Obesity Is Associated With a Decreased Leptin Transport Across the Blood-Brain Barrier in Rats

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Leptin exerts important effects on the regulation of food intake and energy expenditure by acting in the brain. Leptin is secreted by adipocytes into the bloodstream and must gain access to specific regions in the brain involved in regulating energy balance. Its action is mediated by interaction with a receptor that is mainly expressed in the hypothalamus but is also present in other cerebral areas. To reach these target areas, leptin most likely needs to cross the blood-brain barrier (BBB). In this study, we compared the permeability of leptin at the BBB in homozygous lean (*FA*/*FA*), high-fat diet–induced (HFD) obese rats (*FA*/*FA* rats on a high-fat diet), and genetically obese *fa*/*fa* Zucker rats by quantifying the permeability coefficient surface area (PS) product after correction for the residual plasma volume (V_p) occupied by leptin in the vessel bed of different brain regions. The intravenous bolus injection technique was used in the cannulated brachial vein and artery using leptin radioiodinated with 2 isotopes of iodine (^{125}I and ^{131}I) to separately determine the PS and V_p values. The PS for leptin at the BBB in lean *FA*/*FA* rats ranged from 11.0 ± 1.6 at the cortex to 14.8 ± 1.4 × 10^{-6} ml · g^{-1} · ml^{-1} at the posterior hypothalamus. The PS for leptin in HFD obese *FA*/*FA* and obese *fa*/*fa* rats ranged from 3.0- to 4.0-fold lower than in lean *FA*/*FA* rats. The V_p values were not significantly different among the 3 groups studied. SDS-PAGE analysis of the radioiodinated leptin after 60 min of uptake revealed intact protein in the 8 different brain regions. Plasma leptin levels were significantly higher in both obese rat groups compared with those in lean *FA*/*FA* rats. Leptin levels in cerebrospinal fluid were not significantly different among the 3 groups of rats. These findings strongly suggest that the leptin receptor (OB-R) in the BBB can be easily saturated. Saturation of the BBB OB-R in obese individuals would explain the defect in leptin transport into the brain described in this study. *Diabetes* 49:1219–1223, 2000

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BBB, blood-brain barrier; BSA, bovine serum albumin; CNS, central nervous system; CSF, cerebrospinal fluid; HFD, high-fat diet–induced; JAK, janus protein-tyrosine kinase; OB-R, leptin receptor; OB-Ra, short-form OB-R; OB-Rd, long-form OB-R; PS, permeability coefficient surface area; STAT, signal transducer and activator of transcription; TCA, trichloric acetic acid; V_p, residual plasma volume.

The discovery of leptin confirmed the hormonal link between the adipocyte and the brain (1,2). Leptin is mainly secreted by the adipocyte, circulates in part while linked to binding proteins (3), and acts in specific regions of the brain to regulate appetite and energy balance. The vast majority of obese humans, however, have markedly elevated plasma leptin concentrations compared with lean individuals (4). In fact, plasma leptin concentrations strongly correlate with the percentage of body fat (4), and leptin levels are reduced in obese subjects who lose weight (4,5). A higher set point of the cerebral adipostat present in obese individuals may be the result of a relative or absolute insensitivity to leptin. Daily injections of leptin decreased appetite and body weight in both *ob*/*ob* mice and wild-type mice and increased energy expenditure in lean new-born Zucker rats (6-8). Leptin deficiency caused genetic obesity in *ob*/*ob* mice as well as in a recently described family kindred (9).

Leptin is recognized by the leptin receptor (OB-R) (10), which is a product of the *db* gene (11–13). In humans and in rodents, different OB-R isoforms have been found to be widely distributed in many organs, including lung, kidney, adipose tissue, and brain (14–18). The short form (OB-Ra) is the characteristic isoform present in the choroid plexus and is considered to act merely as a transporter (10). The OB-R in the hypothalamus appears to have a long intracellular domain (long form or OB-Rb) that contains putative motifs for janus protein-tyrosine kinase (JAK) and signal transducers and activators of transcription (STATs) (19). This intracellular domain is not present in the short form (10). A specific type of obesity has been described as a consequence of OB-R mutation in *db*/*db* mice (11), and a leptin receptor defect has recently been described in humans (20). The *fa*/*fa* Zucker rat was also shown to have a mutation in the extracellular domain of all OB-R isoforms (12), which may affect leptin transport into the brain.

