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Increase of Mouse Leptin Production by Adipose Tissue after Midpregnancy: Gestational Profile of Serum Leptin Concentration

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The serum concentration of leptin in 10 week old virgin ICR mice assessed by RIA was 1.70 ± 0.08 ng/ml. The serum leptin concentration in the pregnant mice mated at 10 weeks of age significantly increased from day 11 of pregnancy and reached a peak on day 17 of pregnancy (42.2 ± 4.8 ng/ml). After the delivery, the serum leptin concentration rapidly decreased and reached the level of the virgin mouse on the seventh day in the puerperium. Tissue contents of leptin in the placenta, the decidua, the uterus, and the adipose tissue were between 40 to 130 ng/g wet tissue. However, leptin mRNA was expressed only in the adipose tissue and the level of leptin mRNA on days 13 and 17 of pregnancy increased 3- to 5-fold compared with that of virgin mouse. Tissue content of leptin in the adipose tissue significantly increased from day 17 of pregnancy compared with that of the virgin mouse. The m-leptin secretion from the adipose tissue also significantly increased *in vitro*. These results suggest that leptin, which was secreted by adipose tissue, may play important roles in mouse reproduction after midpregnancy.

Leptin, a product of the *ob* gene (1), is a 16-kDa protein and is mainly produced by adipocytes (2). Human placenta also expresses leptin mRNA and produces the leptin protein (3,4). Serum h-leptin concentration increases about 2-3 fold during pregnancy (5). These findings suggest that the source of the increased serum h-leptin during pregnancy is the placenta and that the h-leptin may play important roles in human reproduction.

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Abbreviations: h-leptin, human leptin; m-leptin, mouse leptin.

In rodents, several factors, such as food intake, insulin, and glucocorticoid, are known to regulate serum leptin concentration and to alter leptin expression in the adipose tissue (6-9). But gestational profile of serum m-leptin concentration has not been reported yet. We here demonstrate that the serum m-leptin concentration dramatically increases after midpregnancy and show that adipose tissue, but not placenta, is a possible source of elevated serum m-leptin during gestation.

MATERIALS AND METHODS

Reagents. A RIA kit for mouse leptin was purchased from Linco Research (St. Louis, MO). A cDNA for human ribosomal protein L7/pHE-7 (L7) was generously provided by Dr. J. Campisi (Lawrence Berkeley Laboratories, Berkeley, CA) and leptin cDNA was described previously (10).

Organ culture. Virgin and time-pregnant ICR mice were purchased from Japan SLC Inc. (Hamamatsu City, Shizuoka, Japan). The parametrial adipose tissue was collected from virgin and day 17 pregnant mice. The tissue was cultured in five volumes (wt/vol) of a culture medium (NCTC-109 with 20 mM Hepes, 50 μg gentamicin sulfate/ml) containing 10% fetal calf serum (FCS). The tissues were incubated for 2 h under an atmosphere of 95% air/5% CO_2 at 37 C. The medium was collected and stored at −20 C until assayed.

Tissue extraction. The tissue was homogenized in five volumes (wt/vol) of extraction buffer (100 mM NH_4HCO_3, 10 mM EDTA, 10 mM EGTA, pH 9.3) and centrifuged at 15,000 × g for 15 min. The supernatant was stored at −20 C until assayed.

Leptin RIA. Leptin concentration was determined by RIA using the procedure as described by the manufacturer. The intra- and inter-assay coefficients of variation were 6.8 and 9.2%, respectively.

Northern blot analysis. Total RNAs were isolated from tissues and Northern blot analysis was performed as described previously (10). The RNAs were hybridized with ^{32}P-labeled rat leptin cDNA followed by re-hybridization with ^{32}P-labeled L7 cDNA. Autoradiographs were scanned and band intensities were determined by a densitometer (Imaging Research Inc. St. Catharines, Ontario, Can-

