

Leptin Secretion From Subcutaneous and Visceral Adipose Tissue in Women

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Upper body obesity is a risk factor for type 2 diabetes. Little is known about the regulation of body fat distribution, but leptin may be involved. This study examined the secretion of leptin in subcutaneous and omental fat tissue in 15 obese and 8 nonobese women. Leptin secretion rates were two to three times higher in subcutaneous than in omental fat tissue in both obese and nonobese women ($P < 0.0001$ and $P < 0.001$, respectively). There was a positive correlation between BMI and leptin secretion rates in both subcutaneous ($r = 0.87$, $P < 0.0001$) and omental ($r = 0.74$, $P < 0.0001$) fat tissue. Furthermore, leptin secretion rates in subcutaneous and omental fat tissue correlated well with serum leptin levels ($r = 0.84$, $P < 0.0001$ and $r = 0.73$, $P = 0.001$, respectively), although in multivariate analysis, the subcutaneous leptin secretion rate was the major regressor for serum leptin ($F = 42$). Subcutaneous fat cells were ~50% larger than omental fat cells, and there was a positive correlation between fat cell size and leptin secretion rate in both fat depots ($r = 0.8$, $P < 0.01$). Leptin (but not γ -actin) mRNA levels were twofold higher in subcutaneous than in omental fat tissue ($P < 0.05$). Thus the subcutaneous fat depot is the major source of leptin in women owing to the combination of a mass effect (subcutaneous fat being the major depot) and a higher secretion rate in the subcutaneous than in the visceral region, which in turn could be due to increased cell size and leptin gene expression. *Diabetes* 47:913–917, 1998

Upper body obesity is a major risk factor for type 2 diabetes. It is not known how the distribution of body fat is regulated. However, leptin, a protein produced by adipose tissue, may play an important role in this respect. Results from rodent studies (1–4) have clearly shown that leptin is important in the regulation of appetite and energy balance. Much less is known about the role of leptin in humans. A number of studies have shown that the circulating level of leptin is increased in obese subjects, and that this is more evident in women than in men (3,4). The mechanism for elevated leptin in the circulation of obese

subjects is not well defined. For example, surprisingly little is known about the secretion of leptin from fat cells, the major source of leptin. In a recent study, however, a strong relationship among body weight, fat cell leptin mRNA, and the secretion rate of leptin from fat cells was observed (5), suggesting that increased gene expression, causing accelerated production in fat tissue, is a major factor for elevated circulating leptin levels in obesity.

Which fat depot is the major source of leptin? The subcutaneous adipose tissue is an obvious candidate, since it constitutes about 80% of all fat tissue. However, the metabolism of adipose tissue from various depots in the human body has been shown to differ (6–9). Visceral fat cells are more lipolytic in comparison with subcutaneous fat cells. This regional difference in metabolic activity is believed to be of clinical importance, linking abdominal obesity to various metabolic complications, such as glucose intolerance, hyperinsulinemia, and dyslipidemia, according to mechanisms that have been discussed previously (6–9). It is not known whether there are regional variations in the secretion of leptin from human fat tissue. However, recent studies have indicated that this may be true for leptin gene expression (10, 11). On the other hand, Alessi et al. (12) investigated nonobese subjects and observed that leptin secretion from fat cells did not differ between the fat depots. To obtain more information on potential site-specific differences in leptin secretion, we investigated variations in leptin secretion and leptin mRNA expression between subcutaneous and omental (visceral) fat tissue from nonobese and obese women.

RESEARCH DESIGN AND METHODS

Subjects and blood sampling. The study group consisted of 23 women (ages 24–60 years) who were undergoing either weight reduction surgery with adjustable gastric banding or elective laparoscopic cholecystectomy. In this group, 15 women were classified as obese (BMI 28–60 kg/m²) and 8 as nonobese (BMI 20–27 kg/m²). All subjects were Caucasians born in Sweden. Except for obesity or gallstones, they were all healthy and taking no medication; 9 women were postmenopausal. The study was approved by the Ethics Committee of the Karolinska Institute (Stockholm, Sweden). All subjects gave informed consent to participate in the study.

BMI was measured on the day before surgery after an overnight fast. After a 15-min rest in bed, venous blood samples were taken. Blood serum samples were stored at -70°C for subsequent leptin measurement, for which a commercially available human leptin radioimmunoassay (RIA) kit (Linco, St. Charles, MO) was used.

