Novel Form of Lipolysis Induced by Leptin*

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Hyperleptinemia causes disappearance of body fat without a rise in free fatty acids (FFA) or ketones, suggesting that leptin can deplete adipocytes of fat without releasing FFA. To test this, we measured FFA and glycerol released from adipocytes obtained from normal lean Zucker diabetic fatty rats (+/+) and incubated for 0, 3, 6, or 24 h in either 20 ng/ml recombinant leptin or 100 nM norepinephrine (NE). Whereas NE increased both FFA and glycerol release from adipocytes of +/+ rats, leptin increased glycerol release in +/+ adipocytes without a parallel increase in FFA release. In adipocytes of obese Zucker diabetic fatty rats (fa/fa) with defective leptin receptors, NE increased both FFA and glycolal release, but leptin had no effect on either. Leptin significantly lowered the mRNA of leptin and fatty acid synthase of adipocytes (FAS) (p < 0.05), and up-regulated the mRNA of peroxisome proliferator-activated receptor (PPAR)-α, carnitine palmitoyl transferase-1, (CPT-1), and acyl CoA oxidase (ACO) (p < 0.05). NE (100 nM) also lowered leptin mRNA (p < 0.05) but did not affect FAS, PPARα, ACO, or CPT-1 expression. We conclude that in normal adipocytes leptin directly decreases FAS expression, increases PPARα and the enzymes of FFA oxidation, and stimulates a novel form of lipolysis in which glycerol is released without a proportional release of FFA.

Adenoviral transfer of the leptin gene into normal rats causes rapid loss of all visible body fat within 7 days (1). Unlike the ketogenic fat loss in starvation or insulin deficiency, in which the fatty acids (FA) and glycerol are released proportionately from the adipocytes, hyperleptinemic fat loss is unaccompanied by elevations in plasma free fatty acid (FFA) levels or ketones or by ketonuria (2). One possible explanation for the nonketotic fat loss is that the FAs are oxidized inside the adipocytes. This idea has received further support from the demonstration that the expression of two major enzymes of long chain FA oxidation, acyl CoA oxidase (ACO), and carnitine palmitoyl transferase-1 (CPT-1) are strikingly increased in the adipocytes of hyperleptinemic rats during the disappearance of their fat (3, 4). This finding implies that experimentally induced hyperleptinemia can convert adipocytes from fat-storing cells into fat-burning cells.

Most workers in the field believe that leptin acts largely, if not exclusively, via centers in the hypothalamus, suppressing appetite by inhibiting orexigenic factors such as neuropeptide Y (5) and by increasing thermogenesis via sympathetic innervation of brown adipose tissue (6). It seemed possible, therefore, that the disappearance of the fat of white adipocytes might be the result of leptin-induced, adrenergically mediated activation of lipolysis. Yet there is evidence consistent with the possibility that, at least at high concentrations, leptin can act directly on tissues independently of hypothalamic mediation. First, leptin receptors (OB-R), including the full-length isoform, OB-Rb, are expressed in white adipocytes (10). Second, leptin has been shown in vitro to reduce the expression of lipogenic enzymes in preadipocytes (8) and to increase glycerol release from mature adipocytes (7). Third, if the fat depletion caused by hyperleptinemia is, in fact, caused by norepinephrine through stimulation of sympathetic centers in the hypothalamus, it would be accompanied by a concomitant increase in plasma FFA levels, which did not occur in the hyperleptinemic rats. For these reasons, we suspected that the fat loss of hyperleptinemia involved a novel type of leptin-mediated lipolysis that was independent of catecholamines. The following study was designed to test this possibility.

MATERIALS AND METHODS

Adipocyte Isolation and Culture—Isolation of adipocytes from lean (+/+) and obese (fa/fa) Zucker rats was performed as described previously (9). Briefly, minced epididymal fat pads were digested at 37 °C for 2–3 h in a buffer containing type II collagenase (1 mg/ml), albumin (3.5%), and glucose (0.55 mM). The digestion mixture was swirled and poured through 100-μm nylon mesh into 50-ml conical polypropylene tubes. Cells were washed three times with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5% albumin and cultured for 0, 3, 6 and 24 h at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, antibiotics (penicillin and streptomycin) and with or without recombinant leptin (kindly provided by Dr. Gayle Yamamoto of Zymogenetics, Inc., Seattle, WA) or norepinephrine.

