

Mutations in the Human Leptin and Leptin Receptor Genes as Models of Serum Leptin Receptor Regulation

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A part of serum Ob leptin, an adipocyte-secreted peptide, is bound to a soluble Ob receptor (sObR). Immunoreactive sObR was measured in 125 lean or obese control subjects (group 1), 18 individuals with a mutation in the leptin gene impairing leptin secretion (group 2), and 10 individuals with a mutation in the ObR gene, leading to production of a truncated ObR not anchored to cell membranes (group 3). In group 1, sObR levels were negatively correlated with age and BMI in children and with BMI in adults. sObR levels were also negatively correlated with leptin levels. Leptin binding activity and sObR levels coeluted in gel-filtration chromatography. In group 2, sObR levels did not differ from those in lean control subjects and were not correlated with BMI. A single peak was detected in chromatographic fractions. In group 3, sObR levels were high and positively correlated with BMI. Immunoreactive sObR coeluted with leptin binding activity. These data demonstrate that leptin is not needed for ObR gene expression, and they suggest that leptin plays a role in receptor downregulation because sObR levels are negatively correlated with leptin levels and BMI in control subjects, whereas sObR levels are not depressed in obese leptin-deficient or leptin receptor-deficient individuals. *Diabetes* 51:1980–1985, 2002

Leptin exerts its biological activity through a membrane receptor (ObR) located in various tissues, including the brain (rev. in 1). The lack of leptin due to mutations in the leptin gene and the lack of functional leptin receptors result in early-onset obesity and various endocrine disturbances in both rodents and humans (2–5). In the leptin-deficient *ob/ob* mice as well as in human subjects harboring a mutation in the leptin gene that results in the absence of leptin production, parenteral administration of recombinant leptin reduces food intake and adiposity (6,7). These observations are consistent with an inhibitory role of leptin on food intake and fat mass development (rev. in 8).

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Received for publication 6 February 2002 and accepted in revised form 13 March 2002.

CSF, cerebrospinal fluid.

Serum leptin levels are strongly correlated with BMI or fat mass, as evidenced by a number of studies. That led to the concept of an apparent resistance to leptin in common obesity, since high leptin levels appear unable to reduce fat storage, most likely through a defect in leptin transport to the brain (9).

In serum, leptin circulates mainly as a 16-kDa free peptide and also as a large protein complex (10). The leptin binding proteins were hypothesized to play a role in the availability of leptin to its target cells (11). A soluble form of the extracellular domain of the leptin receptor (sObR) accounted for the majority of the serum leptin binding activity (12,13). Very high levels of leptin binding activity have been reported by us in individuals who were either heterozygous or homozygous for a mutation in the leptin receptor gene responsible for the production of a truncated extracellular receptor not anchored to the cell membrane (14). On the other hand, leptin levels were undetectable or very low in individuals with a mutation in the leptin gene (3,4,15). Serum leptin binding activity has not been reported in these situations.

Because leptin- and leptin receptor-deficient human subjects provide unique models which could, when compared to normal subjects, shed light on the regulatory mechanisms governing the production of the leptin receptor, we investigated the relationship between sObR levels, leptin levels, and BMI in three groups of individuals: subjects with a mutation in the leptin gene or in the ObR gene and lean and overweight control subjects.

RESEARCH DESIGN AND METHODS

Subjects. The subjects in group 1 were 125 individuals of normal weight or with common obesity: 31 girls and 23 boys aged 3–15 years, 37 female subjects aged 17–59 years, and 34 male subjects aged 15–61 years (Table 1). All patients and/or their parents gave informed consent for this study, which was approved by the local ethical committee. The serum leptin receptor was also assayed in all serum chromatographic fractions from eight obese and four nonobese normal female subjects. In group 2, the subjects were 18 individuals from a consanguineous Turkish family with a mutation in the leptin gene, which impairs the normal processing of leptin through the secretory pathway (4). Of these 18 subjects, 4 (1 male and 3 female subjects) were homozygous for the mutation, 13 (6 male and 7 female) were heterozygous for the mutation, and 1 girl was homozygous for the wild-type gene (Table 2). Immunoreactive sObR was also measured in all chromatographic fractions from two homozygotes and two heterozygotes for the mutation. Group 3 consisted of 10 individuals from a consanguineous Kabyle family with a mutation in the leptin receptor gene leading to the production of a truncated receptor not anchored to the cell membrane (5,14). Of these 10 subjects, 3 female subjects were homozygous for the mutation, 3 female subjects were heterozygous, 1 female subject was homozygous for the wild-type gene, 2 male subjects were heterozygous, and 1 male subject had the wild-type gene (Table 3). Immunoreactive sObR was also measured in all chromatographic fractions from three homozygotes, five heterozygotes for the mutation, and the wild-

