Augmented expression of *obese* (*ob*) gene during the process of obesity in genetically obese-hyperglycemic Wistar fatty *(falfa)* rats

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Abstract **Expression of the *obese* (*ob*) gene is up-regulated in the adipose tissue in several obese rodent models. To study the regulation of the *ob* gene expression during the development of obesity, we examined the *ob* gene expression in genetically obese-hyperglycemic Wistar fatty *(falfa)* rats at several stages of obesity. The *ob* mRNA levels in the adipose tissue from Wistar fatty rats was unequivocally augmented and continued to rise in the process of obesity. Furthermore, the *ob* gene expression in this obese model was much more rapidly enhanced in the mesenteric fat than in the subcutaneous fat. Moreover, the *ob* gene expression was more greatly augmented in the mesenteric fat than the lipoprotein lipase gene expression. These results suggest the presence of obesity-linked and region-specific regulation of the *ob* gene expression.**

Key words: obese (*ob*) gene; Obese rodent models; Obesity-linked regulation; Wistar fatty *(falfa)* rat

1. Introduction

Obesity is accompanied by complications such as non-insulin-dependent diabetes mellitus (NIDDM), hyperlipidemia and hypertension [1]. Although the molecular mechanisms for obesity has not been fully elucidated so far, the crucial discovery of the *obese* (*ob*) gene, the mutation of which results in severe obesity in C57BL/6J *ob/ob* mice [2], may provide the key to clarify the mechanisms underlying obesity. We have isolated rat and human *ob* complementary DNAs (cDNAs) and human *ob* gene [3–5] and demonstrated that the *ob* gene is expressed in the adipose tissue in a region-specific manner [3,4,6]. We also showed that the *ob* gene expression is markedly enhanced in the adipose tissue in several obese rodent models such as C57BL/6J *ob/ob* (Shigemoto et al., manuscript in preparation) and C57 BL/Ks *db/db* mice (Ogawa et al., manuscript in preparation), Wistar fatty [6] and Zucker fatty rats [3], and rats fed high-fat diet [7]. However, the regulation of the *ob* gene expression in the development of obesity is poorly understood. In the present study, we examined the alteration of the *ob* gene expression at several stages of obesity in genetically obese-hyperglycemic Wistar fatty (*falfa*) rats. Furthermore, since the lipoprotein lipase (LPL) plays a crucial role in energy storage and utilization in the adipose tissue [8,9], we compared the change of the *ob* gene expression with that of the LPL gene in the development of obesity.

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2. Materials and methods

2.1. Animals and tissue preparation

Four, 12- and 30-week-old male Wistar fatty *(falfa)* rats and their lean littermates *(Fal?)* were used in the present study. Blood was sampled from the tail vein after overnight fasting, and plasma glucose, total cholesterol, triglyceride and insulin levels were measured as previously reported [10]. After rats were anesthetized with ether inhalation, adipose tissue was quickly removed, frozen in liquid N_2, and stored at −80°C until use. White adipose tissue (WAT) was taken from epididymal, mesenteric, subcutaneous abdominal, retroperitoneal fat pads, while brown adipose tissue (BAT) was obtained from the interscapular fat pad.

2.2. Total RNA extraction and Northern blot analysis

Total RNA was extracted as previously described [11]. Northern blot analysis was performed [12] using the ^{32}P-labeled rat *ob* [3] and human LPL [8,13] cDNA fragments as probes.

Autoradiograms were quantitated by densitometric scanning and values were normalized to the β-actin mRNA level to correct for differences in the amount of RNA applied. The *ob* mRNA levels were expressed relative to those in the epididymal WAT from Wistar lean rats. (The mRNA levels in 10 μg of total RNA from the epididymal WAT from Wistar lean rats are defined as 100 unit.)

2.3. Statistical analysis

Data were expressed as the mean ± S.D. (Table 1) and the mean ± S.E.M. (Figs. 1 and 2). The statistical significance of differences between groups was assessed by Student's unpaired *t*-test.

3. Results

3.1. Profiles of 4-, 12-, and 30-week-old male Wistar fatty rats in comparison with their lean littermates

Table 1 summarizes brief profiles of Wistar fatty rats and their lean littermates used in this study. Four-week-old Wistar fatty rats were normoglycemic but have already shown significant body weight gain, mild hyperlipidemia and considerable hyperinsulinemia. By 12 weeks of age, Wistar fatty rats developed marked obesity with hyperglycemia, hypertriglyceridemia and hyperinsulinemia as described [6,10]. These values further increased at the age of 30 weeks.

