MEDICA 16 Inhibits Hepatic Acetyl-CoA Carboxylase and Reduces Plasma Triacylglycerol Levels in Insulin-Resistant JCR:LA-cp Rats

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Intracellular triacylglycerol (TG) content of liver and skeletal muscle contributes to insulin resistance, and a significant correlation exists between TG content and the development of insulin resistance. Because acetyl-CoA carboxylase (ACC) is the rate-limiting enzyme for liver fatty acid biosynthesis and a key regulator of muscle fatty acid oxidation, we examined whether ACC plays a role in the accumulation of intracellular TG. We also determined the potential role of 5′-AMP–activated protein kinase (AMPK) in this process, since it can phosphorylate and inhibit ACC activity in both liver and muscle. TG content, ACC, and AMPK were examined in the liver and skeletal muscle of insulin-resistant JCR:LA-cp rats during the time frame when insulin resistance develops. At 12 weeks of age, there was a threefold elevation in liver TG content and a sevenfold elevation in skeletal muscle TG content. Hepatic ACC activity was significantly elevated in 12-week-old JCR:LA-cp rats compared with lean age-matched controls (8.75 ± 0.53 vs. 3.30 ± 0.18 nmol · min^{-1} · mg^{-1}, respectively), even though AMPK activity was also increased. The observed increase in hepatic ACC activity was accompanied by a 300% increase in ACC protein expression. There were no significant differences in ACC activity, ACC protein expression, or AMPK activity in the skeletal muscle of the 12-week JCR:LA-cp rats. Treatment of 12-week JCR:LA-cp rats with MEDICA 16 (an ATP-citrate lyase inhibitor) resulted in a decrease in hepatic ACC and AMPK activities, but had no effect on skeletal muscle ACC and AMPK. Our data suggest that alterations in ACC or AMPK activity in muscle do not contribute to the development of insulin resistance. However, increased liver ACC activity in the JCR:LA-cp rat appears to contribute to the development of lipid abnormalities, although this increase does not appear to occur secondary to a decrease in AMPK activity.
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ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; DTT, dithiothreitol; PEG, polyethylene glycol; PMSF, phenylmethylsulfonyl fluoride; TG, triacylglycerol.

Obesity, hyperinsulinemia, and hyperlipidemia characterize the insulin resistant syndrome (1). The alterations in glucose and fatty acid metabolism that accompany insulin resistance result in a high risk for development of atherosclerosis, angina, acute myocardial infarction, and congestive heart failure (2,3). Alterations in insulin signaling play an important role in mediating these changes in metabolism, since insulin controls the balance between fuel production and fuel use through its actions on adipose tissue, skeletal muscle, and liver. Skeletal muscle is the primary tissue responsible for glucose disposal in response to insulin (4) and is the major site of peripheral insulin resistance (5).

Several studies have implicated a role for intracellular triacylglycerol (TG) in the development of insulin resistance. The lipid content of muscle, liver, and pancreas significantly correlates with insulin sensitivity (6,7), and a strong negative correlation has been demonstrated in skeletal muscle between insulin-stimulated glucose uptake and local accumulation of TG (5,8–10). In support of this, reduction of skeletal muscle, liver, and islet TG, with either troglitazone or leptin, results in an increase in insulin-stimulated glucose uptake and a decrease in insulin resistance (11,12).

The accumulation of TG in muscle and liver can be the result of increased fatty acid uptake, increased de novo fatty acid synthesis, or decreased fatty acid oxidation. Both fatty acid synthesis and fatty acid oxidation are controlled by two isoforms of acetyl-CoA carboxylase (ACC) (13). The 265-kDa isoform of ACC (ACC-265) is the rate-limiting enzyme for fatty acid biosynthesis in liver and adipose tissue (14), since malonyl-CoA produced by ACC-265 is the precursor for fatty acid biosynthesis in liver and adipose tissue. The 280-kDa isoform of ACC (ACC-280) is a major regulator of muscle fatty acid metabolism (15–17), since malonyl-CoA produced by this enzyme is a potent inhibitor of skeletal muscle carnitine palmitoyltransferase 1, a key enzyme involved in mitochondrial fatty acid uptake (18).

Both isoforms of ACC can be regulated at the level of gene expression, allosteric regulation of the enzyme, and reversible phosphorylation (19,20). In both liver and muscle, AMP-activated protein kinase (AMPK) is capable of phosphorylating and inhibiting ACC activity (21–23). AMPK acts as a metabolic sensor and is activated by

increases in the AMP:ATP and creatine:phosphocreatine ratios (24,25). Once activated, the enzyme switches off ATP-consuming anabolic pathways such as fatty acid synthesis and switches on ATP-producing catabolic pathways such as fatty acid oxidation and glucose uptake. This coordinated control of synthesis and oxidation allows the maintenance of energy balance within the cell. Insulin control of fatty acid biosynthesis and oxidation may also involve AMPK, and in both liver (26) and muscle (27), insulin has been shown to inhibit AMPK activity. Whether alterations in insulin regulation of AMPK are altered in insulin resistance is not known.

The JCR:LA-cp male rat develops insulin resistance between 4 and 12 weeks of age. The *cp* gene results in a mutation in the leptin receptor, resulting in the lack of functional leptin receptors in all tissues of homozygous JCR:LA-cp rats (28). Studies demonstrating the presence of intracellular lipid droplets in the gastrocnemius muscle of 12-week JCR:LA-cp rats support the concept that TG accumulation in the muscle plays a key role in the development of insulin resistance (29). Of importance is that MEDICA 16, an ATP-citrate lyase inhibitor that limits acetyl-CoA supply to ACC, reduces muscle TG content in these insulin-resistant rats (29).