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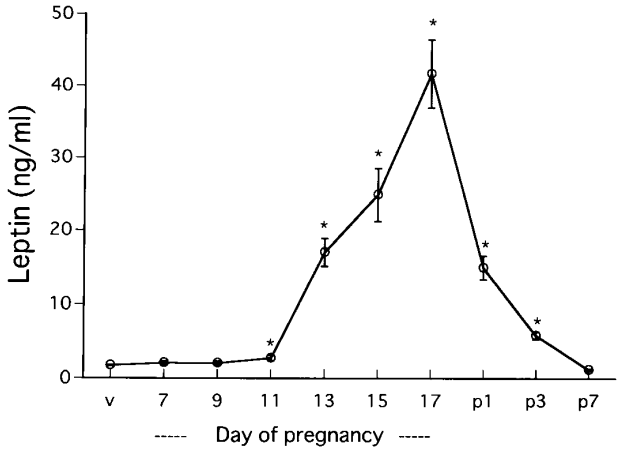

FIG. 1. Serum m-leptin concentration of virgin (v), pregnant, and puerperal (p) mice. Bloods ($n = 8$) were collected from the heart and the serum m-leptin concentration was assessed by the RIA as described in Materials and Method. *, $P < 0.05$ vs virgin

ada). The leptin mRNA band intensity of each lane was normalized by that of the L7.

Statistical analysis. Data were analyzed for homogeneity of variance with Bartlett's test. The data were analyzed by analysis of variance for completely randomized design. Subsequent analysis was carried out with Scheffe's multiple range test, Dunnett's test, and unpaired *t*-test as required. A *P* value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

To determine whether serum m-leptin level changes during pregnancy, blood was collected from virgin, pregnant, and puerperal age-matched mice and serum leptin concentrations were assessed by RIA. Serum leptin concentration of virgin mice was 1.70 ± 0.08 ng/ml ($n = 8$) and the value was not significantly different from that of pregnant mouse on days 7 and 9 of pregnancy. Serum m-leptin concentration increased from day 11 through 17 of pregnancy and the values were significantly higher than that of virgin. Serum m-leptin concentration on day 17 of pregnancy was approximately 25 times higher than that of virgin mouse. After the delivery, serum m-leptin concentration rapidly decreased and reached to the level of the virgin mouse on seventh day of puerperium (Fig. 1). These findings suggested the presence of a specific source for the increased serum m-leptin during gestation. The placenta was the most possible source of serum m-leptin during pregnancy, since human placenta produces leptin and serum h-leptin increases during pregnancy (3,4).

To find out the source of the serum m-leptin during gestation, tissue contents of m-leptin in the placenta, the decidua, the uterus and the parametrial adipose tissue were assessed by RIA. As shown in Table 1, m-leptin concentrations in the adipose tissue from mice of day 17 of pregnancy were about 5 times higher than

TABLE 1

Tissue Content of m-Leptin in the Placenta, Decidua, Uterus, and Adipose Tissue

Tissue	Leptin concentration (ng/g wet tissue)	
	Virgin	Day 17
Placenta	—	46.3 ± 4.0
Decidua	—	94.9 ± 8.1
Uterus	2.30 ± 0.54	92.5 ± 9.8*
Adipose tissue	23.1 ± 3.4	127.4 ± 15.5*

Note. Tissues ($n = 6$) from virgin and day 17 of pregnant mice were homogenized in extraction buffer and leptin concentration was determined by RIA * indicates a significant difference between virgin and day 17 of pregnancy ($p < 0.05$).

that of virgin mouse. Leptin concentration in the uterus of virgin mouse was very low. Leptin concentration in the uterus, the placenta, and the decidua were high on day 17 of pregnancy. These findings suggested that mouse placenta or decidua may produce leptin. To confirm whether these tissues express m-leptin mRNA, Northern blot analysis for m-leptin was performed as described in Materials and Methods. However, mouse placenta and decidua did not express leptin mRNA, although adipose tissue on day 17 of pregnancy expressed leptin mRNA (Fig. 2). Northern blot analysis using poly(A)$^+$RNA from the placenta, decidua, and uterus also did not detect expression of m-leptin mRNA (data not shown), suggesting that adipose tissue is the main source of serum leptin during pregnancy. Presence of high leptin immunoreactivity in the placenta and decidua may suggests that target organs of increased serum leptin are the placenta and decidua during gestation.

