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Induction of Cardiac Uncoupling Protein-2 Expression and Adenosine 5′-Monophosphate-Activated Protein Kinase Phosphorylation during Early States of Diet-Induced Obesity in Mice

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The objective of this work was to characterize the adaptation of cardiac metabolism to a lipid overload in a model of diet-induced obesity (DIO) in mice. After 8 wk dietary treatment, mice receiving a high-fat diet exhibited an increase in the amount of adipose tissue, accompanied by a surge in plasma leptin concentration (from 5.4–16.0 ng/ml). This was associated with: 1) an induction of uncoupling protein-2 (120%), 2) an increase in the phosphorylated form of AMP-activated protein kinase (120%), and 3) a reduction in lactate concentration and lactate dehydrogenase activity in myocardial tissue (40%). Because DIO induces leptin resistance, we analyzed leptin receptor functionality by measuring phospho-signal transducer and activator of transcription 3 in response to acute leptin (1 mg/kg). We observed that leptin receptor signaling remained unaltered within the heart but was fully impaired within the hypothalamus. Taken together, these data show that during DIO development, there is a metabolic shift in the heart aimed at increasing fatty acid oxidation to the detriment of carbohydrates. This effect seems to be leptin-dependent, suggesting that the increased adiposity observed during the onset of obesity might contribute to impairing ectopic lipidic deposition in the heart. (*Endocrinology* 148: 924–931, 2007)

OVERNUTRITION CAUSES ADIPOCYTES to store the surplus fuel in the form of triglycerides (TGs) for retrieval during periods of caloric need. Nonadipose tissues have a limited capacity of TG storage. Indeed, an excessive lipid deposition in organs and tissues such as liver, skeletal muscle, pancreas, or heart has been related to the development of fatty liver, insulin resistance, pancreatic dysfunction, or lipid cardiomyopathy (1). Energy-dissipating mechanisms, together with increased β-oxidation of fatty acids (FAs), are key elements of metabolic adaptation to an elevated intake of fat and are crucial to understand alterations linked to the development of obesity. In this context, uncoupling proteins (UCPs) impair the storage of energy as high-energy phosphates, facilitate heat dissipation, and might contribute to preventing ectopic accumulation of lipids and lipotoxicity (2–7).

High-fat (HF) diets have been shown to induce fat accumulation and overweight in mice prone to diet-induced obesity (DIO). Elevation of plasma leptin concentration is detected early after HF diet instauration (8) in accordance with the lipostatic action of this adipocyte-derived hormone, which acts both on peripheral and central hypothalamic targets. In fact, leptin elicits a satiating effect, up-regulates FA oxidative capacity, and down-regulates the activity of lipogenic enzymes. Leptin also induces the expression of mitochondrial UCPs in different tissues such as brown adipose tissue (9) or β-cells (2). Numerous works carried out in rodents made hyperleptinemic by means of genetic manipulation suggest that leptin might limit ectopic accumulation of lipids, thus protecting lean tissues against lipotoxicity (1, 7, 10–12). Nevertheless, DIO mice develop progressive leptin resistance within the hypothalamus (8, 11, 12), suggesting a desensitization of leptin receptors or leptin transport from the periphery to the brain. Leptin resistance probably also concerns peripheral tissues, but this issue has not been properly established.

Obesity is an independent risk factor for cardiac disease (13) that has been associated with left ventricular hypertrophy, lipid cardiomyopathy, heart failure, and sudden death (14). A substantial amount of work regarding cardiac changes in obese models has contributed to understanding how the heart adapts to obesity. Most data have been obtained by using genetic models of obesity, *i.e.* leptin deficiency (14–16) or leptin signaling disruption (14), as well as genetically engineered rodents (7). However, there are no data regarding the progress of cardiac adaptation to diets containing an elevated fat content, which, in our eyes, more closely resembles the development of human obesity. Our

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* B.S. and R.G. contributed equally to this study.

Abbreviations: AMPK, AMP-activated protein kinase; DIO, diet-induced obesity; FA, fatty acid; HF, high fat; LDH, lactate dehydrogenase; LF, low fat; pAMPK, phosphorylated AMPK; pSTAT3, phosphorylated signal transducer and activator of transcription 3; TG, triglyceride; UCP, uncoupling protein.

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rationale to design this study was that a diet containing an elevated fat content should initially activate mechanisms aimed at buffering the excess lipid intake by increasing: 1) fat stores in adipose tissue and 2) mitochondrial uncoupling and/or oxidative mechanisms in the heart. If leptin plays a role in the development of metabolic changes in the heart, we should detect a parallelism between leptin responsiveness and its ability to activate metabolic uncoupling and to limit lipid deposition. To evaluate early changes in cardiac metabolic adaptation, the study was carried out over a period of 8 wk. We determined cardiac levels of: 1) UCP-2, 2) phosphorylated AMP-activated protein kinase (pAMPK) and AMPK, and 3) lactate content and lactate dehydrogenase (LDH) activity. We also analyzed the eventual contribution of leptin to metabolic changes evoked by a fat overload.

