Liporegulation in diet-induced obesity:
The antisteatotic role of hyperleptinemia

Young Lee1*, May-Yun Wang1*, Tetsuya Kakuma1, Zhuo-Wei Wang1,
Evelyn Babcock2, Kay McCorkle3, Moritake Higa1,
Yan-Ting Zhou1 and Roger H. Unger1,3

1Gifford Laboratories, Touchstone Center for Diabetes Research, Department of Internal
Medicine, University of Texas Southwestern Medical Center, Dallas, Texas 75390-8854;
2Department of Radiology, University of Texas Southwestern Medical Center, Dallas,
Texas 75390-9085; 3Veterans Affairs Medical Center, Dallas, Texas 75216.

* Contributed equally as co-first authors

Running Title: Antisteatotic role of the hyperleptinemia of obesity

Address Correspondence to: Roger H. Unger, M.D.
Center for Diabetes Research
University of Texas Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, TX 75390-8854
Phone: 214/648-3488
FAX: 214/648-9191
E-Mail: runger@mednet.swmed.edu
SUMMARY

To test the hypothesis that the physiologic liporegulatory role of hyperleptinemia is to prevent steatosis during caloric excess, we induced obesity by feeding normal Sprague Dawley rats a 60% fat diet. Hyperleptinemia began within 24 h and increased progressively to 26 ng/ml after 10 weeks, correlating with a ~150-fold increase in body fat (r=0.91; p<0.0001). During this time triacylglycerol (TG) content of nonadipose tissues rose only 1-2.7-fold, implying antisteatotic activity. In rodents without leptin action (fa/fa rats, ob/ob and db/db mice) receiving a 6% fat diet, nonadipose tissue TG was 4-100 times normal. In normal rats on 60% fat peroxisome proliferator-activated receptor-α (PPARα) protein and L-carnitine palmitoyl transferase-1 (L-CPT-1) mRNA increased in liver. In their pancreatic islets, fatty acid (FA) oxidation increased 30% without detectable increase in expression of PPARα or oxidative enzymes, while lipogenesis from [14C]-glucose was slightly below that of the 4% fat-fed rats (p<0.05). Tissue-specific overexpression of wild-type leptin receptors in the livers of fa/fa rats, in which marked steatosis is uniformly present, reduced TG accumulation in liver but nowhere else. We conclude that a physiologic role of the hyperleptinemia of caloric excess is to protect nonadipocytes from steatosis and lipotoxicity, by preventing upregulation of lipogenesis and by increasing FA oxidation.

KEY WORDS: obesity, leptin, hyperleptinemia, steatosis, PPARα, liporegulation, lipotoxicity, leptin receptor (OB-R)
INTRODUCTION

Compelling theoretical considerations, coupled with corroborating experimental evidence, argue against the conventional view that the physiologic role of leptin is to prevent obesity. First, plasma leptin levels of rodents and humans are low in the lean and high in the obese (1), hardly the credentials of an antiobesity hormone. Second, diet-induced obesity is not prevented in hypoleptinemic mice by restoring their plasma leptin levels to normal with recombinant leptin (2). Third, there is no evidence that overnutrition and obesity have ever posed a serious survival threat in evolution; on the contrary, the principal survival threat throughout evolution has been famine, against which obesity provides a measure of protection, as the “thrifty gene” hypothesis maintains (3). Finally, it seems implausible to suggest that hormones evolve for the purpose of preventing the clinical consequences of their own deficiency; just as insulin evolved to confer advantages in nutrient metabolism, rather than to prevent diabetic ketoacidosis, so leptin must have evolved, not to prevent its deficiency syndrome, obesity, (4), but rather to confer a metabolic advantage that has not as yet been identified.

