

Increased Leptin Resistance as Rats Grow to Maturity (44330)

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Abstract. Three or eight-month-old Sprague-Dawley rats were treated intracerebroventricularly (ICV) with 5 µg of rat leptin/d for 5 days to determine the effect of age on leptin's actions in ingestive behavior, adipose tissue cellularity, organ weights, body composition, and blood metabolite profile. Effects of leptin on food intake were greater in young immature rats (22.0 vs. 5.7 g/d) than in mature rats (17.4 vs. 9.3 g/d) with a leptin × age interaction ($P < 0.01$). Leptin results in body weight loss ($P < 0.001$) by 19% and 9% in young and mature rats, respectively. Water intake was reduced by leptin treatment only in young animals ($P < 0.001$). The decrease in carcass weight by leptin treatment ($P < 0.001$) was observed in both young (22%) and mature rats (9%). Leptin treatment greatly reduced retroperitoneal (0.82 vs. 0.11 g, $P < 0.05$) and epididymal fat weight (1.90 vs. 0.48 g, $P < 0.003$), associated with a reduction in total adipocyte cell number, DNA content, and cellular volume in young rats; however, there were no effects of leptin in the mature rats. In addition, young rats also displayed a 60% loss of carcass lipid content. An increase in serum fatty acid levels by leptin treatment was observed also only in young rats ($P < 0.001$). An interaction of leptin by age that was observed for the reduction of serum glucose levels by leptin treatment ($P < 0.04$) further indicated that mature rats showed a leptin insensitivity compared to young rats. In summary, the data suggest that normal rats become resistant to leptin as they age.

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The recently discovered *ob* gene product leptin has been proposed to be an adipose-related satiety factor that reduces food intake and enhances energy expenditure, leading to reduction of fat storage. The identification of leptin and its receptors shows that leptin is an afferent signal in a negative-feedback loop, regulating body weight by regulating the size of the adipose tissue (1, 2). Both the level of plasma leptin and the expression of leptin in adipose tissue are positively correlated with adipose tissue mass (3, 4). Central and peripheral administration of recombinant leptin reduces body weight, food intake, and body fat content and manipulates blood hormonal profiles in the normal animals (5–7).

Halaas *et al.* (5) treated different strains of obese and lean mice with centrally or peripherally administered leptin, and observed a different physiological response. This observation is accounted for by the result of leptin insensitivity or resistance in obese mice. The genotypic differences in response to leptin in lean and *ob/ob* mice, in another study, also demonstrated an increased insensitivity of *ob/ob* mice to leptin (7). The basal level of plasma leptin is increased in obese humans and animals, which indicates that the obese are relatively insensitive to endogenous leptin, and as a consequence become obese (3, 6). Also, a gender difference may also result in a different leptin sensitivity between the male and female since three- to five-fold higher levels of plasma leptin occurred in female than in male mice (8, 9). An increase in dietary fat was also reported to induce resistance to leptin action in normal FVB mice (10).

Animal growth and maturity are complex phenomena that have many metabolic consequences. As humans or animals age, the rates of protein and lipid accretion are altered (11) and associated with a change of endocrine hormone release and function (12–14). Obesity, which was induced by high-fat diets, which shortened life span, and was associated with age (12), was recently reported to be positively correlated with a high level of blood-leptin concen-

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tration (10). Therefore we hypothesized that there is an age-associated change in leptin sensitivity. The results of the study reported here show that normal rats developed an increased leptin resistance as they age and grow to maturity.

Materials and Methods

Experimental Design. Three (309 ± 7 g, immature) and eight-month-old (492 ± 9 g, mature) male Sprague-Dawley rats were implanted with intracerebroventricular (ICV) cannulas (15). A recovery period following surgery extended a week before the rats were subjected to an angiotensin II (ANG II) drinking test for verification of cannula placement. Six rats of each age group were treated with 5 μ g of leptin or vehicle control for 5 days to examine its effects on growth rate and feeding behavior. The rat leptin used in this study was produced with an *E. coli* expression as previously described (15). The purified leptin was shown to be ~98% pure by RP-HPLC and SDS-PAGE.