Adipose tissue biopsies. The subjects fasted overnight. At 8:00 A.M. they were brought to the operating room. General anesthesia was given, as previously described (13). Fat tissue specimens (0.5–2.0 g) were obtained within 30 min after the incision of the abdominal wall from the abdominal subcutaneous and omental fat tissue regions. Before taking the fat biopsies, only saline was administered intravenously. The fat tissue specimens were used immediately for secretion studies and determination of fat cell size. A tissue piece (~500 mg) was immediately frozen in liquid nitrogen and stored at -70°C for subsequent mRNA analysis. In some cases, for technical reasons, it was not possible to obtain enough fat tissue to perform all the analyses. In these subjects, the priority was given to leptin secretion measurements.

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bp, base pair; RIA, radioimmunoassay.

Isolation of fat cells and determination of fat cell size. Fat cells from both the subcutaneous and omental fat tissue regions were isolated from 11 of the obese women and the fat cell size was determined. Fat cells were isolated by collagenase treatment according to the method of Rodbell (14), which has been described elsewhere in detail (13). Using direct microscopy, the diameter of 100 fat cells was determined; the mean fat cell volume could be calculated from these data, assuming that the measured cells were spheres. The fat cell weight was calculated using the density of human fat cell triglyceride (assuming that lipids constitute >95% of a fat cell).

Leptin secretion analysis. Leptin secretion was measured in subcutaneous and omental fat tissue samples from all subjects, as described previously (5). Briefly, the fat tissue specimens were cut into small pieces (~10 mg). All visible vessels and coagulation particles were removed. The remaining tissue was rinsed in 37°C saline and then incubated (300 mg tissue in 3 ml medium) in a Krebs-Ringer phosphate buffer (pH 7.4) supplemented with 40 g/l of defatted bovine serum albumin and 1 g/l of glucose. The incubation was carried out for 2 h at 37°C in a shaking bath, with air as the gas phase. After incubation, 2 ml of the medium was removed, frozen in liquid nitrogen, and stored at -70°C. The fat tissue was immediately homogenized, and the total lipid content of the sample was measured after organic extraction. Leptin secretion was related to the lipid weight and to the number of fat cells (for 11 obese women) in the incubated tissue, which is the total lipid weight divided by the mean fat cell weight. At the time of the analysis, all the frozen medium samples were freeze-dried simultaneously, and the material was then redissolved in 150 µl of distilled water. It was necessary to concentrate the medium in this way to obtain detectable leptin values. The leptin levels in the medium were determined with a human leptin RIA kit (Linco). We have previously shown that 1) the secretion of leptin is linear during an incubation time of at least 3 h, and 2) the recovery of leptin in the incubation medium is complete (5).

Leptin mRNA analysis. Total RNA was prepared from 300 mg of subcutaneous and omental fat tissue samples from eight obese women, using the RNeasy mini kit (Qiagen, Hilden, Germany). The integrity of the RNA was checked by electrophoresis in a 1% agarose gel containing ethidium bromide. The RNA concentration was measured spectrophotometrically. Leptin and γ -actin mRNA were quantified in the RNA samples by solution hybridization, as previously described (15). An RNA probe for leptin was obtained by subcloning a 240 base pair (bp) fragment of leptin cDNA (spanning exons 2 and 3) using polymerase chain reaction primers with flanking *Pst*I and *Xba*I sites (AACTGCAGTCAAGACAATTGTCCACCAGG and GCTCTAGATGGCAGCTCTTAGAGAAGGC, respectively) into a p-Bluescript SK plasmid (Stratagene, La Jolla, CA). A 236-bp γ -actin probe was provided by Mats Gäfvels (KFC Novum, Huddinge, Sweden). The plasmids were used for in vitro synthesis of antisense RNA labeled with [³⁵S]uridine 5'-triphosphate and unlabeled sense RNA, according to Melton et al. (16). Total RNA samples were hybridized in duplicate to the ³⁵S-labeled antisense RNA at 70°C for 24 h in a solution with 0.6 mol/l NaCl, 20 mmol/l Tris-HCl (pH 7.5), 4 mmol/l EDTA, 0.1% SDS, 1 mmol/l dithiothreitol, and 25% formamide. After hybridization, the samples were treated with RNase (40 µg/ml RNase A and 100 U/ml RNase T₁) at 37°C for 45 min. Trichloroacetic acid-precipitated material was collected on Whatman GF/C filters (Whatman, Maidstone, U.K.) and counted in a liquid scintillation counter. A standard curve was obtained by hybridizing known amounts of the unlabeled sense RNA to the ³⁵S-labeled antisense RNA. To exclude possible variations in mRNA-yield among samples, the leptin mRNA levels were expressed as leptin mRNA divided per total RNA and as leptin mRNA divided per γ -actin mRNA. The expression of the γ -actin gene was presumed not to differ between the two fat tissue regions and, therefore, γ -actin mRNA was used as a reference as well. The specificity of the leptin mRNA probe was confirmed by Northern blot analysis (data not shown).