We had previously suggested that the metabolic advantage conferred by the hyperleptinemia of obesity might be prevention of overaccumulation of triacylglycerols (TG) in nonadipose tissues (5). Clearly, leptin does have powerful antilipogenic activity in some such tissues (6). In the absence of leptin action, lipogenesis is increased and fatty acid (FA) oxidation is reduced (7), accounting for the steatosis and lipotoxicity that occurs in such circumstances (7-9). For example, in Zucker Diabetic Fatty (ZDF) rats with a loss-of-function mutation in the leptin receptors (10,11), tissue TG ranges from 10-50 times the normal content (8) and is associated with functional impairment of pancreatic β-cells (12,13) and myocardium (9) and insulin resistance (14). Ultimately,
the progressive overaccumulation of lipids causes death of cells in pancreatic islets and myocardium, resulting in diabetes and myocardial failure, the most serious complications of obesity. It has been proposed that the lipid overaccumulation enlarges the intracellular pool of fatty acyl-CoA beyond the oxidative requirements of the cell (15), thereby providing substrate for potentially destructive nonoxidative pathways such as de novo ceramide formation (16) and lipid peroxidation (17,18).

If the foregoing abnormalities develop in the absence of leptin action, it follows that leptin must be able to prevent them. Certainly hyperleptinemia induced by adenoviral transfer of the leptin gene has remarkable lipopenic and antilipogenic activity in tissues of normal rats, downregulating the expression of genes involved in lipogenesis, while upregulating those genes involved in β-oxidation and thermogenesis (19). Although they are consistent with putative antisteatotic activity of hyperleptinemia, such studies do not prove that the actual physiologic role of adipocyte-derived hyperleptinemia in obesity is to prevent the ectopic accumulation of TG in nonadipose tissues. This study was designed to test this premise.

METHODS

Animals without leptin action: Three groups of rodents were employed. Obese homozygous (fa/fa) Zucker diabetic fatty (ZDF)-drt rats, which are unresponsive to leptin because of a loss-of-function mutation in their leptin receptor (10,11) and lean wild-type (+/+ ) ZDF controls, were bred in our laboratory from [ZDF/Drt-fa (F10)] rats purchased from Dr. R. Peterson (University of Indiana School of Medicine, Indianapolis). Two groups of mice, C57 BL/6J-ob/ob, C57BL/KS-J-db/db and their wild-type controls, C57BL/6J-++/+ and C57BL/KS-J-++/+ mice were purchased from Jackson Laboratory (Bar Harbor, ME).
**Animals with leptin action:** To induce diet-induced obesity in normal rats, Sprague Dawley rats, purchased from Charles River Laboratories, Raleigh, NC, were employed. They were housed in individual metabolic cages (Nalgene, Rochester, NY) with a constant temperature and 12 h of light and 12 h of darkness. Body weight and food intake were measured weekly. Initially all rats were fed standard chow (Teklad 4% Mouse/Rat diet, Madison, WI) *ad libitum* and had free access to water. At 4 weeks of age they were either continued on this diet, which contains 24.8% protein, 4% fat and 3.94 Kcal/g, or they were switched to a high fat diet (Purina test Diet, Purina Mills, Inc., Richmond, IN) containing 60% fat, 7.5% carbohydrate, 24.5% protein and 6.7 Kcal/g in order to produce diet-induced obesity.

**Adenovirus transfer of OB-Rb cDNA to liver of fa/ia ZDF rats:** In *in vivo* experiments containing a total of 1x10^{12} plaque-forming units of recombinant adenovirus containing the cDNA of the leptin receptor, OB-Rb (AdCMV-OB-Rb) or as a control β-galactosidase (AdCMV-β-gal), prepared as described previously (20), were infused into conscious animals over a 10-min period through polyethylene tubing (PE-50, Becton Dickinson) previously anchored in the left jugular vein of 9-week-old ZDF fa/ia rats under sodium pentobarbital anesthesia, as described (20).

