Leptin Activates Cardiac Fatty Acid Oxidation Independent of Changes in the AMP-activated Protein Kinase-Acetyl-CoA Carboxylase-Malonyl-CoA Axis*

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Leptin regulates fatty acid metabolism in liver, skeletal muscle, and pancreas by partitioning fatty acids into oxidation rather than triacylglycerol (TG) storage. Although leptin receptors are present in the heart, it is not known whether leptin also regulates cardiac fatty acid metabolism. To determine whether leptin directly regulates cardiac fatty acid metabolism, isolated working rat hearts were perfused with 0.8 ms [9,10-3H]palmitate and 5 mm [1-14C]glucose to measure palmitate and glucose oxidation rates. Leptin (60 ng/ml) significantly increased palmitate oxidation rates 60% above control hearts (p < 0.05) and decreased TG content by 33% (p < 0.05) over the 60-min perfusion period. In contrast, there was no difference in glucose oxidation rates between leptin-treated and control hearts. Although leptin did not affect cardiac work, oxygen consumption increased by 30% (p < 0.05) and cardiac efficiency was decreased by 42% (p < 0.05). AMP-activated protein kinase (AMPK) plays a major role in the regulation of cardiac fatty acid oxidation by inhibiting acetyl-CoA carboxylase (ACC) and reducing malonyl-CoA levels. Leptin has also been shown to increase fatty acid oxidation in skeletal muscle through the activation of AMPK. However, we demonstrate that leptin had no significant effect on AMPK activity, AMPK phosphorylation state, ACC activity, or malonyl-CoA levels. AMPK activity and its phosphorylation state were also unaffected after 5 and 10 min of perfusion in the presence of leptin. The addition of insulin (100 micromols/ml) to the perfusate reduced the ability of leptin to increase fatty acid oxidation and decrease cardiac TG content. These data demonstrate for the first time that leptin activates fatty acid oxidation and decreases TG content in the heart. We also show that the effects of leptin in the heart are independent of changes in the AMPK-ACC-malonyl-CoA axis.

Leptin is a peptide hormone synthesized by adipocytes (1) that plays a key role in the regulation of appetite and energy expenditure through its actions in the hypothalamus (reviewed in Ref. 2). Accumulating evidence now suggests that leptin can also regulate energy homeostasis through direct actions on peripheral lipid and glucose metabolism (reviewed in Ref. 3). In liver, skeletal muscle, and pancreas, leptin partitions fatty acids toward fatty acid oxidation rather than TG 

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† The abbreviations used are: TG, triacylglycerol; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; FA, fatty acid; HPLC, high performance liquid chromatography; PEG, polyethylene glycol.

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the presence of insulin influenced leptin action on cardiac FA metabolism.

**Experimental Procedures**

**Heart Perfusions**—Isolated working hearts from male Sprague-Dawley rats were subjected to an aerobic perfusion with a modified Krebs-Henseleit solution containing 5 mmol/liter glucose, 0.8 mmol/liter palmitate, 3% bovine serum albumin, 2.5 mM free Ca\(^{2+}\), in the absence or presence of 100 micromolar/milliliter insulin. Spontaneously beating hearts were perfused at an 11.5 mm Hg left atrial preload and an 80 mm Hg aortic afterload. Heart rate, peak systolic pressure, aortic pressure, cardiac output, aortic flow, coronary flow, cardiac work, and oxygen consumption were measured as described previously (23).

The hearts, in which fatty acid oxidation and glucose oxidation rates were measured, were subjected to a 60-min perfusion in perfusate containing [9,10-\(^3\)H]palmitate and [1,13\(^C\)]glucose (23). Leptin (60 ng/ml or 3.8 mmol/liter) or vehicle was added 5 min into the perfusion period. Palmitate and glucose oxidation rates were determined simultaneously by quantitative collection of \(^3\)H\(_2\)O and \(^14\)CO\(_2\) produced by the heart, as described previously (23). Steady-state oxidation rates were calculated from the linear time course as the mean of nanomoles of [\(^3\)H]palmitate-\(_{\text{d}}\)H\(_2\)O\(_\text{d}^{-1}\) or [\(^14\)C]glucose\(_{\text{d}^{-1}}\) for each 10-min sampling time during the 60-min perfusion. The rate of acetyl-CoA production from tricarboxylic acid cycle activity was calculated as described previously (23). Cardiac efficiency was defined as the ratio of cardiac work to the rate of tricarboxylic acid cycle acetyl-CoA produced.