Reverse Transcriptase PCR—mRNA was semiquantified by reverse transcriptase-PCR. Total RNA from adipocytes was extracted using TRIzol Reagent. After treating with RNaseD-Nase I, reverse transcriptase was carried out using 1 μg of total RNA. First strand cDNA was PCR-amplified with sequences specific for leptin (5'-GGAGGATTCCTGCTCCACG-3' and 5'-CTTCTCCGAGAATCCACTG-3'), ACO (5'-GCCCTCAGCTATATTACC-3' and 5'-AGGAATGCTCTCTCAATGC-3'), CPT-1 (5'-TATGGAGATCTGCTTTC-3' and 5'-CTGCCGAAGCATTGCTGCTC-3'), FAS (5'-GCGTGGATGCTCAACCCTT-3' and 5'-TCAACTCCTGAGCCTGCT-3'), and PPARα (5'-GGACCTCCATCGTCGCT-3' and 5'-TCAGAGGTCCCTGAACA-3'). The conditions of PCR were as follows: denaturation for 45 s at 92 °C, annealing for 45 s at 55 °C, and elongation for 1 min at 72 °C with 30 cycles. The PCR products were subjected to electrophoresis on 1.2% agarose gel and were quantified by Southern blot analysis by

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† The abbreviations used are: FA, fatty acid; FFA, free fatty acid; ACO, acyl CoA oxidase; CPT-1, carnitine palmitoyl transferase-1; PCR, polymerase chain reaction; FAS, fatty acid synthase; PPARα, peroxisome proliferator-activated receptor-α; ZDF, Zucker diabetic fatty.
Leptin-induced Lipolysis in Adipocytes

**RESULTS**

**Effect of Leptin on Leptin mRNA**—A dramatic early effect of adenovirus-induced hyperleptinemia *in vivo* is the disappearance of leptin mRNA from the adipocytes (4). To establish the *in vitro* biologic activity of the recombinant leptin, we cultured adipocytes from 20 ng/ml of recombinant leptin, which approximates the levels in hyperleptinemic rats. Leptin mRNA had declined significantly (*p < 0.05*) by 14, 39, and 50% of normal in 3, 6, and 24 h, respectively (Fig. 1A). In adipocytes from *fa/fa* rats, there was no effect on leptin mRNA (Fig. 1B). These results establish a direct action of leptin on mature adipocytes.

Norepinephrine at 100 nM concentration reduced leptin mRNA by 30% (*p < 0.05*) in the adipocytes of either the wild-type (+/+) or *fa/fa* ZDF rats (Fig. 1C). Comparing the levels in hyperleptinemic rats to those in normal or *fa/fa* ZDF rats. After 6 h in culture PPARα mRNA had increased 50% (*p < 0.05*), whereas CPT-1 and ACO mRNA increased ~2-fold compared with controls (*p < 0.05*) (Fig. 1D). In similarly treated adipocytes isolated from obese *fa/fa* ZDF rats, there was no effect on the mRNA of any of the foregoing genes (Fig. 3B).

The presence of 100 nM norepinephrine alone had no effect on PPARα mRNA and caused only a minimal increase in ACO and CPT-1 mRNA (not significant) (data not shown). Norepinephrine could not, therefore, have mediated the *in vivo* effects of leptin on the expression of these enzymes.