TABLE 1
Clinical and biological details in group 1 (subjects with normal weight or common obesity)

	Girls	Boys	Women	Men
<i>n</i>	31	23	37	34
Age (years)	10.5 (3–15)	11 (5–15)	37 (15–59)	31.9 (15–61)
BMI (kg/m ²)	21.5 (11.8–36.1)	22.4 (12.8–34.7)	33.6 (16.6–71.4)	34.4 (16.3–70.4)
Insulin (mIU/l)	8.8 (0.2–34)	6.9 (0.1–22)	16.0 (3–54)	14.8 (1.6–47)
Leptin (ng/ml)	17 (1.8–55)	13.4 (2–43)	37.5 (1.8–180)	25.6 (1–160)
sObR (ng/ml)	42.5 (11.5–97)	50.5 (21.4–116)	37.8 (15–76.9)	38.9 (15–98)

Data are means (range).

type girl. All individuals from the three groups had normal hepatic and renal function, as assessed by conventional analyses.

Methods. Chromatographic fractionations of serum samples were carried out on an Ultrogel AcA column (Biosepra, Villeneuve-la-Garenne, France) as previously described (14). Insulin was measured by a fluoroimmunoassay using AutoDelfia reagents (PerkinElmer, Courtaboeuf, France). Sensitivity was 0.1 mIU/l. Leptin was measured by radioimmunoassay using reagents from Linco Research (St Charles, MO) as previously described (14).

The serum leptin receptor (sObR) was measured by a sandwich enzyme-linked immunosorbent assay composed of two monoclonal antibodies raised against the extracellular domain of the leptin receptor supplied by Biovondor Laboratory Medicine (Brno, Czech Republic). The standard was recombinant human leptin receptor/IgG-Fc fragment dimeric chimera. Intraseries coefficients of variation, for assays in duplicate, were 2.3, 2.1, and 2.2% at the levels 9.7, 41, and 158 ng/ml, respectively. Interseries coefficients of variations, for assays in duplicate, were 3.8% at level 41.9 and 4.4% at level 64.6 ng/ml. Sensitivity was 0.35 ng/ml. The recovery of samples spiked with increasing amounts of the ObR extracellular domain/Fc chimera (R & D Systems, Abingdon, U.K.) was $98 \pm 3\%$ (means \pm SD).

To preclude a hypothetical steric hindrance caused by leptin molecules bound to the soluble receptor, the following experiments were performed: pretreatment in acidic solution, addition of 10–50 ng/ml of recombinant leptin, immunoextraction of leptin before assay, and manipulation of leptin levels by fasting/refeeding in normal individuals. In no case were immunoreactive sObR levels affected by the changes in leptin concentration.

Statistics. Comparisons between groups by means of Student's *t* test and analyses of partial correlations were made using the StatView software from Abacus Concepts (Berkeley, CA).

RESULTS

Group 1: lean and obese control subjects. In girls and boys (Table 1), sObR levels were negatively correlated with BMI: $r = -0.82$ ($P < 0.001$) and $r = -0.58$ ($P < 0.01$), respectively. Significant negative correlations were found with age ($r = -0.62$, $P < 0.001$, and $r = -0.50$, $P < 0.02$) and with leptin levels ($r = -0.77$, $P < 0.001$, and $r = -0.66$, $P < 0.001$) in girls and boys, respectively. However, when partial correlations were computed, sObR levels were correlated only with BMI and age (Table 4).