Shorter perfusion protocols were also performed under identical perfusate conditions as above. The perfusion protocol consisted of a 20-min period of aerobic perfusion to allow for stabilization, followed by the addition of leptin (60 ng/ml or 3.8 mmol/liter) or vehicle and a subsequent 5- or 10-min perfusion period. Hearts were then rapidly frozen at the end of the 5- or 10-min perfusion for biochemical analysis.

**Tissue TG Determination**—TG was extracted from heart tissue according to the method of Folch (24). Briefly, 20 mg of frozen heart tissue was extracted in a 20-fold volume of 2:1 chloroform:methanol, following which 0.2 volume of methanol was added and the extract vortexed for 30 s. The mixture was then centrifuged at 1100 \(\times\) \(g\) for 10 min and the supernatant collected. 0.2 volume of 0.04% CaCl\(_2\) was added to the supernatant and then centrifuged at 550 \(\times\) \(g\) for 10 min. The resulting supernatant was centrifuged at 800 \(\times\) \(g\) for 10 min. The resulting supernatant was used for immunoblotting. Protein content was measured using the Bradford protein assay. Samples were diluted in protein sample buffer, and 50 \(\mu\)g of protein was loaded onto 9% SDS-polyacrylamide mini-gels. Samples were resolved and transferred to polyvinylidene difluoride membranes. Membranes were blocked in 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween and then incubated with phospho-AMPK (Thr-172) antibody (Cell Signaling Technology). The membranes were subsequently incubated in goat anti-rabbit conjugated to horseradish peroxidase and visualized using the ECL\textsuperscript{®} Western blot detection kit.

**Statistical Analysis**—Data are presented as the means \(\pm\) S.E. of the mean. The data were analyzed using the statistical program Instat 2.01\textsuperscript{®}. An unpaired Student’s \(t\) test was used to evaluate the statistical significance of differences between the control and leptin-treated groups. Two-tailed values of \(p < 0.05\) were considered significant.

**Results**

The effect of leptin (60 ng/ml or 3.8 mmol/liter) on cardiac function in isolated working rat hearts subjected to a 60-min period of aerobic perfusion is shown in Table I. This concentration of leptin had no significant effect on heart rate, peak systolic pressure, cardiac output, or cardiac work compared with control hearts. Heart function was stable in both groups throughout the perfusion period and did not differ in any parameter between 5 and 60 min of perfusion (data not shown).

Cumulative palmitate oxidation increased linearly over the 60-min perfusion period in both the control and leptin-treated hearts (data not shown). As demonstrated in Fig. 1A, leptin significantly increased steady-state palmitate oxidation rates 60% above control levels. Total palmitate uptake ([\(^3\)H]palmitate oxidized + [\(^3\)H]palmitate incorporated into TG) into the heart was also significantly increased in the leptin-treated hearts compared with the control hearts (98 \(\pm\) 7 versus 68 \(\pm\) 5 \(\mu\)mol/g dry weight\(^{-1}\)). However, leptin had no effect on glucose oxidation rates in these hearts (Fig. 1B).

Leptin also significantly reduced cardiac TG content to 75% of the level of control hearts by the end of the 60-min perfusion period (Fig. 2A). To investigate whether the leptin-induced decrease in TG content was the result of decreased incorporation of fatty acid into TG or increased TG lipolysis, we also determined the amount of [\(^3\)H]palmitate incorporated into the cardiac TG pool during the perfusion (Fig. 2B). Leptin had no significant effect on the incorporation of [\(^3\)H]palmitate into TG compared with control hearts, suggesting that the leptin-induced decrease in TG content was caused by an increased rate of TG lipolysis.

**Table I**

| Parameter                      | Control (n = 7) | Leptin (n = 6) |
|--------------------------------|----------------|---------------|
| Heart rate (beats \(\cdot\) min\(^{-1}\)) | 228 \(\pm\) 10 | 244 \(\pm\) 8  |
| Peak systolic pressure (mm Hg)  | 128 \(\pm\) 8  | 118 \(\pm\) 3  |
| HR \(\times\) PSP (beats \(\cdot\) min\(^{-1}\) \(\cdot\) mm Hg\(^{-1}\)) | 29 \(\pm\) 1   | 29 \(\pm\) 1   |
| Cardiac output (ml \(\cdot\) min\(^{-1}\)) | 54 \(\pm\) 3   | 60 \(\pm\) 4   |
| Cardiac work (ml \(\cdot\) mm)     | 69 \(\pm\) 5   | 71 \(\pm\) 5   |

13,400 \(\times\) \(g\) for 5 min. The supernatant was removed and subjected to HPLC analysis as described previously (28).