**Comparative Lipolytic Effects of Leptin and Norepinephrine**—The foregoing direct effects of leptin on the expression of enzymes of fatty acid oxidation and synthesis were consistent with the hypothesis that the nonketotic fat loss of adenovirus-induced hyperleptinemia was the consequence of increased oxidation of FA within adipocytes, coupled with a reduction in lipogenesis. To determine whether the direct action of leptin involves a novel type of lipolytic action that differs from that of norepinephrine, we compared the release of glycerol and FFA from normal rat adipocytes cultured for 6 h in 20 ng/ml of leptin, 100 nM norepinephrine, or in buffer alone. As shown in Fig. 4A, norepinephrine and leptin each significantly increased glycerol release from adipocytes isolated from wild-type normal rats (p < 0.05), confirming the observations of Siegrist-Kaiser et al. (7). However, whereas norepinephrine
elicited the expected rise in FFA, leptin did not increase FFA release. In adipocytes of fa/fa rats, by contrast, leptin caused no increase in either glycerol or FA, whereas norepinephrine elicited a robust increase in both (Fig. 4B).

**Dose-Response Characteristics of Leptin-induced Lipolysis**—The foregoing effects of leptin on lipolysis were observed at the unphysiologically high concentration of 20 ng/ml. To determine whether the effect on lipolysis was operative at more physiologic levels of leptin, a dose-response study was done (Fig. 5). Five ng/ml of leptin was the lowest concentration that stimulated release of glycerol (p < 0.05). This concentration is at the upper end of the range of plasma leptin levels of rodents and humans (13, 14). There was no effect of leptin on glycerol release from fa/fa adipocytes at any of the concentrations employed.

**DISCUSSION**

These results provide evidence for multiple direct actions of leptin on mature adipocytes *in vitro*. Previously, leptin had been shown to reduce expression of acetyl CoA carboxylase and FAS in 3T3-L1 cells (8) and to increase glycerol release from normal adipocytes (7). In the present study, the expression of CPT-1 was increased ~2-fold above the base-line level by 20 ng/ml of leptin, whereas that of the lipogenic enzyme, FAS, was lowered by ~90%. It is possible that the up-regulation of ACO and CPT-1 mRNA was mediated by the increase in expression of their transcription factor, PPAR-α, which rose 50%. The mechanism by which leptin up-regulates PPARα is not known.

The changes in mRNA induced by recombinant leptin in isolated adipocytes correspond qualitatively with all those observed previously *in vivo* in adipocytes of rats with adrenovirus-induced hyperleptinemia (4). Because norepinephrine failed to induce any of these changes in the cultured adipocytes, it appears that they are the consequences of direct action of leptin on adipocytes rather than of norepinephrine. It should be stressed, however, that direct effects of leptin on adipocytes were at a concentration at 20 ng/ml, which is far above the physiologic levels in normal humans or rodents. A small but significant increase in glycerol was observed in rat adipocytes in response to 5 ng/ml (p < 0.05), which is in the upper end of the physiologic range (13, 14). However, Siegrist-Kaiser *et al.* (7) reported an effect at 1.8 ng/ml on glycerol release, which is within the physiologic range. Irrespective of the physiologic relevance of these direct effects of leptin upon adipocytes, they do suggest that the pharmacologic strategy of reducing adipocyte fat content by means of direct lipolytic actions of supraphysiologic levels of leptin may be useful (4).

In the earlier reports (7, 15) indicating that leptin increases glycerol release from rodent adipocytes, FFA had not been measured. The finding here that leptin-induced glycerol release is unaccompanied by FFA release explains the *in vivo* observation that fat loss occurring during adrenovirus-induced hyperleptinemia is unaccompanied by a rise in plasma FFA, ketonemia, and ketonuria (2). This and the up-regulation of ACO and CPT-1 support the idea that the FFA are oxidized inside the adipocytes rather than exported to the liver for oxidation to ketoacids. This may provide a valuable therapeutic advantage for leptin treatment of massive obesity, because it permits the rapid removal of fat without the ketoacidosis and hyperuricemia that otherwise complicate rapid weight loss induced by diet restriction.

The mechanism of leptin-induced lipolysis remains to be elucidated. Clearly it requires a functional leptin receptor, because it was completely absent in adipocytes of fa/fa ZDF
rats with defective OB-R, in which norepinephrine elicited a relatively normal lipolytic response. Leptin signal is believed to be transduced via the STAT/JAK pathway (16). It will be of interest to determine whether leptin increases cAMP and whether, like norepinephrine, it activates the hormone-sensitive lipase (17).

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REFERENCES