**Expression of wild-type and mutated OB-Rb in liver and hypothalamus of fa/ia rats:** To compare the expression of wild-type OB-Rb in fa/ia rats with mutated OB-Rb, total RNA of rat liver and hypothalamus was extracted using TRIzol reagent. Reverse transcription of total RNA was carried out after treating RNA samples with RNase free-DNase I. The first strand cDNA was then used to PCR-amplify an OB-R cDNA fragment with OB-R-specific primers encompassing the region with the fa/ia mutation as described (11). The conditions of PCR were as follows: denaturation for 45s at 92° C, annealing for 45s at 55° C, and elongation for 1 min at 72° C. The amplified PCR products were digested with *Msp*I at 37° C for one hour, then run on a 1.2% agarose gel.
**Northern blot analysis:** Total RNA was extracted by the TRIzol isolation method (Life Technologies, Gaithersburg, MD) and Northern blot analysis was carried out as described previously (21). cDNA probes for the oxidative enzymes, acyl CoA oxidase (ACO) and liver-carnitine palmitoyl CoA transferase-1 (L-CPT-1) were prepared by RT-PCR using the following primers: ACO-sense (2891-2910), 5'-GCCCTCAGCTATGGTATTAC-3' and ACO-antisense (3505-3524), 5'-AGGAACTGCTCTCAAAATGC-3' (Gene Bank J02752); L-CPT-1-sense (3094-3113), 5'-TATGTGAGGATGCTGCTTCC-3' and L-CPT-1-antisense (3703-3722), 5'-CTCGGAGAGCTAAGCTTGTC-3' (Gene Bank L07736). The DNA fragment excised after digesting pAC CMV-OB-Rb (13) with KpnI/HindIII restriction enzymes that hybridizes only the intracellular domain of OB-Rb was also used as a probe of OB-Rb. The hybridization signals were analyzed by Molecular Imager GS-363 (Bio-rad, Hercules, CA). Values were normalized to the signal generated with an 18s ribosomal RNA (rRNA) gene probe.

**Multiplex reverse Transcriptase polymerase chain reaction (MPX-RT-PCR):** The procedure used was based on methods described by Jensen et al. (22) and O’Doherty et al. (23). Total RNA (1 µg) was treated with RNase-free DNase (Promega), and first-strand cDNA was generated with the oligo(dT) primer in the first-strand cDNA synthesis kit (Clontech). MPX-RT-PCR was carried out in 25 µl reactions with 1.5 µl of the diluted cDNA reaction as template mixed with 23.5 µl of PCR mix containing 1.25 units of Taq polymerase and buffer (Roche Molecular Biochemicals); 25 µM of dATP, dTTP and dGTP; 2.5 µCi of 2,500 Ci/mmol [α³³P]dCTP (Amersham Pharmacia; 1 Ci=37 GBq); and 5 pmol each primer (Table 1). The standard thermal cycle profile was as follows for lipogenic gene enzyme mRNA (FAS, ACC and GPAT) and β-oxidative enzyme gene mRNA (L-CPT-1 and ACO): denaturation of 94° C for 1 min, annealing at 55° C for 1 min and extension at 72° C for 1 min for 24 cycles in liver and for 26 cycles in islets.
Reaction products were separated on 7 M urea, 1x TBE (0.1 M Tris base/83 mM boric acid/1 mM EDTA) and 6% polyacrylamide gels, dried and PhosphorImager screens were scanned by a Molecular Imager System (GS-363). TATA box binding protein (TBP) mRNA was coamplified as an internal control, and data were expressed as ratios to its signal.

To avoid biased results caused by potential interference between individual amplicons, we analyzed the amplification kinetics of individual amplicons in reactions where several products were coamplified. Representative experiments, in which mRNA encoding lipogenic enzymes and TBP in pancreatic islets was simultaneously amplified, show the noncompetitive amplification of individual products and their almost identical rate of amplification, as indicated by the slopes within the exponential phase observed from a linear regression analysis.

**PPARα Immunoprecipitation:** Fifty mg of liver from the rats was homogenized in 2 ml of lysate buffer with proteinase inhibitors. A total of 100 µg of protein in 0.5 ml of buffer was used for precipitation with 1:500 goat-anti-PPARα (C-20) (Santa Cruz Biotechnology, Inc. Santa Cruz, CA). Protein-A beads from Pharmacia were used for binding. Immunoblotting was carried out with rabbit-anti-PPARα from Calbiochem at 1:1500.