Statistical analysis. Data are means \pm SE. Differences between subcutaneous and omental fat tissue were examined using a paired or unpaired Student's *t* test. The linear regression method (least squares) and stepwise regression analysis were used to analyze correlations. In addition, nonparametrical statistical tests were used (Wilcoxon's, Spearman's, and Kendall's) to study regional differences and correlations. All calculations were made using a commercially available computer program (Statview; Abacus Concepts, Berkeley, CA).

RESULTS

To study possible differences in leptin secretion between fat tissue regions, data from all 23 women were examined. As shown in Fig. 1, the leptin secretion rate was about twofold higher in the subcutaneous than in the omental fat tissue when expressed per gram of fat tissue ($P < 0.0001$).

The relationship between the leptin secretion rate in subcutaneous and omental fat tissue was determined using linear regression analysis (Fig. 2). There was a positive, significant correlation between leptin secretion in the two fat

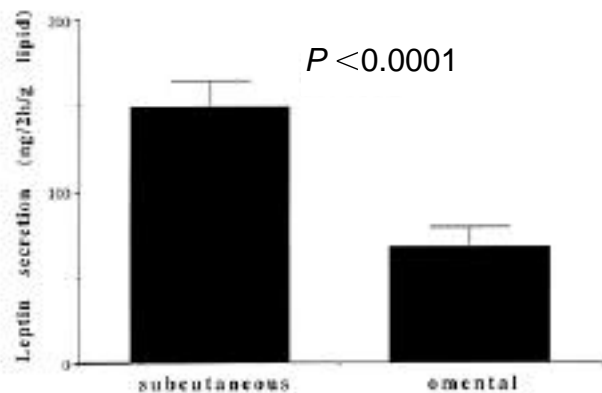


FIG. 1. Leptin secretion rate from subcutaneous and omental fat tissue from 23 subjects. Values are expressed per gram of lipid. Data are means \pm SE. Subcutaneous and omental leptin secretion were compared using a paired *t* test.

depots ($r = 0.86$, $P < 0.0001$). Other regression analyses were also performed. There was also a positive, significant correlation between BMI and the leptin secretion rate in both subcutaneous fat tissue ($r = 0.87$, $P < 0.0001$) and omental fat tissue ($r = 0.74$, $P < 0.0001$) (Fig. 3). Furthermore, the serum leptin level correlated positively and significantly with the leptin secretion rate in both regions ($r = 0.84$, $P < 0.0001$ [subcutaneous fat tissue] and $r = 0.73$, $P < 0.001$ [omental fat tissue]) (Fig. 4). The relative contribution of the leptin secretion rate in subcutaneous and omental fat tissue to the variation in serum leptin was examined by stepwise regression analysis. $F > 4.0$ was considered to reflect a significant contribution to the variation. Only the leptin secretion rate in subcutaneous fat tissue contributed significantly to the variation in serum leptin (partial $F = 42$, adjusted $r^2 = 0.70$).

In Table 1, relevant data on nonobese and obese women are given separately. The leptin secretion rate was about twofold higher in the subcutaneous than in the omental fat tissue from both nonobese and obese women ($P < 0.001$ and $P < 0.0001$, respectively). Furthermore, leptin secretion rates in subcutaneous and omental fat tissue and serum leptin levels were significantly higher in the obese than in the nonobese subjects.