Materials and Methods

Animals and chow

Four-week-old male C57BL/6J mice (CRIFA, Barcelona, Spain) weighing 16–18 g were housed under a 12-h light/12-h dark cycle, in a temperature-controlled room (22 C) with standard food and water *ad libitum*, in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals. After 1 wk, animals were divided into two groups with similar average body weight, housed six to nine per cage, and assigned either to a low-fat (LF) or HF diet. LF (D12450B, 10 kcal % fat, 70 kcal % carbohydrates, and 20 kcal % protein; 3.85 kcal/g) or HF (D12451, 45 kcal % fat, 35 kcal % carbohydrates, and 20 kcal % protein; 4.73 kcal/g) diets were supplied by Research Diets, Inc. (New Brunswick, NJ) and will be referred to as LF and HF, respectively.

Experimental design

HF-fed and their respective LF control mice had free access to food for either 4 or 8 wk. On the last day, mice were killed by decapitation between 1000 and 1200 h. Truncal blood was collected in chilled EDTA-coated polypropylene tubes and heart as well as lumbar, and mesenteric adipose tissue was dissected, weighed, and stored at −80 C.

Plasma measurements

Plasma leptin concentration was analyzed using a specific RIA kit for murine leptin (Linco Research, St. Charles, MO) (4.9% intraassay variation, 3.3% interassay variation). Insulin was determined by means of a specific ELISA kit for mouse insulin (Mercodia, Denmark) (2.2% intraassay variation, 4.9% interassay variation). Glucose was measured by a spectrophotometric method (Glucose Trinder Method, Roche Applied Science, Indianapolis, IN). TGs and free FAs were determined using the GPO (BIOLABO, Maizy, France) and ACS-ACOD (Wako Bioproducts, Richmond, VA) methods, respectively.

Western blot for UCP-2, pAMPK, and phosphorylated signal transducer and activator of transcription 3 (pSTAT3)

UCP-2, pAMPK, and pSTAT3 were measured in whole cardiac left ventricle. Briefly, tissues were homogenized in ice-cold buffer containing 0.42 M NaCl, 20 mM HEPES (pH 7.9), 1 mM $Na_4P_2O_7$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 20% glycerol, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 20 mM sodium fluoride, 1 mM trisodium orthovanadate, and 2 mM phenylmethylsulfonyl fluoride. Tubes containing homogenates were frozen at −80 C and thawed at 37 C three consecutive times, then centrifuged for 10 min at 4 C. Equivalent amounts of proteins (50 μg) present in the supernatant were loaded in Laemli buffer [50 mM Tris (pH 6.8), 10% sodium dodecyl sulfate, 10% glycerol, 5% mercaptoethanol, and 2 mg/ml blue bromophenol] and size-separated in 15% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride membranes (GE Healthcare, Little Chalfont, Buckinghamshire, UK) using a transblot apparatus (Bio-Rad, Hercules, CA). For immunoblotting, membranes were blocked with 5% nonfat dried milk in Tween-PBS for 1 h. Primary antibodies against UCP-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1/100 final dilution), pSTAT3 (Tyr^{705}) (Cell Signaling Technology Inc., Beverly, MA; 1/100 final dilution), STAT3 (Santa Cruz Biotechnology; 1/1000 final dilution), pAMPK-α (Thr^{172}) (Cell Signaling Technology; 1:1000 final dilution), and AMPK-α (Cell Signaling Technology; 1:1000 final dilution) were applied at the convenient dilution overnight at 4 C. After washing, appropriate secondary antibodies (antigoat IgG-peroxidase conjugated) were applied for 1 h at a dilution of 1/15,000. Blots were washed, incubated in commercial enhanced chemiluminescence reagents (GE Healthcare), and exposed to autoradiographic film. To prove equal loadings of samples, blots were reincubated with β-actin antibody (Affinity Bioreagents, Golden, CO). Films were scanned using a GS-800 Calibrated Densitometer (Bio-Rad), and blots were quantified using Quantity One software (Bio-Rad). Values for UCP-2 were normalized with β-actin to account for variations in gel loading. Values for pSTAT3 and pAMPK were normalized with STAT3 and AMPK, respectively.

Determination of LDH activity

Left ventricular tissue (25 mg) was weighed and then homogenized in 0.5 ml ice-cold 0.1 M phosphate buffer (pH 7.8) containing 0.1 mM $MgSO_4$, 2 mM EDTA, and 0.2% BSA. Enzyme activity was determined by the SFBC modified method (BiOLABO). Briefly, homogenates were diluted (1:4) in saline and incubated at 37 C. Absorbance (340 nm) was recorded after 30, 60, and 120 sec and Δ absorbance per minute was calculated. Enzymatic activity was expressed in international units per milligram of protein.