**Magnetic nuclear resonance spectroscopy (MRS) and imaging (MRI):** Using the method of Stein et al. (24), proton magnetic resonance spectroscopy (MRS) and imaging (MRI) data were obtained with a 4.7-T 40-cm-bore system (Omega chemical shift imaging model, Bruker Instruments, Fremont CA) with a six-inch diameter birdcage coil. Anesthetized rats were placed supine within the coil and positioned in the center of the magnet. Proton spectra of the rat were resolved into water and fat resonances, the areas of which were quantified using the nuclear magnetic resonance (NRM-1) software program (Tripos Associates, St. Louis, MO), assuming equal line widths for both resonances. Proton images were obtained from the abdominal region of each rat.
echo transaxial images were acquired with the following parameters: two transients, recycle time (TR) = 500 msec, echo time (TE) = 16 msec, 2 mm slice thickness 2 mm interslice gap, eight slices, a 140 mm FOV, and a 128 * 256 matrix. Images were analyzed using NIH Image software (NIMH, Bethesda, MD).

**TG content of tissues:** Animals were sacrificed under sodium pentobarbital anesthesia. Tissues were dissected and placed in liquid nitrogen. Total lipids were extracted from about 100 mg of tissue by the method of Folch et al. (25), and dried under N\textsubscript{2} gas. TG was assayed by the method of Danno et al. (26).

**Plasma measurements:** Tail vein blood was collected in capillary tubes coated with ethylenediaminetetraacetic acid. Plasma was stored at -20 °C. Plasma leptin was assayed using the Linco leptin assay kit (Linco Research, St. Charles, MO). Plasma glucose was measured by the glucose oxidase method using the glucose analyzer II (Beckman, Brea, CA). Plasma free fatty acids (FFA) were determined using the Boehringer Mannheim kit, (Indianapolis, IN). Plasma TG levels were measured by the GPO-Trinder triglyceride kit (Sigma, St.Louis, MO).

**[^3]H]-Palmitate Oxidation in Pancreatic Islets:** Oxidation of [^3]H]-palmitate by islets were determined as previously described (8). Groups of 100-200 islets were incubated in duplicate with 1 mM 9,10-[^3]H]-palmitate for 3 days. Palmitate oxidation was assessed by measuring tritiated water in the medium. Excess [^3]H]-palmitate was removed by precipitating twice with an equal volume of 10% trichloroacetic acid with 2% BSA. Supernatants in a microcentrifuge tube were placed in a scintillation vial containing unlabeled water and incubated at 50°C for 18 h. Tritiated water was measured as described for use of [^3]H]-glucose (27).

**U-[^{14}C]glucose incorporation into lipids in islets:** Incorporation of U-[^{14}C]glucose (14.6 mmol/l; New England Nuclear, Boston, MA) into lipids was measured in islets as previously described in detail (28). About 200 islets were cultured for 3 days in medium containing 8 mmol/l glucose. After 3 days in culture, lipids were extracted
from the islets according to the method of Bligh and Dyer (29), and counts incorporated into total lipid were determined.

**Statistical analyses:** All values shown are expressed as mean ± SEM. Statistical analysis was performed by two-tailed unpaired Student’s *t*-test by one-way analysis of variance.

**RESULTS**

**Response of leptin levels to caloric excess:** If the function of leptin during caloric excess is to minimize the accumulation of lipids in nonadipose tissues, hyperleptinemia should begin promptly at the start of overnutrition and increase progressively as the overnutrition continues. To test this, a group of 10 normal male Sprague-Dawley rats was fed a diet in which 60% of the calories were derived from fat. Age-matched control rats received a 4% fat diet. Both groups were observed for 70 days. Plasma leptin levels in control rats were relatively unchanged, rising by only 0.04 ± 0.002 ng/ml per day to a level of only 2.80 ± 0.77 ng/ml on the final day of the 70-day study. In rats on a 60% fat diet, by contrast, plasma leptin rose to 4.3 ± 0.2 ng/ml (p<0.001) within 24 h and increased progressively thereafter by 0.37 ± 0.07 ng/ml per day to a level of 26 ng/ml at 70 days (Figure 1A). In this group the rise in plasma leptin levels paralleled the expansion in body fat mass, quantified by magnetic resonance spectrophotometry (MRS) (Figure 1B); there was a highly significant correlation between body fat and the plasma leptin level (*r* = 0.91; *p*<0.0001) (Figure 1C). Thus, leptin levels appear to respond promptly to a caloric excess and they increase in proportion to enlargement of the adipose mass, consistent with the postulated role.

