Original Articles

Fatty Acid Oxidation and the Regulation of Malonyl-CoA in Human Muscle

Peter N. Båvenholm, Jan Pigon, Asish K. Saha, Neil B. Ruderman, and Suad Efendic

Questions concerning whether malonyl-CoA is regulated in human muscle and whether malonyl-CoA modulates fatty acid oxidation are still unanswered. To address these questions, whole-body fatty acid oxidation and the concentration of malonyl-CoA, citrate, and malate were determined in the vastus lateralis muscle of 16 healthy nonobese Swedish men during a sequential euglycemic-hyperinsulinemic clamp. Insulin was infused at rates of 0.25 and 1.0 mU · kg^{-1} · min^{-1}, and glucose was infused at rates of 2.0 ± 0.2 and 8.1 ± 0.7 mg · kg^{-1} · min^{-1}, respectively. During the low-dose insulin infusion, whole-body fatty acid oxidation, as determined by indirect calorimetry, decreased by 22% from a basal rate of 0.94 ± 0.06 to 0.74 ± 0.07 mg · kg^{-1} · min^{-1} (P = 0.005), but no increase in malonyl-CoA was observed. In contrast, during the high-dose insulin infusion, malonyl-CoA increased from 0.20 ± 0.01 to 0.24 ± 0.01 nmol/g (P < 0.001), and whole-body fatty acid oxidation decreased by an additional 41% to 0.44 ± 0.06 mg · kg^{-1} · min^{-1} (P < 0.001). The increase in malonyl-CoA was associated with 30–50% increases in the concentrations of citrate (102 ± 6 vs. 137 ± 7 nmol/g, P < 0.001), an allosteric activator of the rate-limiting enzyme in the malonyl-CoA formation, acetyl-CoA carboxylase, and malate (80 ± 6 vs. 126 ± 9 nmol/g, P = 0.002), an antiporter for citrate efflux from the mitochondria. Significant correlations were observed between the concentration of malonyl-CoA and both glucose utilization (r = 0.53, P = 0.002) and the sum of the concentrations of citrate and malate (r = 0.52, P < 0.001), a proposed index of the cytosolic concentration of citrate. In addition, an inverse correlation between malonyl-CoA concentration and fatty acid oxidation was observed (r = –0.32, P = 0.03). The results indicate that an infusion of insulin and glucose at a high rate leads to increases in the concentration of malonyl-CoA in skeletal muscle and to decreases in whole-body and, presumably, muscle fatty acid oxidation. Furthermore, they suggest that the increase in malonyl-CoA in this situation is due, at least in part, to an increase in the cytosolic concentration of citrate. Because cytosolic citrate is also an inhibitor of phosphofructokinase, an attractive hypothesis is that changes in its concentration are part of an autoregulatory mechanism by which glucose modulates its own use and the use of fatty acids as fuels for skeletal muscle. *Diabetes* 49:1078–1083, 2000

Malonyl-CoA is an inhibitor of carnitine palmitoyl transferase-1 (CPT1), the enzyme that regulates the transfer of long-chain fatty acyl (LCFA) CoA into the mitochondria where they are oxidized (1). A recent study in humans indicated that during a euglycemic-hyperinsulinemic clamp, intracellular fatty acid oxidation is diminished in muscle due to inhibition of long-chain fatty acid entrance into the mitochondria (2); this finding is compatible with inhibition of CPT1 activity resulting from an increase in malonyl-CoA levels. Increases in malonyl-CoA concentrations occur acutely in rat muscle in response to insulin and glucose or inactivity (denervation), and decreases in its concentration occur during exercise (3), which is consistent with the various needs for fatty acid oxidation during these situations (4,5). The regulation of malonyl-CoA concentration by insulin and glucose has not been studied in human skeletal muscle. However, in rat muscle, insulin and glucose have been shown to increase malonyl-CoA levels by increasing the cytosolic concentration of citrate, an allosteric activator of acetyl-CoA carboxylase (ACC), and the precursor of its substrate, cytosolic acetyl-CoA (6).

In this study, the regulation of malonyl-CoA in muscle was evaluated in 16 healthy middle-aged Swedish men who were undergoing a 2-step euglycemic-hyperinsulinemic clamp. The specific aims of the study were to determine whether insulin and glucose acutely increase the concentration of malonyl-CoA as they do in the rat (3) and, if so, whether they concurrently increase the concentrations of citrate and malate. In addition, the relationship between whole-body fatty acid oxidation and malonyl-CoA levels in muscle was explored.

