Pharmacologic Manipulation of ob Expression in a Dietary Model of Obesity*

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Mutation of the obese (ob) gene results in severe hereditary obesity and diabetes in the C57BL/6J and related strains of mice. In this study we examined the expression of the ob gene in a dietary model in which moderate obesity develops in response to fat (58% of calories from fat) without mutation of the ob gene, and in four genetic models of obesity in mice: ob/ob, db/db, tubby, and fat. Several white and brown adipose depots were examined (epididymal, subcutaneous, perirenal, and interscapular). Northern blot analysis shows that levels of ob mRNA are increased in all adipose depots examined in every model of obesity. The average fold increases were 12.0 ± 2.1 (ob/ob), 4.8 ± 1.5 (db/db), 2.8 ± 0.1 (tubby), 2.4 ± 0.3 (fat), and 2.1 ± 0.2 (high fat diet-induced A/J). Moreover, we found that the expression of the ob gene could be manipulated by pharmacologically blocking the development of diet-induced obesity. Supplementation of a high fat diet with a β₃-adrenergic receptor agonist (CL316,243) prevented obesity, but not hypophagia associated with high fat feeding (body weights of high fat-fed A/J mice = 34.0 ± 1.0 g; high fat plus CL316,243-fed mice = 26.8 ± 0.5 g; n = 10). CL316,243-treated, high fat-fed animals contained levels of ob mRNA in all adipose depots that were equal to or less than levels in low fat-fed mice (average levels in high fat plus CL316,243-fed mice relative to low fat-fed mice: 0.93 ± 0.09). Inasmuch as fat cell size, but not number, was increased in a previous study in diet-induced obese A/J mice, these results indicate that expression of the ob gene serves as a sensor of fat cell hypertrophy, independent of any effects on food intake.

The recent cloning of the ob gene has led to renewed interest in the genetic basis of obesity (1). This autosomal recessive mutation is most often expressed on the C57BL/6J (B/6J) background strain and is associated with severe obesity, hyperinsulinemia, hyperglycemia, hypercortisolremia, and infertility. Early studies by Coleman and Hummel (2) and Coleman (3) led to the proposal that ob may encode a diffusible signaling factor, while the product of a separate obesity mutation, db ("diabetic"), residing at a different chromosomal location, has been postulated to receive the signal from the diffusible factor; perhaps encoding a receptor for ob (4). The cloning and sequence analysis of the ob locus suggests that the ob gene indeed encodes a protein that is secreted from adipocytes (1). In the ob/ob mouse, a nonsense mutation in the coding region appears to prevent the production of a functional OB protein, and a marked up-regulation of ob mRNA has been observed in adipose tissue of ob/ob mice (1). From these data, and because it is widely believed that obesity in the ob/ob mouse is caused by a defect in the ob gene (5), it was speculated that this protein normally serves as a feedback regulator of satiety (1). Support for this hypothesis has been provided by several recent studies in which ob/ob or db/db mice were administered recombinant OB protein (6–8). Decreased food intake and body weight were observed in ob/ob mice, but the OB protein had no effect in db/db mice (7, 8). However, in order to demonstrate that the OB protein truly serves a sensor function, the expression of ob protein must be shown to be modulated through alteration of fat mass. We now report that the expression of the ob gene can be increased by diet and adipocyte hypertrophy, and reduced by stimulation of β₃-adrenergic receptors (β₃AR), independent of effects on food intake. These data suggest that changes in ob gene expression do not predict changes in food intake.

EXPERIMENTAL PROCEDURES

Animals—All mice were obtained from The Jackson Laboratory (Bar Harbor, ME). "Obese" (ob/ob) and "tubby" (tub/tub) mutant mice on the C57BL/6J (B/6J) background, and "diabetic" (db/db) and "fat" (fat/fat) mutants on the C57BL/KsJ background, were obtained together with genetically lean (+/+) control animals at 10 weeks of age unless otherwise indicated in the text. These animals were provided free access to Purina mouse chow and water. For dietary obesity studies, the standard protocol of Surwit was followed (9–11). A/J male mice were obtained at 4 weeks of age and were housed five per cage in a temperature-controlled room with reverse 12:12-h light:dark cycle. Water was available ad libitum. The animals were fed one of three diets as follows: 10 mice from each strain were placed on a low fat diet (10.5% calories from fat), a high fat diet (58% calories from fat), or a high fat diet containing 0.001% of the β₃AR agonist CL316,243 as described previously (12, 13). The detailed composition of the diets and their caloric content has been described previously (11). Body weight was assessed biweekly. Food intake measurements for individual animals, 10 per diet group, were performed for 24-h periods at weeks 2, 6, and 16 of the diet regimen. More frequent measurements were not performed due to the stress to the animals as a result of individual housing. All procedures were conducted in accordance with principles and guidelines established by the NIH for the care and use of laboratory animals.