In lean and overweight adults, sObR levels did not differ significantly between female and male subjects: 48.0 ± 3.2 and 49.7 ± 4.1 ng/ml in lean subjects, respectively ($P = 0.87$), and 31.8 ± 2.5 and 25.9 ± 1.6 ng/ml in overweight

subjects, respectively ($P = 0.52$). Serum ObR levels were negatively correlated with BMI (Fig. 1) in both female and male subjects: $r = -0.72$ ($P < 0.001$) and $r = -0.70$ ($P < 0.001$), respectively. The overall correlation for the entire group of male and female subjects was -0.71 . As in children, serum ObR levels were negatively correlated with leptin levels: $r = -0.63$ ($P < 0.001$) in women, and $r = -0.52$ ($P < 0.01$) in men. However, no correlation was found after adjustment for BMI and age (Table 4). Chromatographic fractionation of serum samples from four lean female and eight overweight female subjects confirmed that sObR immoreactivity coeluted with leptin binding activity (Fig. 2A).

As expected, insulin levels were positively correlated with BMI in both children and adults: $P < 0.001$ in girls, $P < 0.02$ in boys, $P < 0.05$ in female subjects, and $P < 0.001$ in male subjects. Insulin levels were negatively correlated with sObR levels, but this was mainly due to the relationship with BMI, since no significant correlations were found after adjustment for BMI (Table 4).

Group 2: leptin-deficient subjects. In the 13 individuals heterozygous for a loss-of-function leptin gene mutation and in the four homozygous individuals (Table 2), sObR levels did not differ from those of lean control subjects from the same geographic area (four male and four female subjects): 69 ± 8.2 , 70.8 ± 5.4 , and 72.8 ± 8.5 ng/ml, respectively. There was no relationship with BMI (Fig. 3B). In the homozygous girl from this family (patient 3), the sObR level was at the upper limit for normal girls (Table 1), although BMI was also at the upper limit of the range of our normal obese girls (Table 2). Leptin levels were low as previously reported, and were negatively correlated with BMI ($r = -0.54$, $P = 0.01$) (Fig. 3A). Chromatographic fractionation of serum from four individuals detected a single peak corresponding to a molecular mass of 380 kDa (Fig. 2B).

Group 3: leptin receptor-deficient subjects. In the three heterozygous female subjects and in the three ho-

TABLE 2
Clinical and biological details in group 2

	Patient number																		Turkish control subjects	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	BMI <25	BMI >35
Sex	M	F	F	F	M	F	M	M	M	F	F	F	M	F	F	F	M	F	2F + 2M	2F + 2M
Status	MM	MM	MM	MM	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	MW	WW		
Age (years)	21	33	6	30	66	65	23	11	27	38	28	50	32	20	20	25	31	25	19–23	20–57
BMI (kg/m ²)	55.8	52.1	32.1	56.7	22.9	29.7	23.4	18.9	27	22.6	22	19.5	27.3	21.6	22.2	23.9	19.9	25	16.3–25	36–51.8
Leptin (ng/ml)	0.65	0.47	0.5	0.58	1.3	2.22	1.54	1.56	1.38	2	1.59	1.29	1.74	4.5	3.17	1.04	0.98	2.65	1.5–4.5	11.1–64
sObR (ng/ml)	39	92	56	69	95	100	42	78	58	66	80	62	66	47	85	47	94	98	51–86	16–36

MM, homozygous for the mutant gene; MW, heterozygous for the mutant gene; WW, homozygous for the wild-type gene.

TABLE 3
Clinical and biological details in group 3

	Patient number										Common obesity (n = 10)
	1	2	3	4	5	6	7	8	9	10	
Sex	F	F	F	F	F	F	M	M	M	F	F
Status	MM	MM	MM	MW	MW	MW	MW	MW	WW	WW	WW
Age (years)	19	13	16	47	24	23	51	16	12	17	48 ± 4
BMI (kg/m ²)	66	68.9	50.1	33	27.3	25.4	27.7	29.8	16.2	33.7	50 ± 8
Total leptin (ng/ml)	640	670	526	362	294	240	155	212	5.6	78	104 ± 23
Free leptin (ng/ml)	92	111	109	52	39	47	3.5	ND	ND	72.2	91 ± 19
sObR (ng/ml)	3,940	4,200	3,700	3,320	2,910	1,920	3,120	2,220	65	30	24.5 ± 1.8

Data are means ± SE unless otherwise indicated. MM, homozygous for the mutant gene; MW, heterozygous for the mutant gene; ND, not done; WW, homozygous for the wild-type gene.

mozygous female subjects (Table 3), sObR levels were extremely high: 1,920–3,320 and 3,700–4,200 ng/ml, respectively. The two heterozygous male subjects had sObR levels in the same range (2,220 and 3,120 ng/ml). Interestingly, in the female subjects, sObR levels were positively correlated with BMI ($r = 0.88$), as were the free leptin levels ($r = 0.88$) (Fig. 4). Chromatographic fractionation of serum from eight individuals detected a single peak perfectly coincident with the high-molecular weight peak of leptin (Fig. 2B).