RESEARCH DESIGN AND METHODS

Subjects. A total of 16 Swedish men (age 48.8 ± 5.9 years) participated in the study. They belonged to a larger population-based control group of healthy middle-aged men, and they were the first group of consecutive control subjects who met the inclusion criteria. Only individuals who had a BMI between 23–32 kg/m^2 and a normal oral glucose tolerance test (OGTT) according to World Health Organization criteria (7) were considered eligible. None of the participants were on med-

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Received for publication 21 May 1999 and accepted in revised form 24 March 2000.

ACC, acetyl-CoA carboxylase; ANOVA, analysis of variance; CPT1, carnitine palmitoyl transferase-1; FFA, free fatty acid; LCFA, long-chain fatty acyl; OGTT, oral glucose tolerance test; R_a, rate of appearance; R_d, rate of disappearance.

ication or had a family history of diabetes. All of the subjects were given both written and oral information about the nature and potential risks of the study, and all of the subjects gave their informed consent. The experimental protocol was approved by the Ethics Committee at the Karolinska Hospital.

Euglycemic-hyperinsulinemic clamp. A sequential 2-step euglycemic-hyperinsulinemic clamp was performed after a 150-min equilibration period. In brief, on the experimental day, a basilic vein of each arm was cannulated, one for sampling and the other for infusion, and both arms were placed into heated (50°C) sleeves. A third catheter introduced into the cephalic vein of the arm was used for infusions and continuous sampling of arterialized blood for glucose measurement by a Biostator (Glucose Controlled Insulin Infusion System; Miles Laboratories, Life Science Instruments, Elkart, IN). Two levels of hyperinsulinemia were induced sequentially, each for 150 min by intravenous infusions of rapid-acting insulin (Actrapid; Novo Nordisk, Bagsværd, Denmark) (0.2 IU/ml with 4 mg/ml of human albumin in saline) at rates of 0.25 and 1.0 mU · kg^{-1} · min^{-1}. Euglycemia was maintained by a Biostator, which infused glucose on the basis of blood glucose measurements during the previous 4 min according to a published algorithm (8). Potassium (0.15 mmol/g of glucose) was added to the infusate.

Indirect calorimetry. The Deltatrac II Metabolic Monitor was used to measure oxygen consumption and carbon dioxide production and from these measurements the rate of fatty acid oxidation was calculated (9). For this purpose, 45 min before the end of the equilibration and 2 of the insulin infusion periods, a transparent plastic hood was placed over the subject's head for 30 min to determine O_2 consumption and CO_2 production. Timed sampling of urine for analysis of urinary urea excretion was performed. After corrections for changes in urea pool size (10), substrate oxidation rates were calculated.

Muscle biopsy. Muscle biopsies (75–100 mg) were obtained from the vastus lateralis portion of the quadriceps femoris muscle using a Weil-Blakesley's conchotome (11). Three biopsies were taken from each individual at the same portion of the vastus lateralis; 1 biopsy was taken at the end of the equilibration period, and the other 2 were taken at the end of the low-dose and high-dose insulin infusions. A trained nurse and a physician (P.N.B.) were used throughout the experiments. First, anesthetics (lidocaine 10 mg/ml) were administered and then a small incision (1 cm) was cut into the skin using a scalpel. In addition, a small incision was cut into the muscle fascia to perform the biopsy without interfering with the fascia. All tissues were immediately frozen (within seconds) in liquid nitrogen and stored at –70°C for subsequent analysis.

Assays. There was no substantial swelling or dehydration of the muscle during the clamp. Muscle was homogenized and deproteinized with 10% perchloric acid, and the filtrate was neutralized as described previously (6). Data were expressed per gram muscle wet weight. Malonyl-CoA was determined radioenzymatically in the neutralized filtrate by the method of McGarry et al. (1); citrate and malate were determined by standard spectrophotometric methods (12); and glucose was determined with the glucose oxidase method using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin (13) and C-peptide (14) values were determined in plasma by radioimmunoassay using an antibody developed in this laboratory and a commercial kit (Novo Research, Bagsværd, Denmark), respectively. Interassay and intra-assay coefficients of variation, respectively, were <3.9 and <3.1% for insulin values and 4.5 and 3% for C-peptide values. Cross-reactivity with proinsulin was 100% in the insulin assay and ~80% in the C-peptide assay. VLDL, LDL, and HDL concentrations were determined by a combination of preparative ultracentrifugation and precipitation (15).

