Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible

William A. Banks and Catherine L. Farrell

1Geriatrics Research, Educational, and Clinical Center, Veterans Affairs Medical Center-St. Louis and St. Louis University School of Medicine, St. Louis, Missouri 63106; and 2Amgen, Inc., Thousand Oaks, California 91320

Submitted 30 October 2002; accepted in final form 28 February 2003

Banks, William A., and Catherine L. Farrell. Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible. Am J Physiol Endocrinol Metab 285: E10–E15, 2003. First published March 4, 2003; 10.1152/ajpendo.00468.2002.—Leptin resistance is a major cause of obesity in humans. A major component of this resistance is likely an impaired transport of leptin across the blood-brain barrier (BBB). The fattest subgroup of otherwise normal 12-mo-old CD-1 mice have severely impaired transport of leptin across the BBB. However, it is unknown whether these mice are born with a BBB impairment or acquire it with aging and obesity. Here, we found within an otherwise normal population of CD-1 mice that the 10% fattest mice gained weight throughout a 12-mo-life span, whereas the 10% thinnest mice gained little weight after 3 mo of age. The fattest mice acquired a progressive impairment in their ability to transport leptin across the BBB, whereas the thinnest mice had a rate of transport that did not change with age. Fasting fat mice for 24 h or treating them with leptin resulted in modest weight reduction and development of transport rates for leptin across the BBB similar to those of thin mice. These results show that, in obese CD-1 mice, the impaired transport of leptin across the BBB develops in tandem with obesity and is reversible with even modest weight reduction.

HUMAN OBESITY represents a resistance state to leptin. Resistance is likely caused by a combination of resistance at the receptor and postreceptor levels as well as a decreased ability of the blood-brain barrier (BBB) to transport circulating leptin into the brain. Several studies have documented various types of evidence for impaired transport of leptin across the BBB. For example, obese humans have a decreased cerebrospinal fluid-to-serum ratio for leptin (8, 22), and some obese rats who no longer respond to peripherally administered leptin can still respond to leptin given directly into the central nervous system (11, 23). Direct impairment of leptin transport across the BBB has been shown in obese rats and mice (5, 7, 9, 17).

The question remains whether defects in leptin transport are inherent in animals destined to become obese or are acquired with the obesity. This question is key to understanding the role of the BBB in leptin resistance and the treatment of obesity. If the leptin transporter is defective before the onset of obesity, then this would suggest that the defect plays a fundamental role in the development of obesity. It would further suggest that the propensity to obesity and the degree to which an individual will become obese can be determined by examination of BBB properties. If the BBB defect is acquired, then this would suggest that some factor, perhaps reversible, that arises with the development of obesity induces the BBB defect. A positive feed-forward cycle of increasing obesity, BBB transport impairment, and leptin resistance could arise. Such an acquired impairment, however, might be reversed if the responsible factor could be identified.

Here, we used the model of obesity of maturity to examine whether the defects in leptin transport are acquired or inherent. This model depends on the natural propensity of a subgroup of otherwise normal 12-mo-old CD-1 mice to gain weight as they age (21). Among 12-mo-old males, the heaviest 10% weighs ∼60% more than the lightest 10% (3). Adiposity, not muscle or bone, accounts for most of this difference in weight (2, 21). These studies also found that, by 12 mo of age, the 10% heaviest mice transport intravenously administered leptin across the BBB at a rate only about one-third that of the 10% lightest mice. In contrast, aging and the increased somatic growth that accompanies aging in mice did not have an effect on leptin transport. This was shown by the similar transport rate for leptin in 12-mo-old mice and 2-mo-old mice selected to represent the median weights for their age groups.

Here, we first confirmed and extended the previous finding by measuring the transport rate of leptin across the BBB for a range of ages between 6 wk and 12 mo. We then examined transport rates for a full range of body weights, not just the top and bottom 10%, within an age group. Finally, we compared the effect on leptin transport of body weight loss induced either by fasting or by administering leptin.

METHODS

Mice and housing. CD-1 male mice (Charles River Labs, Wilmington, MA) were housed in 12:12-h light-dark condi-
BBB TRANSPORT OF LEPTIN IN OBESITY

E11

...tions. Mice were acquired either at the age of 4 wk or as 4-mo-old retired breeders. Mice were between 2 wk and 10 mo at the Amgen facility. Mice received food and water ad libitum. Where indicated, other mice were obtained from the breeding colonies of CD-1 mice at the Veterans Affairs Medical Center (VA) in St. Louis, MO. All studies were performed under approved protocols.