**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 sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 10% w/v glycerol, 1 mM DTT, 0.02% sodium azide, and protease inhibitor mixture (Sigma). After homogenization for 30 s, the mixture was centrifuged at 800 \(\times\) \(g\) for 10 min. The resulting supernatant was used for immunoblotting. Protein content was measured using the Bradford protein assay. Samples were diluted in protein sample buffer, and 50 \(\mu\)g of protein was loaded onto 9% SDS-polyacrylamide mini-gels. Samples were resolved and transferred to polyvinylidene difluoride membranes. Membranes were blocked in 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween and then incubated with phospho-AMPK (Thr-172) antibody (Cell Signaling Technology). The membranes were subsequently incubated in goat anti-rabbit conjugated to horseradish peroxidase and visualized using the ECL\textsuperscript{®} Western blot detection kit.

**Statistical Analysis**—All data are presented as the means \(\pm\) S.E. of the mean. The data were analyzed using the statistical program Instat 2.01\textsuperscript{®}. An unpaired Student’s \(t\) test was used to evaluate the statistical significance of differences between the control and leptin-treated groups. Two-tailed values of \(p < 0.05\) were considered significant.

**Results**

The effect of leptin (60 ng/ml or 3.8 mmol/liter) on cardiac function in isolated working rat hearts subjected to a 60-min period of aerobic perfusion is shown in Table I. This concentration of leptin had no significant effect on heart rate, peak systolic pressure, cardiac output, or cardiac work compared with control hearts. Heart function was stable in both groups throughout the perfusion period and did not differ in any parameter between 5 and 60 min of perfusion (data not shown).

Cumulative palmitate oxidation increased linearly over the 60-min perfusion period in both the control and leptin-treated hearts (data not shown). As demonstrated in Fig. 1A, leptin significantly increased steady-state palmitate oxidation rates 60% above control levels. Total palmitate uptake ([\(^3\)H]palmitate oxidized + [\(^3\)H]palmitate incorporated into TG) into the heart was also significantly increased in the leptin-treated hearts compared with the control hearts (98 \(\pm\) 7 versus 68 \(\pm\) 5 \(\mu\)mol/g dry weight\(^{-1}\)). However, leptin had no effect on glucose oxidation rates in these hearts (Fig. 1B).

Leptin also significantly reduced cardiac TG content to 75% of the level of control hearts by the end of the 60-min perfusion period (Fig. 2A). To investigate whether the leptin-induced decrease in TG content was the result of decreased incorporation of fatty acid into TG or increased TG lipolysis, we also determined the amount of [\(^3\)H]palmitate incorporated into the cardiac TG pool during the perfusion (Fig. 2B). Leptin had no significant effect on the incorporation of [\(^3\)H]palmitate into TG compared with control hearts, suggesting that the leptin-induced decrease in TG content was caused by an increased rate of TG lipolysis.
Previous studies have demonstrated that myocardial TG lipolysis provides an important source of fatty acids for oxidative metabolism (26). Because leptin treatment reduced TG content, total fatty acid oxidation rates must take into account the exogenous palmitate oxidized as well as the endogenous fatty acid released from TG. Thus, we calculated total fatty acid oxidation using the following equation: 

\[ \text{total [3H]palmitate oxidized during the perfusion} \]

\[ / \text{the change in fatty acid content in TG during the perfusion} \]

\[ / \text{total [3H]palmitate oxidized during the perfusion}. \]

This calculation makes the following assumptions: 1) the TG pool size was similar in control and leptin-treated hearts at the onset of leptin administration; 2) the TG pool size remains constant in the control hearts throughout the perfusion period, which we have shown to occur under these perfusion conditions (26); 3) the amount of [3H]palmitate label entering the TG pool equals that of endogenous or unlabelled fatty acid leaving the pool; and 4) all palmitate released from the TG pool is oxidized.

Previous studies of TG turnover and the contribution of TG to energy substrate utilization in isolated working hearts demonstrate that these assumptions are valid (26, 27). Using the above calculation to determine total fatty acid oxidation, we show that hearts exposed to leptin have total fatty acid oxidation rates that were 82% above control hearts (Fig. 3).