Tracer methods. The tracer used in the study, 6-[^{3}H]glucose (DuPont New England Nuclear, Boston, MA) was purified by high-performance liquid chromatography to avoid the underestimation of glucose turnover caused by tritiated nonglucose contaminants. The tracer was diluted with saline to 2 μCi/ml and tested for sterility and pyrogenicity. This solution was then given as a bolus of 18 μCi followed by the continuous infusion of 0.15 μCi/min throughout the study. To minimize changes in plasma glucose–specific activity and to thus decrease errors due to the non–steady-state of the glucose pool, we applied the matched step tracer infusion technique. Consequently, an aliquot of tracer solution was added to the 30% glucose to be infused by the Biostator. The specific activity of the infusate was adjusted according to the formula of Finegood et al. (16) and was modified to allow for incomplete suppression of glucose production (17).

Sampling for plasma-specific activity was performed during the last 30 min of the basal period and the low- and high-insulin infusion periods. Plasma-specific activity remained within 20% of basal levels at all times. Specific activity was measured in plasma samples as previously reported (18). In brief, after deproteinization with $Ba(OH)_2$ and $ZnSO_4$, the supernatant was passed through anion and cation exchange columns (AG 2-X8 and AG 50W-X8, respectively; Bio-Rad Laboratories, Richmond, CA) to remove labeled metabolites of glucose. Additionally, it was lyophilized to remove tritiated water, reconstituted with an aliquot of water and was counted in a β-scintillation counter. Samples of both tracer infusate and glucose infused were measured in the same way after appropriate dilution. The glucose appearance rate was calculated using Steele's non–steady-state equation, as modified by DeBodo (19), with a pool fraction of 0.65 and an extracellular volume of 250 ml/kg (20). Data were smoothed with the optimal segments technique using the optimal error algorithm (21).

TABLE 1
Characteristics of the study population (n = 16)

Age (years)	48.8 ± 5.9
Waist-to-hip ratio	0.93 ± 0.06
BMI (kg/m^2)	26.3 ± 2.2
Cholesterol level (mmol/l)	
Total	5.0 ± 0.9
LDL	3.3 ± 0.8
HDL	1.2 ± 0.4
Triglycerides (mmol/l)	
Total	1.3 ± 0.18
VLDL	1.0 ± 0.19
OGTT	
Fasting blood glucose level (mmol/l)	4.8 ± 0.4
2-h Blood glucose level (mmol/l)	5.6 ± 0.6

Data are means ± SD or SE.

Statistical procedures. All values are presented as means ± SD (or SE). A paired Student's t test was used to determine the significance of the step-wise increase in carbohydrate oxidation and the decrease in fatty acid oxidation during the clamp, whereas repeated measurements of analysis of variance (ANOVA) were used for testing the significance of the increase in malonyl-CoA, citrate, and malate levels. Scheffe's F test was used as a post hoc analysis when the overall F statistics were significant. Regression analysis was used for calculation of correlation coefficients and P values.

RESULTS

Basic characteristics of the study population. The mean age of the study group was 49 years (Table 1). All subjects had normal fasting plasma glucose levels and were not glucose intolerant (22). With the exception of one individual who had mild hypertriglyceridemia, the subjects were normolipidemic. As a group, they were slightly overweight with a mean BMI of 26.3 ± 2.2 kg/m^2 (range 23–32) and a waist-to-hip ratio of 0.93 ± 0.06. None of the study subjects were taking antihypertensive medications or had a resting blood pressure >160/95 mmHg.