3.2. Augmented expression of the ob gene in Wistar fatty rats

To elucidate the physiologic and pathophysiologic roles of the *ob* gene, we analyzed the gene expression in the adipose tissue from Wistar fatty rats and their lean littermates at the age

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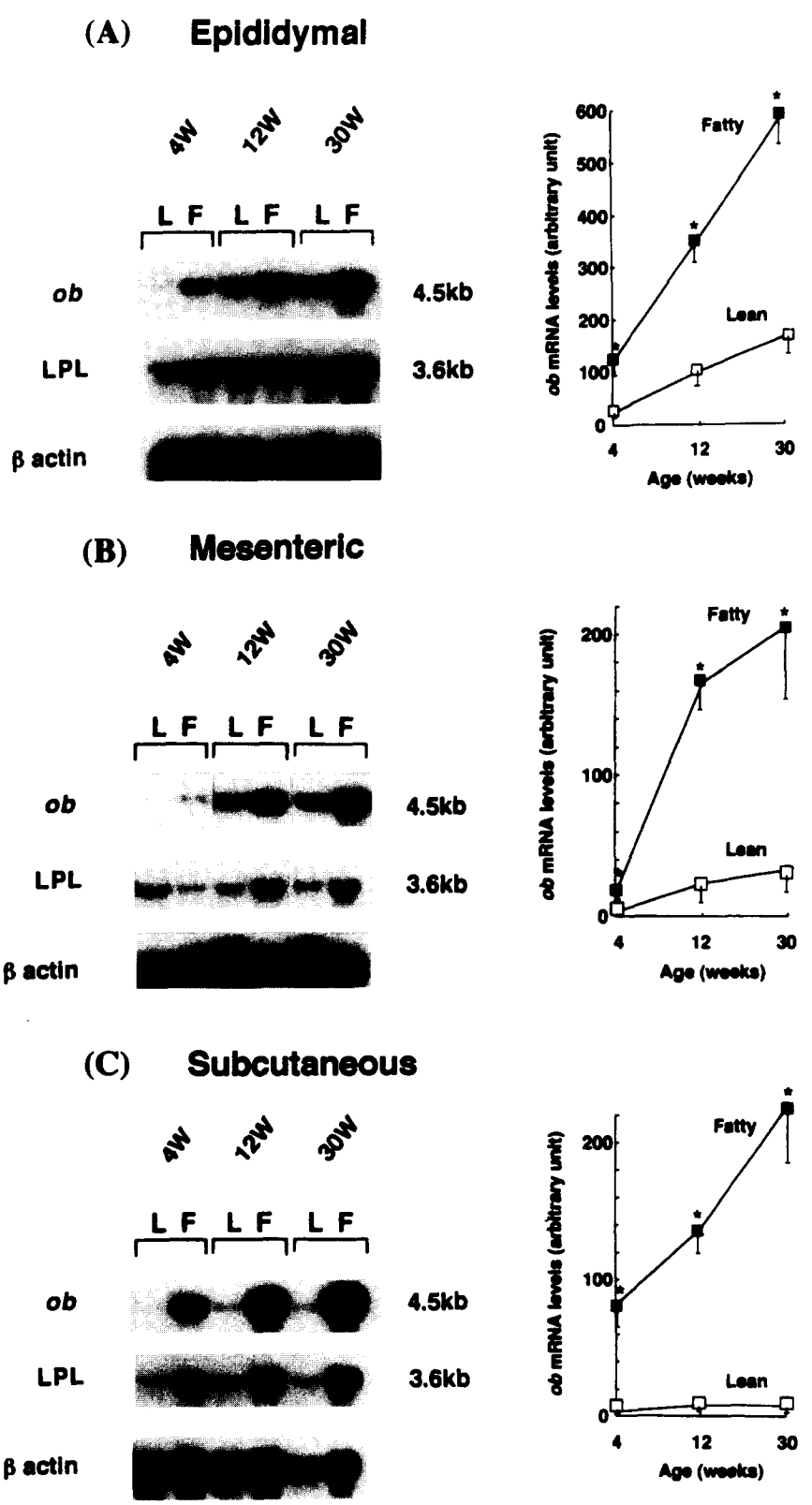

Fig. 1. The *ob* mRNA levels in Wistar fatty rats in comparison with their lean littermates at the age of 4, 12 and 30 weeks. The mRNA levels in Wistar fatty rats and their lean littermates are indicated by closed and open squares, respectively. Values are mean ± S.E.M. ($n = 5$), $^*P < 0.001$ vs. the Wistar lean rats. (A) Epididymal WAT. (B) Mesenteric WAT. (C) Subcutaneous WAT. (D) Retroperitoneal WAT. (E) Interscapular BAT. A representative Northern blot analysis of the *ob* and LPL mRNA is shown in the left panel. F = Wistar fatty rats; L = Wistar lean rats.