DISCUSSION

In normal individuals as well as in individuals with a mutation in the ObR gene, the chromatographic fractionation of serum provided evidence that the immunoreactive sObR material and the leptin binding activity coeluted perfectly. This confirms that sObR is responsible for the majority, if not all, of the serum leptin binding activity, as recently reported (12,13).

Leptin is not needed for the expression of its own receptor. In individuals with a mutation in the Ob gene leading to an impaired secretion of leptin (4), leptin levels and BMI are negatively correlated. Such a relation is described here for the first time, although it was recently reported that heterozygotes for the mutation ΔG133 (deletion of a glycine at residue 133) had lower leptin levels than control subjects (15). In contrast, sObR levels in homozygous and heterozygous adults and children with a mutation in the ObR gene do not differ from those in lean

normal control subjects. This suggests that leptin is not needed for ObR gene expression in humans.

In individuals who produce a truncated leptin receptor lacking the transmembrane and cytosolic domains, the quantitation of the serum immunoreactive material confirms our previous suggestion that huge amounts of the soluble receptor circulate in serum (14). The present results confirm that a leptin signal is not needed for the expression of the leptin receptor gene because individuals homozygous for the mutation failed to deliver any intracellular leptin signal.

Does sObR level reflect ObR gene expression? It was recently reported that sObR is produced in humans by proteolytic cleavage of membrane-anchored receptors (13). Because there has been no evidence so far in rodents and humans that sObR can be directly produced by a splice variant, this finding supports the idea that sObR levels reflect, at least in part, ObR gene expression. Similarly, serum growth hormone binding protein is considered to reflect the expression of the growth hormone receptor gene, another example of a cytokine receptor gene (rev. in 16).

In women the cerebrospinal fluid (CSF)–to–serum leptin ratio is negatively correlated with BMI (17). In rats (18), leptin transfer from serum to CSF is dependent on ObR receptors located in the blood-brain barrier. Obesity is associated with decreased leptin transport, suggesting that the leptin receptor in the blood-brain barrier can be easily

TABLE 4
Analysis of partial correlations between different variables and sObR levels

Variable	Girls	Girls + Boys			Women + Men	
		Boys	Boys	Women	Men	Men
n	31	23	54	37	34	71
Age						
r	-0.505	-0.672	-0.498	0.003	0.019	0.20
P	<0.010	<0.001	<0.001	>0.10	>0.10	>0.10
BMI						
r	-0.453	-0.569	-0.411	-0.464	-0.542	-0.517
P	<0.02	<0.01	<0.01	<0.01	<0.01	<0.001
Insulin						
r	-0.009	-0.168	-0.168	-0.059	-0.323	-0.142
P	>0.10	>0.10	>0.10	>0.10	>0.05	>0.10
Leptin						
r	0.047	0.031	-0.086	-0.010	-0.062	-0.028
P	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10

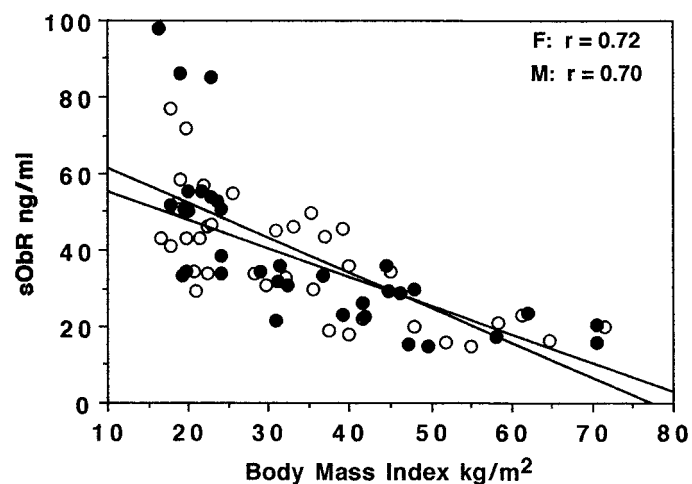


FIG. 1. Correlation of serum leptin receptor levels (sObR) with BMI in lean and obese adult male (●) and female (○) subjects.