Glucose production, oxidation, and nonoxidative glucose disposal during the 2-step euglycemic-hyperinsulinemic clamp. Plasma insulin levels and rates of glucose infusion during the various stages of the protocol are shown in Table 2. To maintain normoglycemia (plasma glucose 5.1 mmol/l), it was necessary to infuse glucose at rates of 2.0 and 8.1 mg · kg^{-1} · min^{-1} (M value) during the low- and high-dose insulin infusions, respectively. Glucose production (the rate of appearance [R_a]) was significantly decreased during the low-dose insulin infusion (P < 0.001) and completely suppressed during the high-dose insulin infusion (Table 3). There was, as expected, an increase in the rate of disappearance (R_d) (P < 0.001), which was equal to the M value during the high-dose insulin infusion (Tables 2 and 3). Glucose oxidation increased from a baseline value of 1.12 to 1.42 mg · kg^{-1} · min^{-1} (P = 0.04) during the low-dose infusion and still further up to 2.41 mg · kg^{-1} · min^{-1} (P < 0.001) when insulin was infused at the higher rate. The calculated rates of nonoxidative glucose disposal during the low- and high-dose insulin infusions (corrected for 0.7 mg · kg^{-1} · min^{-1} of residual hepatic glucose production during the low-dose insulin infusion) were 1.3 and 5.4 mg · kg^{-1} · min^{-1}, respectively (Table 2). Thus, on the assumption that nonoxidative glucose disposal occurs primarily in muscle (22), at least 4 times more glucose was

TABLE 2
Assessment of insulin sensitivity and substrate utilization

	Plasma insulin (pmol/l)	Fat oxidation (mg · kg^{-1} · min^{-1})	Carbohydrate oxidation (mg · kg^{-1} · min^{-1})	Glucose utilization (mg · kg^{-1} · min^{-1})	Nonoxidative glucose disposal (mg · kg^{-1} · min^{-1})
Clamp condition					
Basal	109 ± 9	0.94 ± 0.06	1.12 ± 0.11	—	—
Low-insulin infusion	244 ± 12	0.74 ± 0.07	1.42 ± 0.13	2.0 ± 0.2	1.3 ± 0.1
High-insulin infusion	725 ± 98	0.45 ± 0.06	2.41 ± 0.16	8.1 ± 0.7	5.4 ± 0.6

Data are means ± SE.

taken up by muscle during the high-dose than during the low-dose insulin infusion.

Effect of infusions of insulin and glucose on whole-body fatty acid oxidation and the concentrations of malonyl-CoA, citrate, and malate in muscle. Concurrent with the increases in glucose utilization and oxidation during the 2-step clamp, whole-body fatty acid oxidation decreased from a basal value of 0.94 to 0.74 ($P = 0.005$) and to 0.45 mg · kg^{-1} · min^{-1} ($P < 0.001$) during the low- and high-dose insulin infusions, respectively (Table 2). No increase in the concentration of malonyl-CoA was observed in muscle during the low-dose insulin infusion; however, during the high-dose infusion, its concentration was increased from 0.20 ± 0.01 (basal and low-dose insulin) to 0.24 ± 0.01 nmol/g (high-dose insulin) (Table 4). Although the absolute increase in malonyl-CoA was relatively small, it occurred in all but 2 subjects, and it was highly significant ($P < 0.001$) (Fig. 1). As shown in Fig. 2, when all values during the basal period and the 2 insulin infusions were considered, the concentration of malonyl-CoA was inversely correlated with the rate of fatty acid oxidation ($r = –0.32$, $P = 0.03$) and was positively correlated with glucose utilization ($r = 0.53$, $P = 0.002$).

No increases in the concentrations of citrate or malate were observed during the low-dose insulin infusion. On the other hand, significant increases in the concentrations of both metabolites and the sum of the concentrations of citrate plus malate (182 ± 10, 177 ± 10, and 263 ± 10 nmol/g during the basal and low- and high-dose periods, respectively, $P < 0.001$) were observed during the high-dose insulin infusion. These values correlated inversely with fatty acid oxidation ($r = –0.71$, $P < 0.001$) and correlated positively with the concentration of malonyl-CoA ($r = 0.52$, $P < 0.001$) (Fig. 3) and the rate of glucose utilization ($r = 0.68$, $P = 0.001$).

DISCUSSION

The principal finding of this study is that, in humans, an infusion of insulin and glucose at a high rate is associated with an increase in the concentration of malonyl-CoA in skeletal muscle, which correlates both with increases in the concentrations of citrate and malate and a decrease in whole-body and, presumably, skeletal muscle fatty acid oxidation. Similar increases in malonyl-CoA and citrate have been observed in rat muscle incubated with insulin and glucose (6) and during a euglycemic-hyperinsulinemic clamp (23).