The contribution of glucose and both exogenous and endogenous palmitate to tricarboxylic acid cycle acetyl-CoA production is shown in Fig. 3B. Under the perfusion conditions used, fatty acids were the predominant source of tricarboxylic acid cycle acetyl-CoA in both control and leptin-treated hearts. Leptin treatment resulted in an increase in total tricarboxylic acid cycle acetyl-CoA production. This increase in tricarboxylic acid cycle acetyl-CoA production was specifically caused by an increase in fatty acid oxidation, with no change in the contribution from glucose metabolism. The leptin-treated hearts also consumed significantly more oxygen than the control hearts (Fig. 3C). Although cardiac work was unchanged between control and leptin-treated hearts, the leptin-treated hearts exhibited a significant decrease in cardiac efficiency (i.e., cardiac work/tricarboxylic acid cycle acetyl-CoA produced) (Fig. 3D).

The leptin-induced activation of fatty acid oxidation in skeletal muscle has recently been suggested to be mediated via activation of AMPK (8). However, as demonstrated in Fig. 4A, the leptin-induced activation of fatty acid oxidation in the heart was not accompanied by changes in AMPK activity. We also determined the level of Thr-172 phosphorylation of AMPK, which is indicative of AMPK activation through its upstream kinase, AMPK kinase (28). No difference in the level of Thr-172 phosphorylation of AMPK was observed between control and leptin-treated hearts (Fig. 4A). Representative immunoblots demonstrate variable levels of Thr-172 phosphorylation in the individual hearts, which correlates directly with the level of AMPK activity.

Previous studies have shown that leptin signaling can occur rapidly within minutes (17–19). To determine whether leptin...
may have resulted in an earlier activation of AMPK, which may have been reversed by the end of the 60-min perfusion period, we determined whether leptin could activate AMPK at earlier time points. However, after exposure of hearts to leptin for 5 min (Fig. 4B) or 10 min (Fig. 4C), we were still unable to show any significant difference in AMPK activity or Thr-172 phosphorylation of AMPK.

We also determined whether leptin had any effects on cardiac ACC activity or malonyl-CoA levels, both of which are established downstream targets of AMPK action (10–13). Leptin had no significant effect on cardiac ACC activity or malonyl-CoA (Table II). Increased ratios of AMP:ATP are well known to activate AMPK, but neither AMP nor ATP levels were different between control and leptin-treated hearts (data not shown).

Finally, we investigated whether insulin (100 microunits/ml) had any effect on leptin action in the isolated working rat heart. In hearts perfused with leptin + insulin, no significant effect on the mechanical function was observed compared with hearts perfused with insulin alone (Table III). However, insulin did reduce the leptin-induced activation of exogenous fatty acid oxidation (Table IV). Furthermore, the leptin-induced depletion of cardiac TG was also blocked by insulin (Table IV). Although the presence of insulin significantly increased glucose oxidation rates relative to hearts perfused in the absence of insulin (954 ± 51 compared with 448 ± 51 nmol of [14C]glucose⋅g dry weight−1⋅min−1), glucose oxidative rates were not significantly different between insulin and leptin + insulin hearts (Table IV). Finally, we also demonstrate that in hearts perfused with leptin + insulin, there was no difference in AMPK activity, ACC activity, or malonyl-CoA levels compared with insulin alone (Table IV).

**DISCUSSION**

This study demonstrates for the first time that leptin significantly alters cardiac energy metabolism by preferentially increasing fatty acid oxidation rates and stimulating TG lipolysis. Leptin also increases cardiac tricarboxylic acid cycle activity and oxygen consumption in the absence of any effect on contractile function, resulting in a significant reduction in cardiac efficiency. This suggests that leptin can have important actions on cardiac energy production. We also show that the leptin-induced stimulation of cardiac fatty acid oxidation and mitochondrial metabolism does not occur through the activation of the AMPK-ACC-malonyl-CoA axis. Finally, we show that insulin can prevent the leptin-induced stimulation of fatty acid oxidation and TG lipolysis.

**Leptin Action in the Heart Is Independent of Changes in AMPK**—Recently AMPK was identified as the signaling cascade through which leptin activates fatty acid oxidation in skeletal muscle (8). However, we demonstrate that leptin had no effect on cardiac AMPK activity or AMPK phosphorylation after 5, 10, or 60 min of leptin exposure. After the 60-min perfusion period, there was also no change in the downstream mediators involved in the regulation of cardiac FA oxidation:
ACC activity or malonyl-CoA levels. This suggests that, in the heart, leptin can regulate fatty acid oxidation independent of AMPK.