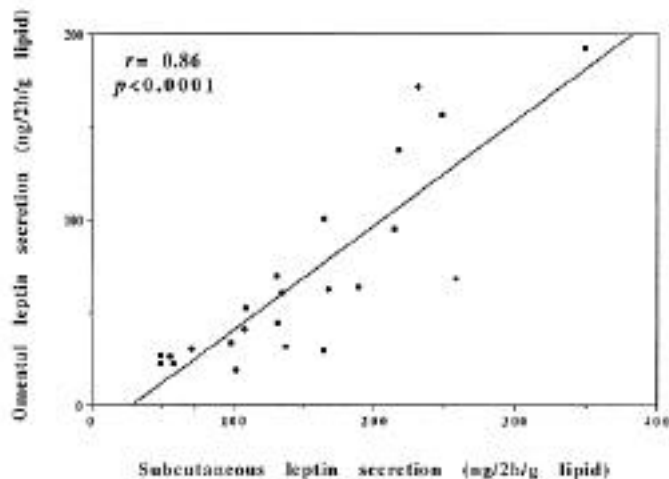


FIG. 2. Relationship of leptin secretion rate in subcutaneous and omental fat tissue. Values were compared by linear regression analysis.

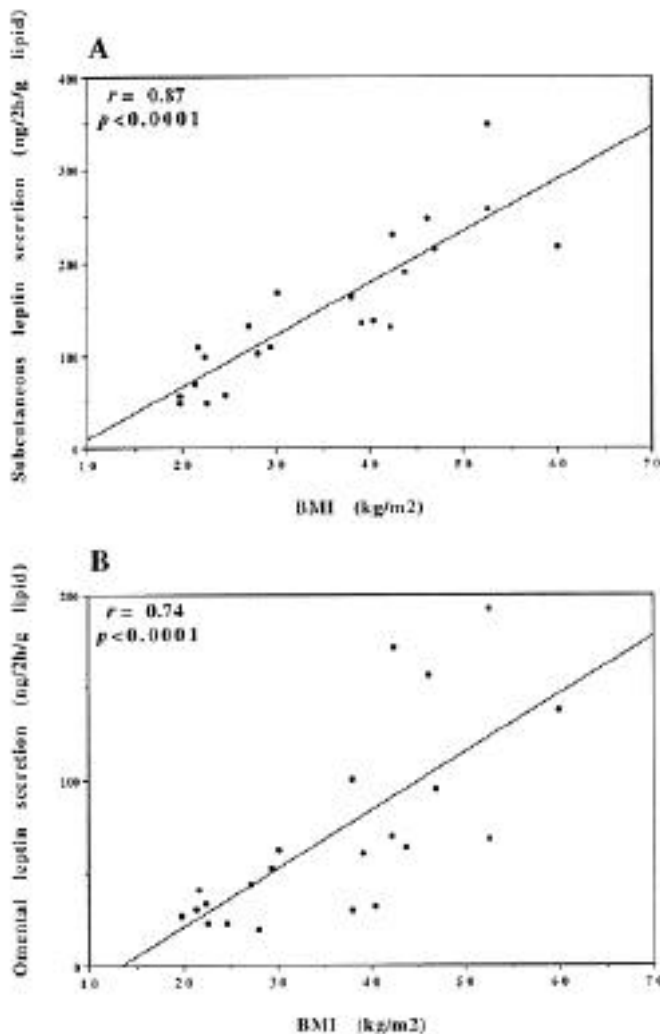


FIG. 3. Relationship between BMI and leptin secretion rate in subcutaneous (A) and omental (B) fat tissue. Values were compared by linear regression analysis.

For 11 obese women, it was possible to isolate subcutaneous and omental fat cells and determine fat cell size (Table 2). Subcutaneous fat cells were about 50% larger than omental fat cells ($P < 0.001$). When leptin secretion was expressed per cell number in these subjects, the secretion rate was significantly higher (by about threefold) in the subcutaneous than in the omental fat tissue ($P < 0.0001$). There was a positive, significant correlation between cell volume and leptin secretion in the two fat regions ($r = 0.82$, $P < 0.01$ [subcutaneous fat tissue] and $r = 0.83$, $P < 0.01$ [omental fat tissue]) (Fig. 5).