Malonyl-CoA has been linked to the regulation of fatty acid oxidation in skeletal muscle in rodents (5,24,25) and mammalian cardiac myocytes (26,27). In humans, its role has not been directly addressed. However, during a euglycemic-hyperinsulinemic clamp similar to that of phase 2 in the present study, Sidossis et al. (2) observed concurrent decreases in whole-body fatty acid oxidation and the con-

TABLE 3
Glucose turnover data

	R_a (mg · kg^{-1} · min^{-1})	R_d (mg · kg^{-1} · min^{-1})
Clamp condition		
Basal	2.0 ± 0.2	—
Low-insulin infusion	0.7 ± 0.5	2.7 ± 0.1
High-insulin infusion	0	7.8 ± 0.8

Data are means ± SE.

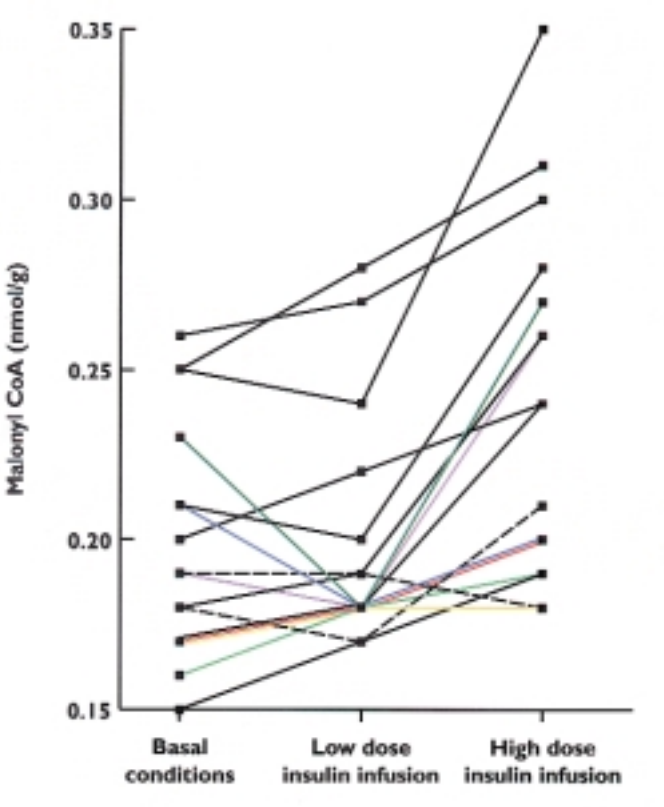

FIG. 1. Concentration of malonyl-CoA in human skeletal muscle during basal conditions and during low- and high-dose insulin infusions (0.25 and 1.0 mU · kg^{-1} · min^{-1}, respectively). Muscle biopsy samples were taken from vastus lateralis at 3 different time points during the clamp: at the end of the calibration period ($t = 0$ min), after 150 min of low-insulin infusion, and finally after a period of high-insulin infusion ($t = 300$ min).

TABLE 4
Effects of insulin and glucose on malonyl-CoA, citrate, and malate levels in human muscle (n = 16)

	Tissue concentration (nmol/g)		
	Malonyl-CoA	Citrate	Malate
Biopsy			
Basal	0.20 ± 0.01	102 ± 6	0 ± 6
Low-insulin infusion	0.20 ± 0.01	100 ± 7	77 ± 9
High-insulin infusion	0.24 ± 0.01*†	137 ± 7†‡	126 ± 9‡§
P	<0.001	<0.001	0.002

Data are means ± SE. *P < 0.001 vs. tissue concentration during basal conditions and high-insulin infusion; †P < 0.001 vs. tissue concentration during low-insulin and high-insulin infusions; ‡P < 0.01 vs. tissue concentration during low-insulin and high-insulin infusions; §P < 0.01 vs. tissue concentration during basal conditions and high-insulin infusion.