**TG deposition in nonadipose tissues in the presence of leptin action:** If the hyperleptinemia induced by high fat feeding does, in fact, protect nonadipose tissues of normal rats from overaccumulation of lipids, their tissue TG content should remain low during the development of obesity, despite the expansion of the adipose tissue mass and
the concomitant rise in plasma lipid levels. To test this, we measured tissue TG content of nonadipose tissues 70 days after the start of the high fat diet, at which point total body fat, measured by MRS, had increased ~150-fold above the pre-diet baseline (Figure 1B) and plasma TG and FFA levels were significantly higher (Figure 2A). However, TG content in nonadipose tissues increased only 1.0 to 2.7 fold above the baseline (Figure 2B). Thus, nonadipose tissues of leptin-responsive hyperleptinemic rats accumulated only a small fraction of the total increase in body fat acquired over 70 days of excessive fat intake, during which time the animals had became grossly obese (Figure 1B).

**Mechanism of antisteatotic protection in liver:** In the liver protection against steatosis might involve, not only increased secretion of VLDL, but also enhanced FA oxidation. In the latter case, an increase in the expression of PPAR\(\alpha\) and its target enzymes, L-CPT-1 and ACO might be expected (30). To determine if the *in vivo* protection against hepatic overaccumulation of TG in normal rats on a high fat diet is mediated by this mechanism, we semiquantified PPAR\(\alpha\) protein and ACO and L-CPT-1 mRNAs in livers of normal rats receiving either a 60% or a 4% fat intake. PPAR\(\alpha\) protein and L-CPT-1 mRNA were both significantly greater in the former group, but ACO mRNA was not different (Figure 3A and B).

**Mechanism of the antisteatotic protection in islets:** Unlike liver, islets cannot export excess FA, which may account for their vulnerability in obesity. To determine the mechanism of the protection against lipid overaccumulation that prevails early in the course of obesity, we measured the rate of oxidation of \[^3H\]-palmitate in isolated islets of Sprague-Dawley rats receiving either a 4% or 60% fat diet. Oxidation was 30% greater in pancreatic islets of rats on the 60% fat diet than in controls on the 4% fat diet (Figure 4A). However, unlike in liver, no change in ACO or L-CPT-1 could be detected by MPX-PCR (data not shown). These findings suggest that the preexisting oxidative machinery of the islets was able to accommodate this increase in oxidation without an increase in expression of genes encoding the enzymes.
We had previously reported that in the absence of leptin activity, as in \textit{fa/}fa ZDF rats, increased lipogenesis was the most important single factor in the ectopic overaccumulation of lipids in islets (7,31). Accordingly, in normal rats the high fat diet should not induce the increase in lipogenesis and lipogenic enzymes that had been observed in fat-laden islets of the leptin-insensitive \textit{fa/}fa rats. As shown in Figure 4B and C, there was no increase in incorporation of $^{14}$C-glucose to lipids or in expression of lipogenic enzymes; in fact, a small but significant decrease in lipogenesis and in FAS mRNA was evident (Figure 4B and C). This was in sharp contrast to the ZDF \textit{fa/}fa rats in which lipogenesis was 2.5 times greater.

\textbf{Ectopic TG deposition in the absence of leptin action:} If the antilipogenic protection observed in normal rats during caloric excess did, in fact, require the action of the accompanying hyperleptinemia, rodent models with either a leptin deficiency (\textit{ob/}ob mice) or a loss-of-function mutation in their leptin receptors (\textit{db/}db mice and ZDF \textit{fa/}fa rats) would be unprotected from lipid overaccumulation. We, therefore, measured the plasma leptin levels (Figure 5A) and the TG content of islets, skeletal muscle, heart and liver of these “unleptinized” rodents (Figure 5B). Although their diet contained only 6% fat, the TG content of their nonadipose tissues ranged from ~4 to ~100-fold above normal controls on the same diet. Thus, when leptin action is lacking, protection from lipid overaccumulation in nonadipocytes is also lacking – even when the dietary fat intake is normal.