Information about mRNA levels in subcutaneous and omental fat tissue from eight of the obese subjects is presented in Table 3. Leptin mRNA relative to total RNA in the fat tissue sample was elevated more than twofold in subcutaneous fat tissue compared with in omental fat tissue ($P < 0.01$). γ -Actin relative to total RNA in the fat tissue sample did not differ between the two fat tissue regions. Leptin mRNA relative to γ -actin mRNA was significantly higher in subcutaneous fat tissue (by about twofold) than in omental fat tissue ($P < 0.05$). No attempts were made to correlate leptin mRNA with leptin secretion, plasma leptin, cell volume, or BMI in this small group of obese subjects.

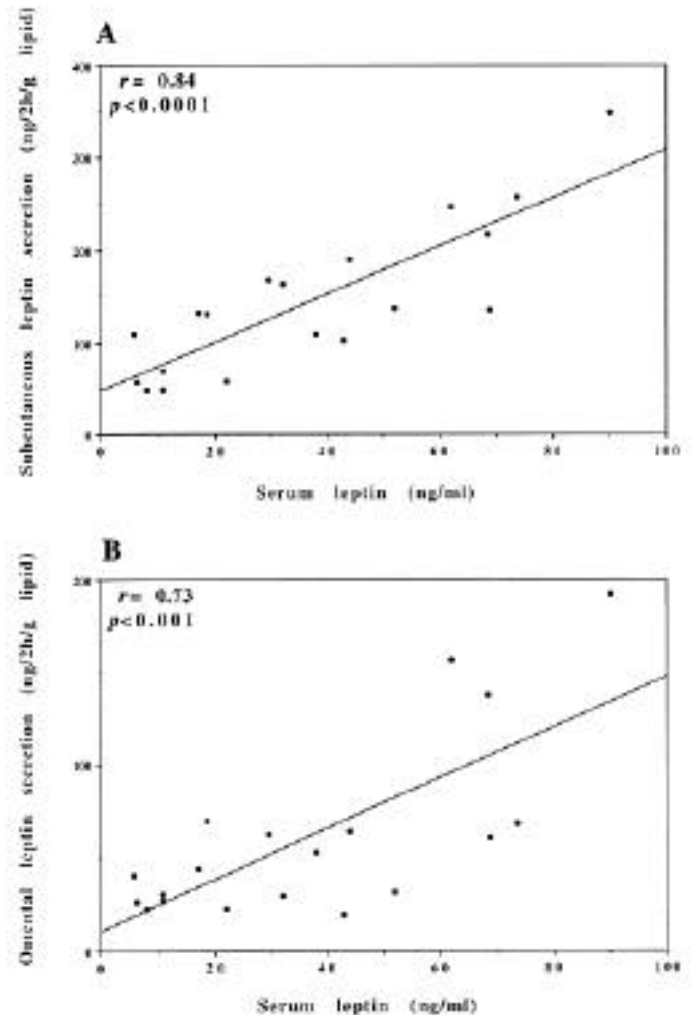


FIG. 4. Relationship between serum leptin levels and leptin secretion rate in subcutaneous (A) and omental (B) fat tissue. Values were compared by linear regression analysis.

There were no significant differences between post- and premenopausal women with regard to leptin secretion in subcutaneous or omental fat tissue (data not shown). Furthermore, the difference in leptin secretion rate between the two fat tissue regions was present in both post- and premenopausal women (paired t test: $P < 0.001$ and $P < 0.0001$, respectively).

The data were also examined using nonparametric statistical tests. The regional differences in leptin secretion were statistically significant using Wilcoxon's test ($P < 0.0001$), and the correlations, presented in Figs. 2–5, were also statistically significant using Spearman's or Kendall's tests ($P < 0.01$).

DISCUSSION

Recently it has been shown that human subcutaneous adipose tissue secretes leptin, and that elevated serum leptin in obese subjects is due to the release of this protein being increased in the obese state, presumably secondary to increased gene expression in the enlarged fat cell (5,17). In the current study, we demonstrated for the first time the differences in leptin secretion rates between subcutaneous and visceral (omental) fat tissue. We found a strong positive correlation between the secretion rate of leptin in subcutaneous and omental fat tissue,

TABLE 1
Comparison of leptin secretion rate from fat tissue and serum leptin values in obese and nonobese women