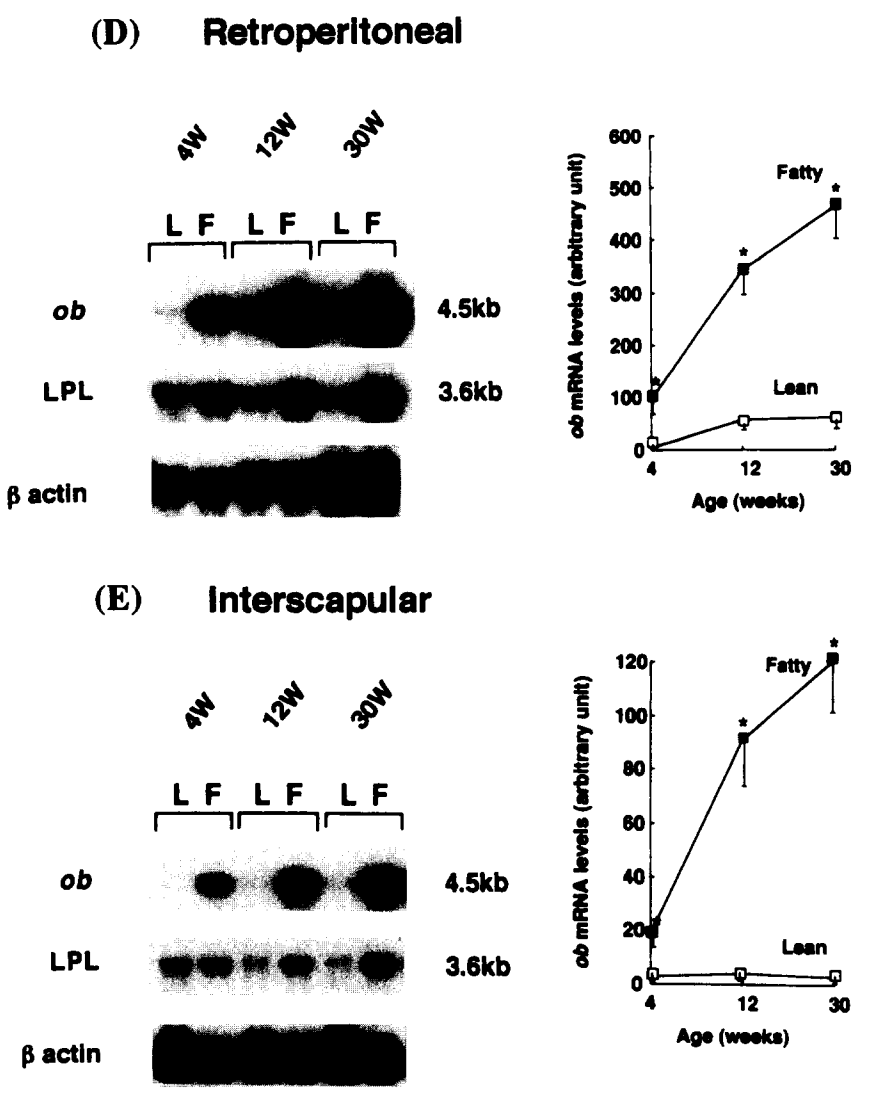

Fig. 1. (*Continued*).

of 4, 12 and 30 weeks. Expression of the *ob* gene was markedly augmented in all the adipose tissue from Wistar fatty rats as compared with their lean littermates at all the ages examined as shown in Fig. 1. Furthermore, there were regional differences in the *ob* gene expression levels in the adipose tissue from Wistar fatty rats. The rank order of the *ob* mRNA level in the adipose tissue was epididymal, retroperitoneal WAT > mesenteric and subcutaneous WAT > interscapular BAT at the stage of established obesity (12th and 30th week).

3.3. Continued rise of the ob gene expression with the advance of obesity in Wistar fatty rats

The *ob* mRNA levels in all the adipose tissue from Wistar fatty rats continued to rise markedly in the process of obesity, while the levels of the *ob* mRNA unaltered or showed a slight increase in their lean littermates (Fig. 1). The rank order of the fold-increase in the *ob* mRNA levels from the 4th week to the 30th week in Wistar fatty rats was mesenteric WAT (12.1) > interscapular BAT (6.4) > epididymal WAT (5.0) > retroperitoneal WAT (4.8) > subcutaneous WAT (2.8). Since the *ob* gene expression was augmented with maximum fold-increase in the mesenteric WAT and with minimum fold-increase in the subcutaneous WAT, we focused on the two regions of adipose tissue (Fig. 2A). In the mesenteric WAT, the *ob* mRNA level increased with 9.8-fold from the 4th week to the 12th week, and 1.2-fold from the 12th week to the 30th week. On the other hand, in the subcutaneous WAT, the *ob* mRNA level increased with 1.7-fold and 1.6-fold, respectively.

