Combined Leptin Actions on Adipose Tissue and Hypothalamus Are Required to Deplete Adipocyte Fat in Lean Rats

IMPLICATIONS FOR OBESITY TREATMENT

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Intense hyperleptinemia completely depletes adipocyte fat of normal rats within 14 days. To determine the mechanism, epididymal fat pads from normal wild-type (+/+ ) and obese (fa/fa) Zucker Diabetic Fatty (ZDF) donor rats were transplanted into normal +/+ and fa/fa ZDF recipients. Hyperleptinemia induced by adenovirus-leptin administration depleted all fat from native fat pads and from fat transplants from +/+ donors but not from transplants from ZDFfa/fa donors with defective leptin receptors. In both native and transplanted +/+ fat pads, large numbers of mitochondria were apparent, and genes involved in fatty acid oxidation were up-regulated. However, +/+ fat pads transplanted into fa/fa recipients did not respond to hyperleptinemia, suggesting lack of an essential leptin-stimulated cohorome(s). In +/+ but not in fa/fa rats, plasma catecholamine levels rose, and both P-STAT3 and P-CREB increased in adipose tissue, suggesting that both direct and indirect (hypothalamic) leptin receptor-mediated actions of hyperleptinemia are involved in depletion of adipocyte fat.

In normal lean rodents, the induction of hyperleptinemia by administration of recombinant adenovirus containing the leptin cDNA (AdCMV-leptin) causes all visible fat to melt away within 7 days (1). In what is probably the most striking effect of this hormone, white adipocytes are transformed into fatless cells filled with small mitochondria (2). In addition, genes such as ucP-1 (uncoupling protein-1) and PGC-1α (peroxisome proliferator-activated receptor-γ-coactivator-1α), normally expressed in brown but not white adipocytes, are expressed at high levels, whereas adipocyte markers, such as leptin and aP2, are profoundly down-regulated (2). Despite their similarities to brown adipocytes, these transformed white adipocytes differ sufficiently to warrant designation as a novel derivative cell, the “post-adipocyte” (2). Although fat depletion of adipocytes by hyperleptinemia occurs only in lean and not in obese rodents, understanding of its mechanisms may have implications for the treatment of obesity.

The fat depletion induced by experimental hyperleptinemia differs from that of naturally occurring catabolic states, such as starvation and insulin deficiency, in which free fatty acids are released from the adipocytes and oxidized in the liver. The fat depletion of experimental hyperleptinemia is unaccompanied by an increase in plasma free fatty acids or ketones (3, 4), and the molecular and morphologic changes (2) imply that the oxidation takes place within the transformed adipocytes.

The pathway by which hyperleptinemia induces transformation of adipocytes to post-adipocytes has not been clearly identified. We know that leptin-responsive centers in the hypothalamus regulate energy metabolism and feeding behavior (3–8), but there is also evidence for direct leptin action on adipocytes (9–11) and on other tissues (12–17). Here we report that the fat-depleting action of hyperleptinemia requires both direct leptin receptor (Lepr)3-mediated action on adipocytes coordinated with Lepr-mediated stimulation of sympathetic and perhaps other centers in the hypothalamus.

EXPERIMENTAL PROCEDURES

Animals—Male obese ZDF homozygous (fa/fa) and wild-type (+/+ ) rats were bred in our laboratory and housed in individual cages with a constant temperature and 12 h of light alternating with 12 h of darkness. All were fed standard chow (Teklad mouse/rat diet, Teklad, Madison, WI) and had access to chow and water ad libitum.