	Obese	Nonobese	P value (obese vs. nonobese)
<i>n</i>	15	8	
Age (years)	43 ± 3	45 ± 3	NS
BMI (kg/m ²)	41.9 ± 2.3	22.3 ± 0.9	<0.0001
Serum leptin (ng/ml)	51.6 ± 6.1	11.6 ± 2.3	<0.001
Leptin secretion (ng · 2 h ⁻¹ · g ⁻¹ lipid)			
Subcutaneous fat tissue	187.6 ± 17.1	77.3 ± 11.1	<0.001
Omental fat tissue	87.2 ± 13.9	30.7 ± 2.9	<0.01
<i>P</i> (subcutaneous vs. omental fat tissue)	<0.0001	<0.001	—

Data are means ± SE. Values from the obese and nonobese subjects were compared using an unpaired *t* test, and values between subcutaneous and omental fat tissue were compared by a paired *t* test.

and also found that the leptin secretion rate was two to three times higher in subcutaneous than in omental fat tissue when leptin secretion was expressed per lipid weight or cell number. These findings contrast those of Alessi et al. (12), who investigated nonobese subjects (four men and three women) and observed a similar secretion rate of leptin from subcutaneous and omental fat tissue. However, it is possible that the number of subjects in the study of Alessi et al. was not large enough to observe a significant difference. Furthermore, most of these subjects were men, whereas we studied only women. There might be sex-specific differences in leptin secretion, as was shown for leptin mRNA expression (17). In addition, we observed the regional variation in both obese and nonobese women.

Which fat depot is quantitatively the most important source of circulating leptin? In this study, we observed a strong correlation between leptin secreted per weight unit in both subcutaneous and omental fat tissue and levels of serum leptin. Thus both fat depots accounted for the variation in circulating leptin levels. According to the adjusted *r*² values, the secretion from subcutaneous and omental fat tissue accounted for about 70 and 50%, respectively, of the variations in serum leptin. However, it is clear from the present data that the subcutaneous fat depot is a much more important source for circulating leptin than the visceral one. First, there is a mass effect, with subcutaneous fat being the major fat depot. Second, there is a true difference in secretion rate (at least *in vitro*) both when expressed per mass unit (twofold) or per fat cell (threefold). Third, in stepwise regression analysis, only the secretion of leptin from the subcutaneous site contributed significantly to the variation in serum leptin.

A positive correlation between BMI and leptin secretion from subcutaneous fat tissue has been shown previously (5). In the current study, BMI was positively correlated to leptin secretion from both subcutaneous and omental fat tissue. According to the adjusted *r*² values, BMI accounted for about 70 and 50% of the variation in subcutaneous and omental leptin secretion, respectively. Comparison of nonobese and obese women revealed that leptin secretion in subcutaneous and omental fat tissue was ~2.5 times higher in the obese than in the nonobese women. However, the regional difference was found in both subgroups of women. These results suggest that the subcutaneous fat tissue is the major site of leptin production in lean as well as in obese subjects.

What is the mechanism behind regional differences in fat cell secretion of leptin? It has been suggested that leptin gene

expression in fat cells regulates the production rate of leptin (5). In this study, it was shown that leptin mRNA expression in subcutaneous fat tissue was higher than in omental fat tissue for obese women, which agrees with previous observations (11). Unfortunately, it was not possible to perform these experiments in nonobese women because of the limited amount of tissue that could be obtained. Masuzaki et al. (10), however, investigated nonobese humans and found higher leptin mRNA in subcutaneous than in omental fat tissue. Furthermore, we observed that the regional difference was specific for leptin mRNA, since mRNA levels of the cytoskeletal protein, γ -actin, did not differ between the fat regions. Thus present and previous results indicate that regional differences in leptin gene expression might be a major factor causing regional differences in leptin secretion rate.

Fat cell size also appeared to be of importance. The subcutaneous fat cells were larger than the omental fat cells, and the regional differences in the secretion of leptin were more pronounced when secretion was related to cell number than when the results were expressed per gram of lipid. Furthermore, there was a strong correlation between fat cell size and leptin secretion rate in both fat depots.

There was no difference in leptin secretion between pre- and postmenopausal women, suggesting that estrogen and progesterone are not of major importance for regional variations in leptin secretion. It remains to be established if there are regional variations in leptin secretion in men.