Determination of lactate concentration

Lactate concentration was measured in the same homogenates prepared for LDH activity determination using an enzymatic colorimetric method (Spinreact, Granada, Spain). Briefly, 2 μl homogenate was diluted in 200 μl PIPES 50 mM (pH 7.5) containing 4-chlorophenol (4 mM), lactate oxidase (800 U/liter), peroxidase (2000 U/liter), and 4-aminophenazone (0.4 mM), then incubated 10 min at room temperature, and absorbance (505 nm) was recorded (2.1% intraassay variation, 3.1% interassay variation). Lactate concentration was expressed in millimoles per milligram of protein.

Determination of heart TGs

TG content in the heart was determined following the method described by Unger (1). Briefly, 20 mg left ventricle was homogenized in a solvent mixture containing 40 μl 2 mM NaCl/20 mM EDTA/50 mM sodium phosphate buffer (pH 7.4), 40 μl *tert*-butylic alcohol, and 20 μl Triton X-100/methanol mixture (1:1). TGs were measured with a Sigma diagnostic kit.

Assessment of leptin resistance

Recombinant murine leptin (1 mg/kg) or saline was administered at 0900 h. After 90 min, mice were killed by decapitation, and hearts and hypothalami were dissected and stored at −80 C until assay. Tissues were prepared for Western blotting as described above.

Statistics

Body weight and food intake variations were analyzed by a two-way ANOVA. The factors of variation were pharmacological treatment and time. Other parameters were analyzed by a one-way ANOVA, followed by Newman-Keuls *post hoc* test. Statistical significance was set at $P < 0.05$.

Results

Effect of a HF diet on body weight, adiposity, and plasma parameters

Overweight was induced in C57BL/6J mice by exposure to a diet in which 45% of the calories were derived from fat.

A control group was fed with LF chow, which only yielded 10% of the calories from fat. The study was carried out over a period of 8 wk to determine early changes in cardiac metabolic adaptation. As illustrated in Fig. 1A, the difference in body weight was already statistically different after 4 wk dietary treatment and progressively increased until wk 8 [two-way ANOVA, $F_{(1,81)} = 18{,}519$; $P < 0.001$ for diet, $F_{(2,81)} = 148{,}362$; $P < 0.001$ for time, $F_{(2,81)} = 3373$; $P < 0.05$ for the interaction]. During this period, food intake (Fig. 1B) was lower in the HF group [two-way ANOVA, $F_{(1,81)} = 207{,}395$; $P < 0.001$ for diet, $F_{(2,81)} = 42{,}967$; $P < 0.001$ for time, $F_{(2,81)} = 19{,}654$; $P < 0.001$ for the interaction], although the average kcal consumption was equivalent both in LF and HF groups (Fig. 1C), indicating that the increase in body weight was related to the type of food consumed rather than to a supplementary intake of kcal. As summarized in Table 1, the HF diet induced a significant gain in adipose tissue, which increased by more than 350% in the case of lumbar adipose tissue after 8 wk HF. Surprisingly, heart weight exhibited a significant increase after 4 wk dietary treatment [$F_{(1,14)} = 9331$; $P < 0.01$) that was not observed after 8 wk of the fat diet [$F_{(1,28)} = 2801$; $P = 0.1$]. Serum analysis revealed that 8 wk HF induced significant hyperleptinemia (Table 2) without changes at this time point in other biochemical parameters, including free FAs. Simple regression analysis revealed a significant correlation [$F_{(1,13)} = 229{,}014$; $P < 0.001$; r = 0.97] between plasma leptin concentration and the amount of lumbar adipose tissue.

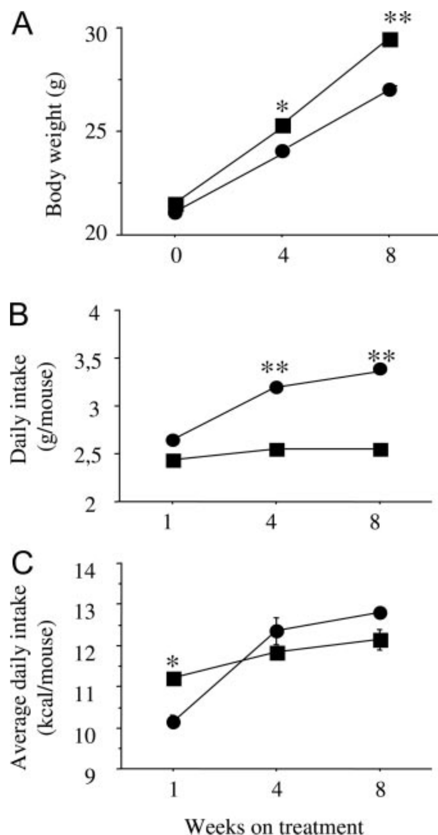

FIG. 1. Time course of body weight evolution and daily food intake. A, Effect of LF (*black circles*) and HF (*black squares*) diets in the evolution of body weight during a period of 8 wk. B, Idem in daily food intake. C, Effect of dietary treatment on kcal intake. Values are means ± SEM of 15–18 individual values. *, $P < 0.05$; **, $P < 0.01$, Newman-Keuls test.