To determine whether the amount of m-leptin mRNA in the adipose tissue increases after midpregnancy, Northern blot analysis for m-leptin was performed. As

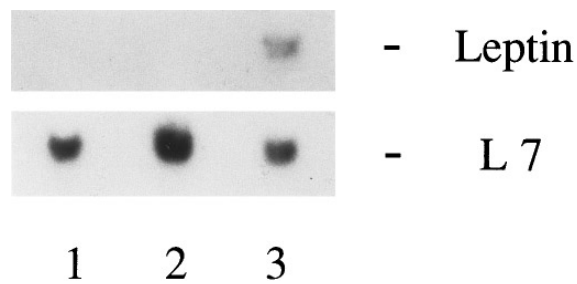

FIG. 2. Expression of m-leptin mRNA in the placenta, decidua, and adipose tissue. Twenty μg of total RNAs from placenta, decidua, and adipose tissue on day 17 of pregnancy were subjected to a Northern blot analysis. Upper rows show the blots hybridized with ^{32}P-labeled leptin probe, and the lower rows show the same blots rehybridized with the ^{32}P-labeled L7 probe. Lane 1: placenta, Lane 2: decidua, Lane 3: adipose tissue

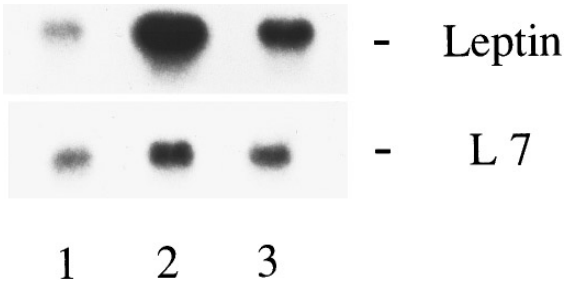

FIG. 3. Levels of m-leptin mRNA expressions by adipose tissues during pregnancy. Twenty μg of total RNAs from parametrial adipose tissues of virgin and days 13 and 17 pregnant mice were subjected to a Northern blot analysis. The upper rows show the blots hybridized with ^{32}P-labeled leptin probe, and the lower rows show the same blots rehybridized with the ^{32}P-labeled L7 probe. The data shown are from a representative experiment. The experiment was repeated three times with similar results. Lane 1: virgin, Lane 2: day 13, Lane 3: day 17

shown in Fig. 3, the band intensity of days 13 and 17 of pregnancy increased 3- to 4-fold compared with that of virgin mouse [m-leptin band intensity normalized by L7 band intensity: virgin, 0.489 $\pm$ 0.058; day 13, 1.61 $\pm$ 0.27; day 17, 1.59 $\pm$ 0.24; $p < 0.05$ vs virgin, $n = 3$ from three indipendent experiments]. To confirm whether the increased production of leptin in the adipose tissue contributes to the increase in serum concentration during gestation, adipose tissues from virgin and day 17 pregnant mice were cultured and m-leptin concentration was assessed in the medium after 2 h incubation of the tissue. The amount of m-leptin secreted in the medium was 14.0 $\pm$ 1.5 ng/g wet tissue ($n = 6$) in the adipose tissue from virgin mice. However, it was 35.4 $\pm$ 3.27 ng/g wet tissue ($n = 6$) in the adipose tissue from day 17 pregnant mice, and was significantly higher than that in virgin mice ($p < 0.05$). These findings suggested that adipose tissue, but not placenta and decidua, is the main source for the increase in serum m-leptin concentration during gestation.

Human placenta expresses leptin mRNA and produces the protein (3,4). Serum h-leptin concentration in pregnant women increases about 3-fold compared with non-pregnant women (12). It is possible that placenta is a source of serum h-leptin. However, this hypothesis is not confirmed since a possible change in the level of h-leptin expression during pregnancy was not examined in the human adipose tissue. Interestingly, serum rat leptin concentration increases only 3-fold during pregnancy compared with virgin rat and gestational profile of serum leptin concentration is similar to that of human's (13 and Kawai, M., Yamaguchi, M., et al., in preparation). Moreover, rat placenta does not express leptin mRNA (Kawai, M., Yamaguchi, M., et al., in preparation). These findings suggest that leptin production may be differently regulated in different species during pregnancy, and that physiological roles of the increased leptin during gestation are different in these species.

In conclusion, we have shown that serum m-leptin level dramatically increases after midpregnancy and adipose tissue, but not placenta, is the main source of serum m-leptin. However, factors which increase the serum m-leptin concentration and the physiological roles of increased serum leptin during gestation remain to be identified.

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