The delivery of leptin into the central nervous system (CNS) seems to represent a crucial step toward the regulation of food intake and energy balance. In the present study, we have evaluated whether *fa*/*fa* and obese rats on a high-fat diet (HFD), as compared with lean rats, might have a defect in leptin access into the brain, which may contribute to the development of their obesity. Like humans, rats fed a high-fat diet become obese despite increased plasma leptin levels (21). This study evaluates the permeability of leptin at the blood-brain barrier (BBB) in lean *FA*/*FA*, HFD obese *FA*/*FA*, and *fa*/*fa* obese rats. Our results indicate a decreased BBB leptin transport in obese rats compared with lean rats, regardless of the etiology of the obesity.

RESEARCH DESIGN AND METHODS

Animals and reagents. All procedures were approved before beginning the study by the Institutional Animal Care and Use Committee at Mayo Clinic and the University of Minnesota. Adult lean *FA*/*FA* rats and genetically obese Zucker *fa*/*fa* rats of 9 weeks were obtained from a colony of Zucker rats maintained at the Hormel Institute. The Hormel Institute Animal Facility is accredited by the American Association for Accreditation of Laboratory Animal Care. The lean *FA*/*FA* rats were divided into 2 groups: 1 group (n = 9) received a control standard diet (Rodent Chow Diet #5001; PMI Nutrition International, St. Louis, MO), and the other group (n = 8) received a high-fat diet (40% fat by wt) (no. TD-97113; Harlan Teklad, Madison, WI) (n = 8) for 10 weeks to induce hypertrophic obesity. The *fa*/*fa* obese group was restricted to receiving 30 g standard diet daily to limit their weight gain. This determination was based on average food intake by the lean *FA*/*FA* group while eating ad libitum. Rooms were temperature- (21°C) and humidity-controlled and maintained with a 12-h light/dark cycle. The rats had free access to water. The leptin was a gift from Eli Lilly Laboratories (Indianapolis, IN). Rat leptin radioimmunoassay kits were obtained from Linco Research (St. Charles, MO).

Protein radioiodination. Aliquots of leptin were labeled with ^{125}I or ^{131}I using the chloramine-T procedure as previously described (22). Free radioactive iodine was separated from the radiolabeled proteins by dialysis against 0.2 mol/l NaI. Purity of the radiolabeled leptin was determined by paper chromatography (22). The amount of radiolabeled leptin that stayed at the origin was always >99%.

Plasma pharmacokinetics of leptin. The integrity of the radioiodinated leptin in rat plasma after intravenous bolus injection was evaluated by trichloric acetic acid (TCA) precipitation and paper chromatography (Tables 1 and 2). Radioiodinated leptin was injected into the brachial vein of every animal. Blood was sampled from the brachial artery over the next 15 min. Aliquots of plasma were removed for protein determination and analyzed by TCA precipitation and paper chromatography. An aliquot of the plasma (0.3 µg protein) was combined with 2% (wt/vol) bovine serum albumin (BSA). An equal volume of 30% TCA was added and centrifuged at 5,000*g*, and the supernatant and pellet were separated to determine the percent TCA precipitation of the radioactive leptin. A separated aliquot (5 µl plasma) was also spotted on paper and chromatographed in a closed chamber with 0.9% NaCl. In this solvent system, intact radiolabeled peptides stay at the origin. Iodotyrosine and Na-^{125}I were determined to migrate at the solvent front. For both the TCA precipitation and paper chromatography, plasma from separate animals was spiked with ^{125}I-leptin, processed in an identical manner, and served as a processing control to determine the extent of in vitro degradation.

