Potential mechanisms and consequences of cardiac triacylglycerol accumulation in insulin-resistant rats

Laura L. Atkinson, Ray Kozak, Sandra E. Kelly, Arzu Onay-Besikci, James C. Russell, and Gary D. Lopaschuk. Potential mechanisms and consequences of cardiac triacylglycerol accumulation in insulin-resistant rats. *Am J Physiol Endocrinol Metab* 284: E923–E930, 2003. First published December 3, 2002; 10.1152/ajpendo.00360.2002.—The accumulation of intracellular triacylglycerol (TG) is highly correlated with the development of insulin resistance, as well as with the severity of insulin resistance (see review in Ref. 16). However, it remains controversial whether the accumulation of TG in muscle is the result of increased fatty acid supply, decreased fatty acid oxidation, or a combination of both. Because abnormal fatty acid metabolism is a key contributor to the pathogenesis of diabetes-related cardiovascular dysfunction, we examined fatty acid and glucose metabolism in hearts of insulin-resistant JCR:LA-cp rats. Isolated working hearts from insulin-resistant rats had glycolytic rates that were reduced to 50% of lean control levels (*P* < 0.05). Cardiac TG content was increased by 50% (*P* < 0.05) in the insulin-resistant rats, but palmitate oxidation rates remained similar between the insulin-resistant and lean control rats. However, plasma fatty acids and TG levels, as well as cardiac fatty acid-binding protein (FABP) expression, were significantly increased in the insulin-resistant rats. AMP-activated protein kinase (AMPK) plays a major role in the regulation of cardiac fatty acid and glucose metabolism. When activated, AMPK increases fatty acid oxidation by inhibiting acetyl-CoA carboxylase (ACC) and reducing malonyl-CoA levels, and it decreases TG content by inhibiting glycerol-3-phosphate acyltransferase (GPAT), the rate-limiting step in TG synthesis. The activation of AMPK also stimulates cardiac glucose uptake and glycolysis. We thus investigated whether a decrease in AMPK activity was responsible for the reduced cardiac glycolysis and increased TG content in the insulin-resistant rats. However, we found no significant difference in AMPK activity. We also found no significant difference in various established downstream targets of AMPK: ACC activity, malonyl-CoA levels, carnitine palmitoyltransferase I activity, or GPAT activity. We conclude that hearts from insulin-resistant JCR:LA-cp rats accumulate substantial TG as a result of increased fatty acid supply rather than from reduced fatty acid oxidation. Furthermore, the accumulation of cardiac TG is associated with a reduction in insulin-stimulated glucose metabolism. AMP-activated protein kinase; glycolysis; acetyl-CoA carboxylase; glycerol-3-phosphate acyltransferase

THE INSULIN-RESISTANT SYNDROME is a pre-type 2 diabetic state characterized by hyperinsulinemia and impaired insulin-stimulated glucose metabolism (27). In muscle, the accumulation of intracellular triacylglycerol (TG) is highly correlated with the development of insulin resistance, as well as with the severity of insulin resistance (see review in Ref. 16). However, it remains controversial whether the accumulation of TG in muscle is the result of increased fatty acid supply, decreased fatty acid oxidation, or a combination of both (16).

Although there is an established link between insulin resistance and cardiovascular disease (10), it is not clear whether the accumulation of TG is associated with insulin resistance in the heart. Elevated cardiac TG content has been associated with depressed contractility, arrhythmias, hypertrophy, heart failure, and apoptosis (6, 7, 28, 42, 43). However, it is not known whether elevated TG content is associated with a reduction in insulin-stimulated glucose metabolism in the heart. Furthermore, there is little information regarding the accumulation of TG and its potential role in the development of cardiovascular disease.

Under normal physiological conditions, the energy requirements of the heart are fulfilled primarily by the oxidation of fatty acids, with the remainder provided by carbohydrate metabolism (24). Several studies have implicated abnormal fatty acid metabolism as an important contributor to the pathogenesis of diabetes-related cardiovascular dysfunction. For example, in untreated type 1 diabetes, elevated plasma fatty acid levels result in a decreased utilization of glucose and an overreliance of the heart on fatty acid oxidation (see review in Ref. 35). Similarly, increased rates of cardiac fatty acid oxidation have been demonstrated in *db/db* mice, a model of type 2 diabetes (4).