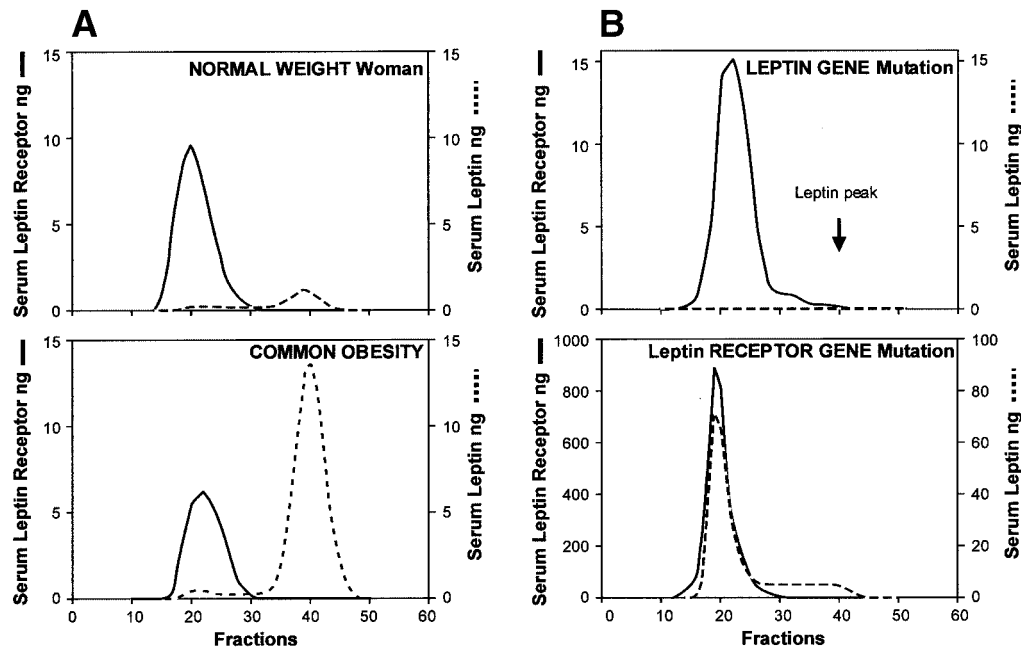


FIG. 2. Typical patterns of serum leptin receptor and leptin levels in chromatographic fractions from AcA gel filtration columns. The ordinate numbers are the amount of immunoreactive material per fraction, adjusted for 1 ml of serum. *A*: Serum from a lean female (top) and an obese female (bottom) subject. *B*: Serum from a female subject homozygous for a mutant leptin gene, leading to impaired secretion of leptin (top), and from a female homozygous for a mutant leptin receptor gene, leading to the production of a truncated leptin receptor not anchored to the cell membrane (note the different ordinate scale) (bottom).

saturated in both humans (9) and rodents (19). On the other hand, in a model of late-onset obesity in rats, hypothalamic leptin receptor protein is reduced in the aged/overweight rats, as is signal transduction in response to centrally administered leptin (20). Neuron-specific disruption of ObR in mice leads to obesity, and in these obese mice, hypothalamic ObR levels are low and serum leptin, insulin, and corticosterone levels are increased (21). In summary, these data give evidence that obesity is associated with low levels of ObR and that, in turn, low levels of ObR can per se lead to obesity.

The striking parallels between the negative correlation of sObR levels with BMI in our control subjects and the negative correlation of CSF-to-serum leptin ratio with BMI in normal lean and obese women supports the assumption that parallel decreases in sObR and ObR levels occur with increases in BMI.