Determination of heart TGs

One-way ANOVA revealed an effect of diet on the amount of TGs in left ventricle, which increased from 1.83 ± 0.24 mg/g tissue to 2.59 ± 0.28 mg/g tissue [$F_{(1,24)} = 4197$; $P < 0.05$] after 4 wk dietary treatment. In 8-wk-treated animals, TG content was similar in control (3.29 ± 0.49 mg/g tissue) and HF (2.90 ± 0.51 mg/g tissue) animals.

Cardiac UCP-2 is increased after 8 wk HF diet

As shown in Fig. 2A, Western blot revealed that the expression of UCP-2 is similar between groups after 4 wk dietary treatment. However, after 8 wk, UCP-2 expression was significantly increased in the HF group compared with the LF diet group [$F_{(1,14)} = 7912$; $P < 0.05$]. Changes in UCP-2 expression after 8 wk positively correlated with the amount of adipose tissue (Fig. 2B) [$F_{(1,14)} = 53{,}425$; $P < 0.001$] and with plasma leptin levels (Fig. 2C) [$F_{(1,6)} = 60{,}658$; $P < 0.01$].

pAMPK is increased in the heart after 8 wk HF diet

As illustrated in Fig. 3, AMPK expression was similar at 4 and 8 wk HF dietary treatment. In contrast, basal phosphorylation (pAMPK-Thr[172]) of this enzyme was significantly increased by about 2.2-fold after 8 wk HF feeding [$F_{(1,13)} = 6361$; $P < 0.01$].

Lactate concentration and LDH activity are decreased in the heart after 8 wk HF diet

Figure 4 illustrates the influence of a HF diet on cardiac lactate concentration and LDH activity. One-way ANOVA revealed a significant increase in lactate content [$F_{(1,32)} = 10{,}035$; $P < 0.01$] 4 wk after dietary treatment (Fig. 4A) which was concomitant with the enhancement of LDH activity [$F_{(1,31)} = 6749$; $P < 0.05$] (Fig. 4B). In contrast, lactate concentration was significantly lower in mice maintained on the HF diet for 8 wk [$F_{(1,31)} = 6749$; $P = 0.058$] (Fig. 4C). Accordingly, LDH activity also decreased in this experimental group [$F_{(1,17)} = 7114$; $P < 0.05$] (Fig. 4D). The reduction in lactate concentration showed a negative correlation with the ratio pAMPK/AMPK [$F_{(1,7)} = 6851$; $P < 0.05$] (Fig. 5A) as well as with the expression of UCP-2 [$F_{(1,8)} = 8.004$; $P < 0.05$] (Fig. 5B) after 8 wk HF diet.

pSTAT3 is increased in the heart after 8 wk HF diet but not in the hypothalamus

To assess the development of leptin resistance after 8 wk HF diet, we analyzed the responsiveness to acute leptin in both cardiac tissue and hypothalamus. Recombinant murine leptin (1 mg/kg) was administered ip to groups of LF- and HF-fed mice and phosphorylated STAT3 measured 90 min after leptin administration by means of Western blot. Two-way ANOVA revealed that the type of diet used had no effect

TABLE 1. Effect of dietary treatment on weight of lumbar and mesenteric adipose tissue, liver, and heart

	4 Wk on diet		8 Wk on diet	
	LF diet	HF diet	LF diet	HF diet
Lumbar adipose tissue (mg)	76.6 ± 4.5	152.1 ± 15.3[b]	99.8 ± 8.8	377.8 ± 25.5[c]
Mesenteric adipose tissue (mg)	297.8 ± 12.0	317.0 ± 19.5	293.2 ± 22.5	397.5 ± 29.5[a]
Liver (g)	1.01 ± 0.02	0.91 ± 0.03	0.92 ± 0.02	0.89 ± 0.04
Heart (mg)	118.1 ± 3.3	138.6 ± 4.9[c]	119.1 ± 1.8	126.3 ± 4.2

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ compared with their corresponding matched control groups. Newman-Keuls test.

on the basal concentration of cardiac pSTAT3 [$F_{(1,11)} = 0.497$; not significant], but all LF- and HF-fed mice exhibited increased values of pSTAT3 after acute treatment with leptin [$F_{(1,11)} = 8799$; $P < 0.05$], without a significant interaction between the type of diet and pharmacological treatment [$F_{(1,11)} = 0.019$; not significant]. These results are shown in Fig. 6, A and B, and demonstrate that leptin receptors in the heart are responsive to leptin. In contrast, peripheral leptin administration had no effect on STAT3 phosphorylation in the hypothalamus of HF-fed mice (Fig. 6C).