\textbf{Overexpression of wild-type OB-Rb in livers of ZDF \textit{fa/}fa rats prevents steatosis:} If the marked hepatic steatosis and hypertriglyceridemia of obese ZDF \textit{fa/}fa rats is the result of lack of direct leptin action on the liver, transgenic overexpression of the wild-type leptin receptor in the liver of these leptin-receptor-defective animals should protect them. Therefore, we infused into 9-week-old ZDF \textit{fa/}fa rats $10^{12}$ plaque-forming units of recombinant adenovirus containing the cDNA of wild-type OB-Rb, the full-length isoform of the leptin receptor (AdCMV-OB-Rb). AdCMV-β-galactosidase (β-gal)
was infused into age-matched ZDF fa/fa rats as a control. The wild-type OB-Rb transgene introduced in vivo with an adenovirus vector was expressed exclusively in the steatotic liver of the ZDF fa/fa rats (Figure 6A). None was detected in any other tissues, including the hypothalamus.

One week after treatment with AdCMV-OB-Rb plasma TG levels of ZDF fa/fa rats declined slightly to below pretreatment levels and remained significantly below the controls for 3 weeks after AdCMV-OB-Rb treatment (Figure 5B). Liver TG content was significantly below that of β-gal controls and untreated controls (Figure 5C), the result of a delay in the increase in liver TG compared to the controls. TG content of heart and skeletal muscle were unaffected (Figure 6B). Food intake in the two groups was identical in the 2 adenovirus-treated groups (29.8 ± 1.4 g/d vs. 29.8 ± 1.5 g/d). Since the liver was the only site of expression of the normal OB-Rb in these ZDF fa/fa rats and the only site of antisteatotic action, we must assume that the elevated endogenous leptin levels, which averaged 24 ± 2 ng/ml in AdCMV-OB-Rb-treated rats, and 28 ± 2 ng/ml in controls, exerted a direct antisteatotic action on the liver. This strongly implies that the function of hyperleptinemia of obesity is to prevent steatosis in tissues with functioning OB-Rb.

**DISCUSSION**

These findings support the concept that a physiologic role of leptin during overnutrition is to confine storage of TG to adipocytes, cells specialized for this role, and thus protect nonadipocytes from the adverse consequences of lipid overaccumulation. This protection begins promptly at the start of overfeeding as the result of progressively increasing hyperleptinemia that continues to rise for the duration of hypernutrition. This appeared to minimize overaccumulation of lipids both by preventing the increase in lipogenesis that occurs in the absence of leptin action (31), and through upregulation of β-oxidative metabolism of the surplus fatty acids (7). Whereas in the liver there was an increase in PPAR-α protein and CPT-1 mRNA, in pancreatic islets no such changes
could be detected, despite a 30% increase in the rate of $^3$H-palmitate oxidation. The
greater induction of FA β-oxidative enzymes in liver than in extrahepatic tissues confirms
a recent observation by Cook et al. (31).

In islets the antilipogenic action of hyperleptinemia appears to be at least as
important as the increase in FA oxidation in protecting islets from the lipid overload;
when leptin action is lacking, as in hyperphagic fa/fa ZDF rats, the fat-laden islets have
a high rate of $^{14}$C-glucose incorporation into lipids, in association with increased PPAR-γ,
ACC and FAS expression on a 6% fat intake (32). By contrast, in normal rats receiving
the 60% fat diet, these remained in the low normal range and the lipogenic rate declined.
When the antilipogenic effect of leptin is lacking, lipogenesis is excessive and cannot be
restrained by lipid excess (32).