Permeability coefficient surface area and residual plasma volume measurements of radioiodinated leptin. Permeability coefficient surface areas (PS) and residual plasma volume (V_p) measurements were performed as previously described (22–24). The intravenous bolus injection technique was used. A bolus of phosphate-buffered saline containing ^{125}I-labeled leptin was rapidly injected into the brachial vein of a pentobarbital anaesthetized rat. Serial blood samples were obtained from the brachial artery over the next 30 min for leptin determination. At 15 s before death, the second isotope of ^{131}I-labeled leptin was then intravenously administered to serve as the residual V_p indicator. After the final blood sample was collected, the rat was immediately killed by cardiac incision, and the brain was removed. The brain was dissected into cortex, anterior, and posterior hypothalamus; caudate-putamen; hippocampus; thalamus; brain stem; and cerebellum. Tissues were placed into preweighed vials. Lyophilized and dry weights were determined with a microbalance. Weights were then converted to their respective wet weights with wet weight–to–dry weight ratios previously determined for the different brain regions. Tissue and plasma samples were assayed for ^{125}I and ^{131}I radioactivity in a 2-channel gamma counter with correction for crossover of ^{131}I activity into the ^{125}I channel for background activity. Calculations were based on a multiple-passage single–time point technique with a 2-compartment analysis as previously described (24).

TABLE 1
Plasma pharmacokinetics of ^{125}I-leptin

	TCA precipitation (%)		
	Lean	HFD	*fa*/*fa*
n	9	8	8
Preinjection	99.0	97.2	89.2
Process control	98.9	93.7	89.3
Plasma washout (min)*			
0.25	98.1 ± 0.5	97.3 ± 0.5	96.2 ± 0.4
0.5	98.6 ± 0.2	97.6 ± 0.3	96.9 ± 0.3
1	98.3 ± 0.4	97.7 ± 0.3	96.2 ± 0.4
3	98.1 ± 0.5	97.3 ± 0.5	96.2 ± 0.4
5	98.1 ± 0.5	97.3 ± 0.5	96.2 ± 0.4
7	98.1 ± 0.3	97.1 ± 0.2	96.2 ± 0.5
10	95.9 ± 0.8	95.4 ± 0.7	94.6 ± 0.8
15	88.7 ± 2.1	91.3 ± 1.5	89.8 ± 1.5

Data are *n*, %, or means ± SE. *Initiated after intravenous bolus injection.

TABLE 2
Plasma pharmacokinetics of ^{125}I-leptin

	Chromatography origin (%)		
	Lean	HFD	*fa*/*fa*
n	9	8	8
Preinjection	83.7	85.0	85.0
Process control	95.8	99.0	86.4
Plasma washout (min)*			
0.25	93.3	95.4	91.4
0.5	94.1	95.4	91.5
1	94.1	95.3	91.4
3	93.3	94.9	91.4
5	92.0	93.7	90.0
7	91.3	92.6	86.3
10	89.9	91.0	83.5
15	82.6	87.5	78.1

Data are *n* or %. *Initiated after the intravenous bolus injection.

Collection of plasma and cerebrospinal fluid for leptin assay. Blood samples were withdrawn from the brachial artery at the outset of the study before the measurement of PS and V_p, were allowed to clot on ice, and were centrifuged for 10 min at 10,000*g* at 4° C; the serum was frozen at –70° C until assayed for leptin. Cerebrospinal fluid (CSF) was obtained by inserting a 1-ml needle into the cisterna magna. CSF (100–150 µl) was gently withdrawn. The samples of CSF were centrifuged to remove possible erythrocyte contamination. Equal volumes of CSF from 4 or 5 CSF samples from rats of the same group were pooled, freeze-dried, and reconstituted to obtain sufficient quantities for assay. The leptin concentration in the CSF was calculated as previously described by Wu-Peng et al. (25).