Experimental Procedures—Epididymal fat pads were isolated from 6-week-old ZDF+/+ or ZDFfa/fa rats under ketamine-xylazine anesthesia. The fat pads were weighed, washed with saline, and trimmed so that each transplant weighed 300 mg. Fat pads from ZDF+/+ donors were transplanted on top of the

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3 The abbreviations used are: Lepr, leptin receptor; ZDF, Zucker Diabetic Fatty; RT, reverse transcription; CREB, cAMP-response element-binding protein; TG, triacylglycerol; FAS, fatty-acid synthase; STAT, signal transduction and activation of transcription.
right anterior rectus abdominis muscle of the ZDF+/+ recipient rats and fat pads from ZDF+/− donors on the left side. The abdominal wound was then closed. In other experiments ZDF+/− rats were used as recipients. Ten days later ZDF+/+ rats were infused with $1 \times 10^5$ plaque-forming units of recombinant adenovirus containing either the leptin cDNA (AdCMV-leptin) or, as an inactive control, the β-galactosidase cDNA (AdCMV-β-gal). Most animals were sacrificed 14 days after the virus injection or in some cases 3 or 7 days after the virus injection. The abdominal wound was reopened, and the transplanted epididymal fat pad grafts were photographed in situ and then excised for weighing. The specimens were divided, and one portion was fixed for morphological studies, and the other was stored in liquid nitrogen for real time RT-PCR analysis.

**Microscopy**—Fat tissue was fixed with 2% glutaraldehyde in cacodylate buffer, post-fixed in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded ethanol, and embedded in Epon 812. Semithin (1-μm-thick) and thin sections were cut with an LKB ultramicrotome (Amersham Biosciences). Semithin sections were photographed under phase contrast using a Zeiss photomicroscope. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM10 electron microscope.

**Quantitative Real Time RT-PCR**—Total RNA was extracted by the Trizol isolation method according to the manufacturer’s protocol (Invitrogen). Total RNA (2 μg) was treated with RNase-free DNase (Invitrogen), and first-strand cDNA was generated with the random hexamer primer in the first strand reaction. Total RNA (2 μg) was treated with RNase-free DNase (Invitrogen), and first-strand cDNA was generated with the random hexamer primer in the first strand reaction. 18 S ribosomal RNA was purchased from Applied Biosystems (Applied Biosystems). Semithin sections were photographed under phase contrast using a Zeiss photomicroscope. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM10 electron microscope.

**Gene Expression**—Total RNA was extracted by the Trizol isolation method according to the manufacturer’s protocol (Invitrogen). Total RNA (2 μg) was treated with RNase-free DNase (Invitrogen), and first-strand cDNA was generated with the random hexamer primer in the first strand reaction. Total RNA (2 μg) was treated with RNase-free DNase (Invitrogen), and first-strand cDNA was generated with the random hexamer primer in the first strand reaction. 18 S ribosomal RNA was purchased from Applied Biosystems (Applied Biosystems). Semithin sections were photographed under phase contrast using a Zeiss photomicroscope. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips CM10 electron microscope.

**RESULTS**

**Direct Lepr-mediated Action on Adipocytes Is Necessary for Depletion of Fat Stores by Hyperleptinemia**—To determine whether normal Lepr on adipocytes are required for the fat-depleting action of hyperleptinemia in vivo, we transplanted into 6-week-old normal, lean ZDF+/+ recipients a fat pad resected from a 5–6-week-old normal lean wild-type (+/+ ) ZDF donor rat with functioning Leprs and a fat pad from an obese ZDF+/− rat with a loss-of-function mutation in the Lepr gene (18). Both fat transplants were readily visible and palpable throughout the first 10 post-operative days. If the fat-depleting effects of hyperleptinemia on adipocytes require normal Lepr, white adipocytes, fat will disappear from the three fat pads with functioning Leprs, i.e. from both native +/+ fat pads of the recipients and from the +/+ transplant but not from the fa/fa transplant. Alternatively, if these effects are mediated by neurotransmitted signals from leptin-responsive hypothalamic centers, only the two intact fat pads will lose their fat.