An important enzyme controlling both fatty acid and glucose metabolism is AMP-activated protein kinase (AMPK). AMPK acts as a metabolic sensor or “fuel gauge” in the mammalian cell and regulates key proteins controlling fatty acid oxidation, TG synthesis (23), glucose uptake (see review in Ref. 11), and glycolysis (20). The regulation of cardiac fatty acid oxidation by AMPK occurs through the phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) (2, 33). ACC
produces malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase I (CPT I), the rate-limiting enzyme for mitochondrial fatty acid uptake (21). The activation of cardiac AMPK reduces ACC activity, lowers malonyl-CoA levels, and stimulates fatty acid oxidation (14, 15). The activation of AMPK also results in the phosphorylation and inhibition of glycerol-3-phosphate acyltransferase (GPAT), the committed step in de novo synthesis of TG (23). Thus, through the coordinated regulation of key enzymes in fatty acid metabolism, AMPK regulates the flux of fatty acids into oxidation rather than TG storage (23).

The male JCR:LA-cp rat is a model of pre-type 2 diabetes that displays complete skeletal muscle insulin resistance by the age of 12 wk (32). The cp gene encodes for a stop mutation in the extracellular domain of the leptin receptor, resulting in the complete absence of leptin receptors (40). The male JCR:LA-cp rat closely resembles the human metabolic syndrome because, unlike other rodent models, it develops spontaneous cardiovascular disease (29). On the other hand, female JCR:LA-cp rats exhibit severe hypertriglyceridemia but do not develop insulin resistance or cardiovascular disease (8). Studies from our laboratory have demonstrated normal cardiac glucose metabolism in the female JCR:LA-cp rat consistent with the absence of insulin resistance (15). However, it is currently not known whether insulin resistance is present in the male JCR:LA-cp rat heart. In this study, we investigated fatty acid and glucose metabolism in isolated working rat hearts from 12-wk-old insulin-resistant male JCR:LA-cp rats. We further investigated whether changes in AMPK activity were responsible for the altered fatty acid and glucose metabolism in the insulin-resistant hearts.

METHODS

Animals. The study was performed on male insulin-resistant (cp/cp) and lean (either +/cp or +/+) male rats at 12 wk of age. Insulin-resistant rats and lean 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 chow (Rodent Diet 5001; PMI Nutrition International) 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 they were killed. At 12 wk of age, male rats were fasted overnight (unless specified) and anesthetized without restraint in a large glass jar with 3% halothane at 1 liter O2/min. Five different groups of 12-wk-old insulin-resistant and lean control rats were used in this study. This total included rats used for 1) plasma collection, 2) isolated working heart perfusions: rats fasted overnight before the experiment, 3) isolated working heart perfusions: rats fed before the experiment, 4) obtaining frozen heart tissue, and 5) fresh hearts used for preparation of mitochondria.

Plasma measurements. Nonfasted insulin-resistant rats and lean controls of one group were anesthetized as we described, and cardiac puncture was performed to obtain a 3-ml plasma sample. Samples were analyzed for plasma glucose with a glucose oxidase technique (Beckman Instruments) and for insulin by radioimmunoassay (32). Plasma free fatty acids (FFA) were measured using a Wako FFA kit, and plasma TG levels were measured using a Wako Triglyceride E kit (Wako Pure Chemical Industries for both kits).

Heart perfusions. Isolated working hearts from fasted lean and insulin-resistant JCR:LA-cp rats were subjected to an aerobic perfusion with a modified Krebs-Henseleit solution containing 0.8 mM palmitate, 5 mM glucose, 3% bovine serum albumin, 2.5 mM free Ca2+, and 500 μU/ml insulin. This perfusion condition is referred to as “high fat/low glucose/low insulin.” Spontaneously beating hearts were perfused at a left atrial preload of 11.5 mmHg and an aortic afterload of 80 mmHg. Heart rate, peak systolic pressure, aortic developed pressure, cardiac output, aortic flow, coronary flow, and cardiac work were measured as described previously (17).