3.4. Comparison of the change between the ob and LPL mRNA levels in the development of obesity

Since the LPL gene expression in the adipose tissue has been reported to be enhanced in obesity [9,14], we also examined the LPL gene expression in the adipose tissue from Wistar fatty rats and their lean littermates at the age of 4, 12 and 30 weeks, and compared the alteration of the LPL gene expression with that of the *ob* gene expression in the development of obesity. The LPL gene expression was also augmented in all the adipose tissue examined in Wistar fatty rats at the age of 4, 12 and 30 weeks as compared with their lean littermates. In Wistar fatty rats, the LPL gene expression mildly increased in all the adipose tissue examined during the process of obesity (Figs. 1 and 2).

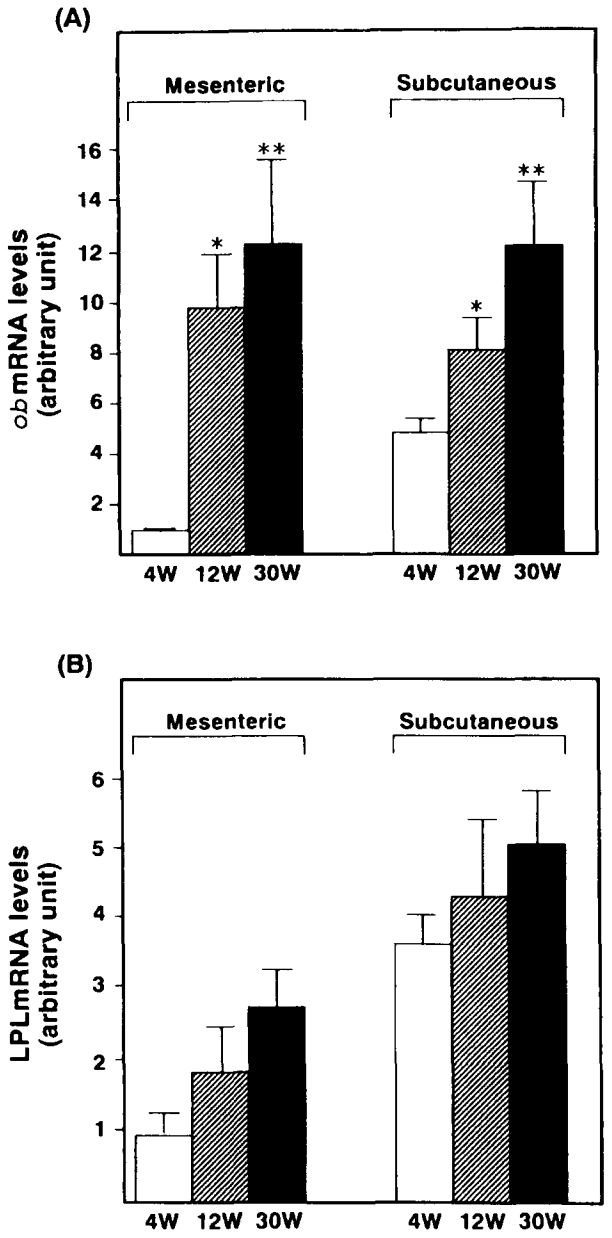

Fig. 2. (A) The *ob* mRNA levels in the mesenteric and subcutaneous WAT from Wistar fatty rats at the age of 4, 12 and 30 weeks. The mRNA levels in Wistar fatty rats at the 4th, 12th, and 30th weeks are indicated by open, hatched, and closed boxes, respectively. Values are mean ± SEM of the densitometric measurement of five experiments. $*P < 0.01$ vs. rats at the 4th week, $**P < 0.05$ vs. rats at the 12th week. (B) The LPL mRNA levels in the mesenteric and subcutaneous WAT from Wistar fatty rats at the age of 4, 12 and 30 weeks.

As we demonstrated the regional differences in the fold-increase of the *ob* gene expression during the course of obesity (maximum fold-increase in the mesenteric WAT and minimum fold-increase in the subcutaneous WAT), we compared the augmentation of the LPL mRNA levels with those of the *ob* mRNA in the mesenteric and subcutaneous WAT. The fold increase in the LPL mRNA levels from the 4th week to the 30th week was 2.8 in the mesenteric WAT, while that in the *ob* mRNA levels was 12.1. On the other hand, the LPL mRNA increased 1.4-fold in the subcutaneous WAT from the 4th week to the 30th week, while the *ob* mRNA levels were augmented 2.8-fold (Fig. 2).