Leptin may downregulate leptin receptor gene expression. In our control subjects, leptin levels are positively correlated with BMI, as has been extensively reported. On the other hand, sObR levels in these subjects are inversely correlated with BMI and also with age in children. This is consistent with the observation that serum leptin binding capacity (22) and sObR levels (23) decline with age. Because the percentage of fat mass increases with age, particularly in girls and women, the decline in sObR with age and the negative correlation with BMI are in agreement. These results suggest that in overweight subjects, the production of sObR is downregulated. Furthermore, free leptin is positively correlated with sObR levels in subjects harboring the ObR gene mutation. Such an unexpected relationship in individuals whose leptin is ineffectual suggests that leptin exerts a downregulating effect on either ObR gene expression or sObR production.

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Chinese hamster ovary cells expressing the leptin receptor isoforms ObRa or ObRb exhibited ligand-induced receptor downregulation when exposed to leptin (24). By measuring hypothalamic leptin activity, Wang et al. (25) found that in rats made hyperleptinemic by overfeeding, fat mass was abundant and hypothalamic leptin activity increased poorly relative to adenovirus-leptin-treated rats, demonstrating the development of a resistance to leptin by overfeeding. Martin et al. (26) induced a decrease in body mass for the first 2 weeks of a long-term administration of leptin in rats and then found that a resistance to leptin developed for the final 2 weeks, and that the hypothalamic leptin receptor mRNA was downregulated.

Is leptin the main factor of sObR downregulation? Insulin levels, like leptin levels, are negatively correlated with sObR in overweight subjects. However, a downregulating effect of insulin on the production of sObR is unlikely because the high insulin levels observed in the homozygous individuals from groups 2 and 3 (4,5) seem to have no inhibitory effect on sObR production in these models. It is unlikely that changes in the metabolic clearance rate of sObR might explain the changes in sObR levels, because no renal or hepatic abnormalities were shown in either the control group or the pathological groups. On the other hand, because BMI is the main factor related to sObR levels in our cohort of overweight subjects, some factors related to obesity, outside leptin, may be involved in the regulation of sObR production.

Leptin levels, although negatively correlated with sObR levels when computing a simple correlation, are no more closely correlated after adjustment for BMI and age. This

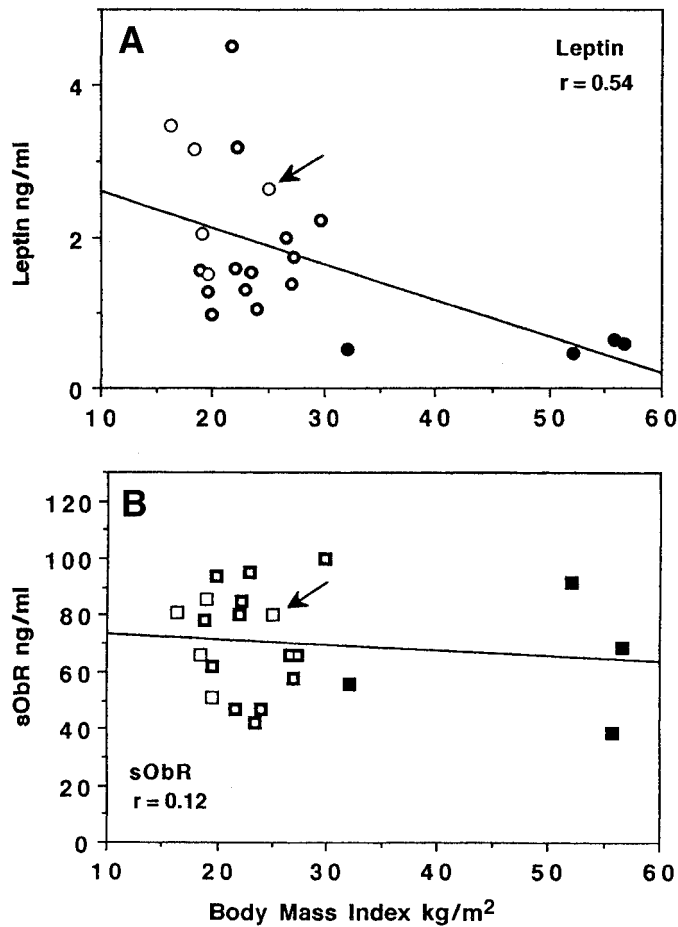


FIG. 3. Correlation of leptin levels (A) and serum leptin receptor (sObR) levels (B) with BMI in leptin-deficient individuals from a consanguineous family harboring a mutation in the leptin gene impairing the secretion of leptin. The arrows point to the wild-type subject from the affected family. ○ and □, lean control subjects from the same geographic area; bold open circles and squares, individuals heterozygous for the mutant gene; ● and ■, individuals homozygous for the mutant gene.

reflects an interindividual difference in the sensitivity to leptin and a marked variation in the inhibitory set point among a population of overweight subjects, depending on various factors, including the duration of hyperleptinemia, as suggested by the experiments in rats.

