Vol. 216, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 2, 1995 Pages 355-358

AUGMENTED EXPRESSION OF THE *OBESE* GENE IN THE ADIPOSE TISSUE FROM RATS FED HIGH-FAT DIET

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Received September 28, 1995

Summary: Expression of the *obese* (*ob*) gene is augmented in the adipose tissue in several rodent models of genetic obesity. In the present study, we examined the *ob* gene expression in a rodent model of acquired obesity obtained by pure overfeeding of normal rats. Male Sprague-Dawley rats at 8 weeks of age were fed standard diet or high-fat diet. Rats fed high-fat diet developed moderate degree of obesity, hyperglycemia, and hyperlipidemia as compared with rats fed standard diet. Northern blot analysis revealed that the *ob* gene is expressed abundantly in the adipose tissue obtained from the epididymal, mesenteric, subcutaneous, retroperitoneal, and interscapular fat pads in rats fed standard diet. Expression of the *ob* gene was augmented in all the adipose tissue examined in rats fed high-fat diet. The present study demonstrates that the *ob* gene expression is augmented in the adipose tissue in diet-induced obesity, thereby suggesting the pathophysiologic roles of the *ob* gene in acquired obesity.
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Obesity, characterized by increased mass of adipose tissue, arises from complex interactions between environmental and genetic factors (1). The molecular pathogenesis of obesity is, however, largely unknown. The crucial discovery of the *obese* (*ob*) gene by Friedman and colleagues has provided the key to understand the molecular mechanisms underlying obesity (2). The *ob* gene encodes a 166-/167-amino-acid polypeptide with a putative signal sequence, and expression of the *ob* gene occurs abundantly and specifically in the adipose tissue in mice (2). We and others have also isolated rat and human *ob* complementary DNAs (cDNAs) and the human *ob* gene (3-8), and revealed the regional differences in the *ob* gene expression in the adipose tissue in rats and humans (3,4,9).

The *ob* gene expression is markedly up-regulated in the adipose tissue in several rodent models of genetic obesity; C57BL/6J *ob/ob* and C57BL/KsJ *db/db* mice (2,10), and Zucker fatty (*fa/fa*) and Wistar fatty (*fa/fa*) rats (3,6,9). Furthermore, the augmentation of the *ob* gene expression is also region-specific in the adipose tissue from Zucker fatty (*fa/fa*) and Wistar fatty (*fa/fa*) rats (3,9). These observations suggest the pathophysiologic significance of the *ob* gene in genetic obesity. Indeed,

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0006-291X/95 $12.00
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nonsense mutation in the *ob* gene was regarded as the obesity-causing mutation in C57BL/6J *ob/ob* mice (2). However, the regulation of the *ob* gene expression in acquired obesity is poorly understood. In this context, in the present study, we examined the *ob* gene expression in a rodent model of acquired obesity obtained by pure overfeeding of normal rats.

MATERIALS AND METHODS

Animals: Eight-week-old male Sprague-Dawley rats were used in the present study. Rats were housed in a temperature-, humidity-, and light-controled room with free access to water and standard rat chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan; 50 kcal/day). These animals were fed standard diet (50 kcal/day) (n=5), or high-fat diet (80 kcal/day) (n=5) for 2 weeks as described (11). A 0.2-ml blood was sampled from the tail vein, and the plasma glucose, total cholesterol, triglyceride, and insulin levels were measured.

Tissue preparation: After rats were anesthetized with ether inhalation, the adipose tissue was immediately removed from rats as described (3,9). The white adipose tissue (WAT) was obtained from the epididymal, mesenteric, subcutaneous, and retroperitoneal fat pads, while the brown adipose tissue (BAT) was from the interscapular fat pad. Tissue samples were frozen in liquid nitrogen and stored at -70 °C until use.

Total RNA extraction and Northern blot analysis: Total RNA was extracted as previously described (3,9). Northern blot analysis was performed (12) using the ^{32}P-labeled rat *ob* cDNA fragment as a probe (3). A human β-actin genomic probe (Wako Pure Chemical Inc., Osaka, Japan) was used to monitor the amount of total RNA in each sample. The *ob* mRNA levels (arbitrary units) were expressed relative to those in the epididymal WAT from control rats fed standard diet (The mRNA levels in 10 μg of total RNA from the epididymal WAT are defined as 100 units).

Statistical analysis: All data were expressed as the mean ± SD. The statistical significance of differences in mean values was assessed by unpaired Student's *t* test.

RESULTS

Profiles of rats fed standard diet and high-fat diet

Table 1 summarizes the brief profile of rats fed standard diet and those fed high-fat diet. Rats fed high-fat diet weighed 7 % more than control rats fed standard diet, and developed moderate hyperglycemia and hyperinsulinemia. The weight of the epididymal, mesenteric, and subcutaneous

Table 1. Profiles of rats fed standard diet and those fed high-fat diet

	S	H	
Body weight (g)	297.7 ± 8.2	321.8 ± 6.9	**
Fat weight (g)			
Epididymal	2.7 ± 0.4	4.7 ± 0.7	*
Mesenteric	1.8 ± 0.2	3.8 ± 0.5	*
Subcutaneous	1.6 ± 0.3	2.8 ± 0.4	**
Glucose (mg/dl)	94 ± 14	115 ± 10	**
Total cholesterol (mg/dl)	39 ± 4	41 ± 4	
Triglyceride (mg/dl)	70 ± 13	63 ± 11	
Insulin (μU/ml)	14.7 ± 2.3	32.7 ± 6.1	**

Values are mean ± SD (n=3), S; rats fed standard diet, H; rats fed high-fat diet.
**P<0.05; *P<0.01 vs. rats fed standard diet.

inguinal fat pads from rats fed high-fat diet increased significantly as compared with those from rats fed standard diet (1.7-fold, 2.1-fold, and 1.8-fold, respectively).