4. Discussion

The present study demonstrates that the *ob* mRNA level in the adipose tissue from Wistar fatty rats continues to rise markedly in the process of obesity, while the level of the *ob* mRNA remains unchanged or shows a slight increase in their lean littermates. Since body weight increase greatly in Wistar fatty rats in the development of obesity, the *ob* gene expression may be regulated by obesity-linked mechanisms such as adipose cell size or content of intracellular triglyceride. Alternatively, humoral factors such as insulin, which also increase greatly during the course of obesity, may be responsible for the augmented *ob* gene expression. Further studies are required for the elucidation of the augmentation of the *ob* gene expression during the development of obesity.

We also demonstrate the regional specificity in the fold-increase of the *ob* gene expression in Wistar fatty rats (Fig. 2). Although the *ob* mRNA levels in the mesenteric fat were the same as those in the subcutaneous fat at the 30th week, the fold-increase of the *ob* mRNA levels from the 4th week to the 12th week in the mesenteric WAT was 9.8, while that in the subcutaneous WAT was 1.7. Funahashi et al. [15] have recently shown that the *ob* gene expression was induced with more rapid rate in the mesenteric WAT than in the subcutaneous WAT in ventromedial hypothalamus (VMH)-lesioned rats. Together with their results, the greater fold-increase of the *ob* mRNA levels in the mesenteric WAT in Wistar fatty rats suggests that the *ob* gene expression is more rapidly enhanced in the mesenteric WAT than in the subcutaneous WAT in the process of obesity. Therefore, the *ob* gene expression may be regulated partly by region-specific mechanism.

In the state of obesity, elevated adipose-tissue LPL synthesis has been reported [9,14]. LPL is a rate-limiting enzyme in the uptake of free fatty acid into the adipose tissue and plays important roles in certain metabolic disorders including obesity

Table 1
Profiles of 4-, 12-, and 30-week-old male Wistar fatty rats and their lean littermates

	Lean	Fatty
(A) *4 weeks*		
Body weight (g)	101.7 ± 9.1	131.6 ± 11.4*
Glucose (mg/dl)	145 ± 4	148 ± 12
Triglyceride (mg/dl)	89 ± 7	104 ± 6*
Total cholesterol (mg/dl)	99 ± 7	128 ± 17*
Insulin (μU/ml)	18.2 ± 7.0	153.3 ± 41.4*
(B) *12 weeks*		
Body weight (g)	384.9 ± 0.9	489.9 ± 18.0*
Glucose (mg/dl)	127 ± 4	381 ± 24*
Triglyceride (mg/dl)	83 ± 3	355 ± 48*
Total cholesterol (mg/dl)	107 ± 12	126 ± 8
Insulin (μU/ml)	56.6 ± 13.0	853.7 ± 73.5*
(C) *30 weeks*		
Body weight (g)	452.9 ± 20.0	653.8 ± 62.8*
Glucose (mg/dl)	120 ± 9	379 ± 28*
Triglyceride (mg/dl)	85 ± 9	380 ± 144*
Total cholesterol (mg/dl)	113 ± 11	126 ± 8
Insulin (μU/ml)	62.3 ± 11.1	1083.3 ± 168.7*

Values are maen ± S.D. ($n = 5$), $*P < 0.01$ vs. the len littermates.

[9,13,14]. Therefore, we compared the alteration of the LPL gene expression with that of the *ob* gene expression in the process of obesity. The fold-increase of the *ob* mRNA level was much greater in the mesenteric WAT from the 4th week to the 12th week than that of the LPL mRNA level (9.8 vs. 1.2), while the *ob* gene expression was induced in the subcutaneous WAT as much as the LPL gene expression (1.7 vs. 1.2). The regulation of the *ob* gene expression in the mesenteric fat in obesity might be different from that of the LPL gene expression.

In conclusion, the present study demonstrates that the *ob* gene expression in the adipose tissue from Wistar fatty rats is enhanced and continues to rise during the process of obesity. The current study also demonstrates the regional specificity in the enhancement of the *ob* gene expression. The *ob* gene expression is more greatly enhanced in the mesenteric WAT than in the subcutaneous WAT with the advance of obesity. Further studies investigating the mechanisms responsible for the 'region-specific' regulation of the *ob* gene expression in the adipose tissue may provide fresh insight into the pathophysiology of obesity.

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