Leptin. Leptin (Amgen, Thousand Oaks, CA) was radioactively labeled by the enzyneboid method (Pierce, Rockford, IL) with $^{125}$I purchased from Amersham Pharmacia (Piscataway, NJ). Radioactively labeled leptin (I-Lep) was separated from free iodine on a Sephadex G-10 column. The I-Lep had a specific activity of $\sim 100–125$ Ci/g and was stored at 4°C until use. Albumin was labeled with $^{131}$I by the chloramine-T method and purified on a column of Sephadex G-10.

Measurement of leptin transport rate into brain. The unidirectional influx rate ($K_i$) from blood to brain was measured by multiple-time regression analysis (6, 20). Mice were anesthetized with urethane, and the left jugular vein and right carotid artery were exposed. Mice were given an injection into the proximal trunk of 0.2 ml of lactated Ringer (LR) solution containing 1% bovine serum albumin (BSA) and $\sim 10^5$ cpm of I-Lep. Blood from the carotid artery was obtained between 1 and 10 min after intravenous injection and centrifuged for 10 min at 5,000 g at 4°C, and the radioactivity level in the serum was determined. Immediately after collection of the arterial blood, the mouse was decapitated, and the level of the radioactivity in the whole brain (with the pituitary and the pineal gland removed) was determined. The brain-to-serum (brain/serum) ratios of I-Lep in units of microliters per gram ([counts/min / (cpm/g/µl serum)] were plotted against exposure time (Expt, in min)

$$\text{Expt} = \left[ \int_0^t C_p(t) \cdot \tau \cdot \text{d}t \right] / C_p(t)$$

where $C_p$ is the level of radioactivity in serum at time $t$ (6, 20). The slope of the linear portion of the relation between the brain/serum ratios and Expt was calculated by the least squares method and measures the $K_i$ from blood to brain; it is reported with its standard deviation of the residuals.

Capillary depletion. This method is used in rats (22a) and mice (10) to determine the extent to which a substance taken up by the BBB is either being retained by capillaries or completely crosses the capillary wall to enter the brain parenchyma. We determined whether thin and fat mice differed in this regard. The 10% heaviest or 10% lightest mice from a group aged 8 wk (from St. Louis VA) were selected and anesthetized with urethane. The mice were given an intravenous injection of $1 \times 10^6$ cpm, and 10 min later brain and arterial blood was collected as we have described. Serum (50 µl) was mixed thoroughly with 0.25 ml of LR-BSA and 0.25 ml of 30% TCA. It was centrifuged at 5,000 g at 4°C for 10 min, and the resulting pellet and supernatant were counted. Each whole brain was mechanically homogenized in 2 ml of water containing 0.25 mM each of EDTA, t-thyroxine, N-ethylmaleimide, and 1,10-phenanthroline. The homogenate was centrifuged at 5,000 g for 10 min, and the supernatant was collected. Brain supernatant (0.25 ml) was vigorously mixed with 0.25 ml of 30% TCA and centrifuged at 5,000 g for 10 min; the resulting supernatant and pellet were collected. To determine degradation of I-Lep that occurred ex vivo (processing controls), 100 µl of I-Lep in LR-BSA solution were placed on the surface of a nonradioactive mouse brain or in a tube used to obtain carotid blood, and the samples were processed as described above. The percentage of radioactivity precipitated by acid was calculated as the percentage of total cpm (supernatant + pellet cpm) found in the pellet.

Leptin transport with onset of obesity of maturity. Groups of 100 mice were housed until 6 wk or 2, 3, 6, 9, or 12 mo of age. The mice were then weighed, and the 10% heaviest (Fat) and 10% lightest (Thin) mice were selected. $K_i$ was determined as described above.

Relation between body weight and leptin transport at 5 mo of age. Retired breeder mice aged 5 mo ($n = 100$) were weighed and assigned numbers from 1 (lightest) to 100 (heaviest) and segregated into groups of 10. The mice were measured in the following groups: 1–10 (smallest), 21–30 (next smallest), 41–50 (low medium), 51–60 (high medium), 71–80 (next largest), and 91–100 (largest).

Effects of fasting and leptin administration. Retired breeder mice aged 5 mo (from St. Louis VA) were weighed, and the 10% heaviest (Fat) and 10% lightest (Thin) mice were selected. The heaviest mice were divided into three groups, whereas only one of every three of the lightest mice was used. All mice received an intraarterial injection of phosphate-buffered solution in the morning for four consecutive days (days 1–4). Leptin (10 mg/kg) was included in the injections of one of the groups of heavy mice (Fat-Leptin group). Another heavy group was fasted for 24 h starting on the morning of day 4 (Fat-Fasted group). The other heavy group (Fat-Control group) and the light group (Thin-Control group) received the intraperitoneal injections of PBS but no leptin; they were not fasted. Leptin uptake was studied on the morning of day 5 by injecting I-Lep intravenously and collecting brain and serum samples 10 min later, as described above.