Concluding remarks. These results provide evidence that leptin is not needed for the expression of leptin receptor gene, and they support the assumption that in common obesity, BMI is the main factor governing the production of sObR and maybe that of ObR. Leptin, alone or in conjunction with other factors related to adiposity, may play an inhibitory role in the regulation of the serum leptin receptor gene expression by regulating either the cleavage process or receptor gene expression (if sObR levels reflect ObR production). Following this assumption, the decrease in leptin receptor expression secondary to the increase in adiposity would impair the access of leptin to the hypothalamus. The apparent resistance to leptin would therefore be a consequence of obesity itself rather than a primary mechanism: obesity, through this vicious circle, might be self-maintained, an assumption consistent with the concept of the “thrifty phenotype” (27).

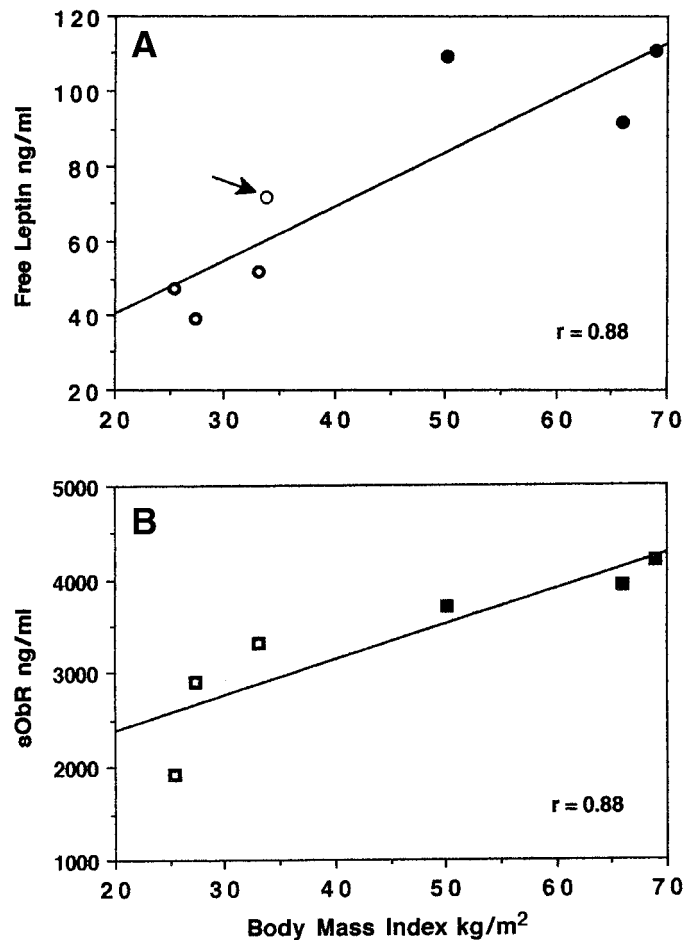


FIG. 4. Correlation of free leptin levels (A) and serum leptin receptor (sObR) levels (B) with BMI in leptin receptor-deficient female subjects from a consanguineous family harboring a mutation in the leptin receptor gene that leads to the production of a truncated receptor not anchored to the cell membrane. The arrows point to the wild-type girl from the affected family. ○ and □, individuals homozygous for the wild type gene; bold open circles and squares, individuals heterozygous for the mutant gene; ● and ■, individuals homozygous for the mutant gene.

ACKNOWLEDGMENTS

This study was supported by Institut de Recherche Endocrinienne et Metabolique (Paris, France).

The authors are indebted to Yvette Le Bihan and Josiane Le Fourn for skillful technical assistance and to Sharon Bowman for the revision of the manuscript. They also wish to thank the Biovendor team for fruitful discussions and help.

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