The most compelling evidence in support of the antisteatotic role for leptin was
the in vivo demonstration in leptin-unresponsive fa/fa ZDF rats that transgenic
overexpression of the wild-type receptor in their livers prevented the severe hepatic
steatosis and hypertriglyceridemia that otherwise occurred. These findings are congruent
with earlier evidence of the antisteatotic action of recombinant leptin (33) and of
transplanted fat tissue in “fatless” mice with congenital lipodystrophy (34). Furthermore,
in our experiments the wild-type leptin receptors were expressed only in the liver and not
in the hypothalamus or anywhere else; it follows, therefore, that the endogenous
hyperleptinemia of those obese fa/fa rats must have acted directly via the transgenic OB-
Rb to prevent the lipid overaccumulation.

The prompt rise of plasma leptin levels on the very first day of the high fat diet,
and their high degree of correlation with the expanding body fat, are all consistent with
the response of an antilipogenic hormone with a physiologic liporegulatory mission,
namely to maintain FA homeostasis in nonadipocytes during overnutrition. This
protection may account for the fact that in hyperleptinemic rats and humans the lipotoxic
complications of diet-induced obesity do not appear until late in life, when leptin
effectiveness wanes (35,36). When leptin is absent, as in congenital generalized lipodystrophy (33), or when leptin receptors are congenitally defective, as in ZDF rats, these complications appear in severe form early in life.

It should be emphasized that we do not suggest that the direct antisteatotic activity ascribed to the endogenous hyperleptinemia of obesity occurs in normal lean animals. It appears to be a factor only during overnutrition when plasma leptin levels approach or exceed the threshold for transport across the blood-brain barrier, which is probably in the vicinity of 10 ng/ml (34). In the absence of overnutrition plasma levels are below 5 ng/ml and leptin action is presumed to be largely on the hypothalamic centers for control of food intake and thermoregulation (35).

ACKNOWLEDGMENTS

The authors wish to thank Susan Kennedy for superb secretarial services. We acknowledge the grant support of the Department of Veterans Affairs Institutional Support (SMI 821-109), The National Institutes of Health (DK02700-37), The National Institutes of Health/Juvenile Diabetes Foundation Diabetes Interdisciplinary Research Program and Novo-Nordisk Corporation. We thank Cai Li, Ph.D., and Daniel Foster, M.D. for critical review of this manuscript.
REFERENCES


17

Obesity 19, 804-810


FIGURE LEGENDS:

Figure 1: Relationship of plasma leptin to total body fat in Sprague-Dawley rats receiving diets with a fat content of 4% △ or 60% ●. A) Plasma leptin levels in the two groups; the inset provides a view of the daily leptin profile for the first week of the study. B) Total body fat measured by MRS. Representative spectral tracings and transrenal images are displayed. C) The relationship between plasma leptin levels and body fat.

Figure 2: A) Comparison of the mean (± S.E.M.) plasma leptin, TG and FFA levels in normal Sprague-Dawley rats fed a diet containing either 4% □ (N=6) or 60% ■ (N=6) fat for 10 weeks. *p<0.001 B) The mean (± S.E.M.) tissue TG content of 6 normal Sprague-Dawley rats on a 60% fat diet at 4 weeks of age before starting the high fat diet □ and at 14 weeks of age after 10 weeks on the 60% fat intake ■. TG content in islets is expressed as ng/islet; in liver, heart and skeletal muscle, it is expressed as mg/g of wet weight of tissue. Body fat, as determined by MRS, is expressed as g/animal. *p<0.001

Figure 3: Mechanism of antisteatotic protection of liver during high fat feeding of normal rats. A) PPARα protein measured by immunoprecipitation in liver of 4 rats fed a diet containing 4% fat and 4 rats fed a 60% fat diet. B) mRNA of enzymes of fatty acid β-oxidation, ACO and L-CPT-1, quantified by northern hybridization. *p<0.01

Figure 4: Mechanism of antisteatotic protection of islets during high fat feeding of normal rats. A) Comparison of rates of oxidation of [H³]-palmitate in rats receiving a diet containing either 4% □ or 60% ■ fat. *p<0.01. B)
Comparison of the rates of incorporation of [U-14C]-glucose into lipids in normal Sprague-Dawley (SD) rats on a 4% or 6% fat intake. These are significantly less than in islets of hyperphagic fa/fa ZDF rats on a 6% fat intake. *p<0.01; §p<0.05.