Statistics. Data are presented as means ± SE. Statistical evaluations were performed using the Student's 2-tailed paired t test with significance accepted at $P < 0.05$.

RESULTS

Rat weight, serum, and CSF leptin levels. Body weights of lean *FA*/*FA*, HFD obese *FA*/*FA*, and obese *fa*/*fa* rats are summarized in Table 3. HFD obese *FA*/*FA* rats gained a significant amount of body weight compared with the *FA*/*FA* lean rats fed a standard diet ($P < 0.001$). The obese *fa*/*fa* group was significantly heavier compared with lean *FA*/*FA* ($P < 0.001$) and HFD obese *FA*/*FA* rats ($P < 0.001$). The obese *fa*/*fa* rats had been food-restricted to limit their weight gain. Less obese *fa*/*fa* rats would have lower plasma leptin levels and would therefore be less likely to exhibit transport saturation phenomena. The HFD obese *FA*/*FA* and the obese *fa*/*fa* rats had significantly higher plasma leptin concentrations than the lean *FA*/*FA* rats ($P < 0.001$). Plasma leptin concentrations were also significantly different between the 2 obese groups ($P < 0.001$) (Table 3). CSF leptin concentrations were not significantly different among groups.

PS and V_p values for leptin. PS and V_p values for leptin across the BBB in lean *FA*/*FA*, HFD obese *FA*/*FA*, and obese

TABLE 3
Body weight and serum and CSF leptin levels in lean *FA/FA*, HFD obese, and *fa/fa* rats

	Lean *FA/FA*	Obese HFD	*fa/fa*
Body weight (g)	440.0 ± 53.2	547.0 ± 34.9*	686.6 ± 42.6*
Serum leptin levels (ng/ml)	8.8 ± 9.6	14.3 ± 7.1*	45.4 ± 16.3*
CSF leptin levels (ng/ml)	0.19 ± 0.03	0.21 ± 0.08	0.24 ± 0.06

Data are means ± SE. To obtain sufficient CSF for leptin assay, equal volumes of 4 or 5 CSF samples from rats of the same group were pooled and run in duplicate. *P < 0.001 vs. lean *FA/FA* rats.

fa/*fa* rats are shown in Table 4. The PS for leptin at the BBB in lean *FA*/*FA* rats ranged from 11.0 ± 1.6 at the cortex to $14.8 \pm 1.4 \times 10^{-6}$ ml · $g^{-1} \cdot s^{-1}$ at the posterior hypothalamus. No significant differences in leptin PS values were found among the different brain areas. HFD obese *FA*/*FA* rats had a significantly lower leptin PS in all the different brain regions evaluated when compared with lean *FA*/*FA* rats. The HFD obese *FA*/*FA* rats were significantly heavier and also had higher levels of plasma leptin. The second model of obesity, the *fa*/*fa* Zucker rat, also showed a PS for leptin across the BBB that was 2- to 3-fold lower than in lean *FA*/*FA* rats, but was no different compared with HFD obese *FA*/*FA* rats. The V_p values were not significantly different among the 3 groups.

DISCUSSION

In animals and humans, leptin appears to be a major signal to the brain of peripheral fat deposits. Leptin exerts its actions mainly in the hypothalamus (14,15), and these responses are mediated by interaction with leptin's specific OB-R (10).

How leptin gains access to specific regions in the brain is still a controversial issue. Different studies have shown the presence of specific OB-R in the brain capillaries (26–28) as well as binding of ^{125}I-leptin to human (26) and mouse (29) brain capillaries. We also recently confirmed the presence of the OB-Ra and, to a lesser extent, OB-Rb in the endothelium of the human brain (30). The presence of a specific OB-R in the endothelial cells would allow leptin to gain access through the capillary wall to the specific hypothalamic nuclei and other cerebral areas in order to exert its effects.