RNA Preparation and Analysis—Adipose tissue was collected from the gonadal, subcutaneous, perirenal, and interscapular regions. Total cellular RNA was prepared from freshly isolated adipose tissue by the cesium chloride gradient method as previously detailed (14). For Northern blot hybridization, RNA was denatured by the glyoxal procedure, fractionated through 1.2% agarose gels, and blotted onto Biotrans nylon membranes (ICN, Irvine, CA) as described previously (15). A probe for the murine ob gene sequence was prepared by reverse transcriptase-

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1 The abbreviations used are: β₃AR, β₃-adrenergic receptor; PCR, polymerase chain reaction.

2 T. H. Claus, personal communication.
polymerase chain reaction (PCR). The primers used were 5'-AAAACCTCTCACATACAGACC-3' (sense) and 5'-CAACTGGTTGAAGATTCC-3' (antisense) corresponding to nucleotide 209–226 and 598–580 of the sequence reported by Zhang et al. (1). The reaction conditions were as follows. Epididymal adipose tissue RNA from C57BL/6j mice was incubated in a 20-μl reaction containing 50 μM Tris, 10 μM MgCl₂, and 1 unit of RNase-free DNase (U. S. Biochemical Corp., Cleveland, OH) for 30 min at 37°C. The DNase was denatured by heating to 95°C for 5 min. Two hundred and fifty ng of the RNA were then annealed to the antisense primer in a 20-μl reaction containing Moloney murine leukemia virus reverse transcriptase and 5 μM MgCl₂, and incubated at 42°C for 15 min, followed by 99°C for 5 min to denature the enzyme. PCR was initiated by addition of the sense strand primer, 2.5 units of AmpliTaq polymerase and 10 × PCR buffer (final concentrations: 10 μM Tris, 50 μM KCl, 1.5 μM MgCl₂); 94°C for 30 s, 57.5°C for 30 s and 72°C for 1.5 min; 35 cycles. Amplification reactions without the initial reverse transcriptase step were included as controls. The 390-nucleotide PCR product was purified by agarose gel electrophoresis and subcloned into the plasmid vector T7-Blue (Novagen, Madison, WI). A probe for mouse glyceraldehyde 3-phosphate dehydrogenase was obtained from Clontech (San Diego, CA) and a rat cDNA probe for cyclophilin was obtained from J. Douglas. Radiolabeled probes for hybridization were prepared by nick-translation with [32P]dCTP to a specific activity of between 3 and 7 × 10⁶ dpm/μg of DNA. Blots were hybridized and washed as described previously (16). Quantitation of hybridizing species was by exposure to Molecular Dynamics PhosphorImager screens. Permanent data records were generated by exposing the blots to Kodak X-AR film at −80°C.

RESULTS AND DISCUSSION

As previously reported (1, 17), ob gene expression was elevated in obese, diabetic ob/ob and db/db mice (Fig. 1). In the ob/ob mice, the increase ranged from 10- to 14-fold, while the increase in db/db mice was 4- to 8-fold. These values are somewhat less than reported by other investigators but consistent with their findings. Based upon the early studies by Coleman (2, 3) he proposed that ob encodes a diffusible signaling factor and that the db locus might encode the receptor for ob. Thus, persistently up-regulated expression of ob mRNA in the db/db diabetic mouse would be consistent with this model, in which db would be incapable of receiving the signal from ob. Support for this original hypothesis has recently been obtained from studies in which recombinant OB protein was administered to ob/ob and db/db mice (6–8). In these studies, decreased food intake and body weight were observed in ob/ob and db/db mice, but the OB protein had no effect in db/db mice (7, 8). These latter results support the notion that the db locus is downstream in the pathway from ob. However, we and others have also observed significantly increased expression of the ob gene in other genetic models of obesity that do not possess mutations in the ob locus. For example, the levels of expression and nucleotide sequence of the ob gene from the Zucker “fatty” (fa/fa) rat have been examined (18, 19). While similar increases in ob mRNA are observed in obese fa/fa rats, unlike ob/ob mice the ob gene from the Zucker fa/fa is not mutated. More recently, increased expression of ob in overweight humans without mutations of the ob gene has also been reported (20). We examined ob expression in several white and brown adipose tissues from “tubby” (tub/tub) and “fat” (fat/fat) mutant mice (21), including gonadal, subcutaneous, perirenal, and interscapular. The fat locus has recently been identified as carboxypeptidase E (22), but the nature of the mutation in tubby has not yet been reported. These two animal models develop obesity more gradually with age and do not possess the severe hypercorticoidism that is such a prominent feature in ob/ob or db/db mice (21). As shown in Fig. 2, ob mRNA was significantly increased in adipose tissue from tubby and fat mice as compared with genetically lean (“+”) littersmates, although the degree of overexpression is less dramatic than in ob