Discussion

This study documents the importance of dietary composition on changes in cardiac metabolism detected early after the onset of an elevated intake of saturated fat. We demonstrate that a HF diet up-regulates the expression of UCP-2, enhances the activity of metabolic pathways involved in FA oxidation, and decreases the oxidation rate of carbohydrates in the heart. These effects are not apparently linked to a caloric surplus because daily caloric intake was similar in LF- and HF-treated animals, but seem to be related to the hyperleptinemia induced by the HF diet. The major new finding of this study is that cardiac metabolic adaptation to HF occurs in parallel with the increase in plasma leptin concentration. Interestingly, cardiac responsiveness to leptin was fully conserved 8 wk after HF diet, whereas leptin resistance within the hypothalamus appeared early during the development of DIO (12; this study). Data presented in this work demonstrate that physiopathological mechanisms regulating hypothalamic and peripheral leptin receptor responsiveness are different and suggest that leptin plays a key role in adaptation to a lipid overload in the heart by an extrahypothalamic mechanism.

UCPs act as proton carriers regulating mitochondrial membrane potential. In the case of UCP-1, which is mainly expressed in brown adipose tissue, this effect results in heat dissipation. In addition to the role of UCP-2 in thermogenesis, numerous studies suggest the involvement of these proteins in adaptive mechanisms aimed at improving FA use (2, 4; for review, see Ref. 17) and at limiting fat accumulation in non adipose tissues (1; for review, see Ref. 7). For example, mitochondrial uncoupling appears to play an important role in cardiac metabolic adaptation in newborn animals as a consequence of the elevated lipid/carbohydrate ratio in milk compared with the fetal diet (18). A similar metabolic adaptation to a lipid overload might be occurring in our dietary treatment.

In postprandial periods, about 60–90% of cardiac ATP production is derived from FA oxidation and only 10–40% from lactate and glycolysis. Lactate uptake is a major source of pyruvate, which is oxidized by LDH yielding pyruvate (19). The relative proportion between FA and glucose/lactate as a source of ATP is dependent on arterial substrate concentration and hormone levels (Ref. 20 and references cited therein). In consequence, an excess of dietary lipids would enhance FA metabolism to the detriment of lactate and glucose. Our data suggest that this metabolic adaptation occurs gradually. We observed that both cardiac lactate concentration and LDH activity are increased after 4 wk dietary treatment. In later stages (8 wk), lactate uptake and oxidation seem to decline, suggesting that the ability of the heart to manage FA is adapted to the HF diet. Interestingly, at this time point, lactate concentration negatively correlated with UCP-2 expression (r = 0.73), suggesting improved FA use. As further confirmation, we also observed an increase of pAMPK. AMPK is a key enzyme in energetic metabolism, and an increase in pAMPK leads to the stimulation of β-oxidation and inhibition of lipogenesis. Basal pAMPK is also increased in skeletal muscle after HF dietary treatment (21). As occurs with UCP-2, lactate concentration also correlated negatively with pAMPK (r = 0.72), indicating that the decline in lactate uptake occurs when the ability of the heart to

TABLE 2. Effect of dietary treatment on plasmatic parameters

	4 Wk on treatment		8 Wk on treatment	
	LF diet	HF diet	LF diet	HF diet
Insulin (μg/liter)	0.75 ± 0.07	0.98 ± 0.09	0.70 ± 0.08	0.89 ± 0.06
Leptin (ng/ml)	6.4 ± 0.8	8.8 ± 3.9	5.4 ± 0.3	16.0 ± 1.7[a]
Glucose (mg/dl)	148.5 ± 12.4	157.7 ± 4.3	157.4 ± 3.9	157.7 ± 3.9
TGs (mg/dl)	85.2 ± 10.6	65.4 ± 6.2	64.1 ± 6.0	69.5 ± 3.6
Free FAs (mM)	0.78 ± 0.06	0.67 ± 0.02	0.70 ± 0.06	0.61 ± 0.02
Glycerol (μM)	680.7 ± 41.6	623.3 ± 38.2	622.5 ± 46.2	747.5 ± 44.5

[a] $P < 0.01$ compared with the corresponding matched control groups. Newman-Keuls test.