The present investigation showed that PS for leptin in HFD obese *FA*/*FA* rats and in obese *fa*/*fa* rats was lower than the PS observed in lean *FA*/*FA* rats, suggesting that obesity is associated with a decreased leptin transport at the BBB. The OB-R in the BBB appears to be easily saturated. The reduced leptin uptake in obese rats is probably due to increased competition from higher concentrations of endogenous leptin. This hypothesis is supported by the observation that the reduction of PS in HFD obese rats compared with that in lean *FA*/*FA* rats is equivalent (2-fold in the hypothalamus) to the increase in plasma leptin levels. The PS for leptin in obese *fa*/*fa* rats, which exhibited significantly higher plasma leptin levels than the HFD obese group, was not significantly lower than that in the HFD obese group. The obese *fa*/*fa* rat has a mutation in the extracellular domain of the OB-R, and the lack of lower PS in this group compared with the obese HFD *FA*/*FA* is probably secondary to a floor effect. The saturation of the OB-R in the obese HFD *FA*/*FA* group would explain the similarities in leptin PS levels between this group and the obese *fa*/*fa* model.

TABLE 4
PS and V_p at the BBB for leptin

		Lean vs. HFD		Lean vs. *fa/fa*		HFD vs. *fa/fa*
	Lean	HFD	*P*	*fa/fa*	*P*	*P*
PS (ml · $g^{-1} \cdot s \times 10^{-6}$)						
Anterior hypothalamus	11.7 ± 1.9	5.9 ± 1.2	<0.05	5.4 ± 2.2	<0.05	NS
Posterior hypothalamus	14.8 ± 1.4	6.1 ± 1.1	<0.01	6.7 ± 1.9	<0.01	NS
Cortex	11.0 ± 1.6	3.2 ± 0.7	<0.001	2.5 ± 0.8	<0.001	NS
Caudate-putamen	11.2 ± 1.2	3.1 ± 0.6	<0.001	2.7 ± 0.6	<0.001	NS
Hippocampus	12.0 ± 1.5	4.4 ± 0.7	<0.001	4.7 ± 0.7	<0.001	NS
Thalamus	11.7 ± 1.4	4.6 ± 0.7	<0.001	4.2 ± 1.2	<0.001	NS
Brainstem	14.4 ± 1.7	5.5 ± 1.3	<0.001	5.5 ± 1.3	<0.001	NS
Cerebellum	14.3 ± 1.7	4.8 ± 0.9	<0.001	5.5 ± 1.6	<0.001	NS
V_p (μl/g)						
Anterior hypothalamus	14.1 ± 1.3	13.2 ± 0.6	NS	14.8 ± 0.9	NS	NS
Posterior hypothalamus	14.1 ± 1.2	14.9 ± 1.9	NS	16.6 ± 1.6	NS	NS
Cortex	15.1 ± 1.2	16.5 ± 1.4	NS	17.8 ± 1.4	NS	NS
Caudate-putamen	8.5 ± 0.4	8.1 ± 0.6	NS	11.3 ± 1.2	NS	NS
Hippocampus	10.6 ± 0.9	10.8 ± 1.0	NS	12.1 ± 1.3	NS	NS
Thalamus	14.5 ± 1.5	13.9 ± 1.1	NS	18.9 ± 3.2	NS	NS
Brainstem	23.2 ± 2.1	20.5 ± 1.6	NS	27.0 ± 2.7	NS	NS
Cerebellum	17.2 ± 1.3	18.1 ± 2.4	NS	26.3 ± 1.7	<0.01	<0.05

Data are means ± SE. NS, not significant.