At 10 days after the surgery, the recipients received an intravenous injection of recombinant adenovirus containing either the leptin cDNA (AdCMV-leptin) or the β-galactosidase cDNA (AdCMV-β-gal). The diet of the latter control rats was matched to that of the AdCMV-leptin-treated group. Plasma leptin levels rose in the AdCMV-leptin-treated group from 1.0 ± 0.2 to 81 ± 35 ng/ml during the 1st week, food intake declined by 43%, and body weight of the +/+ recipients fell within 14 days from 267 ± 33 before AdCMV-leptin treatment to 223 ± 27 g 10 days after treatment. On external inspection and palpation, transplanted epididymal fat pad grafts were photographed in situ and then excised for weighing. The specimens were divided, and one portion was fixed for morphological studies, and the other was stored in liquid nitrogen for real time RT-PCR analysis.
(Fig. 1B) was observed (Fig. 1D). On dissection of the AdCMV-leptin-treated +/+ recipients, fat tissue could not be identified in either the +/+ transplant, the native fat pads, or anywhere else in the body other than in the fa/fa transplant (Fig. 1C). The native epididymal fat pads appeared as slender strands of vascular tissue devoid of visible fat, whereas the ~300 mg +/+ fat transplant was reduced to a flat hypervascular red patch resembling the remnant of fat-depleted native fat pads (Fig. 1C). Only 67 ± 13 mg or 22% of the original weight of +/+ transplants could be recovered from their transplantation sites (Table 1, part A). By contrast, in the normoleptinemic AdCMV-β-gal-treated control rats pair-fed to the leptinized rats, 273 ± 16 mg or 91% of the +/+ transplants was recovered (Table 1, part A). The TG/protein ratio of the various transplants corresponded to the change in the weight of the transplant (Table 1, part A). Thus, hyperleptinemic fat depletion in the denervated +/+ fat pad transplants with normal Lepr seemed to parallel that in the intact +/+ native fat pads with normal Lepr and intact neurocircuity (Fig. 1C).

By contrast, the Lepr-defective fa/fa fat pads transplanted into +/+ recipients did not respond to hyperleptinemia. The rounded, elevated mass that was visible and palpable through the abdominal wall immediately postoperatively was still present (Fig. 1C). When excised, the weight of fa/fa transplants averaged 224 ± 24 mg or 75% of the original weight. Again the TG content of the transplants corresponded to their weight (Table 1, part A). These results indicate that depletion of TG in adipocytes requires the presence of functioning Lepr in the fat tissue.

To exclude the possibility that the unresponsiveness of the adipocytes from obese, age-matched fa/fa rats to hyperleptinemia was the nonspecific result of their much larger size, we transplanted into +/+ recipients fat pads from pre-obese 4-week-old fa/fa donor rats in which adipocyte diameters approximated those of 6-week-old lean rats. These fat pads also failed to respond to hyperleptinemia, evidence that receptor dysfunction, rather than adipocyte size, was the cause of the unresponsiveness (data not shown).

**Comparison of Morphologic Transformation of Adipocytes to Post-adipocytes by Hyperleptinemia in Transplanted +/+ and fa/fa Fat Pads—Microscopic examination of the native and transplanted fat pads in +/+ recipient rats made hyperleptinemic by AdCMV-leptin treatment 14 days earlier confirmed the impression gained by gross examination. Phase contrast views**

**TABLE 1**

*Effects of hyperleptinemia on epididymal fat transplants*

Plasma leptin and body weight change of hyperleptinemic and normoleptinemic wild-type (+/+) (part A) and obese (fa/fa) ZDF recipients (part B) are compared 9 days after treatment with either AdCMV-β-gal or AdCMV-leptin, together with the weight and TG/protein ratio of the transplants. All fat pads weighed 300 mg at the time of transplantation. AdCMV-β-gal-treated rats were diet-matched to the AdCMV-leptin-treated rats.

