for proteolysis of activated PKC (2). The maintenance of total levels of activated PKC-ε is most likely a reflection of the specific resistance of this kinase to proteolytic degradation (18, 44).

We have determined PKC localization in our study groups after normalization of plasma glucose and insulin levels, to avoid secondary effects due to prevailing insulin and/or glucose levels (3, 17, 42), which might have confounded between-group comparisons. However, it is not known whether LCA-CoA levels are also reduced under these conditions. Therefore, any postulated effect of LCA-CoA on PKC translocation (22, 26) might have been lessened over the 2-h normalization period, and so it would not be detected in our study. However, it is unlikely that the downregulation of a specific PKC, such as of PKC-δ as seen in high fat-fed rats (39), could be reversed in 2 h, and so the absence of such an effect here is probably not an artifact of this normalization procedure.

The translocation of PKC-ε seen in this study has also been observed in previous work concerning other animal models of diabetes and insulin resistance (3, 39). However, in the diabetic GK rat, membrane levels of several other PKC isozymes were also found to be higher than those of control rats, and it was concluded that hyperinsulinemia played a role in such translocation (3). We have observed more specific changes suggestive of chronic activation of PKC-ε and PKC-δ in the high-fat-fed rat model of insulin resistance, which does not exhibit overt hyperinsulinemia or hyperglycemia (39), and these correlated with muscle lipid levels, suggesting that increased lipid availability might play a primary role in insulin resistance through PKC activation. The current study further supports such a role for PKC-ε, as secondary effects of insulin and glucose were again discounted.

Consistent with the effects of elevated muscle lipids in high fat-fed rats (38), insulin resistance induced by lipid accumulation during glucose infusion was not associated with alterations in the activation state of PKC-α. Recent distribution studies by Schmitz-Peiffer et al. (38) and others (3) have not suggested a role for the atypical PKC-ζ, also present in skeletal muscle (37), and this isozyme was therefore not investigated here.

In conclusion, the concentration of LCA-CoA, the metabolically active form of lipid, is increased in muscle of insulin-resistant chronically glucose-infused rats. Elevated muscle malonyl-CoA levels during glucose infusion may influence the development of insulin resistance by increasing the cytosolic LCA-CoA concentration. A dual mechanism of 1) malonyl-CoA inhibition of LCA-CoA transport into mitochondria and 2) turnover of an increased triglyceride mass may operate to increase LCA-CoA in the cytosol. Alteration in the subcellular distribution of PKC-ε in muscle is consistent with the possibility that the lipid-induced activation of this isozyme mediates inhibitory effects on insulin action during glucose infusion. Taken with other studies (8, 38, 39, 41), it is apparent that there are
similarities (muscle triglyceride and LCA-CoA accumulation and isozyme-specific PKC activation) accompanying muscle insulin resistance induced by both systemic lipid and glucose oversupply in rats, suggestive of common, or overlapping, mechanisms. This would be consistent with causative roles of LCA-CoA accumulation and PKC activation in muscle insulin resistance, but further work is necessary to establish this and to elucidate protein substrates involved in PKC-dependent mechanisms.

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