Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in human skeletal muscle

Blake B. Rasmussen,1,2 Ulf C. Holmbäck,1,3 Elena Volpi,4,5 Beatrice Morio-Liondore,1,6 Douglas Paddon-Jones,1 and Robert R. Wolfe1

1Department of Surgery, University of Texas Medical Branch, Galveston, Texas, USA
2Department of Kinesiology, University of Southern California, Los Angeles, California, USA
3Department of Medicine, Nutrition, Uppsala University Hospital, Uppsala, Sweden
4Department of Internal Medicine, University of Texas Medical Branch, Galveston, Texas, USA
5Department of Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA
6Unité Métabolismes Protéique et Energétique, Unité Mixte de Recherche, INRA/Université d’Auvergne, Laboratoire de Nutrition Humaine, Clermont Ferrand, France

Physiological hyperglycemia with hyperinsulinemia reduces fat oxidation in skeletal muscle. The mechanism responsible for this decrease in fat oxidation in human muscle is not known and may contribute to the development of insulin resistance. We hypothesized that the transfer of long-chain fatty acids (LCFAs) into the mitochondria via carnitine palmitoyltransferase-1 (CPT-1) is inhibited by increased malonyl coenzyme A (malonyl-CoA) (a known potent inhibitor of CPT-1) in human muscle during hyperglycemia with hyperinsulinemia. We studied six healthy subjects after an overnight fast and during an induced 5-hour period of hyperglycemia with hyperinsulinemia. Muscle fatty acid oxidation was calculated using stable isotope methodology combined with blood sampling from the femoral artery and vein of one leg. Muscle functional CPT-1 activity was assessed by concurrently infusing an LCFA tracer and a CPT-independent medium-chain fatty acid tracer. Muscle biopsies were obtained from the vastus lateralis after the periods of fasting and hyperglycemia with hyperinsulinemia. Hyperglycemia with hyperinsulinemia decreased LCFA oxidation, but had no effect on LCFA uptake or medium-chain fatty acid oxidation across the leg. Malonyl-CoA concentration significantly increased from 0.13 ± 0.01 to 0.35 ± 0.07 nmol/g during hyperglycemia with hyperinsulinemia. We conclude that hyperglycemia with hyperinsulinemia increases malonyl-CoA, inhibits functional CPT-1 activity, and shunts LCFA away from oxidation and toward storage in human muscle.


Introduction
Insulin resistance and type 2 diabetes are characterized by hyperglycemia with hyperinsulinemia, elevated plasma FFA levels, a reduced ability to oxidize fat, and an accumulation of fat within skeletal muscle (1, 2). The increase in muscle fat content is highly associated with insulin resistance (3, 4). Therefore, a better understanding of the mechanisms that lead to these metabolic alter-

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Address correspondence to: Blake B. Rasmussen, Department of Kinesiology, University of Southern California, University Park, PED 107, Los Angeles, California 90089-0652, USA. Phone: (213) 821-2846; Fax (213) 740-7909; E-mail: blakeras@usc.edu.

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Nonstandard abbreviations used: long-chain fatty acid (LCFA); carnitine palmitoyl transferase-1 (CPT-1); malonyl coenzyme A (malonyl-CoA); acetyl coenzyme A carboxylase (ACC); University of Texas Medical Branch (UTMB); oral glucose tolerance test (OGTT); 5-aminoimidazole-4-carboxamide-1β-D-ribofuranoside (AICAR); AMP-activated protein kinase (AMPK).
diately in the synthesis of LCFA. Malonyl-CoA allosterically binds to CPT-1, inhibiting the enzyme and the transfer of LCFA into the mitochondria (7). An accumulation of malonyl-CoA in liver and adipose tissue stimulates LCFA synthesis while concomitantly inhibiting LCFA oxidation. McGarry et al. (7) have also identified malonyl-CoA in non-lipogenic tissues such as heart and skeletal muscle. Since LCFAs are not significantly synthesized in these tissues, malonyl-CoA appears to play a role in energy balance by affecting CPT-1 and consequently the rates of LCFA oxidation and/or reesterification into muscle triglyceride (8–10).

In a recent study (11) reports that mice lacking the ACC enzyme (and thus having no malonyl-CoA) have elevated rates of fat oxidation and reduced fat storage, suggesting that ACC and malonyl-CoA play an important role in overall energy balance. Despite these recent advances, it is still not known whether malonyl-CoA regulates fatty acid oxidation in human skeletal muscle.