The hearts in which fatty acid oxidation and glycolytic rates were measured were subjected to a 40-min perfusion with perfusate containing [1-14C]palmitate and [5-3H]glucose. Palmitate oxidation rates were determined simultaneously by quantitative collection of 14CO2 and 3H2O produced by the hearts, as described previously (17, 18). Steady-state fatty acid oxidation and glycolytic rates were calculated from the linear time course as the mean of the nanomoles of [14C]palmitate per gram dry wt per minute or the mean of the micromoles of [3H]glucose per gram dry wt per minute, respectively, for each 10-min sampling time point during the 40-min perfusion. At the end of the perfusions, heart ventricles were quickly frozen with Wollenberger clamps cooled to the temperature of liquid N2.

In another series of perfusions, hearts were taken from nonfasted (fed) JCR:LA-cp rats and perfused in Krebs-Henseleit solution containing 0.4 mM palmitate, 11 mM glucose, and 2,000 μU/ml insulin to mimic plasma levels of fatty acids, glucose, and insulin of fed rats. This perfusion condition is referred to as “low fat/high glucose/high insulin.” Hearts were subjected to the exact perfusion protocol as above, except that the perfusate contained [9,10-3H]palmitate, and palmitate oxidation was measured by collecting 3H2O produced by the heart (17).

Frozen heart isolation. Fed insulin-resistant and lean control rats of another group were anesthetized as described by (1). Once surgical plane was reached, hearts were rapidly isolated, frozen immediately in liquid N2, and stored at −80°C until analysis. Visible adipose tissue was quickly dissected from the heart before freezing.

Tissue TG determination. Twenty milligrams of frozen heart tissue were extracted in a 20-fold volume of 2:1 chloroform-methanol, following which an 0.2 volume of methanol was added, and the extract was vortexed for 30 s. The mixture was then centrifuged at 1,100 g for 10 min, and the supernatant was collected. An 0.2 volume of 0.04% CaCl2 was added to the supernatant, which was then centrifuged at 550 g for 20 min. The upper phase was then removed, and the interface was washed three times with pure solvent upper phase consisting of 1.5 ml chloroform, 24.0 ml methanol, and 23.5 ml water. The final wash was removed, and 50 μl of methanol was added to obtain one phase. The samples were then dried under N2 at 60°C and redissolved in 50 μl of 9.2% tributyl alcohol-Triton X-100. Cardiac TG was then quantitated colorimetrically with an enzymatic assay (Wako Pure Chemical Industries).

Isolation and measurement of ACC and AMPK activity. A cytosolic fraction containing ACC and AMPK was isolated from frozen heart tissue according to the polyethylene glycol
(PEG) precipitation method (14). Protein content was measured by using the Bradford protein assay (Bio-Rad) (1). The “CO₂ fixation technique” was used to measure ACC activity in the 6% PEG fraction (14). AMPK activity of the 6% PEG precipitate was measured as incorporation of "³²P into the synthetic peptide HMSAMSGLHLVKRR (14).

GPAT activity determination. Whole cell homogenates were obtained by homogenizing 50 mg of frozen heart tissue in homogenization buffer containing 0.1 M Tris-HCl (pH 7.5 at 4°C), 50 mM NaF, 5 mM Na pyrophosphate, 1 mM EDTA, 1 mM EGTA, 10% wt/vol glycerol, 1 mM DTT, 0.02% sodium azide, and protease inhibitor cocktail (Sigma Chemical). After homogenization for 30 s, the mixture was centrifuged at 800 g for 10 min. The resulting supernatant was used to determine GPAT activity. Protein content was measured using the Bradford protein assay (Bio-Rad). GPAT activity was assayed by following the incorporation of [³²P]glycerol-3-phosphate into lysophosphatidate, as described by Swanton and Saggerson (36). Reactions were performed at 30°C in solutions containing 1 mM [³²P]glycerol-3-phosphate and 40 μM palmitoyl-CoA in the absence or presence or 10 mM N-ethylmaleimide (NEM) to inhibit the microsomal isoform. Mitochondrial GPAT activity is expressed as the activity determined in the presence of 10 mM NEM.