The *ob* gene expression in rats fed high-fat diet

Northern blot analysis using the rat *ob* cDNA probe identified a single mRNA species of 4.5 kilobase in size in the adipose tissue obtained from the epididymal, mesenteric, subcutaneous, retroperitoneal, and interscapular fat pads in 8-week-old Sprague-Dawley rats fed standard diet (Figure 1A). The rank order of the *ob* mRNA level in the adipose tissue was epididymal and retroperitoneal WAT > mesenteric and subcutaneous WAT > interscapular BAT. These results were consistent with our previous reports using 12-week-old Sprague-Dawley and Wistar rats (3,9). The *ob* gene expression was augmented in all the adipose tissue examined in rats fed high-fat diet. In rats fed high-fat diet, the *ob* mRNA levels in the epididymal, mesenteric, subcutaneous, and retroperitoneal WAT and in the interscapular BAT were 2.2-fold, 3.2-fold, 2.0-fold, 2.1-fold, and 3.0-fold higher than those in rats fed standard diet, respectively (Figure 1B). The rank order of the *ob* mRNA level in the adipose tissue was, therefore, epididymal, mesenteric, and retroperitoneal WAT > subcutaneous WAT and interscapular BAT in rats fed high-fat diet.

DISCUSSION

The present study demonstrates that adipose tissue expression of the *ob* gene is augmented during overfeeding in normal rats. Furthermore, we have recently observed the down-regulation of the *ob* gene expression during fasting in normal mice, when the body weight is reduced (Ogawa et al., manuscript in preparation). These findings suggest that adipose tissue expression of the *ob* gene is up- or down-regulated, depending upon the peripheral nutritional status. Considering that the weight of fat pads examined is significantly increased in rats fed high-fat diet as compared with those fed standard diet, it is tempting to speculate that adipose tissue expression of the *ob* gene reflects the adipose tissue stores. Further studies are required to elucidate the physiologic significance of the up- or down-regulation of the *ob* gene expression during feeding or fasting.

The present sutdy provides the direct evidence that the *ob* gene expression is up-regulated in the adipose tissue without genetic alterations. These findings are consistent with the previous report that the

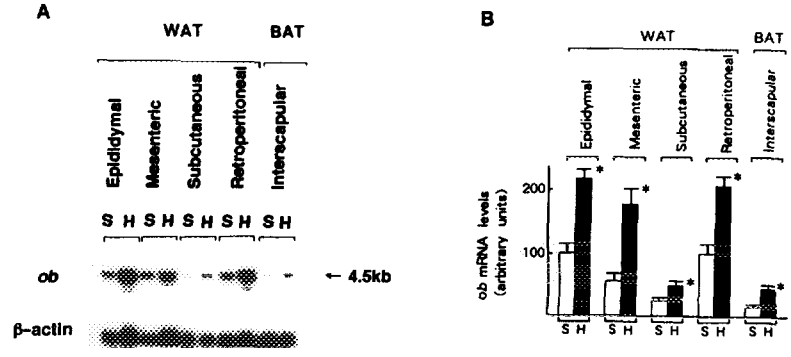

Figure 1. (A) Northern blot analysis of rat *ob* mRNA in rats fed standard diet (S) and those fed high-fat diet (H). Total RNA (10 μg/lane) was analyzed. (B) The rat *ob* mRNA levels in the adipose tissue from rats fed standard diet (S) and those fed high-fat diet (H). *P<0.05 vs. rats fed standard diet. *ob*, *obese*; kb, kilobase; WAT; white adipose tissue; BAT; brown adipose tissue.

ob gene expression is increased in human obesity despite the absence of the *ob* mutation (5). It has been demonstrated that adipose tissue expression of the *ob* gene is augmented in several rodent models of genetic obesity (2,3,6,9,10). The molecular mechanisms by which adipose tissue expression of the *ob* gene is augmented are unclear at present. Nonsense mutation in the *ob* gene apparently encodes an inactive ob protein in C57BL/6J *ob/ob* mice (2). The defect in the putative ob protein receptor has been suggested in C57BL/KsJ *db/db* mice (13), Zucker fatty (*fa/fa*) and Wistar fatty (*fa/fa*) rats (14). Augmented expression of the *ob* gene in these genetically obese animals might be due to the disregulation of the negative feedback loop formed by the ob protein and its receptor. Since, in the present study, expression of the *ob* gene can be increased during the process of the body weight increase, the augmentation of the *ob* gene expression observed in C57BL/6J *ob/ob* and C57BL/KsJ *db/db* mice, and Zucker fatty (*fa/fa*) and Wistar fatty (*fa/fa*) rats is due at least in part to the marked obese phenotype *per se*.

In conclusion, the present study demonstrates for the first time that the *ob* gene expression is augmented in the adipose tissue in a rodent model of obesity obtained by pure overfeeding of normal rats, thereby suggesting the pathophysiologic roles of the *ob* gene in acquired obesity.

ACKNOWLEDGMENTS

We thank Ms. M. Kawakatsu and Ms. C. Kawahara for their secretarial assistance. This work was supported in part by research grants from the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare, Yamanouchi Foundation for Research on Metabolic Disorders, and Japan Diabetes Foundation.

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