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Plasma leptin</th>
<th>Body weight change</th>
<th>Transplant</th>
<th>+/+ donors</th>
<th>fa/fa donors</th>
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<tr>
<td></td>
<td>mg/ml</td>
<td></td>
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<td>mg</td>
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<td>Tg/protein</td>
<td>Tg/protein</td>
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<td>A. ZDF+/+</td>
<td></td>
<td></td>
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<tr>
<td>AdCMV-leptin</td>
<td>81 ± 35</td>
<td>↓44 ± 11</td>
<td>67 ± 13 &lt;b&gt; 78%&lt;/b&gt;</td>
<td>20 ± 4 &lt;b&gt;75%&lt;/b&gt;</td>
<td>224 ± 24 &lt;b&gt;25%&lt;/b&gt;</td>
</tr>
<tr>
<td>AdCMV-β-gal</td>
<td>1.1 ± 0.2</td>
<td>↑5 ± 5</td>
<td>273 ± 16 &lt;b&gt;9%&lt;/b&gt;</td>
<td>438 ± 384 &lt;b&gt;16%&lt;/b&gt;</td>
<td>253 ± 33 &lt;b&gt;15%&lt;/b&gt;</td>
</tr>
<tr>
<td>B. ZDF&lt;sup&gt;fa/fa&lt;/sup&gt;</td>
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<tr>
<td>AdCMV-leptin</td>
<td>122 ± 12</td>
<td>↑88 ± 2</td>
<td>570 ± 56 &lt;b&gt;190%&lt;/b&gt;</td>
<td>585 ± 169 &lt;b&gt;33%&lt;/b&gt;</td>
<td>283 ± 38 &lt;b&gt;6%&lt;/b&gt;</td>
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<tr>
<td>AdCMV-β-gal</td>
<td>24 ± 6</td>
<td>↑115 ± 12</td>
<td>725 ± 104 &lt;b&gt;242%&lt;/b&gt;</td>
<td>577 ± 155 &lt;b&gt;3%&lt;/b&gt;</td>
<td>290 ± 48 &lt;b&gt;3%&lt;/b&gt;</td>
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<sup>a</sup>p < 0.01 versus ZDF<sup>fa/fa</sup> and AdCMV-leptin.

<sup>b</sup>p < 0.01 versus ZDF<sup>fa/fa</sup> and AdCMV-β-gal.

<sup>c</sup>p < 0.01 versus ZDF<sup>fa/fa</sup>.
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A  Native: +/+ Lept.

B  Transplant: +/+ Lept.

C  Native: +/+ Lept.

D  Transplant: +/+ Lept.

E  Transplant: fa/fa Lept.

F  Transplant: +/+ BetaGal
of semithin sections of the intact native +/+ fat pads (Fig. 2A) and fat pads transplanted from lean ZDF+/+ donors (Fig. 2B) revealed in both the same fatless, shrunken post-adipocytes in the intact native fat pads of hyperleptinemic +/+ rats described in detail previously (2). At the ultrastructural level both native (Fig. 2C) and transplanted cells (Fig. 2D) contained the same apparent abundance of distinctive mitochondria with an electron-dense matrix (Fig. 2D, inset). By contrast, phase contrast views of semithin sections of the intact native fat pads of hyperleptinemic +/+ recipients contained normal-appearing white adipocytes with a typical lipid droplet (Fig. 2E), as did +/+ fat pads transplanted into normoleptinemic +/+ control recipients that had been treated with AdCMV-β-gal instead of AdCMV-leptin, and diet-matched to the leptinized rats (Fig. 2F). In addition, the molecular changes observed in the +/+ recipients and in the native +/+ fat pads of hyperleptinemic rats (Fig. 3) were not present in these normoleptinemic controls (data not shown). The foregoing findings demonstrate that the action of hyperleptinemia on the fat metabolism of white adipocytes in lean rats requires the presence of wild-type (+/+ Lepr) adipocytes but does not require neural connections to the hypothalamus.