Malonyl-CoA determination. Approximately 20 mg of frozen heart tissue were extracted in 200 μl of 6% perchloric acid containing 1 mM DTT (to prevent oxidation during isolation). After homogenization, samples were left on ice for 10 min and then centrifuged at 13,400 g for 5 min. The supernatant was removed and subjected to HPLC analysis, as described previously (13).

Immunoblotting. Whole cell homogenates were obtained by homogenizing 50 mg of frozen heart tissue in homogenization buffer containing 0.1 M Tris-HCl (pH 7.5 at 4°C), 50 mM NaF, 5 mM Na pyrophosphate, 1 mM EDTA, 1 mM EGTA, 10% wt/vol glycerol, 1 mM DTT, 0.02% sodium azide, and protease inhibitor cocktail (Sigma Chemical). After homogenization for 30 s, the mixture was centrifuged at 800 g for 10 min. The resulting supernatant was used for immunoblotting. Protein content was measured using the Bradford protein assay (Bio-Rad). Samples were resolved by 9% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in BSA-Triton X-100 and incubated with anti-H-FABP (Santa Cruz Biotechnology). Western blots were visualized using the enhanced chemiluminescence Western blot detection kit.

Preparation of mitochondria from fresh hearts. Hearts from insulin-resistant and lean rats were excised and finely minced with scissors in 10 ml of homogenization buffer (0.25 M sucrose, 5 mM Tris-HCl, and 1 mM EGTA, pH 7.4). The crude homogenate was centrifuged at 800 g for 10 min at 4°C. The resulting pellet was washed in two volumes of homogenization buffer and was then recentrifuged at 800 g for 10 min; this step was repeated twice to maximize the yield of mitochondria. The combined supernatants were centrifuged at 6,000 g for 15 min. The pellet was carefully resuspended in 2 ml of homogenization buffer and centrifuged at 6,000 g for 15 min. The resultant pellet (crude mitochondria) was gently resuspended in 2 ml of homogenization buffer. The crude mitochondrial fraction (0.5 ml) was layered onto 9 ml of 30% Percoll and centrifuged at 50,000 g for 60 min at 4°C. The bottom mitochondrial protein band was collected and used for CPT I activity determination.

CPT I activity. CPT I activity was assayed by measuring acyl carnitine formation, with palmitoyl-CoA and carnitine as substrates. Final concentrations in the assay were 75 μM palmitoyl-CoA, 500 μM carnitine (including 1-[¹⁴C]carni-
tine), 4 mM ATP, 4 mM MgCl₂, 0.25 mM glutathione, 40 μg/ml rotenone, 2 mM KCN, 15 mM KCl, 1% (wt/vol) BSA, and 105 mM Tri-HCl, pH 7.4.

Statistical analysis. All data are presented as means ± SE. The data were analyzed with the statistical program Instat 2.01. Unpaired Student’s t-tests were used to determine the statistical significance of differences between the insulin-resistant JCR:LA-cp and lean control rats. Two-tailed values of P < 0.05 were considered significant.

RESULTS

Characteristics of the insulin-resistant JCR:LA-cp rat. Table 1 depicts some characteristics of 12-wk-old insulin-resistant JCR:LA-cp and lean control rats. Body weights were significantly greater in insulin-resistant rats compared with the lean controls. Heart weights were similar between the insulin-resistant rats and lean controls. The large increase in body weight in the insulin-resistant rats resulted in a slight decrease in heart weight-to-body weight ratio in the insulin-resistant rats compared with the lean controls. Fasting plasma insulin levels were significantly elevated in the insulin-resistant rats compared with the lean controls, demonstrating the presence of insulin resistance. However, the insulin-resistant rats remain normoglycemic, as indicated by similar plasma glucose levels.

Cardiac function in insulin-resistant rat hearts. Mechanical function was monitored continuously over the 40-min perfusion period in the spontaneously beating isolated working hearts. All hearts were subject to a constant left atrial preload of 11.5 mmHg and an aortic afterload of 80 mmHg. As shown in Table 2, heart rate was slightly but nonsignificantly lower in the insulin-resistant hearts compared with lean control hearts, whereas no differences were evident in peak systolic pressure or cardiac output. Both coronary and aortic flows were also continuously monitored, which allowed for the determination of cardiac work (the product of cardiac output and peak systolic pressure) as an index of mechanical function. There was no significant difference in cardiac work (Table 2) between the insulin-resistant and lean control hearts.