![Fig. 1. Overexpression of ob mRNA in genetically obese ob/ob and db/db mice. Northern blots containing 40 μg of total RNA from epidymal white adipose tissue (EWAT), or interscapular brown adipose tissue (IBAT) were prepared as pools from two or three animals as described under “Experimental Procedures” and hybridized successively with 32P-labeled probes for the mouse ob coding region and glyceraldehyde 3-phosphate dehydrogenase (gapdh). The position of the ob or gapdh mRNA is indicated by the arrowhead. A, comparison of genetically lean (+) C57BL/6j mice and obese (ob) mice. In the data shown, the level of ob mRNA is increased 14-fold in the ob/ob mutant (average of two experiments = 12.0 ± 2.1-fold increase). B, comparison of genetically lean (+/+ ) C57BL/KsJ mice and diabetic (db/db) mice. In the data shown, the level of db mRNA is increased 4-fold in EWAT and 8-fold in the IBAT of the db/db mutant. The data shown are representative of two experiments.](http://www.jbc.org/)
the diet (Fig. 3). In fact, they tended to weigh even less than animals consuming the low fat diet.

We next examined the levels of ob mRNA in epididymal, subcutaneous, and perirenal white adipose tissue and interscapular brown adipose tissue from these animals (Fig. 4). Levels of ob mRNA in high fat-fed obese mice increased 1.48 ± 0.07-fold in epididymal, 2.45 ± 0.32-fold in subcutaneous, and 1.78 ± 0.13-fold in perirenal depots compared to low fat-fed control animals. In interscapular brown adipose tissue from high fat-fed animals some samples showed a modest elevation, but overall there was no significant increase (1.07 ± 0.19-fold) compared with low fat-fed mice. Most likely the levels of ob expression observed in this brown adipose depot are derived from small amounts of white adipocytes present in the tissue that we were unable to separate by visual inspection alone. By contrast to these increases seen in adipose tissue from the high fat-fed mice, these same adipose tissue regions from mice consuming the high fat diet supplemented with the β3AR agonist expressed levels of ob mRNA that were equal to, or slightly less than, that observed in animals fed the low fat diet (0.93 ± 0.09-fold). Thus, these data suggest that we were able to manipulate the expression of the ob gene by pharmacologically blocking the development of diet-induced obesity, despite the consumption of a calorically rich diet.

In our studies as well as those of other investigators, the fold increases in ob mRNA observed in either diet-induced obese A/J mice, or the severely obese ob/ob or db/db, are consistently greater than would be predicted based upon increase in total body weight alone. For example, we observe a ∼25% increase in body weight of A/J mice consuming a high fat diet, but there is a 50–250% increase in ob mRNA levels. Similarly, ob/ob mice generally display a 75–100% increase in body weight (4), but a 10–20-fold increase in ob mRNA levels (1, 17) (Fig. 1). When we examined fat pad weights in A/J mice raised on these three diets, the average increase over all depots in high fat fed animals increased by 2.30 ± 0.30 (n = 3), while in the presence of the β3AR agonist, average fat pad weights were essentially identical to low fat-fed animals (fold change = 1.03 ± 0.13; n = 3). These values are very similar to the changes observed in ob
mRNA levels in this study. In a previous study we have shown that high fat feeding in A/J mice causes adipocyte hypertrophy, but not hyperplasia (11). Therefore, the differences in weight in A/J mice raised on high fat diets with and without the β3AR agonist CL316,243 are most likely due to differences in adipocyte size between the groups, but not in number. Thus, the difference in ob mRNA in these mice, compared to those raised on fat alone, suggests that OB is a "sensor" of fat cell size presumably sending a signal, either autocrine, paracrine, or endocrine to elicit some metabolic response; possibly to control energy partitioning and utilization. Interestingly, A/J animals consuming the high fat diet supplemented with the β3AR agonist, CL316,243, also display increases in brown adipose tissue content and βAR gene expression.4

Recent research has emphasized the role of leptin in satiety (6–8). In these studies decreased food intake and body weight were observed in ob/ob mice, but the OB protein had no effect in db/db mice. Campfield et al. (8) also found that administration of recombinant OB protein to diet-induced obese AKR mice causes reductions in food intake and body weight, although to a more modest degree than in ob/ob mice. To understand the relationship between food intake and ob gene expression in our dietary model and the effects of a β3AR agonist, we examined food intake in individual animals from all three diet groups at three times during the study period (Fig. 5). These data show that the caloric intake of animals on either of the high fat diets was equivalent and greater than that consumed by animals fed the low fat diet. However, despite this greater caloric density of the high fat diet, animals supplemented with CL316,243 actually weighed less than low fat fed animals. Therefore, our experiments appear to highlight a disassociation between ob gene expression and caloric consumption. In summary, we have shown that A/J mice consuming a high fat diet produce more leptin and become moderately obese, while mice consuming the high fat diet supplemented with CL316,243 do not become obese or show increases in ob gene expression, despite the fact that they consumed as much, or more, food than animals on high fat alone. It is possible that treatment with CL316,243 may serve to mimic effects of OB protein involved in enhancing metabolic and/or thermogenic activity, as recently speculated (28). Therefore, food intake in animals fed high fat diets with and without the β3AR agonist was similar, these results indicate that there is no apparent obligatory effect of OB protein on satiety.

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