Molecular Responses to Hyperleptinemia in Intact Versus Transplanted Fat—We reported previously that the transformation of white adipocytes induced by hyperleptinemia was accompanied by an equally striking array of molecular changes (2). For example, pgc-1α, a gene that regulates mitochondrial biosynthesis in brown adipocytes (19), but is not normally expressed in white adipocytes, is greatly up-regulated by hyperleptinemia, whereas the normally high expression level of lipogenic enzymes, such as acetyl-CoA carboxylase-2 (ACC2), fatty-acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1), is profoundly suppressed in adipose tissue after hyperleptinemia (2). To determine whether the hyperleptinemia-induced expression profile in the fat pads transplanted from normal rats matched that of intact fat pads, we compared the mRNA of ACC2 FAS, SCD-1, and PGC-1α by means of real time RT-PCR (Fig. 3). PGC-1α was increased and ACC2, FAS, and SCD-1 were reduced in both the intact native fat pads and in the wild-type fat transplants from hyperleptinemic wild-type rats but not in fa/fa fat pads from Lepr-defective obese ZDF/+/fa rats. However, the magnitude of the PGC-1α response in the +/+ transplant fat was less than in the native +/+ fat pad, possibly reflecting the loss of normal innervation and circulation.

Phospho-CREB and Phospho-STAT3 Are Both Increased in Fat Tissue during Fat Depletion by Hyperleptinemia—Lepr-mediated leptin signaling is transduced by STAT3 (20), whereas catecholamine signaling is transduced by CREB (21).

To determine at the tissue level if both direct leptin-mediated and indirect catecholamine-mediated actions on adipocytes could be involved in hyperleptinemic fat depletion, we compared the activation of the respective transcription factors during fat depletion of intact native adipose tissue of normal mice and rats. We observed a major increase in P-STAT3 to a peak at 3 days after induction of hyperleptinemia, followed by a decline at 7 days (Fig. 4, A and B), consistent with direct leptin action. P-CREB remained at base-line levels at 3 days but was markedly increased at day 7 (Fig. 4, A and B), consistent with indirect leptin action via hypothalamic sympathetic centers. At day 7 no adipocyte fat was detected microscopically in the fat pads (Fig. 4C).

STAT3 and CREB activation was also observed in fat transplants from normal rats with hyperleptinemia (Fig. 4A, center panels) but not in fa/fa rats (Fig. 4A, right panels). However, the increases in P-STAT3 and P-CREB/CREB ratio were less than in the intact fat pads, perhaps reflecting the surgical disruption of the normal circulation. This combination of up-regulation of the adipose tissue oxidative machinery, in concert with adrenergic enhancement of lipolysis, could explain how fat stores are depleted by hyperleptinemia without the increase in plasma free fatty acid levels that characterizes other forms of fat depletion (11, 13).

Fat Pads from +/+ Donors Do Not Respond to Hyperleptinemia When Transplanted into Obese fa/fa Recipients—The foregoing results indicate that direct Lepr-mediated action of leptin on adipose tissue is necessary for fat depletion, but they do not...
indicate whether or not direct action is sufficient for the effect.

To determine whether leptin action on the regulatory centers of the hypothalamus might also be required, we repeated the foregoing transplantation experiments using leptin-unresponsive, obese ZDF\textsuperscript{fa/fa} rats as recipients. When fat pads were transplanted into \textit{fa/fa} recipient rats, equivalent hyperleptinemia had no fat-depleting action; in fact, they accumulated more TG than the \textit{fa/fa} transplants (Table 1, part B, and Fig. 5A). Their unresponsiveness could signify either lack of a required cohormone normally stimulated by leptin (22–25) via hypothalamic sympathetic centers, such as catecholamines, and/or a blockade of the direct action of leptin on the fat pad transplant by a circulating factor present in the plasma of the \textit{fa/fa} recipients. The greater accumulation of fat in \textit{fa/fa} fat pads could reflect the fact that normal adipocytes are able to undergo hypertrophy when transplanted into a hyperlipidemic environment, whereas the obese adipocytes from hyperlipidemic \textit{fa/fa} donors had already undergone maximum hypertrophy before transplantation.