Interestingly, despite significantly higher plasma leptin levels in both obese rat groups compared with lean *FA*/*FA* rats, the CSF leptin levels were not significantly different among the 3 groups. In humans, leptin concentrations in the CSF are reported to be correlated, in a nonlinear manner, with plasma leptin levels and BMI (31). Two reports have shown that the ratio of CSF-to–serum leptin levels was 4 times higher in lean individuals compared with obese subjects, suggesting that obesity in humans could be secondary to a central resistance to leptin action rather than to an inadequate production of leptin (32,33). The lack of leptin access into the brain could then be responsible for obesity (32–35). Several authors have suggested that leptin crosses the BBB by a saturable transport mechanism (33,36). The presence of saturable transport mechanisms between the blood and the CSF could explain why a significant increase in the levels of plasma leptin, as occurs in obesity, corresponds with smaller changes of leptin levels in the CSF. We hypothesize that the brain in hyperleptinemic obese individuals may not be exposed to high concentrations of leptin. The physiological brain response to hyperleptinemia would suppose a disadvantage in a situation of famine. Hyperleptinemia is a relatively young phylogenetic phenomenon, and it is possible that the human brain has not yet learned to deal with it (34,35).

Banks et al. (29) demonstrated an insulin-independent saturable transport for radioiodinated leptin into the brain in mice after intravenous administration. Leptin does not leave the bloodstream until reaching the particular target areas by this mechanism (29). In agreement with these studies, Karonen et al. (36) showed that the in vivo uptake of ^{123}I-leptin occurred in the choroid plexus, lung, and kidney of rabbits. In support of this concept, the expression of the OB-Ra and OB-Rb mRNA in rat brain microvessels was recently described by Bjorbaek et al. (27) using in situ hybridization and reverse transcriptase–polymerase chain reaction analysis (27). This short isoform probably participates in the transport of leptin across cellular barriers, such as the choroid plexus and the BBB endothelium (10).

Boado et al. (37) have recently shown that OB-Ra is the most abundant OB-R isoform at the BBB. Furthermore, they found that BBB OB-Ra transcript content was markedly increased in rats fed a high-fat diet compared with controls. It is intriguing that despite higher levels of OB-Ra at the BBB, rats that consumed the high-fat diet gained significantly more weight than those that consumed the control diet. OB-Ra is most likely involved in the regulation of leptin availability to brain cells; however, its capacity to perform these functions may be limited in certain circumstances, such as those involving a high-fat diet or hyperleptinemia. Higher OB-R levels at the BBB in response to high caloric intake might be a physiological response to allow leptin to gain access to specific hypothalamic/pituitary areas involved in growth and reproduction. The decreased leptin BBB permeability that we have seen in the present studies might be secondary to the higher levels of circulating leptin present in both obese groups. This hyperleptinemia is most likely saturating the OB-R at the BBB level. In the study of Boado et al. (37), rats that consumed the high-fat diet had levels of leptin similar to those in the control group (37).

The transport of leptin into the CNS represents a crucial step toward the regulation of food intake and energy balance, and it may possibly occur by more than one route. In addition to the BBB, the choroid plexus, the ventricular system, and the circumventricular organs could also be involved in leptin transport (38).

A recent report by Wiesner et al. (39) has suggested the possibility that the human brain produces leptin. They noticed that concentrations of leptin in the internal jugular vein were significantly higher than arterial levels in both lean women and obese men. However, their study did not reveal any regionalization of brain leptin secretion. Our group has recently shown that leptin is expressed by most human anterior pituitary cell types and that there is a decreased leptin protein immunoreactivity in pituitary adenomas compared with that in normal pituitary tissues (40). Studies are currently being performed to address if other brain areas are involved in leptin production and whether obese subjects may have a defect of cerebral leptin production.

Studies evaluating the existence of a putative autocrine/paracrine loop involving leptin and other appetite neuromodulators may help to understand leptin's actions in the brain and explain why obese individuals become overweight despite high circulating levels of plasma leptin.

This study presents conclusive evidence of a defect of leptin transport at the BBB associated with both dietary and genetic obesity. Our data suggest that the leptin transport system is saturated near physiological concentrations in lean individuals, which implies that the elevated leptin levels observed in obesity can produce no biological effects because the system is already saturated. Saturability of the leptin system seems to be a major explanation for the apparent leptin resistance associated with obesity.

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