The reason why leptin activates AMPK in skeletal muscle and not in heart may be related to differences in AMPK subunit expression and regulation between these tissues. AMPK is a heterotrimeric complex comprising an α catalytic subunit and two regulatory subunits (β and γ) (29). AMPK can be activated through phosphorylation of Thr-172 by AMPK kinase (30) and through increases in AMP:ATP (31) and creatine-phosphocreatine (Cr:PCR) ratios (32). Several isoforms of each subunit have been identified with varying tissue distribution (33). The α2β2γ1 and α2β2γ2 complexes are highly expressed in both cardiac and skeletal muscle (29, 34). However, the α2β2γ3 complex is exclusively expressed in skeletal muscle (34). In skeletal muscle, direct activation of α2-AMPK by leptin was accompanied by an increase in AMP levels and Thr-172 phosphorylation (8). However, in isolated working rat hearts, we observed no change in AMP levels or Thr-172 phosphorylation. Although further investigation is required, it is possible that the activation of AMPK by leptin is specific to skeletal muscle and is mediated through the γ3 isoform. Because isoform specific AMPK activity was not determined in this study, we cannot completely rule out the possibility that α2-AMPK activity was increased by leptin. However, if α2-AMPK was activated, changes in Thr-172 phosphorylation, ACC activity, and malonyl-CoA levels would be expected, and these changes were not observed.

**Leptin Has No Effect on Cardiac Mechanical Function but Decreases Cardiac Efficiency**—In the isolated working rat heart, leptin had no significant effect on any measured index of cardiac function (peak systolic pressure, cardiac output, or cardiac work). However, leptin treatment increased myocardial fatty acid oxidation 60% above control hearts and decreased TG content by 33%. Studies of triglyceride turnover in the heart have demonstrated that myocardial TG pools undergo substantial turnover, i.e., continuous lipolysis and synthesis (26). Thus, we determined the amount of [3H]palmitate label in the TG pool and demonstrated that the leptin-induced decrease in TG content was the result of an increase in TG lipolysis rather than a decrease in palmitate incorporation into TG. Previous studies have also demonstrated that myocardial TG lipolysis provides an important source of fatty acids for oxidative metabolism (26). We further determined that the total (exogenous and endogenous) fatty acid oxidation in leptin-treated hearts was elevated 82% above control hearts. Thus, we have demonstrated that leptin increases the oxidation of palmitate derived from exogenous sources as well as from the lipolysis of endogenous myocardial TG.

In addition to an increase in fatty acid oxidation, leptin also increased tricarboxylic acid cycle activity, and oxygen con-
Leptin and Cardiac Fatty Acid Oxidation

TABLE IV

| Table IV: The effect of leptin + insulin on oxidative rates, TG content, and AMPK-ACC-malonyl-CoA | Insulin (n = 7) | Leptin + insulin (n = 6) |
| Palmitate oxidation (nmol [3H]palmitate·g dry weight⁻¹·min⁻¹) | 1108 ± 279 | 1429 ± 291 |
| Glucose oxidation (nmol [14C]glucose·g dry weight⁻¹·min⁻¹) | 954 ± 88 | 1141 ± 249 |
| TG content (μmol·g dry weight⁻¹) | 22 ± 2 | 21 ± 3 |
| Palmitate incorporation into TG (μmol·g dry weight⁻¹) | 6 ± 1 | 6 ± 1 |
| AMPK activity (pmol·min⁻¹·mg⁻¹) | 1133 ± 142 | 941 ± 116 |
| ACC activity (nmol·min⁻¹·mg⁻¹) | 7.3 ± 0.8 | 5.9 ± 1.2 |
| Malonyl-CoA (nmol·g dry weight⁻¹) | 5.8 ± 0.5 | 3.3 ± 1.1 |

values are means ± S.E. measured in hearts subjected to the 60-min perfusion period.

consumption. This resulted in a significant decrease in cardiac efficiency in the leptin-treated hearts. One possible explanation for the decrease in cardiac efficiency is a leptin-induced increase in uncoupling protein 2 (UCP2) and/or UCP3 activity. We did not observe any increases in either UCP2 or UCP3 protein expression (data not shown) but this does not rule out a leptin-induced increase in uncoupling protein activity. Leptin induction of skeletal muscle fatty acid oxidation has been suggested to be the result of an increase in UCP3 activity (35). Further studies are required to determine whether leptin induces mitochondrial uncoupling by increasing UCP2 and/or UCP3 activity in the heart.