Ratio = (cpm Fr)/(w)(cpm/µl serum)

where cpm Fr is the cpm in either the parenchyma or supernatant fraction, $w$ is the weight of the cortex, and cpm/µl serum is the level cpm in a microliter of serum.

Identification of radioactivity in serum and brain. Previous work has shown that acid precipitation can be used to accurately determine how much of the radioactivity in serum and brain I-Lep represents (4). Here, we determined whether the radioactivity recovered from the serum and brains of thin and obese mice differed in its I-Lep representation. The 10% heaviest or 10% lightest mice from a group aged 8 wk (from St. Louis VA) were selected and anesthetized with urethane. The mice were given an intravenous injection of $1 \times 10^6$ cpm, and 10 min later brain and arterial blood was collected as we have described. Serum (50 µl) was mixed thoroughly with 0.25 ml of LR-BSA and 0.25 ml of 30% TCA. It was centrifuged at 5,000 g at 4°C for 10 min, and the resulting pellet and supernatant were counted. Each whole brain was mechanically homogenized in 2 ml of water containing 0.25 mM each of EDTA, t-thyroxine, N-ethylmaleimide, and 1,10-phenanthroline. The homogenate was centrifuged at 5,000 g for 10 min, and the supernatant was collected. Brain supernatant (0.25 ml) was vigorously mixed with 0.25 ml of 30% TCA and centrifuged at 5,000 g for 10 min; the resulting supernatant and pellet were collected. To determine degradation of I-Lep that occurred ex vivo (processing controls), 100 µl of I-Lep in LR-BSA solution were placed on the surface of a nonradioactive mouse brain or in a tube used to obtain carotid blood, and the samples were processed as described above. The percentage of radioactivity precipitated by acid was calculated as the percentage of total cpm (supernatant + pellet cpm) found in the pellet.
To determine what factors other than loss of fat might have accounted for a decrease in body weight, randomly selected retired breeders (from St. Louis VA) were treated as above (control, 24-h fast, lepin treatment). On the morning of day 5, mice were given an intravenous injection of albumin radioactively labeled with $^{131}$I ($2.5 \times 10^5$ cp/mouse). Ten minutes later, arterial blood was taken from the carotid artery, and the level in serum of radioactive albumin was determined. The vascular space was calculated as the cpm injected intravenously ($2.5 \times 10^5$ cp/mouse) divided by the cpm present in a milliliter of arterial serum. Leptin levels were also measured in serum with the murine leptin ELISA kit (Linco, St. Charles, MO). The entire gastrointestinal tract was removed, weighed, washed free of food and fecal material, and reweighed.

**Statistics.** Means are presented with their SE, and the number of mice in the statistical cell is given (n). Means were compared by ANOVA followed by the Newman-Keuls range test when appropriate. Regression lines were calculated by the least squares method and compared statistically with the software package in Prism 3.0 (GraphPad, San Diego, CA).

**RESULTS**

**Leptin transport and body weight.** Mice between the ages of 6 wk and 12 mo were studied. The rate at which the 10% thinnest mice gained weight decreased greatly after ~3 mo of age, so that 12-mo-old thin mice weighed only ~10% more than 3-mo-old thin mice (Fig. 1A). In comparison, the fat mice continued to gain weight, so that 12-mo-old fat mice weighed 46% more than 3-mo-old fat mice.

The rate of entry ($K_i$) of I-Lep did not change with aging in the thin mice (Fig. 1B), with values ranging from 0.45 to 0.62 $\mu$g $g^{-1} \cdot min^{-1}$. In obese mice, the $K_i$ changed significantly as a function of age, decreasing from a high at 6 wk of 0.482 $\mu$g $g^{-1} \cdot min^{-1}$ to a low at 12 mo of 0.176 $\mu$g $g^{-1} \cdot min^{-1}$ ($r = 0.900, n = 6, P < 0.05$). The results in Fig. 1B were submitted to two-way ANOVA, with age and weight as independent variables and $K_i$ as the dependent variable. The results showed a significant effect for weight ($F(1,87) = 9.77, P < 0.005$) but not for age or interaction.

Capillary depletion (Table 1) showed no difference between thin and fat mice in the ability of I-Lep to completely cross the capillary wall of the BBB once it was taken up by brain endothelial cells. Recovery of radioactivity from brain and serum did not differ between thin and fat mice. Table 1 shows that, in both thin and fat mice, essentially all of the recovered radioactivity was identified as I-Lep.