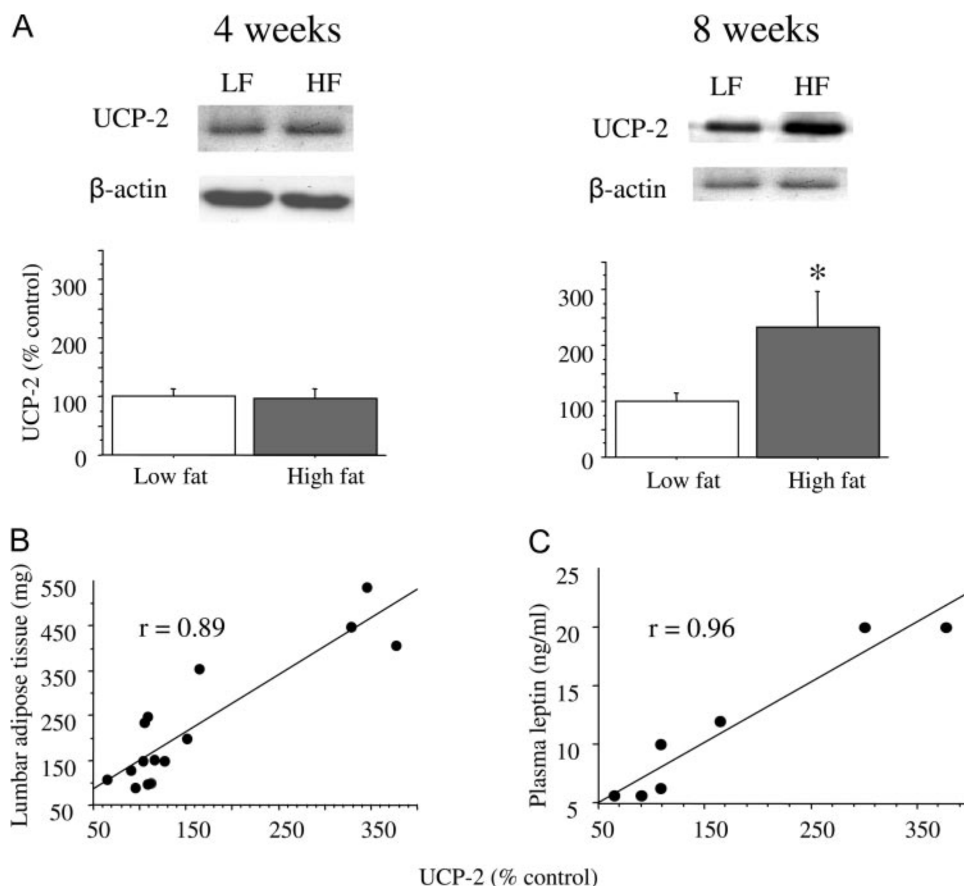

FIG. 2. A, Effect of a HF diet on protein expression of cardiac UCP-2, measured after 4 and 8 wk dietary treatment. *Microphotographs* illustrate representative Western blots of UCP-2 (n = 6–8 animals/treatment). *Diagram bars* show the result of densitometric analysis of UCP-2 immunoblots, expressed as percentage of UCP-2 in the control group. Data are presented as means ± SEM. Statistical analyses were performed with one-way ANOVA followed by a Newman-Keuls test. *, $P < 0.05$ compared with the control group. B, Correlation between UCP-2 expression and the amount of lumbar adipose tissue. C, Correlation between UCP-2 expression and plasma leptin concentration. Individual values in B and C correspond to animals receiving a HF diet during 8 wk.

metabolize FA increases. Interestingly, cardiac TGs were slightly increased after 4 wk HF diet but returned to control values after 8 wk. These changes occurred in parallel with changes in cardiac weight. This normalization is coincident with the increase in all plasma leptin, adiposity, AMPK phosphorylation, and UCP-2 expression. Taken together and despite the lack of direct functional measures of fat oxidation, all these changes suggest that β-oxidation becomes a more important source of energy in HF than in LF-fed mice to the detriment of pyruvate oxidation. As additional support for the role of leptin, studies carried out in leptin-deficient mice, which exhibit elevated content of cardiac TGs (1), have provided evidence that leptin repletion reverses cardiac hypertrophy (14), suggesting that leptin is a relevant mediator for cardiac homeostasis.

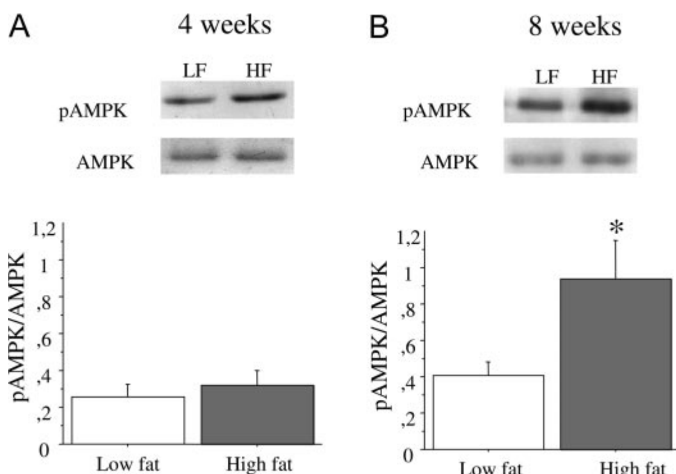

FIG. 3. Cardiac expression of AMPK and phosphorylated AMPK-Thr[172] (pAMPK) after 4 (A) and 8 (B) wk of LF or HF diet. *Top*, Representative Western blots of phospho-AMPK and total AMPK (n = 6–7 animals/treatment). *Bottom*, Level of phosphorylation of AMPK expressed as the mean ± SEM of the ratio pAMPK/AMPK (n = 7–9 animals). Statistical analyses were performed with one-way ANOVA followed by a Newman-Keuls test. *, $P < 0.05$ compared with the control group.

A main finding in this work is the link between the surge in both adiposity and plasma leptin concentration and changes in cardiac UCP-2, pAMPK, and TGs. In this study, hyperleptinemia associated with the HF diet might be responsible, at least partially, for metabolic uncoupling in the heart. The positive correlation between plasma leptin concentration and the expression of UCP-2 (r = 0.98) detected 8 wk after the HF diet strongly supports a role for leptin in the metabolic adaptation observed in this study. Indeed, UCP-2 remains unaltered after 4 wk of dietary treatment, when plasma leptin values were still similar both in LF- and HF-fed mice. In fact, HF diets are known to induce UCP-2 expression in the heart (22), and a role for leptin in modulating UCP-2 expression in adipose tissue and skeletal muscle has been suggested in models of transitory hyperleptinemia induced by exogenous administration of leptin (23). Expression of UCP-2 has been shown to be up-regulated in rats made hyperleptinemic by means of leptin gene transfer, both in

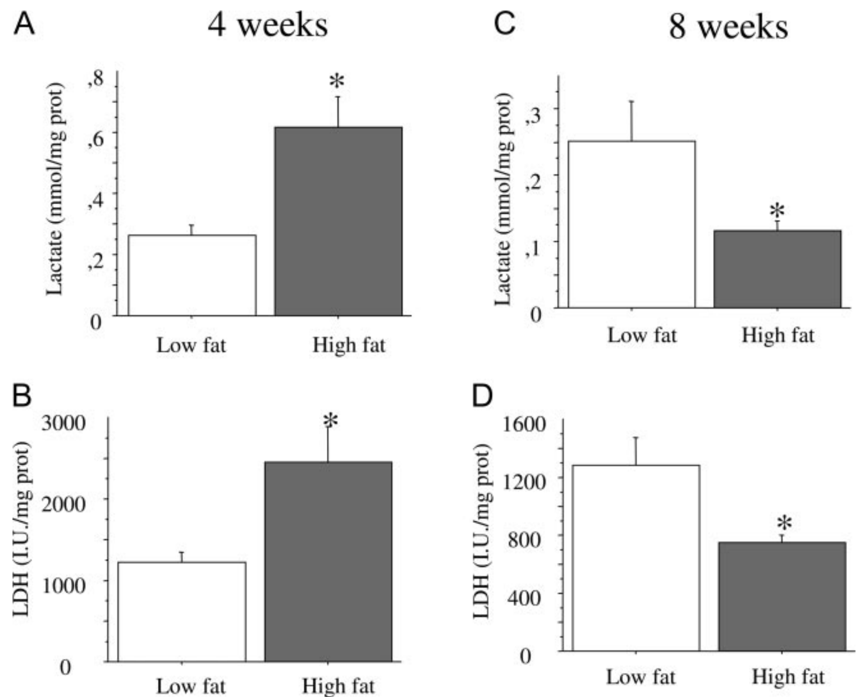

FIG. 4. Effect of a HF diet on cardiac lactate concentration (millimoles per milligram of protein) and LDH activity (international units per milligram of protein). A and B, Effect of 4 wk dietary treatment. C and D, Effect of 8 wk dietary treatment. Results are expressed as means ± SEM of 15–17 individual values. Statistical analyses were performed with one-way ANOVA followed by a Newman-Keuls test. *, $P < 0.05$ compared with the control group.

β-cells (2) and adipocytes (24). Nevertheless, the role of leptin in regulating UCP-2 expression is far from being well characterized and seems to depend on the experimental conditions used. For example, studies carried out in *ob/ob* (leptin-deficient) mice suggest that the elevated expression of UCP-2 observed in the adipose tissue of these animals is linked to the absence of leptin signal (25). As a further confirmation, Commins *et al.* (26) have reported a lack of effect of exogenous leptin in UCP-2 expression in white adipose tissue of *ob/ob* mice. As an alternative, biochemical changes detected in HF mice might be linked to the type of food rather than to the increase of fat mass and/or leptin. However, studies carried out after 72 h of dietary treatment (at this time point, both adipose tissue and plasma leptin remain unchanged) did not evidence changes in cardiac TGs, lactate, or LDH activity (data not shown). This supports the importance of adipocytokines in the metabolic changes characterized in this study. In any case, mechanistic studies are needed to provide a definitive cause-effect relationship between plasma leptin concentration and UCP-2 expression.

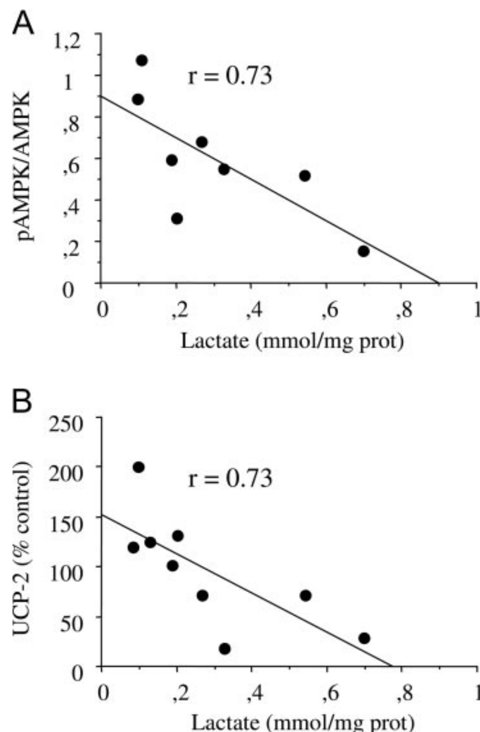

FIG. 5. Correlation between cardiac lactate concentration and pAMPK/AMPK ratio (A) and UCP-2 expression (B) in mice treated with HF diet for 8 wk.

In addition to the correlation between leptin and UCP-2 expression, our study yields additional data supporting the role of leptin. Leptin receptors are expressed in cardiac myocytes (27, 28), and we have demonstrated that these receptors are functionally coupled to the JAK/STAT signaling pathway (this study). Interestingly, although leptin signaling within the hypothalamus is disrupted early after HF instauration (12; this study), responsiveness of leptin receptors remains unaltered after 8 wk HF. This result demonstrates dissociation between central and peripheral resistance to leptin. Studies carried out in DIO models together with clinical data suggest that leptin resistance is linked to the impairment of leptin transport from the periphery to the brain, thus involving Ob-Ra rather than Ob-Rb receptors. Our results fit well with this hypothesis; thus, peripheral Ob-Rb receptors, which are accessible to circulating leptin, conserve leptin responsiveness longer than central receptors. It has to be noted nevertheless that the lack of leptin inputs in the central nervous system can also affect peripheral signals, *i.e.*

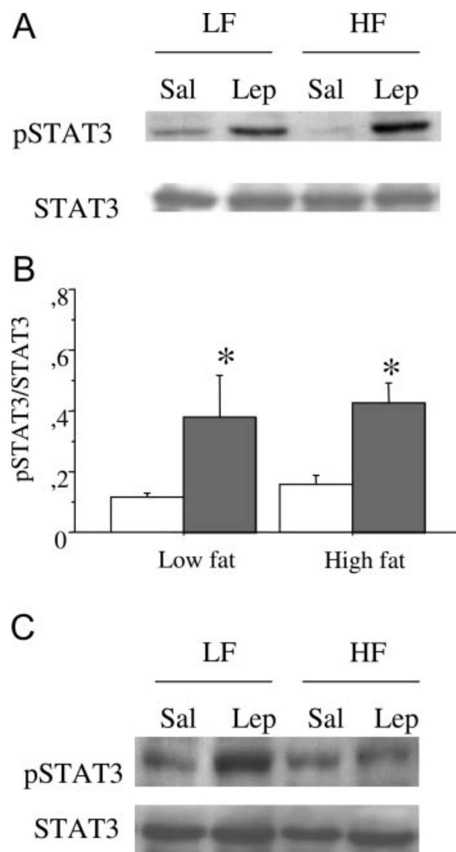

FIG. 6. Cardiac expression of STAT3 and pSTAT3 90 min after leptin injection (1 mg/kg ip) to mice treated during 8 wk with HF diet. A, Immunoblot of both STAT3 and pSTAT3 obtained from left ventricle. B, Effect of leptin on STAT3 phosphorylation as the mean ± SEM of the ratio pSTAT3/STAT3 (n = 5–7 animals). *, $P < 0.05$ compared with the control group. C, Effect of acute leptin (1 mg/kg ip) on STAT3 phosphorylation in the hypothalamus of both LF and HF mice.

autonomic nervous system, modulating metabolism in the heart.

Taken together, our data strongly suggest that during early states of DIO, there is a metabolic adaptation in the heart shifting from pyruvate to FA oxidation. The absence of cardiac leptin resistance, together with the positive correlation between plasma leptin and UCP-2 expression, suggest that this shift is mediated, at least in part, by leptin. The increase in plasma leptin occurring in parallel with the increase in adipose tissue has the physiological significance of protecting nonadipose tissue and, in our context, the heart, against ectopic lipidic deposition and lipotoxicity. Indeed, it has been reported that leptin deficiency in ob/ob mice is accompanied by the accumulation of TGs in cardiac myocytes (15) and that leptin reverses morphological alterations in the ob/ob mouse (14). However, the participation of other adipokines, such as adiponectin (Ref. 1 and references cited therein), cannot be excluded.

In summary, we demonstrate that, with diets containing elevated fat, the heart cannot initially metabolize the excess dietary lipids and, instead, stores them as TGs. However, a subsequent surge in plasma leptin concentration, linked to an increased adiposity, induces the expression of UCP-2 and phosphorylates AMPK, leading to a normalization of cardiac TG content together with a shift to FA as an energy source. Our work shows that all these changes could be advantageous because they contribute to impairing ectopic fat deposition in the heart and suggest that further resistance to leptin might be responsible for cardiac lipotoxicity.

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