We next examined the P-STAT3 and P-CREB in the unresponsive \textit{+/+} fat pads transplanted into the \textit{fa/fa} recipients, treated with AdCMV-leptin. As expected, neither STAT3 nor CREB in native intact fat pads or in \textit{fa/fa} transplants were activated by hyperleptinemia (Fig. 5B). The lack of an increase in P-STAT3 in the \textit{fa/fa} fat transplants was unexpected and could signify a circulating blocker of direct leptin action on adipocytes in the plasma of the \textit{fa/fa} recipients. The lack of an increase in P-CREB was expected and is attributed to leptin unresponsiveness of hypothalamic sympathetic nuclei in \textit{fa/fa} recipients.

Catecholamines as Cohormones for Hyperleptinemic Fat Depletion—The hyperleptinemia-induced increase in P-CREB in \textit{+/+} fat tissue suggested that catecholamines could play a cohormonal role in the fat depletion observed in normal rats by

<table>
<thead>
<tr>
<th>A</th>
<th>INTACT FAT</th>
<th>TRANSPLANTED FAT</th>
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<tr>
<td>Lean (+/+)</td>
<td>Lean (+/+)</td>
<td>Fatty (fa/fa)</td>
</tr>
<tr>
<td>β-gal</td>
<td>β-gal</td>
<td>Leptin</td>
</tr>
<tr>
<td>Leptin</td>
<td>P-STAT3</td>
<td>P-CREB</td>
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<tr>
<td></td>
<td>STAT3</td>
<td>CREB</td>
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FIGURE 4. A, representative immunoblots for P-STAT3, STAT3, P-CREB, and CREB from intact fat of lean \textit{+/+} rats (far left) and transplanted fat pads from \textit{+/+} (center) and \textit{fa/fa} (right) donors 3 and 7 days after induction of hyperleptinemia. B, ratio of mean densitometric readings of P-STAT3/STAT3 and P-CREB/CREB ratios. β-Tubulin was employed throughout as a loading control. C, representative sections of intact fat pads of lean \textit{+/+} rats before (0) and at 3 and 7 days after induction of hyperleptinemia (left) and transplanted fat pads from \textit{+/+} (center) and \textit{fa/fa} (right) donors. The unleptinized group is marked control rather than “0” because they received AdCMV-β-gal treatment 3 days before their fat was obtained. *, \(p < 0.01\); **, \(p < 0.05\); #, \(p < 0.03\).
stimulating lipolysis to provide substrate for the leptin-up-regulated oxidative machinery of the white adipocytes (2). If so, leptin-stimulated catecholamine levels in plasma of lean +/+ rats should be higher than in leptin-unresponsive obese fa/fa rats. In fact, we observed that hyperleptinemia stimulated an increase in plasma levels of both norepinephrine and epinephrine (ng/ml) in +/+ and fa/fa ZDF rats. In fact, we observed that hyperleptinemia stimulated an increase in plasma levels of both norepinephrine and epinephrine (ng/ml) in +/+ and fa/fa ZDF rats. It is not clear why this was not accom-
panied by a higher basal level of P-CREB. Nevertheless, basal norepinephrine levels in the rats were higher than leptin-stimulated +/+ rats (Table 2), confirming earlier work by others (22–25). Nevertheless, basal norepinephrine levels in the fa/fa rats were higher than leptin-stimulated levels in the +/+ rats. It is not clear why this was not accom-
panied by a higher basal level of P-CREB.

Circulating Blockers of Hyperleptinemia—The lack of an increase in P-STAT3 in response to hyperleptinemia in +/+ fat pads transplanted into fa/fa rats could be because of a circulating blocker of leptin action. At least three potential circulating blockers of leptin action have been reported. Two of these, the soluble leptin receptor and C-reactive protein, have already been shown to be increased in the circulation of fa/fa ZDF rats (26, 27) and to impair the action of leptin (27, 28).