The recent classification of obesity as an independent risk factor for cardiovascular disease and characteristic elevated leptin levels in obesity has led to the suggestion that leptin may be the link between obesity and cardiovascular disease (36). This study demonstrates that acute leptin treatment increases myocardial fatty acid oxidation rates and reduces cardiac efficiency. Elevated rates of fatty acid oxidation have previously been demonstrated to reduce cardiac efficiency and the recovery of mechanical function following acute ischemia (37). Although further studies are necessary, it seems likely that long term elevation of plasma leptin levels may result in cardiac dysfunction by stimulating fatty acid oxidation and decreasing cardiac efficiency.

In this study we show that the leptin-induced increase in tricarboxylic acid cycle activity is specifically caused by an activation of fatty acid oxidation originating from exogenous palmitate and endogenous TG with no change in the contribution from glucose metabolism. Although elevated rates of fatty acid oxidation are associated with reduced cardiac efficiency as discussed above, they may be important in preventing the “lipotoxicity” or toxic effects associated with TG accumulation (4, 5, 38). Indeed, in the heart, TG accumulation is associated with depressed contractile function, arrhythmias, hypertrophy, heart failure, and apoptosis (39–44). The reduction of myocardial TG content by troglitazone treatment results in the normalization of cardiac function (39). Thus, elevated leptin levels in obesity may play a protective role in limiting TG accumulation and its associated cardiac dysfunction. However, the potential anti-steatotic benefit of leptin in the heart must be weighed against the elevated rates of fatty acid oxidation and decrease in cardiac efficiency.

Insulin Reduces Leptin-induced Activation of Fatty Acid Oxidation and TG Depletion—Several studies have shown that leptin and insulin signaling pathways interact (17–19) and, further, that AMPK can be activated by leptin (8) and inhibited by insulin (20–22). In this study we demonstrate that the leptin-induced activation of fatty acid oxidation and decrease in TG content is reduced by insulin. We also show that acute exposure to leptin does not interfere with the ability of insulin to stimulate glucose metabolism in the heart. The mechanism by which insulin inhibited the actions of leptin is not clear. However, our data do show that insulin reduced the leptin-induced activation of FA oxidation and TG depletion without changes in AMPK activity, ACC activity, or malonyl-CoA levels.

Other Potential Mediators of Leptin Action—Leptin has also been shown to induce reactive oxygen species generation by increasing fatty acid oxidation in aortic endothelial cells through the activation of protein kinase A, which results in the phosphorylation of ACC and a reduction of ACC activity and malonyl-CoA levels (45). It is therefore possible that leptin may be acting through cAMP-dependent protein kinase activation. However, the elevation of cAMP levels in the heart is associated with increases in contractile function that were not observed in the presence of leptin. Furthermore, there was no change in ACC activity or malonyl-CoA levels. Thus, although cAMP-dependent protein kinase activity was not measured, it seems that leptin does not mediate the activation of fatty acid oxidation in the heart through this mechanism.

The mechanism by which leptin increases cardiac fatty acid oxidation remains unclear. It is possible, however, that increased mitochondrial uncoupling protein activity may play a role. For example, the leptin-treated hearts had an increase in fatty acid oxidation and oxygen consumption. The induction of mitochondrial uncoupling protein activity has been demonstrated to increase oxygen consumption (46). Furthermore, the induction of fatty acid oxidation by leptin in skeletal muscle has been suggested to be the result of an increase in uncoupling protein activity (35). Further studies are required to determine whether leptin can induce mitochondrial uncoupling activity in the heart and whether the induction of mitochondrial uncoupling activity is responsible for the elevation of fatty acid oxidation.

Summary—In conclusion, this study demonstrated for the first time that leptin specifically alters cardiac metabolism by preferentially increasing fatty acid oxidation and TG lipolysis. This occurs independently of changes in the AMPK-ACC-malonyl-CoA axis. Leptin also increased myocardial oxygen consumption and significantly reduced cardiac efficiency. Given that elevated rates of fatty acid oxidation and decreased cardiac efficiency can contribute to contractile dysfunction and cardiovascular disease, future studies are clearly needed to establish whether leptin is indeed the link between obesity and cardiovascular disease.

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