Substrate metabolism in insulin-resistant rat hearts. Isolated working hearts from fasted insulin-resistant rats were perfused with Krebs-Henseleit solution containing 0.8 mM palmitate, 5 mM glucose, and 500 μU/ml insulin. This perfusion condition is termed high

<table>
<thead>
<tr>
<th>Table 1. Characteristics of 12-wk-old lean and insulin-resistant rats</th>
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<tr>
<td>Lean (n = 5)</td>
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<tr>
<td>Body wt, g</td>
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<tr>
<td>Heart wt, g</td>
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<tr>
<td>Heart wt/body wt (×1,000)</td>
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<tr>
<td>Plasma insulin, pmol/l</td>
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<td>Plasma glucose, mmol/l</td>
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Values are means ± SE. *Significantly different from lean controls.
Table 2. Mechanical function of isolated working rat hearts from 12-wk-old lean and insulin-resistant rats

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<th>Lean (n = 5)</th>
<th>Insulin Resistant (n = 6)</th>
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<tr>
<td>Heart rate, beats/min</td>
<td>263 ± 7</td>
<td>229 ± 15</td>
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<tr>
<td>Peak systolic pressure, mmHg</td>
<td>102 ± 2</td>
<td>106 ± 2</td>
</tr>
<tr>
<td>HR × PSP, beats·min⁻¹·mmHg⁻¹·10⁻³</td>
<td>27 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>46 ± 6</td>
<td>48 ± 7</td>
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<tr>
<td>Cardiac work, ml·mmHg⁻¹·min⁻¹·10⁻²</td>
<td>47 ± 7</td>
<td>51 ± 7</td>
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Values are means ± SE. Hearts were perfused with Krebs-Henseleit solution containing 5 mM glucose, 0.8 mM palmitate, 3% albumin, and 500 μU/ml insulin. Hearts were subjected to a 11.5 mmHg left atrial preload and 80 mMg aortic afterload. HR × PSP, heart rate × peak systolic pressure.

In this study, we demonstrate that hearts from insulin-resistant JCR:LA-cp rats have a substantial accumulation of TG. We further demonstrate that this appears to be the result of an increased supply and uptake of myocardial fatty acids as opposed to a decrease in fatty acid oxidation rates. The increase in intracellular TG content was also associated with a significant decrease in insulin-stimulated glucose metabolism. However, although the substrate preference of the hearts was dramatically altered, there was no significant difference in AMPK activity, a key regulator of glycolysis, fatty acid oxidation, and TG content.

DISCUSSION

In this study, we demonstrate that hearts from insulin-resistant JCR:LA-cp rats have a substantial accumulation of TG. We further demonstrate that this appears to be the result of an increased supply and uptake of myocardial fatty acids as opposed to a decrease in fatty acid oxidation rates. The increase in intracellular TG content was also associated with a significant decrease in insulin-stimulated glucose metabolism. However, although the substrate preference of the hearts was dramatically altered, there was no significant difference in AMPK activity, a key regulator of glycolysis, fatty acid oxidation, and TG content.
rat hearts have a 50% reduction in glycolytic rates compared with lean controls. This observation is in agreement with studies in the obese Zucker rat, another rodent model of insulin resistance, which also show that cardiac glycolytic rates are reduced by ~50% (22). We further demonstrate that the cardiac TG content was significantly increased in the insulin-resistant hearts compared with those of lean controls. In contrast, there was no significant difference in palmitate oxidation rates between the insulin-resistant and lean control hearts perfused under either high fat/low glucose/low insulin or low fat/high glucose/high insulin conditions. The lack of difference in fatty acid oxidation rates is consistent with a previous study from our laboratory in insulin-resistant JCR:LA-cp rat hearts perfused under low fat/high glucose/high insulin conditions (18). Although glycolytic rates were not measured in this previous study, we demonstrated similar rates of glucose oxidation between control and insulin-resistant hearts (18). Because glucose oxidation is more dependent on fatty acid oxidation rates than glycolysis, this previous study is consistent with a lack of difference in fatty acid oxidation rates between control and insulin-resistant hearts.