Obesity is associated with a number of adverse outcomes, including type 2 diabetes. This relationship is most apparent in subjects with accumulation of fat in the intra-abdominal depot (7,8). It has been speculated that leptin from omental fat tissue may be a less important contributor to the long-term

TABLE 2
Leptin secretion rate from subcutaneous and omental fat tissue in 11 obese women in relation to fat tissue cellularity

	Subcutaneous	Omental	P value
Fat cell volume (pl)	951 ± 68	636 ± 48	<0.001
Leptin secretion (ng · 2 h ⁻¹ × 10 ⁻⁷ cells)	1,861.6 ± 272.9	650.4 ± 135.9	<0.0001

Data are means ± SE. Subcutaneous and omental fat cell volumes and leptin secretion rates were compared using paired *t* tests.

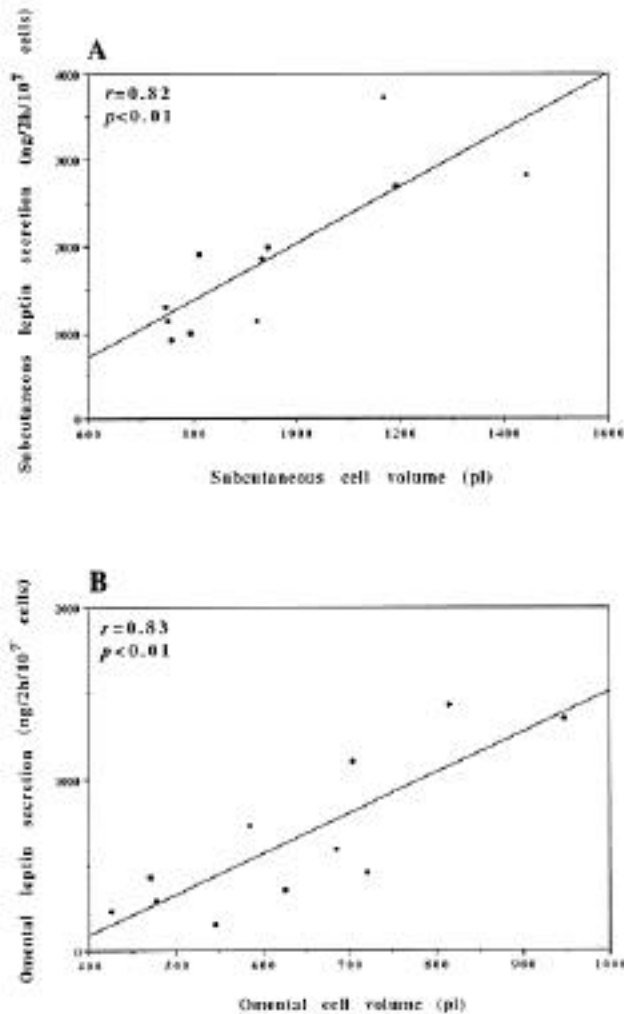


FIG. 5. Relationship between cell volume and leptin secretion rate in subcutaneous (A) and omental (B) fat tissue. Values were compared by linear regression analysis.

feedback loop, which controls metabolic rate and appetite, than leptin from subcutaneous fat tissue (11). Thus low leptin production from omental fat cells might contribute to the development of visceral obesity.

In summary, the present findings suggest that the subcutaneous fat depot is the most important source of adipose-derived leptin. First, there is a mass effect, since subcutaneous fat is the major fat depot. Second, the subcutaneous fat tissue showed a higher secretion rate of leptin than the omental fat tissue due to enlarged cell size and increased expression of the leptin gene. It is possible that these site variations in leptin expression and secretion are involved in the regulation of fat distribution.

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TABLE 3
mRNA values in subcutaneous and omental fat tissue from eight obese women

	Subcutaneous	Omental	P value
γ -actin mRNA/total RNA	180 \pm 16	184 \pm 12	NS
Leptin mRNA/total RNA	91 \pm 12	40 \pm 11	<0.01
Leptin mRNA/ γ -actin mRNA	0.50 \pm 0.04	0.22 \pm 0.06	<0.05

Data are means \pm SE. Leptin and γ -actin mRNA are expressed as attomoles per microgram of total RNA or as a ratio between leptin and γ -actin. Subcutaneous and omental fat tissue values were compared using a paired *t* test.

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