C) Comparison of expression of the lipogenic enzymes acetyl CoA carboxylase (ACC), fatty acid synthase (FAT) and glycerol-PO₄ acyltransferase (GPAT) in islets of normal Sprague-Dawley (SD) rats fed either 4% or 60% fat diet. As an internal control for enzyme mRNA, TATA box-binding protein (TBP) was employed. *p<0.005; ¶p<0.01; §p<0.05.

Figure 5: A) Comparison of mean (± SEM) plasma leptin, TG and FFA levels in rodents that are either leptin-deficient (ob/ob mice) or unresponsive to leptin because of loss-of-function mutation in its receptor gene (db/db mice and fa/fa rats) and the corresponding wild-type (+/+ ) controls. *p<0.01; **p<0.001  B) Comparison of TG content in tissues of these rodents. All animals received a diet containing 6% fat. *p<0.001

Figure 6: A) Effect of intravenous infusion of obese ZDF fa/fa rats with AdCMV-OB-Rb or AdCMV-β-gal on the expression of wild-type receptor in the liver and hypothalamus. B) Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of OB-Rb in liver and hypothalamus of a lean wild-type (+/+) ZDF rat, an untreated obese (fa/fa) ZDF rat and an obese fa/fa rat 4 weeks after treatment with either AdCMV-βgal or AdCMV-leptin. Whereas in the untreated and AdCMV-βgal-treated fa/fa rat only mutated OB-Rb is present, in the AdCMV-leptin-treated fa/fa rat the OB-Rb matches that of the +/+ rat. C) Plasma
TG after AdCMV-OB-Rb ▲ or AdCMV-βgal ▯. **p<0.01. D) Triacylglycerol (TG) content of liver in obese ZDF fa/fa rats after treatment with AdCMV-OB-Rb ■ or AdCMV-βgal □. *p<0.05. E) Heart TG and skeletal muscle TG after AdCMV-OB-Rb ■ or AdCMV-βgal □. TG content in tissues of lean ZDF +/+ rats □ are shown for comparison.
Table 1. Primers employed for multiplex RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primer (5’-3’)</th>
<th>Antisense primer (5’-3’)</th>
<th>Predicted size, Bp</th>
<th>Species</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>ACAGTGAAGGCTTACGTCTG</td>
<td>AGGATCCTTACAACCTCTGC</td>
<td>242</td>
<td>Rat</td>
<td>J03808</td>
</tr>
<tr>
<td>FAS</td>
<td>TGCTGTGGACCTCATACATA</td>
<td>TGGATGATGTGGATGATAGAC</td>
<td>297</td>
<td>Rat</td>
<td>M76767</td>
</tr>
<tr>
<td>GPAT</td>
<td>CCTCTGAACCTGGAGAAGTGA</td>
<td>AGACAGTATGTGGCCTCTGC</td>
<td>287</td>
<td>Rat</td>
<td>AF021348</td>
</tr>
<tr>
<td>TBP</td>
<td>ACCCTTACCAATGACTCCTATG</td>
<td>TGACTGCAGCAAATCGCTTGG</td>
<td>186</td>
<td>Mouse</td>
<td>D01034</td>
</tr>
</tbody>
</table>
A. PPARα protein

B. mRNA of enzymes of FA oxidation

ACO

CPT-1
A. Oxidation of [³H]-palmitate.

B. Lipogenesis ([U-¹⁴C]-glucose to lipids)

C. Expression of Lipogenic Enzymes

- ACC/TBP mRNA
- FAS/TBP mRNA
- GPAT/TBP mRNA
A. Liver OB-Rb mRNA

B. PCR-RFLP for OB-Rb

C. Plasma TG

D. Liver TG

E. Heart (H)/Skeletal muscle (SM)
Liporegulation in diet-induced obesity: The antisteatotic role of hyperleptinemia
Young Lee, May-Yun Wang, Tetsuya Kakuma, Zhuo-Wei Wang, Evelyn Babcock, Kay
McCorkle, Moritake Higa, Yan-Ting Zhou and Roger H. Unger

J. Biol. Chem. published online November 28, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M008553200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts