Expression of ob mRNA and its Encoded Protein in Rodents

Impact of Nutrition and Obesity

Robert C. Frederich, Bettina Löllmann, Andreas Hamann, Antonella Napolitano-Rosen, Barbara B. Kahn, Bradford B. Lowell, and Jeffrey S. Flier

Division of Endocrinology, Beth Israel Hospital (Research North), Boston, Massachusetts 02215

Abstract

The mutant gene responsible for obesity in the ob/ob mouse was recently identified by positional cloning (Zhang Y., R. Proenca, M. Maffel, M. Barone, L. Leopold, and J. M. Friedman. 1994. *Nature (Lond.)* 372:425). The encoded protein was predicted to be an adipocyte-derived secreted protein and to represent an "adipostat" signal reflecting the state of energy stores. We confirm that the adipocyte is the source of ob mRNA and that the predicted 16-kD ob protein is present in rodent serum as detected by Western blot. To evaluate the hypothesis that it might represent an adipostat, we assessed serum levels of ob protein and expression of ob mRNA in adipose cells and tissue of rodents in response to a variety of perturbations which effect body fat mass. Both ob protein and ob mRNA expression are markedly increased in obesity. The levels of ob protein are ~ 5–10-fold elevated in serum of db/db mice, in mice with hypothalamic lesions caused by neonatal administration of monosodium glutamate (MSG), and in mice with toxigene induced brown fat ablation, (UCP-DTA). Very parallel changes are observed in adipocyte ob mRNA expression in these models and in ob/ob mice. As predicted however, no serum ob protein could be detected in the ob/ob mice. By contrast to obesity, starvation of normal rats and mice for 1–3 d markedly suppresses ob mRNA abundance, and this is reversed with refeeding. Similarly, ob protein concentration in normal mice falls to undetectable levels with starvation. In the ob/ob, UCP-DTA and MSG models, overexpression of ob mRNA is reversed by caloric restriction. These data support the hypothesis that expression of ob mRNA and protein are regulated as a function of energy stores, and that ob serves as a circulating feedback signal to sites involved in regulation of energy homeostasis. (*J. Clin. Invest.* 1995. 96:1658–1663.) Key words: fat • adipocyte • mice • rats • obesity

Address correspondence to Jeffrey S. Flier Division of Endocrinology, Beth Israel Hospital (Research North), 330 Brookline Ave, Boston, MA 02215. Phone: 617-667-2151; FAX: 617-667-2927; e-mail: jflier@bih.harvard.edu. A. Napolitano-Rosen's present address is Instituto Semeotica Medica, Padova, Italy.

Received for publication 16 March 1995 and accepted in revised form 2 June 1995.

J. Clin. Invest.

0021-9738/95/09/1658/06 $2.00
Volume 96, September 1995, 1658–1663

Introduction

Energy homeostasis requires the integration of physiologic systems regulating energy intake, expenditure, and storage. Adipocytes, the primary site for energy storage and energy release in response to changing energy needs of the organism, play a key role in this process (1). In addition to their well described role in energy storage, it has been suspected for many years that adipocytes also play a more active, regulatory role in fuel homeostasis. Specifically, it was hypothesized that adipocytes produced a signal whose intensity is linked to the adequacy of nutrient stores. This hypothetical signal was proposed to act, directly or indirectly, at the level of the hypothalamus to regulate nutrient intake and other processes linked to fuel homeostasis (1–4). The existence of such a closed loop "adipostat" feedback system has been supported by several lines of experimentation (for review see reference 3), including parabiosis, but a definitive molecular mediator was never identified. Recently, the "obesity" gene, mutation of which is responsible for a severe syndrome of obesity with insulin resistance in the ob/ob mouse, was identified through positional cloning (5). The gene encodes a fat-specific mRNA and a predicted protein with characteristics of a secreted protein, making it a strong candidate to be a, or the, adipostatic signal from fat (5). In this report, we present evidence that expression of ob mRNA is regulated in adipose cells or tissue of the rodent in response to diverse nutritional, pharmacologic, and disease perturbations that influence adipose cell mass. We also provide evidence that the ob protein is present as a 16-kD protein in the serum of normal mice. The circulating level of ob falls in response to starvation, is increased in several models of murine obesity, including db/db, brown adipose tissue deficient mice, and mice with monosodium glutamate (MSG)[1] induced obesity, and is undetectable in serum of ob/ob mice. Our results suggest that the level of expression of the ob mRNA and the circulating protein that it encodes reflect the state of energy stores in a manner consistent with the proposed role of this molecule as an adipostat.

Methods

Animals. Two- or three-month-old male Sprague-Dawley rats (CD strain), or 30–35 gram CD-1 mice were obtained from Charles River

1. *Abbreviations used in this paper:* ^{32}P-dCTP, alpha-^{32}P deoxycytidine 5′-triphosphate; BAT, brown adipose tissue; MSG, monosodium glutamate; UCP-DTA, uncoupling protein-diphtheria toxin A chain; WAT, white adipose tissue.

Laboratories (Wilmington, MA), and maintained with ad libitum access to standard lab chow (Purina Rodent Chow #5008: 6.5% fat, 49% carbohydrate and 23% protein, wt/wt, 3.5 kcal/gram) and water except as indicated. Animals fasted for 12 h had food removed at night and were sacrificed the following morning. Animals fasted for 24 h had food removed in the morning and were sacrificed the following morning. The ob/ob genotype mice on the C57BL/6J background, the db/db mice on a C57BL/KSJ background and their lean (+/?) littermates were obtained from Jackson Laboratory (Bar Harbor, ME) and maintained, 4–5 per cage at 25°C with a 12:12 h light/dark cycle, in accordance with institutional guidlines. The brown fat deficient (UCP-DTA) mice, a new transgenic model of obesity based on brown fat ablation by toxigene expression (6) were obtained from the colony maintained at Beth Israel Hospital. The MSG model of obesity was produced by daily injection of CD-1 mice with MSG during the first week of life according to the method of Djazayery et al. (7). A group of MSG mice were treated for 6 wk with ephedrine (1 grams/kg chow; Sigma Chemical Co., St. Louis, MO) and caffeine (1.4 grams/kg chow; Sigma Chemical Co.) mixed in the diet (8). A second group of MSG mice were fed ad libitum for 10 weeks and then food restricted, receiving ~ 60% of the normal chow consumed by control animals.

Tissue. For all mouse samples and the indicated rat samples, fat pads were surgically removed and frozen in liquid N_2 or solid CO_2. Total RNA was extracted with guanidium isothiocyanate and centrifuged over CsCl as previously described (10), or in the case of the UCP-DTA mice by the RNAzol™ (Biotecx Inc, Houston, TX) method. In selected experiments rat adipocytes were first isolated by digestion with 1.5 mg/ml collagenase in Hepes buffered KRB (pH 7.4) with 1% BSA and subsequent floatation as previously described (11). An aliquot was taken for measurement of cell number and size by osmic acid fixation and Coulter electronic method (11). Total RNA was then isolated as for intact tissue.

Northern analysis. RNA quantity and purity was estimated by 260 and 280 nm OD correcting for 320 mm OD background. Equal amounts of total RNA (or in part of Fig. 2, RNA from an equal number of adipocytes) were electrophoresed on 1.2% formaldehyde agarose gels. RNA integrety and equal RNA loading were further confirmed by ethidium bromide staining and ultraviolet transilumination. The RNA was blotted to nylon filters (Magnagraph, MSI, Westboro, MA) via standard capillary techniques (9), and fixed with ultraviolet irradiation (120 mJoules). A probe for the predicted translated region of the ob mRNA was obtained by PCR amplification of mouse adipocyte RNA after reverse transcriptase reaction (Superscript™; Life Technologies, Gaithersburg, MD). The primers used (5′-AGGAAAATGTGCTGGAGACC-3′) and (5′-CTTCAGCATTCAGGGCTAAC-3′) span the region from bases 110 to 621 in the published sequence (5). The identity of the appropriately sized product was confirmed by mapping with multiple restriction endonucleases and sequencing.

The ob probe was labeled with ^{32}P-dCTP by the random priming (Boehringer Mannheim, Indianapolis, IN) method, and hybridized to the blotted nylon filters using QuickHyb® (Stratagene, La Jolla, CA). The labeled filters were analyzed by exposure to x-ray film. Quantification of ob gene expression was performed using densitometry or phosphoimaging using Image Quant Software (Molecular Dynamics, Benton, NJ).

Western blotting for ob protein. Polyclonal rabbit antibodies were raised against NH_2-terminal (QKVQDDTKTLIKTIVTRIND) and COOH-terminal (RLQGSLQDILQQLDVSPEC) peptides of the predicted ob protein (5). Antibody against the COOH-terminal peptide was purified using the Sulfolink® antibody purification kit (Pierce, Rockford, IL) using the manufacturers recommended procedure. Briefly, the peptide was coupled to the Sulfolink® gel which was then incubated with 1 ml of antiserum overnight. The gel was then drained and washed with PBS until no eluted protein could be detected at 280 nm OD. The purified antibody was then eluted with 0.2 M glycine (pH 2.5). The collected fractions were neutralized with 1 M Tris (pH 10.5) and the appropriate peak was identified by 280 nm OD, and confirmed to be IgG by appropriate size on 7.5% SDS-PAGE gels. Western blots using this affinity purified antibody identified a 16-kD band in the media of 293 cells that had been transfected with the pRc/CMV vector (Invitrogen, San Diego, CA) into which had been cloned a cDNA encoding the predicted translated region of the ob mRNA (from bases 110 to 621). The specificity of the antibody was confirmed by the absence of the 16-kD band when pre-immune serum was used, its disappearance when the rabbit serum was preincubated with excess peptide antigen, and its absence from ob/ob mouse serum, in which both alleles have a nonsense mutation that truncates the protein prior to the COOH-terminal peptide.

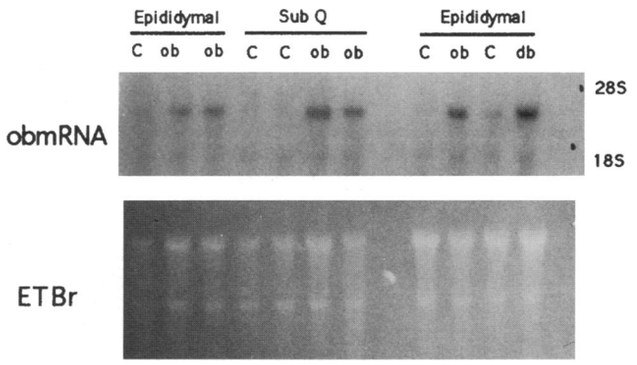

Figure 1. The ob mRNA in white adipose tissue of ob/ob mice and lean controls (+/?). Total RNA from epididymal or subcutaneous WAT depots was prepared from 4-mo-old mice and analyzed by Northern blot with an ob cDNA probe. Each lane represents 5 μg. Similar results for epididymal fat were obtained in three additional experiments. The final two lanes represent the RNA from fat pads of db/db mice (*db*) or their lean (+/?) controls (*C*). A picture of the UV transilluminated, ethidium bromide gel (*ETBr*) is presented to allow assessment of amount and quality of the RNA probed.

Sera from the various mouse models and their controls were run on 10 or 16% tricine-SDS-PAGE gels. The proteins were then transferred to PVDF (Immobilon-P, Millipore, Bedford, MA) membranes by electroblotting in 39 mM Glycine, 48 mM Tris, and 20% methanol (pH 8.3) at 240 mA for 40 min. The membranes were then blocked with 5% nonfat dry milk + 1% BSA. The blots were then incubated for one hour with the purified anti-ob COOH-terminal peptide antibody (1/100 or 1/50 dilution), washed in TBS-0.05% tween, and then 1 hour with goat anti–rabbit IgG horseradish peroxidase conjugate (BIO-Rad, Hercules, CA). After a second wash with TBS-0.05% tween, the immunocomplexes were visualized with the ECL system (Amersham, Buckinghamshire, UK).

Physiologic characterization. Body and fat pad weights were obtained using an analytical balance.

Reagents. All chemicals were reagent grade. ^{32}P-dCTP was obtained from ICN (Costa Mesa, CA).

Calculation and statistical analysis. Statistical analysis was done using the unpaired *t* test calculated with the Statview® program.

Results

ob mRNA is expressed in multiple fat depots and is over-expressed in ob/ob mice. In their initial report on the cloning of the ob gene, Zhang et al. (5) demonstrated that ob mRNA is expressed exclusively in RNA derived from adipose tissue and that the mutant ob mRNA is markedly overexpressed in ob/ob mice. We confirm this overexpression of ob mRNA in ob/ob mice, and find that it is also seen in epididymal, subcutaneous (Fig. 1), and parametrial (not shown) WAT. ob mRNA is also markedly over expressed in BAT of ob/ob mice (not shown) to a level comparable to that seen in WAT of ob/ob mice.

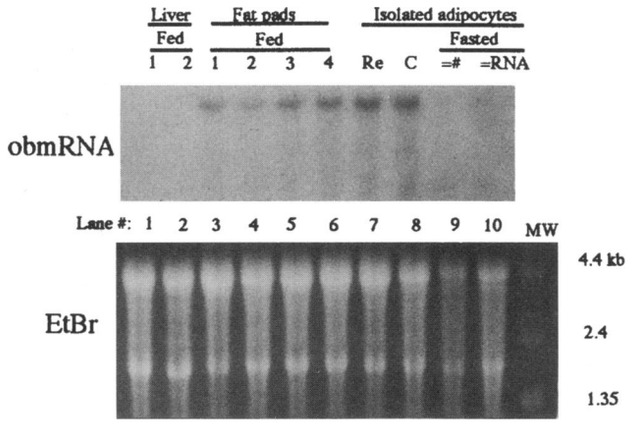

Figure 2. Effect of fasting and refeeding on ob mRNA expression. 280 grams (~ 3 mo old) CD rats were fed ad lib, fasted for 3 d (with salt supplementation), or fasted for 2 d and refed for 6 d before sacrifice. The first 6 lanes (*1–6*) represent 14 μg of total RNA from liver or epididymal fat pads of fed rats. Lanes *7–10* had RNA prepared from isolated adipocytes. Lane *7* (refed) contained 13.3 μg of RNA, lane *8* had 14 μg RNA from chow fed, and lanes *9* an *10* had 8.5 or 14 μg RNA from fasted animals, representing loading per equal cell number or per equal RNA level. A picture of the UV transilluminated, ethidium bromide stained gel (*EtBr*) is presented to document RNA quantity and quality. The values for the molecular weight markers (*MW*) are indicated.

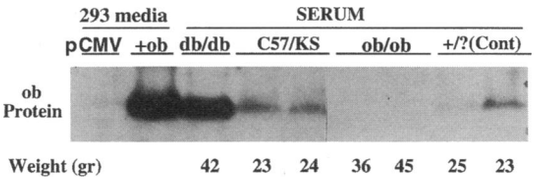

Figure 3. Antibody against the COOH-terminal epitope of the ob protein identifies a 16-kD protein on Western blots that is elevated in serum of db/db and undetectable in ob/ob mice. Immunopurified antibody against the COOH-terminal peptide was used to identify the ob protein by Western blot. The first two lanes represent 4 μl of media from 293 cells transfected with pRc/CMV with (*+ob*) or without (*pCMV*) the ob cDNA. 4 μl of 6-wk-old male db/db serum (*db/db*), or 8 μl of serum from age and sex matched C57BL/KSJ (*C57/KS*), ob/ob (*ob/ob*), and lean litermates of the ob/ob mice ("+/? lean controls") were also evaluated. Unpurified antisera containing antibodies directed against both the NH_2- and COOH-terminal ob peptides gave very similar results. The body weights of the animals listed at the bottom of the figure demonstrate the obesity of the db/db and ob/ob mice.

By contrast ob mRNA is barely detectable in control BAT by Northern analysis (not shown). Similar to the findings in ob/ob mice, overexpression of ob mRNA is also seen in the db/db mouse model (Fig. 1), consistent with the hypothesis that db/db mice are resistant to an adipostatic signal that is produced by normal mice, and absent in ob/ob mice (12). We also confirm the finding that ob mRNA is not observed in other tissues such as liver (Fig. 2), kidney, heart, or placenta (the latter not shown).

The adipocyte is the major source of ob mRNA in fat tissue. Most of the cells in the fat pad are not adipocytes (13). Thus only a fraction of RNA from fat tissue is derived from adipose cells. To investigate whether the adipocyte is the source of ob mRNA expression in RNA derived from fat pads, we compared equal amounts of total RNA prepared from entire pads (Fig. 2 lanes *3–6*), to that obtained from isolated adipocytes (Fig. 2 lanes *7–10*). Compared to whole fat pads, the ob mRNA signal from adipocytes isolated from the contralateral epididymal pad of the same animals is concentrated 1.74-fold in RNA from the isolated adipocytes relative to RNA from whole fat pads from the same animals (Fig. 2 lanes *1–4* vs lane *6*). This confirms, as expected, that the adipocyte is the primary source of ob mRNA expression in fat.

Immunodetection of the ob protein in the serum of mice. We developed an antiserum to the ob protein by immunizing rabbits with predicted NH_2- and COOH-terminal peptides of the mouse ob protein, and we used this serum or an antibody that was affinity-purified from this serum using the COOH-terminal immunizing peptide to detect an abundant 16-kD protein in the sera of db/db mice on Western blots. A much lower intensity signal of the same mobility is seen in the serum of control C57 mice and ob/+ mice, and no signal was detected in the serum of ob/ob mice (Fig. 3). This presumed ob signal comigrates precisely with a signal detected in medium conditioned by cells transfected with an expression vector that was engineered to drive expression of the ob cDNA, but not in serum from cells transfected with the empty vector (Fig. 3). This band was not seen when the blots were probed with preimmune sera, or when the antiserum was preincubated with the immunizing peptides (data not shown), confirming its identity as ob. Under these conditions of blotting and detection, loading increasing volumes of serum from db/db mice produced a linearly proportional signal ($r = 0.99$) on Western blots (Fig. 4), which allows us to conclude that db/db mice have ninefold higher levels of the ob protein than do fed control mice.

The effect of fasting on the level of ob. If, as hypothesized, the protein encoded by the ob mRNA functions as a feedback signal reflecting the size of fat stores, then the expression of the protein must be regulated by conditions which alter the size of those stores. Fasting is a physiologic conditions which would be anticipated to be important in this regard. As seen in Fig. 4,

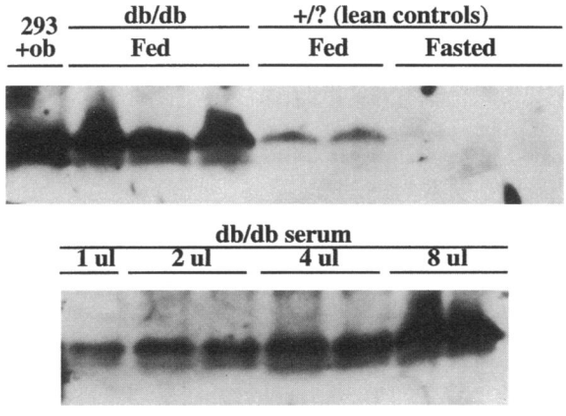

Figure 4. Fasting reduces circulating ob protein concentration. The upper panel represents ob protein in 8 μl of serum from individual 6-wk-old fed male db/db (*db/db*) or lean litermates (*+/? lean controls*) in the fed (*fed*) or 24-h fasted (*fasted*) condition. The first lane contains 10 μl of media from 293 cells transfected with the ob-pRc/CMV vector. The bottom panel demonstrates the linearity of the ob message signal ($r = .99$) with increasing amounts of db/db serum over an eightfold range.

fasting of lean littermates of db/db mice (+/?) for 24 h caused a marking reduction in the ob signal, to a level beneath detection in this assay. If this regulation of ob protein with fasting occurs at the level of transcription or mRNA stability, ob mRNA should be responsive to fasting. In fact, with 3 d of fasting in rats, the ob mRNA falls dramatically. In the experiment shown in Fig. 2 (lanes *7* and *8*), fasting levels of ob mRNA were reduced to 12% of fed control levels. In two other experiments (not shown), ob mRNA became undetectable with 3 d of fasting.

This effect is fully reversible with refeeding. After two days of fasting and six days of refeeding the mean ob mRNA level in rats was 128% of control levels (Fig. 2). This small but significant elevation with refeeding ($P = 0.04$), occurred in the face of epididymal fat pads that are still smaller than those of control fed animals (pad weight 1.05 vs 1.56 grams $P = 0.002$ in the experiment shown) consistent with the fact that the adipocytes of 6-d refed rats are still slightly smaller using this protocol (14). Qualitatively similar effects were seen with fasting and refeeding in lean, control mice (not shown).

ob mRNA expression in two distinct models of murine obesity. To extend our understanding of the regulation of ob gene expression, we studied two models of obesity whose etiology is probably independent of the ob/ob gene or its product: MSG and UCP-DTA mice.

The MSG model. The MSG model is produced by daily injections of monosodium glutamate during the first week of life. This treatment results in a hypothalamic lesion with stunting, hypogonadism, and moderate obesity apparently related to decreased brown adipose tissue and metabolic rate and not associated with hyperphagia (15). We determined that the level of circulating ob protein was markedly increased in MSG mice compared to controls at 8, 12 weeks and 1 year of age (Fig. 5), and the level at the latter time points was similar in magnitude to that of 6-wk-old male db/db mice. We also determined ob mRNA expression in this model at several ages (Fig. 5). Interestingly, there was no expression of ob mRNA in fat pads from 2-wk-old animals, even though other adipocyte specific genes are expressed at this point (8). At 3 wk ob mRNA is easily observed in both control and MSG mice, and the level in MSG-treated mice is 126% of controls. The over expression of ob mRNA is even greater at 5 mo (fourfold) and one year (threefold) at which time obesity in this model is more marked.

The overexpression of ob mRNA in MSG obese mice can be partially overcome by 6 wk of food restriction sufficient to induce an 11 gram weight loss (Fig. 5). Similarly, 6 wk of treatment with the "thermogenic cocktail" (7) of ephedrine and caffeine which resulted in a 12 gram weight loss, caused ob mRNA level to fall by 60% into nearly the normal range.

The UCP-DTA model of obesity. The UCP-DTA model of murine obesity was produced by transgenically expressing the A chain of diphtheria toxin under the uncoupling protein promotor (6). The resulting animals have marked selective ablation of brown adipose tissue (6). These animals become progressively obese and later become hyperphagic. However, in contrast to ob/ob and db/db mice, UCP-DTA animals do not have elevated levels of corticosterone, nor decreased body protein, and they have normal or increased lean body mass and are fertile (unpublished observations). As demonstrated in Fig. 6, these mice have a marked increase in the level of ob protein in the blood compared to control mice. As with the db/db and MSG mice, this increased level of circulating ob protein is associated with

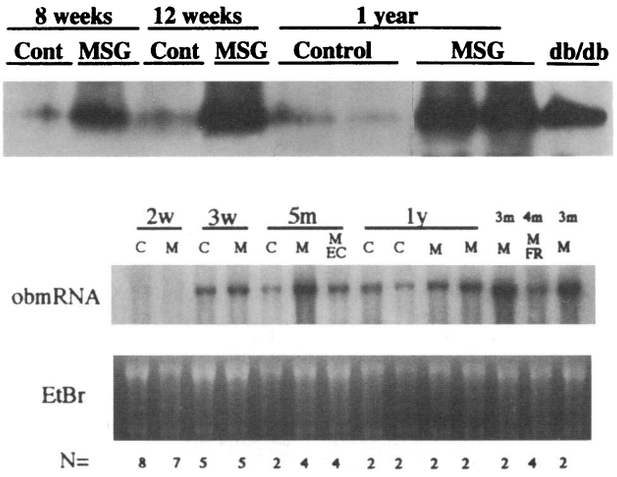

Figure 5. Increased ob protein and mRNA expression in the MSG mouse model of obesity. The upper panel is a Western blot measuring ob protein in 8 μl of serum pooled from six untreated CD-1 (*Cont*) or age matched MSG-treated (*MSG*) mice at 8 and 12 wk, and from individual mice at 1 yr. The last lane represents 4 μl of serum from a 6-wk-old male db/db mouse for comparison. The body weights of the MSG mice were not significantly different from controls at 8 and 12 wk and at one year were 38.6 and 40.2 grams for controls and 50.4 and 57.7 grams for MSG. The lower panel represents ob mRNA by Northern blotting of 1.5 μl of total RNA from pooled epididymal fat pads from MSG treated (*M*) or untreated CD-1 (C) mice sacrificed at two (*2w*), or 3 (*3w*) weeks, 3 (*3m*), 4 (*4m*), or 5 (*5m*) mo or one year (*1y*). The number of animals from which RNA was pooled is expressed beneath the picture of the UV transiluminated, ethidium bromide stained gel (EtBr). The indicated MSG mice were treated with ephedrine and caffeine (*EC*) or food restricted (*FR*) for 6 wk.

an approximate fourfold increase in ob mRNA expression in WAT (Fig. 6). Interestingly this elevated ob mRNA falls with 12 h of overnight fasting to approximate the level of fed control animals (though remaining much higher than fasted controls) despite no significant weight loss. Preliminary results suggest serum concentrations show the same pattern (not shown). This suggests that while adipocyte size and total body fat mass are strongly correlated with serum ob protein concentration and adipocyte ob mRNA, other factors may also have an influence.

Discussion

The molecular cloning of the obese (*ob*) gene in the mouse (5) has created new avenues for research into the regulation of body weight. The mRNA encoded by ob appeared to be expressed specifically in adipose tissue, was predicted to encode a secreted protein, and was overexpressed in ob/ob mice that harbor a nonsense mutation, suggesting the operation of a feedback loop (5). It was therefore proposed that the ob protein is a fat derived signalling molecule whose levels reflect the state of adipose energy stores, and whose actions involve (either directly or indirectly) a suppression of food intake and other adaptations that limit fat stores, such as activation of sympathetic nervous system activity (5). Elaboration and proof of the ob hypothesis will require many additional studies, including reversal of the syndrome by replacement of the missing protein, and discovery of its molecular targets and mode of action. In parallel, it will also be necessary to identify the factors that regulate expression

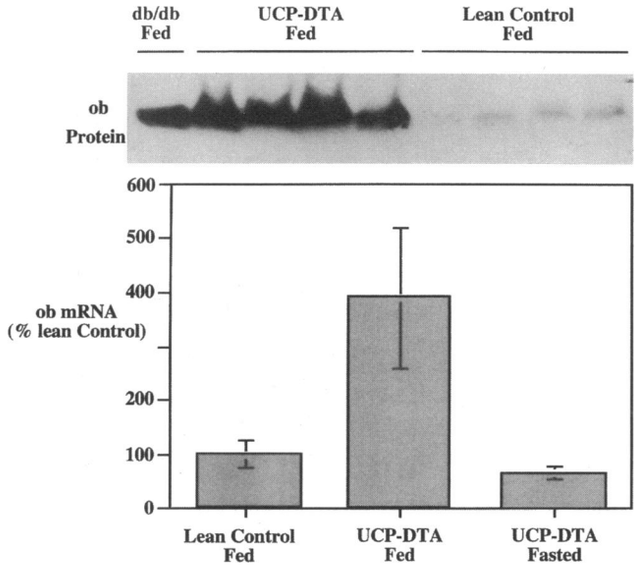

Figure 6. Increased ob protein and mRNA expression in obese UCP-DTA mice. The upper panel represents Western blotting for the ob protein using immunopurified antibody. The first lane (*db/db*) demonstrates the ob protein present in 4 μl of 6-wk-old male db/db mouse serum. The next 8 lanes represent ob protein in 8 μl of fed 12-wk-old male mice carrying the UCP-DTA transgene (*UCP-DTA*), or their fed lean, age matched, FVB/N controls (*lean controls*). Similar results were obtained in a second experiment.

The bottom panel represents 12-wk-old fed or 12-h fasted UCP-DTA mice, and their fed lean controls. 20 μg of total RNA from parametrial fat was analyzed for ob mRNA by Northern analysis. Each bar represents densitometric analysis of three mice presented as the mean±SEM. Comparable results for the overexpression of ob mRNA in fed UCP-DTA mice were observed in two additional experiments.

of ob mRNA and protein in normal physiology and in diverse models of obesity, in part to determine whether the observed regulation is consistent with the expectations for an "adipostatic" signal. The latter were the primary goals of the present study.

The findings reported here support the proposed model for the function of ob (5). Thus, using an anti-peptide antibody based on the predicted protein sequence of ob, we have demonstrated a protein with the predicted molecular mass of ob in the circulation of normal mice, and in the media conditioned by cells transfected with the ob cDNA. Thus, the prediction that ob is a fat derived circulating peptide is fulfilled. It is also shown that db/db mice, in whom we found the ob mRNA to be markedly overexpressed, have markedly increased levels of the ob protein as well, consistent with the prediction that these mice are resistant to the ob protein (12). Although the level of ob mRNA is similarly increased in fat of ob/ob mice and db/db mice, the ob signal is not seen in sera from ob/ob mice, consistent with the observation that these mice harbor a missense mutation in the ob sequence (5).