**Relation between body weight and leptin transport at 5 mo of age.** The heaviest mice weighed 48% more than the lightest mice. Figure 2 shows the values for $K_i$ for the six groups. The inset shows the correlation between $K_i$ and body weight, which was statistically significant ($r = 0.926, n = 6, P < 0.01$).

**Effects of fasting and leptin administration.** ANOVA showed differences among the four groups for both body weights [$F(3,34) = 65.6, P < 0.001$] and leptin uptake rates [$F(3,34) = 8.53, P < 0.001$]. Each group contained 10 mice except the Fat-Fasted group, which contained 8 mice. The Fat-Control group weighed ~12.5 g (~34%) more than the Thin-Control group ($P < 0.001$; Fig. 3A). The Fat-Fasted mice lost ~5.5 g; this group was statistically different from both the Fat-Control ($P < 0.001$) and Thin-Control ($P < 0.001$) groups. The Fat-Leptin group lost about one-half this much weight and did not differ statistically from the Fat-Control group. In comparison, both fasting and

### Table 1. Capillary depletion and acid precipitation in thin vs. fat mice

<table>
<thead>
<tr>
<th></th>
<th>Thin</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary depletion</td>
<td>$n = 7$</td>
<td>$n = 4$</td>
</tr>
<tr>
<td>Parenchyma</td>
<td>2.73 ± 0.34</td>
<td>2.19 ± 0.33</td>
</tr>
<tr>
<td>Capillary</td>
<td>4.03 ± 0.42</td>
<td>2.83 ± 0.52</td>
</tr>
<tr>
<td>Acid precipitation</td>
<td>$n = 2$</td>
<td>$n = 2$</td>
</tr>
<tr>
<td>Serum</td>
<td>90.3 ± 1.1</td>
<td>93 ± 0.1</td>
</tr>
<tr>
<td>Brain</td>
<td>85.4 ± 1.6</td>
<td>96.0 ± 2.1</td>
</tr>
</tbody>
</table>

Values for parenchymal and capillary compartments (capillary depletion, means ± SE) are in μl/g, %P, % cortical labeled leptin (I-Lep) found in parenchymal compartment. Distribution did not differ with body wt. Recovery of radioactivity (acid precipitation, means ± SE) did not differ significantly between thin and fat mice and was mainly identified as I-Lep. Control values for serum and brain were 89 and 83.9%, respectively.
leptin administration increased the uptake of leptin (Fig. 3B), so that each group differed from the Fat-Control group \(P < 0.001\) but not from the Thin-Control group.

In other randomly selected retired breeders, both fasting and leptin administration significantly reduced body weight. However, these treatments had no statistically significant effect on vascular space or gastrointestinal weights (Table 2), thus ruling out the possibilities that weight loss was caused by dehydration or lack of contents in the gastrointestinal tract rather than loss of fat. There was no significant difference among serum leptin levels. This shows that the increase in transport of I-Lep cannot be explained by a decrease in the competition from elevated endogenous levels of serum leptin.

**DISCUSSION**

These results show that the defect in leptin transport is acquired concurrently with increasing body weight in an outbred strain of mouse. The propensity to gain weight with aging was indicated by initial body weight at 6 wk of age. The heaviest and lightest groups of mice experienced a growth spurt until about 3 mo of age. After that, the lightest mice gained little weight over the next 9 mo, whereas the heaviest mice continued to gain weight throughout the study.

As CD-1 mice age, they gain both lean and adipose tissues. However, the differences in body weight within an age group largely reflect differences in body fat. Previous studies have shown that such differences in weight among aged CD-1 mice are caused almost exclusively by differences in adiposity and not by differences in lean body mass (2, 21). Those studies also showed that use of body mass index, which corrects for differences in lean mass by dividing body weight by body length, produces results for leptin transport almost identical to those that use body weight (3). Therefore, body weight can be used within an age group to differentiate fat from thin CD-1 mice.

We found that, whereas \(K_i\) differed little among thin mice at various ages, fat mice showed a steady decrease in \(K_i\). The lack of change in the \(K_i\) for thin mice over time suggests that the defect in leptin transport is not caused by differences in body fat mass. However, the lack of change in \(K_i\) for thin mice could be due to differences in other factors, such as leptin receptor expression or binding affinity.