The accumulation of intracellular TG is strongly associated with the presence of skeletal muscle insulin resistance (16). In this study, we demonstrate a twofold increase in the cardiac TG content of insulin-resistant rats. The 50% increase in cardiac TG content was directly proportional and correlated to the 50% decrease in glycolytic rates. Although these data provide only correlative evidence to the relationship of cardiac TG content and reduced glucose utilization, several studies in skeletal muscle have demonstrated this same relationship (25, 26, 37). Importantly, previous studies have demonstrated that elevated muscle TG in the JCR:LA-cp rat is strongly associated with the onset of insulin resistance (32). Furthermore, we have shown that treatment of insulin-resistant rats with a lipid-lowering agent, MEDICA 16, prevents the accumulation of muscle TG along with the development of insulin resistance (1, 32).

The accumulation of TG could result from increased fatty acid uptake, decreased fatty acid oxidation, or both. Because fatty acid oxidation rates in the isolated working hearts were not significantly different, we determined whether the supply and uptake of fatty acids into the insulin-resistant rat heart were increased. Indeed, plasma fatty acid and TG levels were significantly elevated in the insulin-resistant rats, indicating that there is an increased supply of fatty acids to the heart. Furthermore, cardiac FABP protein expression was significantly higher in the insulin-resis-

![Fig. 2. Palmitate oxidation rates in isolated working hearts from fasted (A) or fed (B) lean and insulin-resistant rats. Fasted hearts were perfused with Krebs-Henseleit solution containing 0.8 mM palmitate, 5 mM glucose, and 500 µU/ml insulin. Fed hearts were perfused with Krebs-Henseleit solution containing 0.4 mM palmitate, 11 mM glucose, and 2,000 µU/ml insulin, as described in METHODS. Values are means ± SE of 5–6 hearts/group.](http://ajpendo.physiology.org/)

Table 3. Plasm lipid levels and cardiac FABP protein expression in 12-wk-old lean and insulin-resistant rats

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<th>Lean (n = 5)</th>
<th>Insulin Resistant (n = 6)</th>
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<tr>
<td>Plasma fatty acids, mmol/l</td>
<td>0.27 ± 0.02</td>
<td>0.37 ± 0.06*</td>
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<tr>
<td>Plasma triacylglycerol, mmol/l</td>
<td>1.76 ± 0.15</td>
<td>6.44 ± 0.54*</td>
</tr>
<tr>
<td>Heart-FABP expression, relative units</td>
<td>0.91 ± 0.03</td>
<td>1.08 ± 0.02*</td>
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Values are means ± SE. Plasma fatty acid and triacylglycerol measurements were performed on samples obtained from nonfasted lean and insulin-resistant rats. Heart-fatty acid-binding protein (FABP) expression was determined by using immunoblotting and densitometric analysis as described in METHODS. *Significantly different from lean controls.

![Fig. 3. Cardiac triacylglycerol content in lean and insulin-resistant rats. Values are means ± SE of 5–6 hearts/group. *Significantly different from lean controls.](http://ajpendo.physiology.org/)

AJP-Endocrinol Metab • VOL 284 • MAY 2003 • www.ajpendo.org
tant rats compared with the lean controls, suggesting that the insulin-resistant rat hearts are also capable of increased fatty acid uptake. This is consistent with studies demonstrating that skeletal muscle from high fat-fed rats accumulates TG as a result of an enhanced ability of the muscle to take up fatty acids (12). Although we have not directly measured fatty acid uptake in the present study, our data are consistent with studies demonstrating both increased cardiac fatty acid uptake and increased FABP mRNA expression in obese Zucker rat hearts (5, 19).

AMPK is considered a novel therapeutic target for the treatment of obesity, insulin resistance, and type 2 diabetes (38) because of its key role in the regulation of glucose and fatty acid metabolism (11). However, although there was an apparent trend for AMPK activity to decrease in the insulin-resistant rat hearts, we were unable to demonstrate significant changes in AMPK activity. We also did not observe any relationship between AMPK activity and the increased TG content and decreased glycolysis observed in the insulin-resistant rat hearts. Several studies have suggested that AMPK is inhibited by insulin (3, 9, 39) and by high glucose in pancreatic β-cells (34). In hyperglycemic db/db mouse hearts we have observed a significant reduction in AMPK activity, suggesting that hyperglycemia may be a prerequisite for the downregulation of AMPK activity (Atkinson LL, Severson D, and Lopaschuk GD, unpublished observation).

We also measured the activities of enzymes involved in the control of fatty acid oxidation and TG synthesis: ACC activity, CPT I activity, and GPAT activity. However, we were unable to demonstrate any significant difference in these key enzymes that are established downstream targets of AMPK. Thus these data suggest that alterations in AMPK do not mediate the primary alterations occurring in cardiac substrate metabolism in insulin resistance. However, one may speculate that reductions in AMPK activity result when hyperglycemia and type 2 diabetes develop. Further studies are required to elucidate potential changes in AMPK activity that may occur in type 2 diabetes.

In a recent study, Young et al. (41) investigated whether impairments in fatty acid oxidation result in cardiac TG accumulation and contractile dysfunction.

Fig. 4. Cardiac AMP-activated protein kinase (AMPK) activity in lean and insulin-resistant rats. Values are means ± SE of 5–6 hearts/group.

Fig. 5. Cardiac acetyl-CoA carboxylase (ACC) activity (A), malonyl-CoA levels (B), carnitine palmitoyltransferase (CPT I) activity (C), and glycerol-3-phosphate acyltransferase (GPAT) activity (D) in lean and insulin-resistant rats. Values are means ± SE of 5–6 hearts/group. Enzyme activities were determined as described in METHODS.
in obese Zucker rats. They demonstrate that the obese Zucker rat heart is unable to elevate fatty acid oxidation in response to increased fatty acid availability (41). Consistent with the observations in the obese Zucker rat heart, we demonstrate that the JCR:LA-cp rat hearts have an increased fatty acid supply as well as elevated TG content. However, our data suggest that the JCR:LA-cp rat heart has no difference in fatty acid oxidation rates. Although this observation is consistent with that of Young et al., in which fatty acid oxidation rates were similar in fed control and insulin-resistant rats, our data do not suggest that fatty acid oxidation is depressed in hearts from fasted insulin-resistant rats.

One possible explanation for the differences between our study and that of Young et al. (41) may relate to the age of the animals used and the relative level of TG accumulation. For instance, studies in 7- and 14-wk-old obese Zucker hearts have demonstrated the presence of TG accumulation without changes in cardiac function (42). However, the continued accumulation of TG eventually results in depressed cardiac function by 20 wk of age in obese Zucker hearts (42). At this age, troglitazone treatment leads to a reduction in myocardial TG content and the normalization of cardiac function (42), suggesting that there may be a threshold level of TG accumulation that appears to be age dependent.

On the basis of body weight, the obese Zucker animals in the study by Young et al. (41) appear to be older than those used in the present study. Furthermore, the obese Zucker rat hearts exhibited contractile dysfunction, whereas the JCR:LA-cp rat hearts had no difference in mechanical function or cardiac work. Although the absolute levels of cardiac TG in the obese Zucker heart cannot be compared with the level in the JCR:LA-cp heart because of differences in experimental determination, it may be that the initial accumulation of TG in the insulin-resistant heart results from an elevated supply of fatty acids to the heart. Subsequently, impairments in fatty acid oxidation lead to the continued accumulation of TG and contractile dysfunction. Although purely speculative, these data collectively present an interesting hypothesis that clearly requires further investigation.

In summary, we have demonstrated that insulin-resistant JCR:LA-cp rat hearts have a dysregulation in fatty acid metabolism whereby substantial TG accumulates due to increased fatty acid supply with no change in fatty acid oxidation rates. Whether the accumulation of cardiac TG has a role in the development of cardiovascular disease remains to be answered. However, it is interesting to note that the lowering of plasma lipids, which should reduce cardiac TG accumulation, results in protection against the development of cardiovascular disease in the insulin-resistant JCR:LA-cp rat (31, 32).

We thank Ken Strynadka for performing HPLC analysis of CoA esters.

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