This study was designed to determine whether simulating the metabolic profile of insulin resistance by inducing physiological hyperglycemia (156 ± 4 mg/dl) and hyperinsulinemia (43 ± 7 µU/ml) while maintaining constant FFA levels (0.56 ± 0.10 mM) would: (a) increase human muscle malonyl-CoA content; (b) inhibit LCFA entry into the mitochondria of muscle (i.e., inhibit functional CPT-1 activity); and (c) shunt LCFA away from oxidation and toward intramuscular storage.

**Methods**

**Subjects.** Six volunteers (four males and two females) gave informed written consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch (UTMB) at Galveston. Each subject was screened for determination of health status and given an oral glucose tolerance test (OGTT) at the General Clinical Research Center at UTMB prior to the study. Subjects were not insulin resistant (i.e., had a normal OGTT response) and refrained from physical exercise for at least 24 hours prior to participating in the study. Subject characteristics, lipid profile, and OGTT results are listed in Table 1. Leg volume was calculated using an anthropometric method as previously described (12).

**Study protocol.** A schematic for the study protocol is shown in Figure 1. Briefly, subjects arrived at the UTMB General Clinical Research Center the evening prior to the study and consumed an evening meal at approximately 9 pm. The study began the next morning at approximately 6 am with the insertion of femoral arterial and venous catheters in one leg and placement of one catheter in each arm. Shortly after insertion of the catheters, background blood and breath samples were obtained, a priming dose of $^{13}$C and $^{14}$C bicarbonate was given, and a continuous infusion of an LCFA (1$^{13}$C-octanoate) and a medium-chain fatty acid (1$^{14}$C-octanoate) was initiated. Breath was collected throughout the experiment, and blood samples were obtained from the femoral artery and the femoral vein at 75, 90, 105, and 115 minutes. The first sampling period was labeled the “fasting period,” and also included the infusion of indocyanine green to measure blood flow. At the end of the fasting period (120 minutes after infusion), a muscle biopsy was taken from the lateral portion of the vastus lateralis muscle, about 20 cm above the knee, using a 5-mm Bergström biopsy needle (Stille, Stockholm, Sweden). Following the biopsy, the tracer infusions were stopped and a 20% dextrose infusion was initiated and varied to achieve blood glucose values at approximately 150 mg/dl. In addition, heparin and Intralipid (Baxter, Deerfield, Illinois, USA) were started in order to prevent the expected decrease in plasma FFA. Insulin was not infused, and the increase in plasma insulin concentrations during the dextrose infusion was the result of endogenous stimulation. Three hours after the dextrose infusion was initiated, new blood and breath background samples were obtained and the bicarbonate pool was primed with another bolus injection of $^{13}$C and $^{14}$C bicarbonate. Following this, the oleate and octanoate tracer infusions were restarted as described previously for the fasting period. Blood and breath collection during the period of physiological hyperglycemia with hyperinsulinemia was done identically as during the fasting period, at 375, 390, 405, and 415 minutes after initial tracer infusion. Blood flow was also measured and another muscle biopsy obtained at 420 minutes.

**Infusates.** The bicarbonate pools were primed with NaH$^{12}$CO$_3$ (3 µmol/kg; Cambridge Isotope Laboratories Inc., Andover, Massachusetts, USA) and NaH$^{14}$CO$_3$ (25 nCi/kg; NEN Life Science Products Inc., Boston, Massachusetts, USA) prior to the fasting period and the period of hyperglycemia with hyperinsulinemia. The infusion rate of $^{13}$C-octanoate (Cambridge Isotope Laboratories Inc.) was 0.04 µmol/kg/min in both periods. Prior to infusion.

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**Table 1**

<table>
<thead>
<tr>
<th>Subject characteristics, lipid profile, and OGTT data</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
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<tr>
<td>Height (m)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
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<tr>
<td>Leg volume (l)</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
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<tr>
<td>Plasma triglycerides (mg/dl)</td>
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<tr>
<td>OGTT basal (mg/dl)</td>
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<tr>
<td>OGTT 2-hour (mg/dl)</td>
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<td>OGTT 6-hour (mg/dl)</td>
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OGTT consisted of a 75-gram bolus ingestion of sucrose. OGTT basal is blood sampled prior to the bolus, and OGTTs 2-hour is blood sampled 2 hours after ingestion. Blood samples for cholesterol and triglyceride measurements were obtained following a 12-hour overnight fast. BMI, body mass index. Values are mean ± SEM.
of oleate, the oleate tracer was bound to 5% human albumin to form a solution with an oleate concentration of approximately 2 µmol/ml. A primed (16 nCi/kg), continuous infusion (0.4 nCi/kg/min) of 1-14C-octanoate (NEN Life Science Products Inc.) was also infused along with the oleate tracer. A variable infusion of 20% dextrose (6.7 ± 0.6 mg/kg/min) was performed to clamp blood glucose concentration at approximately 150 mg/dl. A primed (7 U/kg), continuous infusion of heparin (7 U/kg/h) and an infusion of lipids (Intralipid, 0.7 ml/kg/min) was used to prevent the expected insulin-induced decrease in plasma FFA concentration. To measure leg blood flow, a continuous infusion of indocyanine green was given at a rate of 0.5 mg/min.

**Isotope analysis.** For determination of plasma arterial and venous oleate enrichments, FFAs were extracted using a solid-phase extraction method and derivatized to their methyl esters (13). Oleate concentration was measured on a gas chromatograph with flame ionization detection using heptadecanoic acid as an internal standard. On average, the arterial oleate concentration was 33% ± 7% and 28% ± 3% of total FFAs during the periods of fasting and physiological hyperglycemia with hyperinsulinemia, respectively. Oleate enrichment (tracer/tracee ratio) was determined by gas chromatography/mass spectrometry (Hewlett-Packard, Palo Alto, California, USA) by selectively monitoring ions mass-to-charge 296 and 297.

Breath samples were collected, and 10 ml of expired air was injected into evacuated tubes. Breath enrichment was analyzed using an isotope ratio mass spectrometer (SIRA; VG Isotech, Cheshire, United Kingdom). To determine 14C-octanoate specific activity in femoral artery and vein samples, 1 ml of 6 N HCl was added to 1 ml of plasma. After waiting for all CO2 to be eliminated, scintillation fluid was added and octanoate specific activity was determined with a liquid scintillation counter.

The specific activity of 14CO2 in expired breath samples was determined after trapping CO2 by bubbling expired air through 3 ml of a 1:4:9 solution of phenolphthalein, benzethonium hydroxide, and absolute ethanol, titrated to trap 1 mmol of CO2 as previously described (13). Ten milliliters of scintillation fluid was added to the solution and then counted in a liquid scintillation counter. Femoral arterial and venous 14CO2 specific activity was measured by a method described previously (14). Briefly, a filter paper (1 × 2 cm) was glued to a cap from a 20-ml glass liquid scintillation vial. The filter paper was saturated with 75 µl of ethanolamine immediately before blood sampling. Femoral arterial and venous blood (5 ml) was added to the vials, which contained 1 ml of 4.5 M lactic acid. Vials were immediately capped and incubated for 24 hours to allow the CO2 to be trapped on the filter paper. Following the 24-hour incubation, the filter paper was quickly transferred to another scintillation vial, 10 ml of scintillation fluid was added, and the vials were kept in the dark for 2 days before being analyzed in a liquid scintillation counter.

**Kinetic calculations.** Whole-body fat and glucose oxidation rates were calculated from VO2 and VCO2 measurements obtained from indirect calorimetry (Vmax 229; SensorMedics Corp., Yorba Linda, California, USA) and incorporation of these values into established stoichiometric equations (13). Total fatty acid oxidation was determined by converting the rate of total fat oxidation to its molar equivalent and assuming that the average molecular weight of triglyceride is 860 g/mol. The molar rate of triglyceride oxidation was then multiplied by 3 because each triglyceride molecule contains 3 mol of fatty acids. Whole-body plasma oleate and octanoate oxidation rates were calculated from the equation: oxidation (µmol/kg/min) = (ECO2 × VCO2)/Eo × a, where ECO2 is the enrichment or specific activity of breath CO2, Eo is the arterial enrichment or specific activity of oleate

![Figure 1](http://www.jci.org)  
**Figure 1**  
Study protocol consisting of a fasting period and a period of physiological hyperglycemia with hyperinsulinemia (PHGI). ICG, indocyanine green.
Table 2

<table>
<thead>
<tr>
<th>Substrate and hormone concentrations during the fasting period and during physiological hyperglycemia with hyperinsulinemia</th>
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<tbody>
<tr>
<td><strong>Fasting</strong></td>
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<tr>
<td>Blood glucose (mg/dl)</td>
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<tr>
<td>Glucose uptake (mg/min)</td>
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<tr>
<td>Plasma FFA (mmol/l)</td>
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<tr>
<td>β-hydroxybutyrate (mg/dl)</td>
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<tr>
<td>Plasma insulin (µIU/ml)</td>
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Values are mean ± SEM. *Significantly different from the fasting period (P < 0.05). Glucose uptake values are for one leg.