centration of long-chain acylcarnitine in skeletal muscle. They suggested that the latter could be due to inhibition of CPT1 as a result of an increase in the concentration of malonyl-CoA. The results of the present study support this contention. However, 2 somewhat unexpected findings need to be explained. First, no increase in malonyl-CoA was observed during phase 1 of the sequential clamp (plasma insulin 244 pmol/l), even though whole-body fatty acid oxidation was significantly diminished (0.94 to 0.74 mg · kg^{-1} · min^{-1}, P = 0.005). Thus, either fatty acid oxidation was predominantly diminished in tissues other than skeletal muscle, such as liver and heart, or it was diminished by other factors (e.g., a decrease in free fatty acid [FFA] availability) during the low-dose insulin infusion. Future studies in which levels of palmitoylcarnitine in muscle and FFA in plasma are measured during such a low-dose insulin infusion should address such possibilities. A second unexpected finding was that the increase of malonyl-CoA during the high-dose insulin infusion, although it occurred in nearly every subject, was only 20%. In contrast, the corresponding decrease in fatty acid oxidation (0.74 to 0.44) was 41%. This could be explained if, as has been suggested by previous studies in the rat (28,29), malonyl-CoA in muscle is compartmentalized and its concentrations in the vicinity of CPT1 and in whole muscle do not totally reflect each other (28,29). Given this context, it is worth noting that the concentration of malonyl-CoA in human muscle is ~10% of that in the rat (30) (Table 4) and that both absolute and relative changes, due to such factors as insulin and glucose (present study) and exercise (30), appear to be smaller and may be difficult to detect. For instance, we have found 12–17% decreases in malonyl-CoA levels in human muscle during exercise vs. 35% in the rat, but, in both species, we found large (50–80%) decreases in ACC activity (30). Thus, changes in the pool of malonyl-CoA (presumably cytosolic) that regulates CPT1 may not be reflected as readily by changes in whole-cell malonyl-CoA in the human as they are in the rat (A.K.S., N.B.R., unpublished data).

Increases in the concentration of malonyl-CoA in rat muscle produced by insulin and glucose both in vitro (6) and in vivo (23) are associated with increases in the concentrations of citrate and malate and, to an even greater extent, in the sum of the whole-cell concentration of these metabolites (29). In the present study, we also observed a highly significant correlation between increases in citrate plus malate and malonyl-CoA. Thus, a mechanism for increasing malonyl-CoA similar

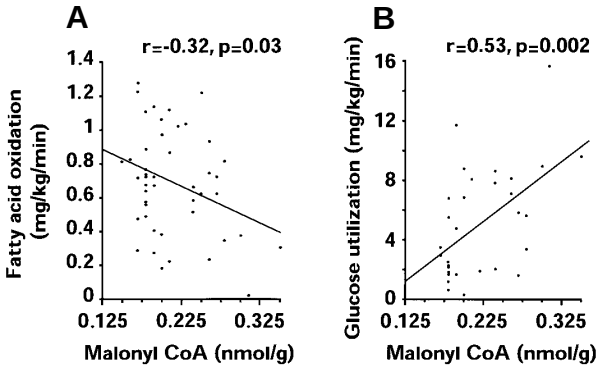

FIG. 2. Relationships between the concentration of malonyl-CoA in human skeletal muscle and the rate of whole-body fatty acid oxidation (*A*) (using indirect calorimetry) and glucose utilization (*B*) during a 2-step euglycemic-hyperinsulinemic clamp.

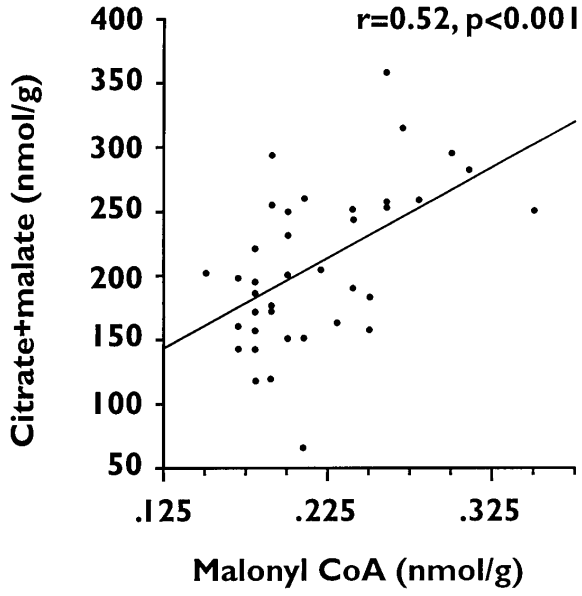

FIG. 3. Relationship between concentration of malonyl-CoA and the sum of the concentrations of citrate plus malate in human skeletal muscle during a 2-step euglycemic-hyperinsulinemic clamp.

to that in the rat is operative in humans. However, an increase in the concentration of citrate was not observed in the muscle of normal human volunteers during a euglycemic-hyperinsulinemic clamp in the study of Boden et al. (31). This could be related to the fact that during the second phase of the clamp, when we infused insulin at a rate of 1.0 mU · kg^{-1} · min^{-1}, insulin concentrations were 76% higher than those reported by Boden et al. (725 vs. 413 pmol/l). The 2 study populations also differed: our subjects were older (age 49 vs. 25 years) and had a higher BMI (26.3 vs. 25 kg/m^2).

A noteworthy finding was that the increase in the sum of the concentrations of citrate and malate (i.e., presumed indicator of cytosolic citrate) in muscle of the study subject correlated closely with their rates of whole-body fatty acid oxidation and glucose utilization. Similar results have been previously reported in perfused rat heart (32), incubated rat muscle (6,33), and rat muscles in vivo (23,34). This apparent involvement of cytosolic citrate in the regulation of fatty acid oxidation links malonyl-CoA regulation by insulin and glucose to the glucose–fatty acid cycle concept proposed by Randle et al. (35) on the basis of studies in rat heart. According to the glucose–fatty acid cycle concept, enhanced fatty acid or ketone-body oxidation increases the concentrations of acetyl-CoA and NADH in mitochondria, which leads to inhibition of glucose oxidation at pyruvate dehydrogenase and, in the presence of glucose, to increases in the mitochondrial and subsequently the cytosolic concentration of citrate. The latter effect, in turn, allosterically inhibits phosphofructokinase, thereby restraining the use of additional glucose for fuel metabolism. As reviewed elsewhere (29), the common involvement of cytosolic citrate in the glucose–fatty acid cycle and the malonyl-CoA fuel-sensing mechanism has led to the hypothesis that an increase in cytosolic citrate is not a unique feature of the glucose–fatty acid cycle, but rather a more general signal to the muscle cell that it has an excess of fuel for its immediate needs. According to this hypothesis, when glucose is present in excess, it both autoregulates its own use (via phosphofructokinase) and exerts inhibition of CPT1 and fatty acid oxidation via malonyl-CoA. As reviewed by Prentki and Corkey (36), a similar regulatory mechanism appears to be present in the pancreatic β-cell and may play a key role in the regulation of insulin secretion. The results presented here strongly suggest that this mechanism can also operate in human muscle.

The participants in the present study were moderately overweight (mean 26.3 ± 2.2 kg/m^2). Two individuals had a BMI <25 kg/m^2 (23.4 and 23.0, respectively), and 2 individuals had a BMI >30 kg/m^2 (31.1 and 31.6, respectively). The correlations observed between fatty acid oxidation and malonyl-CoA, between glucose utilization and malonyl-CoA, and between citrate plus malate and malonyl-CoA (Figs. 2 and 3) remained entirely unchanged after exclusion of the 2 obese subjects (data not shown). The same was true when the 2 subjects with a BMI <25 kg/m^2 were excluded from the analysis.

Finally, sustained increases in the concentration of malonyl-CoA in muscle have been linked to insulin resistance in rodents (37), possibly by virtue of secondary increases in the cytosolic concentration of LCFA CoA and diacylglycerol–protein kinase C signaling. It has also been demonstrated that increases in plasma FFA are more likely to produce insulin resistance in this setting (29,38). In humans, insulin resistance can be produced experimentally by increasing plasma FFA levels (by infusing a lipid emulsion) during a euglycemic-hyperinsulinemic clamp (39–42). It remains to be determined whether the increase in malonyl-CoA and inhibition of fatty acid oxidation described in this article is necessary for FFAs to produce insulin resistance in humans.

In conclusion, the data suggest that, in humans, an infusion of insulin and glucose inhibits fatty acid oxidation in skeletal muscle, at least in part, by increasing the cytosolic concentration of citrate and, secondarily, the concentration of malonyl-CoA. They also suggest that cytosolic citrate is a component of a glucose-autoregulatory mechanism that could restrain the further use of glucose as a fuel (via phosphofructokinase inhibition) when it is presented to the muscle cell in excess of its needs.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (K99-72X-00034-35A), the Karolinska Institute, the Novo Nordisk Insulin Foundation, the National Institutes of Health (DK-19514 and DK-49147), and the Juvenile Diabetes Foundation.

The authors especially wish to express their appreciation to the nurses Annica Clark and Kajsa Sundquist of the Department of Nutrition and Metabolism at the Karolinska Hospital for their help in performing the experiments, to the personnel at the laboratory who performed the hormone measurements, and, finally, to all of the volunteers who participated in the study. We also thank Holly Courture of Boston University for technical assistance.

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