**Table 2. Effects of fasting and leptin treatment on body wt, gastrointestinal wt, vascular space, and serum leptin levels**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>24-h Fast</th>
<th>IP Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>31.5 ± 4.7</td>
<td>22.6 ± 3.9</td>
<td>29.8 ± 2.8</td>
</tr>
<tr>
<td>%Body wt lost, g</td>
<td>0.60 ± 1.37</td>
<td>9.32 ± 0.48*</td>
<td>4.08 ± 0.63*</td>
</tr>
<tr>
<td>GI wt, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.23 ± 0.57</td>
<td>4.83 ± 0.35</td>
<td>4.89 ± 0.33</td>
</tr>
<tr>
<td>GI only</td>
<td>2.57 ± 0.19</td>
<td>2.81 ± 0.21</td>
<td>2.53 ± 0.15</td>
</tr>
<tr>
<td>Contents</td>
<td>2.67 ± 0.40</td>
<td>2.03 ± 0.15</td>
<td>2.36 ± 0.28</td>
</tr>
<tr>
<td>Vascular space, ml</td>
<td>2.25 ± 0.16</td>
<td>1.98 ± 0.16</td>
<td>1.78 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 6\) for all groups. %Body wt lost, change in body wt between day 1 and day 5 of study. GI Total wt, food and fecal material (Contents) in gastrointestinal (GI) tract. GI Only wt, GI tract washed free of these contents. *\(P < 0.05\) vs. control.
shows that aging up to 12 mo does not itself affect leptin transport across the BBB. This is consistent with other work showing that CD-1 mice of mean body weight for a given age do not have age-related alterations in leptin transport until after 12 mo of age (3). Fat mice, in contrast, had a dramatic decrease in Kᵢ with age and increasing adiposity. Capillary depletion and acid precipitation showed that this decreased uptake was not caused by leptin being sequestered by the capillaries, an inability of leptin to cross the BBB once taken up by brain endothelial cells, or by the accumulation of degradation products in brain or blood.

The relation between body weight and Kᵢ was also evident within a group of mice aged ~5 mo. The fattest mice weighed ~50% more than the thinnest mice and had a Kᵢ that was only about one-third that of the thin mice.

The obesity-related reduction in the Kᵢ for intravenously injected leptin consists of two components (3). About one-third of the decrease is attributed to inhibition by the higher serum leptin levels seen in obesity. About two-thirds of the decrease is caused by a reduced capacity of the leptin transport system. The nature of this leptin transport system has yet to be worked out in detail. Evidence strongly suggests that the short form of the leptin receptor is a component of this system (12, 13). Other evidence shows that leptin transport can occur independently of leptin receptor function (5, 17, 24). Work with various models of obese rodents having defects in the leptin receptor or in leptin protein expression suggests that there are inducible and noninducible components to the leptin transport system (19). These findings raise the question of whether reversal of obesity would lead to recovery of leptin transport function.

We therefore reduced the weight of fat mice either by fasting or by treating the mice with leptin. We ruled out dehydration by measuring the serum vascular space for albumin and loss of gastrointestinal contents as possible explanations for body weight loss. Mice were only fasted for 24 h, as fasts of 72 h or more inhibit the leptin transporter (14). Either treatment increased the transport of intravenously injected I-Lep to levels seen in thin mice. Fasting for 24 h can reduce serum leptin levels (18). Because the last dose of leptin was given 24 h before study of I-Lep transport, and because leptin has a short half-life, all of the exogenous leptin would have been cleared from blood in the Fat-Leptin group. Because these mice were studied by intravenous injection rather than by brain perfusion, both the saturation and capacity components of inhibition were being assessed. Decreases in serum leptin levels with either fasting or leptin treatment did not reach statistical significance. This means that capacity must have recovered to a large degree to account for the recovery in leptin transport.

Neither leptin treatment nor fasting reduced body weights to those of thin mice despite the increase of leptin transport to rates seen in thin mice. This mismatch of a modest weight reduction with a robust increase in transport suggests that some factor, perhaps a circulating one associated with obesity or weight reduction, may be modulating the leptin transporter. Consistent with this is work showing that the leptin transporter is modulated by α₁-adrenergics (1), starvation (14), sex steroids (16), glucose, and insulin (15).

In conclusion, these studies show that CD-1 mice acquire a defect in the transport of leptin across the BBB as adiposity increases. This was seen in both an aging population and within an age group. Aged CD-1 mice that did not become obese with aging did not develop a defect in leptin transport. With moderate reductions in body weight, the leptin transport rate increased to levels seen in thin mice. These results show that the obesity-related defects in leptin transport across the BBB are acquired and that they can be reversed with reductions in body weight induced by either fasting or leptin treatment.

We thank Mike Niehoff for technical assistance.

This study was supported by Veterans Affairs Merit Review, Amgen, and National Institutes of Health Grants R01 NS-41863 and R01 AA-12743.

REFERENCES