Arterial insulin concentrations were measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, California, USA) and were averaged over the last hour of each period. Blood glucose was determined by a glucose oxidase method immediately after sampling using a YSI 2700 analyzer (Yellow Springs Instruments, Yellow Springs, Ohio, USA). Plasma FFA concentrations were measured using an enzymatic, colorimetric kit (Wako NEFA; Wako Chemicals GmbH, Neuss, Germany) following blood collection in tubes containing a lipase inhibitor (0.4 mM K3-EDTA). This procedure has been previously shown to inhibit in vitro lipolysis of plasma triglycerides in human subjects given heparin (16). Plasma β-hydroxybutyrate was determined enzymatically (Sigma-Aldrich, St. Louis, Missouri, USA). Leg blood and plasma flow was determined from blood samples collected during a continuous infusion of indocyanine green (17). Sera from the blood samples were analyzed in a spectrophotometer with absorbance set at 805 nm. Blood CO2 concentration was measured immediately after sampling by using a 965 Ciba Corning CO2 analyzer (Ciba-Corning Diagnostics Corp., Medfield, Massachusetts, USA).

Blood glucose uptake across the leg was calculated by multiplying the blood flow by the arterial-venous difference of glucose.

Statistical analysis. The effects of physiological hyperglycemia with hyperinsulinemia on the substrate, hormone, and malonyl-CoA concentrations and the fatty acid kinetic variables were assessed using the two-tailed, paired Student t test. Data are expressed as mean ± SEM. Differences were considered significant at P < 0.05.

Results

Substrate and insulin data are reported in Table 2. Physiological hyperglycemia with hyperinsulinemia significantly increased arterial glucose concentration, glucose uptake across the leg, and plasma insulin levels (P < 0.05). Plasma FFA levels remained constant because of the simultaneous infusion of lipid and heparin throughout the period of physiological hyperglycemia with hyperinsulinemia, whereas the ketone β-hydroxybutyrate was significantly decreased (P < 0.05).

Oleate enrichments in the femoral artery during the fasting period and during the period of physiological hyperglycemia with hyperinsulinemia were in steady state (1.9% ± 0.5% and 1.4% ± 0.2%, respectively). Octanoate specific activity in the femoral artery was 75 ± 9 dpm/ml of blood during the fasting period and 89 ± 7 dpm/ml during the physiological hyperglycemia with hyperinsulinemia period (P > 0.05). Breath 13CO2 enrichments were 0.007% ± 0.001% and 0.0007% ± 0.0003% during the fasting and physiological hyperglycemia with hyperinsulinemia periods, respectively (P > 0.05). Breath 13CO2 specific activity was 2,710 ± 109 dpm/mmol of CO2 during the fasting period and 2,460 ± 146 dpm/mmol during the physiological hyperglycemia with hyperinsulinemia period (P < 0.05).
Whole-body total fatty acid oxidation and the respiratory quotient (VCO₂/VO₂) as measured by indirect calorimetry are presented in Table 3. Physiological hyperglycemia with hyperinsulinemia significantly increased the respiratory quotient and decreased total fat oxidation (P < 0.05). On the other hand, whole-body total carbohydrate oxidation increased significantly during the period of physiological hyperglycemia with hyperinsulinemia (Table 3, P < 0.05).

Table 3
Whole-body plasma and total fatty acid oxidation during the fasting period and during physiological hyperglycemia with hyperinsulinemia

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>PHGI</th>
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<tr>
<td>Indirect calorimetry:</td>
<td></td>
<td></td>
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<tr>
<td>Respiratory quotient</td>
<td>0.77 ± 0.01</td>
<td>0.94 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total fat oxidation</td>
<td>3.6 ± 0.2</td>
<td>0.5 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total carbohydrate oxidation</td>
<td>4.2 ± 0.9</td>
<td>18.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isotopic determination:</td>
<td></td>
<td></td>
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<tr>
<td>Plasma oleate oxidation</td>
<td>1.0 ± 0.2</td>
<td>0.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma FFA oxidation</td>
<td>3.0 ± 0.3</td>
<td>0.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma octanoate oxidation</td>
<td>4.9 ± 0.2</td>
<td>5.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. All oxidation rates are in µmol/kg/min, except for octanoate (pmol/kg/min). *Significantly different from fasting values (P < 0.05).

Discussion
The primary finding of the present study is that human skeletal muscle malonyl-CoA is increased during physiological hyperglycemia with hyperinsulinemia and that this increase is associated with the inhibition of functional CPT-1 activity and reduced muscle LCFA oxidation. Nonetheless, fatty acid uptake was not inhibited, meaning that intramuscular esterification of LCFA was accelerated. The simultaneous infusion of an LCFA tracer (oleate) and a CPT-independent medium-chain fatty acid tracer (octanoate) in combination with the limb balance technique allowed us to assess, in vivo, functional CPT-1 activity in human skeletal muscle.