We have also shown that in several physiologic states, diseases, and therapeutic interventions, expression of ob mRNA and the level of the circulating protein declines as adipose mass and cell size fall, and rises as adipose mass returns towards normal. Caloric restriction is the most relevant maneuver to evaluate the regulation of ob. It is clear that fasting of mice for a period of 24 h causes a substantial fall in the levels of circulating ob. This regulation is at least partially at the pre-translational level, since fasting of both normal rats and mice is accompanied by a major reduction of ob mRNA expression in white adipose cells or tissue. Refeeding restores the expression of ob mRNA to or somewhat above control levels.

We have also gained insights from studies of ob expression in two models of murine obesity distinct from ob/ob and db/db mice. Mice with obesity due to neonatal treatment with monosodium glutamate do not have hyperphagia, but become obese as a result of impaired energy expenditure, due to an MSG-induced defect in hypothalamic control of energy expenditure (15). We have found that mice with MSG-induced obesity have increased expression of ob mRNA in adipose tissue and increased circulating ob protein, suggesting that the enlarged adipocyte in this model can respond in an appropriate manner at the level of ob expression. Whether the absence of hyperphagia in these mice is due to the high levels of the ob protein acting to produce satiety, or to an MSG-induced hypothalamic lesion that impairs the capacity to generate hunger signals cannot be determined from these studies. It is notable that two maneuvers that reduced the obesity of these mice through different mechanisms, caloric restriction and treatment with the thermogenic cocktail of ephedrine and caffeine, returned the level of ob mRNA expression towards normal.

We recently created a new model of murine obesity through transgenic ablation of brown adipose tissue using a tissue specific toxigene (6). These mice develop obesity, initially due solely to efficient metabolism (with no hyperphagia), and eventually with both efficient metabolism and hyperphagia (6). These mice, when obese, also have a markedly increased circulating level of ob protein, due at least in part to overexpression of the ob mRNA, and this falls after caloric restriction as it does in normal, MSG, and ob/ob mice (the latter not shown). Thus, brown fat deficient mice appear to regulate ob expression at the level of the adipocyte in a normal manner (i.e., they increase it as the adipocyte enlarges). It appears, however, that these mice are resistant to the actions of the ob product, or at least those actions related to the control of food intake, since despite increased circulating levels of ob, they are clearly hyperphagic. This suggests, as we earlier surmised (6), that BAT may participate in a mechanism that regulates food intake that is distinct from the ob system. In this regard, it should be noted that brown adipose tissue of normal mice has low but detectable expression of the ob mRNA, and that this expression can be enhanced, since the level of ob mRNA expression is markedly elevated in BAT of ob/ob mice. The nature of a putative signal from BAT that might regulate satiety is unknown at this time.

The data presented here builds importantly upon the critical discovery of the obese gene (5) and its predicted role in metabolic control. Many important aspects of this evolving story remain unknown, however. For example, more detailed analyses of the relationship between adipocyte size, adipose mass, energy stores and ob expression will need to be determined, and many further investigations of the intracellular signals, and possibly extracellular factors, involved in ob mRNA and protein expression, will be required. It will also be necessary to determine the nature of the receptor for ob and the sites of it's actions in the hypothalamus or elsewhere in the body.

In conclusion, these studies have demonstrated that the ob protein is present in the circulation of mice as a 16-kD protein, and that the level of this protein is regulated in a manner expected for an adipostatic signal, i.e., falling with starvation and

rising with obesity. Studies of ob mRNA expression in adipose tissue and cells of rodents under a variety of physiologic and disease states reveal that the regulation of ob occurs to a substantial degree at a pretranslational level. These data support the idea that ob is a circulating physiologic signal that reflects energy stores in adipose tissue. In addition, our findings with the brown fat deficient mouse suggest that hyperphagia in this model is due to the ability of deficient BAT function to cause resistance to ob, or to interfere with a satiety mechanism that operates downstream from or independent of ob.

Acknowledgments

We wish to acknowledge the excellent technical assistance of Brandon Ayres, and Dr. Eleftheria-Maratos Flier for the db/db mouse sera.

This work was supported by National Institutes of Health grants: P30DK46200, K08 HL-02564, K08 DK-02119, R37 DK-28082, RO1 DK-46930, and RO1 DK-43051.

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