The purpose of this study was to determine the involvement of ACC and AMPK in the accumulation of TG in the liver and skeletal muscle of the JCR:LA-cp insulin-resistant rat. This was studied in JCR:LA-cp rats during the critical period in which insulin resistance develops (4–12 weeks after birth). We also determined whether MEDICA 16 treatment affected liver and skeletal muscle ACC and AMPK activity in these animals.

RESEARCH DESIGN AND METHODS

Animals. The study involved the use of obese JCR:LA-cp (*cp/cp*) and lean age-matched controls (+/*cp* or +/+), at either 4 or 12 weeks of age. JCR:LA-cp rats and lean age-matched controls were bred in our established colony at the University of Alberta and were maintained in a controlled environment at 20°C and 50–55% relative humidity, with a 12:12 h light-dark cycle. Rat food (Rodent Diet 5001; PMI Feeds, St. Louis, MO) and distilled water were available ad libitum. Care and treatment of the rats conformed to the guidelines of the Canadian Council on Animal Care and were subject to prior institutional approval as provided for in the guidelines.

Rats were handled twice a week from birth to ensure minimal stress at the time of experiments. Rats were anesthetized without restraint in a large glass jar with 3% halothane at 1 l/min O_2. This procedure allowed minimal stress to the animal as well as minimal muscle activity before tissue sampling. The time to reach surgical plane with halothane varied slightly, with heavier animals taking slightly longer. Cardiac puncture was performed to obtain 1.5- to 3-ml plasma samples (depending on the age and size of the animal). AMPK can be artificially activated if tissues are not isolated rapidly and freeze-clamped in liquid nitrogen; extreme care was taken to minimize the activation of AMPK. Liver and gastrocnemius muscle were harvested within 30 s after cardiac puncture, frozen immediately in liquid N_2, and stored at −80°C until analysis. Visible adipose tissue was quickly dissected from the gastrocnemius before freezing.

In another series of experiments, JCR:LA-cp rats were treated for 6 weeks with 0.25% MEDICA 16 in food from age 6 to 12 weeks. All animals were studied in the nonfasted state at the end of the dark phase.

Plasma measurements. Samples were analyzed for plasma glucose using a glucose oxidase technique (Beckman Instruments, Brea, CA) and for insulin by radioimmunoassay (29). Plasma free fatty acid levels were measured using a Wako FFA kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma TG levels were measured using a Wako Triglyceride E kit (Wako Pure Chemical Industries). Plasma leptin levels were measured using immunoassay (R&D Systems, Minneapolis, MN).

Tissue TG determination. Aliquots of frozen skeletal muscle and liver were homogenized at 4°C using a Polytron homogenizer in 2:1 chloroform:methanol. The mixture was centrifuged at 3,500 rpm, and the supernatant was applied to a silicic acid column. TG was eluted in 10 volumes chloroform, dried under N_2 at 60°C, and quantitated colorimetrically as glycerol using an enzymatic assay (Wako Pure Chemical Industries).

Isolation of ACC and AMPK. A cytosolic fraction containing ACC and AMPK was isolated from frozen tissue according to the polyethylene glycol (PEG) precipitation method (22). Frozen tissue (200 mg) was homogenized in homogenization buffer containing 50 mmol/l Tris-HCl (pH 7.5 at 4°C), 50 mmol/l NaF, 5 mmol/l Na^+ pyrophosphate, 1 mmol/l EDTA, 1 mmol/l EGTA, 0.25 mol/l mannitol, 1 mmol/l dithiothreitol (DTT), and the following protease inhibitors: 1 mmol/l phenylmethylsulfonyl fluoride (PMSF), 4 μg/ml soybean trypsin inhibitor, and 1 mmol/l benzamidine. After homogenization for 30 s, the mixture was centrifuged at 14,000*g* for 20 min. The resulting supernatant was made to 2.5% PEG, vortexed for 10 min, and centrifuged for 10 min at 10,000*g*. The resulting supernatant was then made to 6% PEG, vortexed for 10 min, and centrifuged for 10 min at 10,000*g*. The resulting pellet was resuspended in buffer containing 0.1 mol/l Tris-HCl (pH 7.5 at 4°C), 50 mmol/l NaF, 5 mmol/l Na^+ pyrophosphate, 1 mmol/l EDTA, 1 mmol/l EGTA, 10% wt/vol glycerol, 1 mmol/l DTT, 0.02% sodium azide, and the following protease inhibitors: 1 mmol/l PMSF, 4 μg/ml soybean trypsin inhibitor, and 1 mmol/l benzamidine. Protein content was measured using the Bradford protein assay.

Measurement of ACC activity. The CO_2 fixation technique (22) was used to measure ACC activity in the PEG fraction. Briefly, 12.5 μg protein was added to a reaction mixture (final volume 165 μl) containing 60.6 mmol/l Tris acetate, 1 mg/ml BSA, 1.32 μmol/l ATP, 2.12 mmol/l β-mercaptoethanol, 5 mmol/l magnesium acetate, 1.06 mmol/l acetyl CoA, 18.08 mmol/l $NaH^{14}CO_3$, and 0 or 10 mmol/l magnesium citrate. Samples were incubated at 37°C for 4 min, and the reaction was stopped by adding 25 μl of 10% perchloric acid. Samples were spun at 3,500 rpm for 20 min, and then 160 μl supernatant was placed in glass vials and dried under medium heat. The samples were resuspended in 100 μl distilled deionized water (ddH_2O) and 4 ml scintillation fluid and then counted for the presence of radiolabeled malonyl CoA. ACC activity is expressed as nanomoles of malonyl CoA produced per minute per milligram of protein.

Measurement of AMPK activity. AMPK activity of the PEG precipitate was measured by following the incorporation of ^{32}P into the synthetic peptide SAMS (22). The assay mixture contained 40 mmol/l HEPES, pH 7.0, 80 mmol/l NaCl, 0.8 mmol/l EDTA, 1 mmol/l DTT, 0.2 mmol/l [γ-^{32}P]ATP, 5.0 mmol/l $MgCl_2$, 0.2 mmol/l SAMS, 8% glycerol, 0.01% Triton X-100, and 2 μg of the PEG precipitate in the presence or absence of 200 μmol/l AMP. Incorporation of ^{32}P into SAMS was measured at 30°C for 5 min. An aliquot of the reaction was then blotted onto phosphocellulose paper and washed four times in H_3PO_4 and once in acetone. The phosphocellulose was dried and counted in 4 ml scintillant. AMPK activity is expressed as picomoles of ^{32}P incorporated per minute per milligram of protein.

Western blot analysis. Samples were resolved by 5% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in Tris-buffered saline with BSA and Triton X-100 and incubated with peroxidase-labeled streptavidin. Western blots were visualized using an enhanced chemiluminescence Western blot detection kit.

Statistical analysis. All data are presented as means ± SE. The data were analyzed with the statistical program Instat 2.01. ANOVA was used to evaluate the statistical significance of differences among groups. When necessary, data were transformed to test for statistical significance. Two-tailed values of $P < 0.05$ were considered significant.

RESULTS

Characteristics of the JCR:LA-cp rat. Insulin resistance in the JCR:LA-cp rat occurs between the ages of 4 and 12 weeks (29). Body weight and various plasma measurements for 4- and 12-week JCR:LA-cp rats and their lean age-matched controls are shown in Table 1. At 4 weeks of age, there was no difference in body weight between the JCR:LA-cp and lean age-matched controls. At 12 weeks of age, both JCR:LA-cp and lean age-matched control rats had significantly higher body weights compared with 4-week rats. The JCR:LA-cp rats also had a significantly elevated body weight compared with the lean age-matched controls. Plasma insulin levels began to rise at 4 weeks of age in the JCR:LA-cp rats, with a profound hyperinsulinemia ensuing by 12 weeks of age. In contrast, plasma glucose levels were not significantly different in the JCR:LA-cp rats in either the 4- or 12-week-old rats, compared with lean age-matched controls.

TABLE 1
Body weight and plasma measurements of 4- and 12-week lean and JCR:LA-cp rats

	4-week rats		12-week rats	
	Lean	JCR:LA-cp	Lean	JCR:LA-cp
n	6	6	4	8
Body weight (g)	87 ± 5	78 ± 4	324 ± 6†	399 ± 11*†
Insulin (pmol/l)	57.7 ± 5.2	431.0 ± 42.9*	272.1 ± 27.1†	2746.1 ± 438.5*†
Glucose (mmol/l)	8.6 ± 0.2	8.5 ± 0.2	9.5 ± 0.4	10.1 ± 0.7
FFA (mmol/l)	0.18 ± 0.01	0.16 ± 0.01	0.27 ± 0.02†	0.37 ± 0.06*†
Triglyceride (mmol/l)	1.4 ± 0.1	3.2 ± 0.1*	1.8 ± 0.2	6.4 ± 0.5*†
Leptin (ng/ml)	1.3 ± 0.4	16.5 ± 0.8*	1.2 ± 0.3†	36.9 ± 1.8*†

Data are means ± SE. *Significantly different from lean age-matched controls; †significantly different from 4-week genotype matched rats. FFA, free fatty acid.

Plasma free fatty acid levels were not significantly different between JCR:LA-cp rats at 4 weeks of age and lean age-matched controls. However, at 12 weeks of age, free fatty acid levels were significantly elevated in the JCR:LA-cp rats compared with age-matched controls. Free fatty acid levels also increased slightly in the lean rats with age. Plasma TG levels were significantly elevated at 4 weeks of age in the JCR:LA-cp rats compared with lean age-matched controls. By 12 weeks, plasma TG levels were almost double that of the 4-week JCR:LA-cp rats and were significantly higher than the 12-week lean age-matched controls. Plasma TG levels in the lean rats did not rise significantly with age.

At 4 weeks of age, leptin levels in the JCR:LA-cp rats were elevated 13-fold compared with lean age-matched controls. Leptin levels continued to increase and were 30-fold greater than those of the lean age-matched controls at 12 weeks. Plasma leptin levels in the lean rats did not increase with age or body weight.

Liver and skeletal muscle TG content. Figure 1 shows that at 4 weeks of age, JCR:LA-cp rats have a threefold greater liver TG content than lean age-matched controls. There was no further increase in TG content in the liver of 12-week-old JCR:LA-cp rats compared with lean age-matched controls. The gastrocnemius of the 4-week JCR:LA-cp rats also contained a threefold greater amount of TG compared with the lean age-matched controls. By 12 weeks of age, however, the TG content was more than sevenfold higher than that of lean controls.

Hepatic ACC activity and expression. At 4 weeks of age, ACC activity (Fig. 2) was already significantly elevated in the JCR:LA-cp rats compared with lean age-matched controls. A further increase was seen at 12 weeks in the JCR:LA-cp rats, with ACC activity being twofold higher than in 12-week lean age-matched controls. Calculation of the citrate-dependent activity (subtraction of basal ACC activity from the citrate-stimulated activity) gave the same result (data not shown). The increased ACC activity in the 12-week JCR:LA-cp rats was accompanied by a significant increase in ACC-265 protein expression compared with the 12-week lean age-matched controls. The increased ACC activity in the 4-week JCR:LA-cp rats was also accompanied by a slight increase in protein expression, although this increase did not reach statistical significance compared with the 4-week age-matched controls.

Hepatic AMPK activity. To determine if the increase in hepatic ACC in the JCR:LA-cp was due to alterations in AMPK control of ACC, we measured AMPK activity in 4- and 12-week-old JCR:LA-cp and lean age-matched control rats (Fig. 3). No significant difference in AMPK activity was seen in the 4-week JCR:LA-cp rats compared with the lean age-matched controls. However, AMPK activity in the 12-week JCR:LA-cp rats was increased compared with the

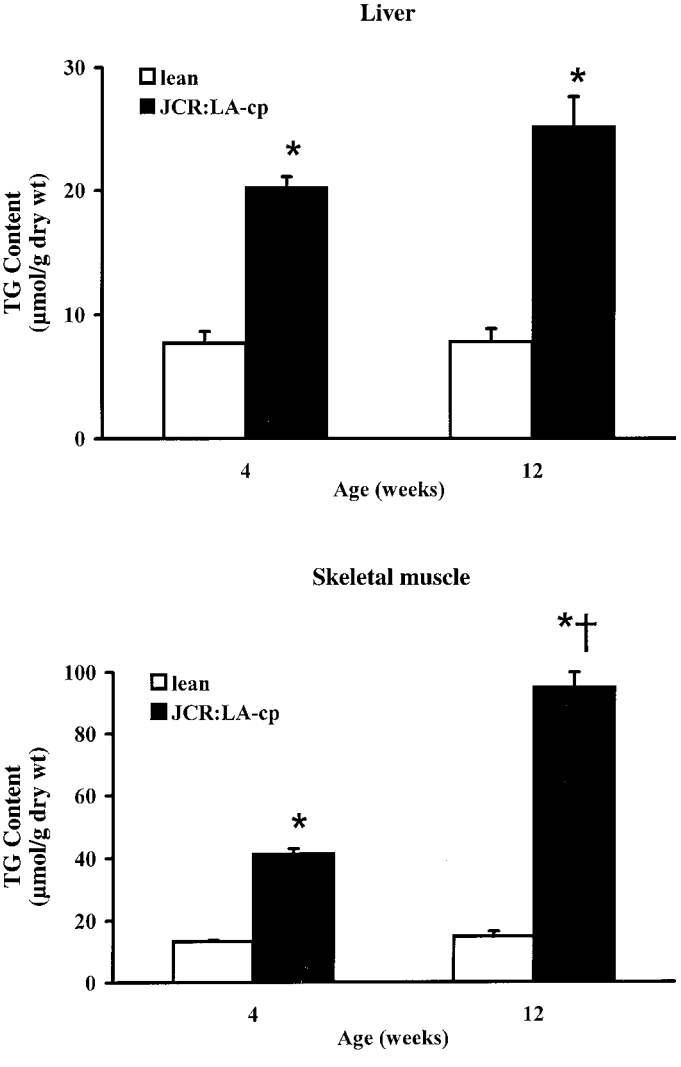

FIG. 1. TG content of liver and skeletal muscle of JCR:LA-cp rats and lean age-matched controls. Values are means ± SE of groups (n = 6). *Significantly different from lean age-matched controls; †significantly different from 4-week JCR:LA-cp rats.

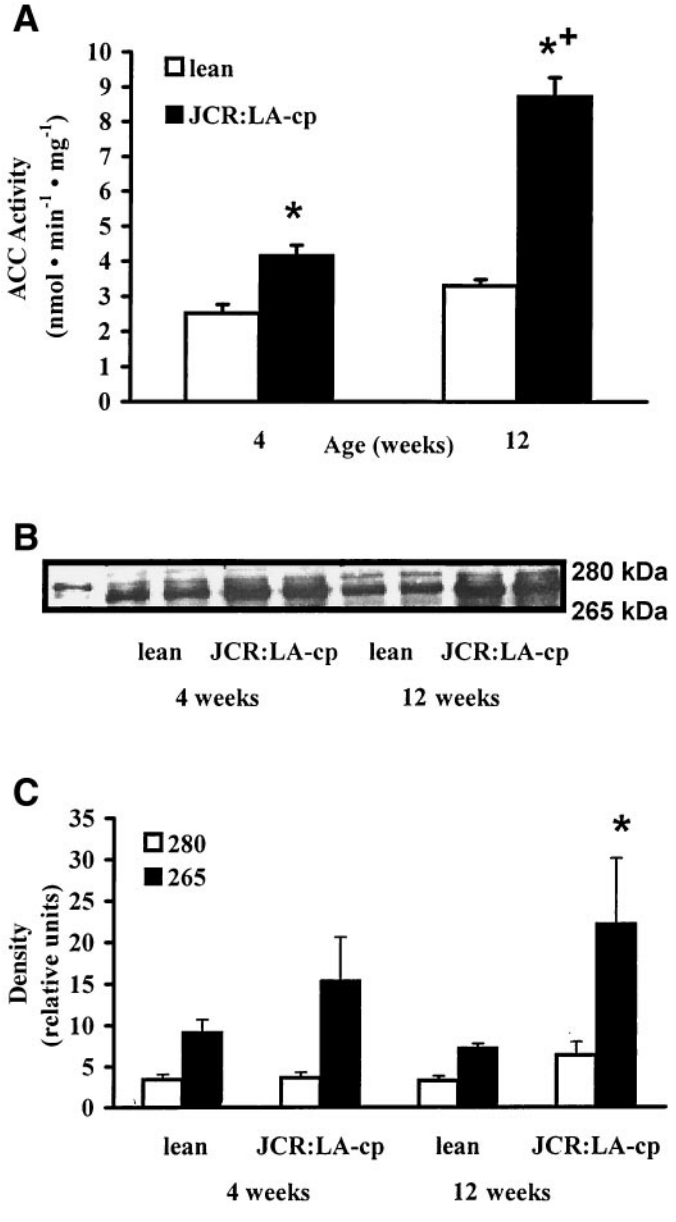

FIG. 2. ***A*****: Hepatic ACC activity of JCR:LA-cp rats and lean age-matched controls. ACC activity was measured in the presence of 10 mmol/l citrate.** ***B*****: Protein expression of hepatic ACC-280 and ACC-265 isoforms in JCR:LA-cp rats and lean age-matched controls. Rat heart was loaded in** ***lane 1*** **to serve as a standard.** ***C*****: Relative density of ACC isoforms determined using a densitometry program. Values are means ± SE of groups (*n* = 6–11). *Significantly different from lean age-matched controls; +significantly different from 4-week JCR:LA-cp rats.**

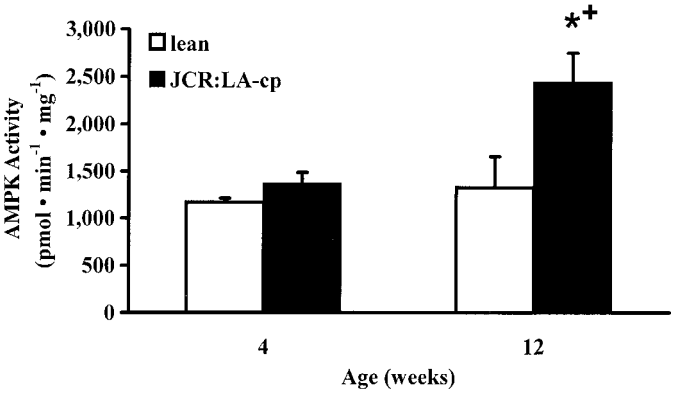

FIG. 3. Hepatic AMPK activity measured in JCR:LA-cp rats and lean age-matched controls. AMPK activity was measured in the presence of 200 μmol/l AMP. Values are means ± SE of groups (*n* = 6–11). *Significantly different from lean age-matched controls; +significantly different from 4-week JCR:LA-cp rats.

4-week JCR:LA-cp rats and was significantly increased compared with the 12-week lean controls.

Skeletal muscle ACC and AMPK activity. Skeletal muscle has been implicated as an important site of insulin resistance (5), and TG accumulation within the muscle has been implicated as contributing to insulin resistance (8). We therefore measured ACC activity and protein expression in gastrocnemius muscle (Fig. 4). However, no differences were seen in ACC activity between JCR:LA-cp and lean age-matched control rats at either 4 or 12 weeks of age. Although a trend for a decreased expression of the ACC-280 isoform occurred in the 12-week JCR:LA-cp rats compared with the lean age-matched controls, this difference was not statistically significant. The decrease did, however, result in the expression of the two isoforms being relatively equal in the JCR:LA-cp rats, rather than the 280-kDa isoform predominating. There was no difference in AMPK activity in the JCR:LA-cp rats or lean age-matched controls at either age (Fig. 5).

Treatment with MEDICA 16. MEDICA 16 (β,β′-tetramethyl hexadecanedioic acid) is a long-chain fatty acyl analog developed as a hypolipidemic and antiobesity-antidiabetogenic compound (30). Previous studies have demonstrated that MEDICA 16 significantly reduces intracellular TG content in gastrocnemius muscle, and this reduction is accompanied by an increase in insulin sensitivity (29). We therefore treated JCR:LA-cp rats with MEDICA 16 for a 6-week period. This treatment protocol did not significantly change body weight or plasma glucose levels (Table 2). The modest loss of body weight was associated with a slight decrease in adipose tissue mass and a nonsignificant decrease in food intake. Plasma insulin levels did decrease, although they remained higher than values in the lean age-matched controls (Table 2).

As shown in Fig. 6, MEDICA 16 treatment significantly decreased liver ACC activity to levels in the lean age-matched controls. Interestingly, hepatic AMPK activity also decreased with MEDICA 16 treatment. In skeletal muscle, there was no significant change in either ACC or AMPK activity. However, we have observed that MEDICA 16 does not readily penetrate muscle tissue (J.B.-T., unpublished observations), which may explain the lack of effect in muscle.

DISCUSSION

At 4 weeks of age, JCR:LA-cp rats have normal body weights, as well as normal plasma glucose and fatty acid levels. At that age, muscle glucose uptake in response to insulin is identical to lean controls (29). However, we demonstrate that increased levels of plasma insulin, TG, and leptin are already apparent before the onset of muscle insulin resistance. By 12 weeks of age, the JCR:LA-cp rats displayed all the symptoms of insulin resistance, including obesity, hyperinsulinemia, and hyperleptinemia. In addition, plasma TG levels were markedly elevated in the JCR:LA-cp rats, as were both hepatic and skeletal muscle

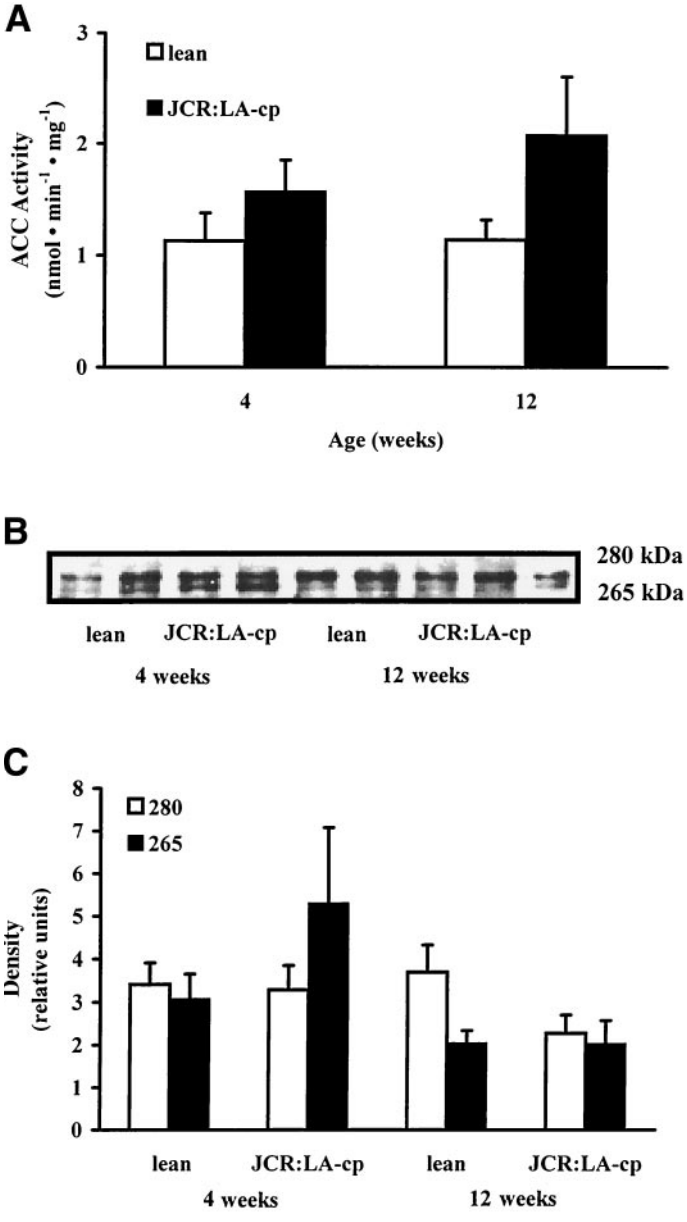

FIG. 4. *A*: Skeletal muscle ACC activity of JCR:LA-cp rats and lean age-matched controls. *B*: Protein expression of skeletal muscle ACC-280 and ACC-265 isoforms in JCR:LA-cp rats and lean age-matched controls. Rat heart was loaded in *lane 9* to serve as a standard. *C*: Relative density of ACC isoforms determined using a densitometry program. Values are means ± SE of groups (n = 6–9).

TG levels. In this study, we demonstrate that increases in hepatic ACC activity and expression may be primarily responsible for the increase in TG content, whereas skeletal muscle ACC activity/expression did not correlate with the changes in TG content.

Several studies have claimed a link between TG content and the development of insulin resistance (6–10). In this study, we confirm that both the liver and muscle of insulin-resistant rats contain elevated levels of TG. The measurement of intramuscular triglyceride is often artifactually elevated because of the presence of extramuscular adipose tissue (31). To address this issue, visible adipose tissue was quickly dissected from the gastrocnemius muscle before freezing. Furthermore, the presence of intracellular triglyceride in the gastrocnemius of JCR:LA-cp rats

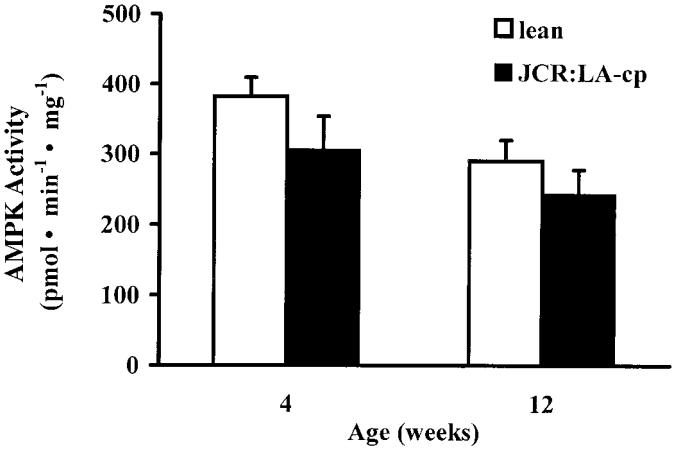

FIG. 5. Skeletal muscle AMPK activity measured in JCR:LA-cp rats and lean age-matched controls. Values are means ± SE of groups (n = 6–9)

has been previously demonstrated using electron microscopy and oil red O stain (29). Thus, although we cannot completely rule out the possibility that extracellular adipose tissue contributed to our measurements, our results are in agreement with several studies demonstrating a link between triglyceride accumulation and the development of insulin resistance.

The liver TG content of JCR:LA-cp rats was approximately threefold higher than that in lean age-matched controls, at both 4 and 12 weeks of age. However, in gastrocnemius muscle, TG levels rose from threefold higher to sevenfold higher than in lean age-matched controls between the ages of 4 and 12 weeks. This occurred despite the observation that at 4 weeks of age skeletal muscle from these rats is not insulin resistant, as demonstrated by normal insulin-mediated glucose uptake (29). This suggests that substantial accumulation of TG is an early event in the development of insulin resistance. It is tempting to speculate that there is a threshold level of TG accumulation within muscle before the development of insulin resistance. If so, this raises the possibility that continued accumulation of TG within the muscle may indeed play a causative role in the development of insulin resistance.

ACC not only catalyzes the rate-limiting step in fatty acid biosynthesis (14), it also has an important role in the regulation of muscle fatty acid oxidation, secondary to the formation of malonyl-CoA, a potent inhibitor of mitochondrial fatty acid uptake (15–17). In JCR:LA-cp rats, hepatic ACC activity almost doubled by 4 weeks of age compared with lean age-matched controls and increased even further by 12 weeks of age. This increase in ACC activity most

TABLE 2
Body weight and plasma measurements of 12-week JCR:LA-cp and MEDICA 16–treated JCR:LA-cp rats

	JCR:LA-cp	Medica 16
n	4	8
Body weight (g)	465 ± 9	420 ± 10
Insulin (pmol/l)	2,746.1 ± 438.5	1,120.0 ± 160.7*
Glucose (mmol/l)	13.3 ± 1.0	12.4 ± 1.0

Data are means ± SE. *Significantly different from control JCR:LA-cp rats.

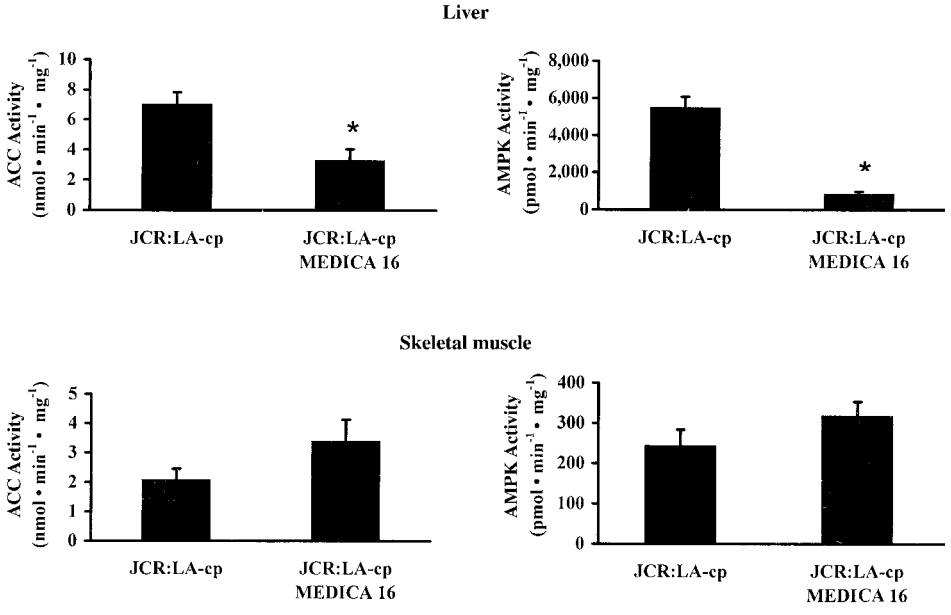

FIG. 6. Effect of MEDICA 16 treatment on liver and skeletal muscle ACC and AMPK activity. Values are means ± SE of groups (n = 5–6). *Significantly different from untreated 12-week JCR:LA-cp rats.

likely contributes to the elevated plasma TG levels at 4 weeks of age, as well as the previously reported increase in plasma VLDL (32). The elevated ACC activity at 12 weeks was also accompanied by an increase in ACC-265 isoform expression, suggesting that increases in ACC activity and expression in the liver contribute to the abnormal plasma lipid levels and elevated hepatic TG content. The increase in ACC activity at 4 weeks was not accompanied by significant changes in protein expression. The reported increase in ACC activity was measured in the presence of 10 mmol/l citrate, which represents maximally stimulated ACC activity. Under basal assay conditions (0 mmol/l citrate), there was no change in ACC activity in JCR:LA-cp rats compared with lean controls (data not shown). Whether a decrease in phosphorylation was responsible for the increase in ACC activity was not addressed in this study.

We initially hypothesized that the increase in ACC activity in the liver was due to a decrease in AMPK activity in the JCR:LA-cp rats. However, the increase in hepatic ACC in the insulin-resistant rats was associated with an increase in AMPK activity. Since AMPK and ACC can form a complex (33), the increase in AMPK may be a reflection of the increase in ACC expression. A recent study demonstrating that metformin activates hepatic AMPK may provide an alternative explanation (34). It is possible that the increase in hepatic AMPK is a compensatory mechanism to inhibit the increase in hepatic glucose production. This may provide an explanation for the lack of hyperglycemia in these animals.

A very large TG accumulation was seen within the gastrocnemius muscle of 12-week-old JCR:LA-cp rats (29). Despite this, however, we observed no significant changes in skeletal muscle ACC activity. Previous studies have demonstrated that obese rats and mice have increased muscle malonyl-CoA and triglyceride levels that are not associated with an increase in ACC activity (35). The increase in malonyl-CoA was attributed to an increase in cellular citrate levels (measured as citrate plus malate), an allosteric activator of ACC. Citrate and malate levels were not determined in this study but may provide an explanation for the elevation in muscle TG content without changes in ACC activity. Furthermore, the enzymatic measurement of ACC activity cannot differentiate between the contributions of the different ACC isoforms. Thus, we also measured protein expression. Of interest was the observation that 12-week lean rats express almost twice the amount of the 280-kDa isoform as the 265-kDa isoform, whereas the JCR:LA-cp rats showed a decrease in 280-kDa expression such that the isoforms were expressed in almost a 1:1 ratio.

We also observed no changes in AMPK activity in the gastrocnemius of the JCR:LA-cp rats compared with the lean age-matched controls. AMPK activity was determined in a fed state at the end of the dark phase. However, these measurements do not indicate changes in AMPK activity that may occur throughout the day. While we are not aware of studies demonstrating a change in muscle AMPK activity in response to fasting and feeding, it has been demonstrated that hepatic AMPK is maximal during fasting and its activity is rapidly diminished during feeding (36). Because the time of sampling may influence AMPK activity, all samples were taken at the same time in a fed state. Furthermore, potential changes in AMPK expression or contraction-induced AMPK activity require further investigation.

Given the important role of AMPK in regulating glucose uptake and fatty acid oxidation in muscle (37), we were surprised that no changes in AMPK activity were seen in insulin-resistant muscle. AMPK has at least two isoforms of the α catalytic subunit (α1 and α2). The α2 isoform represents the majority of AMPK activity in muscle (38). At present, little is known about the specific roles of the isoforms and whether specific isoforms are responsible for the control of fatty acid oxidation and glucose uptake. In insulin-resistant muscle, glucose uptake is completely blunted (29). The absence of changes in AMPK activity may suggest a defect in the upstream signaling or possibly several signals affecting AMPK activity. Clearly, further studies are required to address this important issue.

MEDICA 16 is an ATP-citrate lyase inhibitor, and chronic treatment with MEDICA 16 has previously been

shown to reduce plasma TG concentrations by 80% and blunt the development of insulin resistance in the JCR:LA-cp rat (29) as well as in the related Zucker rat (39). Several studies have also shown acute effects of MEDICA 16. Acute treatment with MEDICA 16 results in a significant decrease in plasma VLDL cholesterol and triglyceride (40) and a significant inhibition of hepatic lipogenesis (41). In vitro, MEDICA 16 inhibits ACC activity (42) and induces a decrease in mitochondrial proton motive force that is accompanied by an increase in cellular respiration (43). In this study, we demonstrate that chronic treatment of JCR:LA-cp rats for 6 weeks with MEDICA 16 dramatically reduced liver ACC activity. This result supports the concept that increased hepatic ACC activity contributes to the elevated plasma TG levels in insulin-resistant JCR:LA-cp rats. Further support comes from studies using hydroxycitrate, another inhibitor of ATP-citrate lyase. Hydroxycitrate treatment of Zucker rats was also shown to reduce elevated lipogenic rates and plasma TG levels (44). Skeletal muscle ACC activity was unaffected by MEDICA 16 treatment, which may be because of the inability of MEDICA 16 to get into muscle tissue (J.B.-T., unpublished observations).

Interestingly, the decrease in ACC activity with MEDICA 16 treatment resulted in a dramatic reduction in hepatic AMPK activity. This result suggests dissociation in the changes in ACC activity from changes in AMPK activity in JCR:LA-cp rats but supports the concept of a parallel change in ACC and AMPK activity. Although it has been suggested that activation of the AMPK signaling cascade may be effective in correcting insulin resistance (39), our data suggest that hepatic AMPK is activated in insulin resistance. However, if hepatic AMPK activity is increased to reduce hepatic glucose production, the use of AMPK activators may be useful in type 2 diabetes, a situation where hyperglycemia exists. It is thus important to characterize changes in AMPK signaling in models of type 2 diabetes.

In summary, our data show that an increase in liver ACC activity appears to contribute to the elevated plasma TG levels and probably the increased liver and muscle TG accumulation that occur during insulin resistance. We recognize that these studies provide correlative evidence rather than a cause-effect relationship with regard to an increase in hepatic ACC activity and TG content. However, other studies have demonstrated that high-fat feeding results in an increase in hepatic ACC activity that is correlated with an increase in TG content (45). Furthermore, the inhibition of hepatic ACC activity with a fatty acid analog is associated with a decrease in TG content and reduction in VLDL secretion (46). Of interest is the observation that these changes in TG and ACC precede the development of insulin resistance, suggesting a possible causative role of ACC and TG accumulation in the development of insulin resistance. We also demonstrate that changes in AMPK activity do not mediate this increase in ACC activity and TG accumulation. Finally, treatment with MEDICA 16, which decreases TG accumulation (29) and the symptoms of insulin resistance (29,39), results in a decrease in hepatic ACC activity. This supports the concept that increased ACC activity and increased TG accumulation have important roles in the development of insulin resistance.

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