Another potential candidate blocker is hyperlipidemia, which is believed to impede leptin transport across the blood-brain barrier (29). It seemed possible that the marked hyperlipidemia of obese ZDF fa/fa rats might also block direct action of hyperleptinemia on peripheral tissues. To test this possibility, we administered gemfibrozil to lower plasma TG of the fa/fa recipients beginning 7 days prior to inducing the hyperleptinemia. Although the plasma TG levels of the gemfibrozol-treated ZDF fa/fa recipients declined from 530 ± 174 to 106 ± 32 mg/dl, hyperleptinemia still failed to reduce either the weight or TG content of +/+ fat transplants in fa/fa recipients (data not shown), and there was no increase in PGC-1α or UCP-1 or -2 expression in +/+ fat pads transplanted into fa/fa recipients. These negative findings exclude hyperlipidemia as the cause of a block of hyperleptinemic action on the hypothalamus and point instead to the previously established elevations of soluble Lepr and C-reactive protein as a possible cause of the leptin unresponsiveness.

**DISCUSSION**

These findings indicate that fat depletion of adipocytes by hyperleptinemia requires a combination of direct leptinergic and indirect hypothalamic actions of leptin. The direct action, which is mediated, at least in part, through activation of STAT3, up-regulates the oxidative machinery of white adipocytes and enhances oxidation of the fatty acyl-CoA (2). The indirect leptin action is presumed to stimulate hypothalamic sympathetic centers to release catecholamines (22) and activate CREB in the fat tissue. The fact that hyperleptinemia stimulated plasma epinephrine and norepinephrine in leptin-responsive +/+ rats, but not in the leptin-unresponsive fa/fa rats, is consistent with a cohoromonal role for catecholamines in the fat depletion. The lipolytic action of catecholamines on adipocytes would increase the availability of fatty acyl-CoA for mitochondrial oxidation, while reducing stored TG (Fig. 6). The uncou-
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...leptinemia when they had been transplanted into certain neurons (32), is also a prime candidate for a cohormonal role. Catecholamines or ketones, and weight loss is confined to fat tissue with no loss of lean body mass. This is probably the result of marked insulin sensitivity despite hypoinsulinemia.

However, because there is no current evidence to prove that catecholamines are required for hyperleptinemic depletion of adipocyte fat, other hypothalamic factors could also be involved. Cocaine- and amphetamine-regulated transcript (30), previously shown to be up-regulated by intense hyperleptinemia in normal but not in fa/fa rats (31) and to increase P-CREB in certain neurons (32), is also a prime candidate for a cohormonal role. Cocaine- and amphetamine-regulated transcript tends to increase lipid oxidation (33) and may therefore contribute to the fat-depleting action of intense hyperleptinemia.

The resistance of +/+ fat pads to hyperleptinemia when transplanted into obese fa/fa ZDF recipient rats could result from a circulating blocker of direct hyperleptinemic action on the fat transplant, or from lack of one or more leptin-stimulated cofactors essential for hyperleptinemic fat depletion, or both. Hypertriglyceridemia, one of the circulating factors proposed to block the hypothalamic action of leptin (29), was excluded as the cause of the unresponsiveness of the +/+ fat pads to hyperleptinemia when they had been transplanted into fa/fat recipients. However, soluble Lepr remains a highly likely candidate as a circulating blocker in fa/fa rats because it is increased in the ZDF fa/fat rats (26) and is known to impair leptin action (28). More recently, elevated C-reactive protein levels of obesity (34) can perhaps be explained by the underexpression of Lepr-b on adipocytes of diet-induced obesity, as recently reported in overfed rodents (35).

In summary, it appears that in normal, lean rodents the rapid disappearance of body fat caused by intense hyperleptinemia involves both direct action of leptin on the adipocytes and indirect action of leptin on leptin-responsive hypothalamic centers. Reversal of obesity with leptin therapy may require recognition of this dual requirement for the lipid-depleting action of leptin on adipocytes.

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