Physiological hyperglycemia with hyperinsulinemia, like elevated FFA levels, is common in obese and type 2 diabetic humans. The muscles from these subjects have a decreased capacity to oxidize fat and significantly increased levels of intramuscular triglyceride stores (3). It has been proposed that elevated fat stores within muscle may play a role in the development of insulin resistance (4, 18, 19), although a precise mechanism has yet to be identified. Our data provide the first evidence in humans that physiological hyperglycemia with hyperinsulinemia increases muscle malonyl-CoA concurrently with the decrease in LCFA oxidation. We also found that the fractional extraction of LCFA did not change. This suggests that the increase in malonyl-CoA shunted LCFA away from oxidation and toward storage inside the muscle cell, thus providing a likely explanation for the mechanisms underlying the effects of physiological hyperglycemia with hyperinsulinemia on fat metabolism. In fact, we have estimated that in one leg, the total amount of LCFA shunted away from oxidation and toward reesterification and storage during the 5 hours of physiological hyperglycemia with hyperinsulinemia is significant and amounts to approximately 6.5 mmol/leg. We realize that not all LCFA uptake across the leg can be attributed to skeletal muscle, but it is reasonable to assume that the extent of suppression of oxidation corresponds to the amount of LCFA shunted into the intramuscular triglyceride pool. In agreement with our results, using the proton nuclear magnetic resonance (¹H-NMR) spectroscopy technique, it has been shown that a 4-hour hyperinsulinemic, euglycemic clamp with
Skeletal muscle malonyl-CoA concentration during conditions of fast-resistance during conditions of hyperglycemia (25). In Figure 3, we may play a role in the development of muscle insulin resistance (22, 23) and during refeeding following fasting (28, 29), during electrical hind limb stimulation (30, 31), during exercise (15, 32), during the post-exercise recovery state (33), and when exposed to 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an AMP analogue (14, 34). The decrease in muscle malonyl-CoA during these conditions relieves the inhibition of malonyl-CoA on CPT-1 and allows for increased rates of LCFA oxidation. Muscle contraction and AICAR administration lower malonyl-CoA by activating AMP-activated protein kinase (AMPK), which phosphorylates and inactivates ACC (35). On the other hand, fasting appears to lower malonyl-CoA by allosterically inhibiting ACC (due to an increased concentration of acyl-CoA and a reduction in citrate levels) (24). Recent studies have measured human muscle malonyl-CoA following exercise. Two of the studies reported no change in malonyl-CoA concentration (36, 37), whereas a more recent study has reported a significant decrease in malonyl-CoA and ACC activity following exercise (38). The latter study appears to be consistent with studies reporting an increase in human muscle AMPK activity with exercise (39, 40) and supports the notion that a decrease in malonyl-CoA during exercise allows for an increase in the rate of LCFA oxidation.

A well-known paradox regarding malonyl-CoA should be acknowledged. For example, the IC₅₀, (the malonyl-CoA concentration required for 50% inhibition of CPT-1 activity) for malonyl-CoA is much lower than the physiologically reported values, and one would expect CPT-1 to be continually inhibited regardless of any change in muscle malonyl-CoA concentration. One possible explanation is that there are two pools of malonyl-CoA in muscle (i.e., cytoplasmic and mitochondrial), with the cytoplasmic pool being much smaller than the mitochondrial. There is recent evidence suggesting that ACC is embedded in the outer mitochondrial membrane and may be in close proximity to CPT-1 (41). Thus, as suggested recently by McGarry (42), the concentration of...
malonyl-CoA in the direct vicinity of the CPT-1/ACC complex may be the true regulator of CPT-1 activity, thus explaining this apparent paradox. This observation further stresses the importance of in vivo studies with the measurement of functional enzyme activity, as the anatomic and biochemical conditions in the live organism may be significantly different from the artificial conditions in the in vitro setting.

In summary, we have demonstrated that during physiological hyperglycemia with hyperinsulinemia and maintained FFA concentrations (i.e., a condition that mimics the insulin-resistant state), human skeletal muscle malonyl-CoA concentrations are significantly increased and are directly associated with a reduction in LCFA oxidation and functional CPT-1 activity. Also, LCFA uptake is not altered during these conditions, leading to a shunting of LCFA away from oxidation and towards reesterification and storage as intramuscular triglyceride. We conclude that malonyl-CoA in human skeletal muscle plays a significant role in the regulation of fatty acid oxidation.

Acknowledgments

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