After 5 days of treatment, rats were sedated with CO₂ and decapitated. Organs and tissues were removed and weighed. Carcasses were kept at -70°C for further determination of body composition. Epididymal fat pads were stored at -70°C for the later determination of adipose cellularity and DNA contents. Trunk blood was collected, allowed to clot, and kept on ice. Serum was harvested by centrifugation and stored at -20°C for future blood metabolite analysis. All surgical and experimental procedures performed in this study were conducted in accordance with the NIH *Guidelines* and were approved by the Animal Care and Use Committee for The University of Georgia prior to initiating the study.

Adipose Tissue Cellularity and DNA Content.

For adipose tissue cellularity, 50-mg osmium-fixed sections of epididymal fat pads in duplicate were determined as described by Mersmann and MacNeil (16). Adipocyte number and size distribution in the range of 20–240 μm were measured electronically using a Coulter Counter (Coulter Electronics, Hialeah, FL). Another 50 mg of epididymal fat tissue was also used to determine DNA contents. After treatment of tissue samples with lysis buffer (50 mM Tris, pH 8.0; 100 mM EDTA, pH 8.0; 100 mM NaCl, and 1% SDS), the DNA content was quantitated using a bisbenzimidazole fluorescence assay (17).

Body Composition.

Chemical composition of the eviscerated, headless carcass was determined as previously described (18). Carcasses were placed in covered containers and autoclaved for 30 min at 120°C to soften bones. Three volumes of distilled water were added to each carcass and homogenized. While the carcass was blending, two sets of samples were taken in triplicate for the determination of fat, water, and ash. The amount of body fat was determined by chloroform-methanol extraction. The amount of carcass water was determined by drying the samples for 72 hr at 85°C . These samples were then heated at 600°C for 12 hr to determine the amount of ash. The amount of protein was determined by the difference. Total body water, fat, protein,

and ash were determined by multiplying the ratio of each component by the carcass weight.

Blood Metabolite Analysis. Serum samples were assayed for insulin, glucose, urea nitrogen, free fatty acids, triiodothyronine (T3) and thyroxine (T4) using commercially available kits. Insulin, T3, and T4 were determined using the respective ¹²⁵I radioimmunoassay (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Glucose was determined colorimetrically using the glucose oxidase method (Sigma Chemical Co., St. Louis, MO). The urea nitrogen was assayed using urease and colorimetric procedures (Sigma, St. Louis, MO). The free fatty acids were measured using acyl-CoA synthetase and colorimetric procedures (Wako Chemical, Dallas, TX).

Statistical Analysis. Data were analyzed using the general linear models procedure in SAS (1997), with the mean effect of leptin and age in the model. When the main effect of leptin and/or age were significant or an interaction existed ($P < 0.05$), the least significant difference method was used to determine differences between means. Results are reported at least squares means \pm pooled SEM.

Results

Growth Rate and Feeding Behavior. ICV leptin treatment of both young and mature age groups caused a reduction in body weight after the first day of treatment ($P < 0.001$), and continued treatment with leptin led to further body weight reduction (Fig. 1). After 5 days of the experimental period, total body weight loss was 58.3 and 46.6 g by leptin treatment, respectively, for young and mature rats, whereas body weight was respectively increased by 6.8 and 6.3 g in control young and mature rats.

Food intake was decreased by leptin treatment in both age groups ($P < 0.001$) (Fig. 2). The reduction of food intake was much greater in young rats than in mature rats

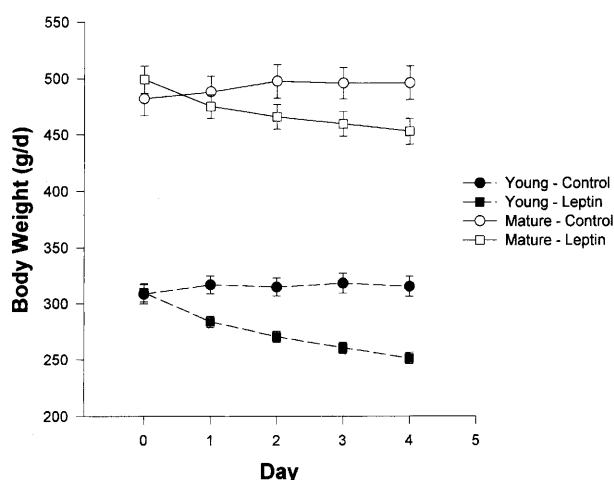


Figure 1. Effect of leptin on body weights in young and mature rats. Six young (3-month-old) and mature (8-month-old) rats were treated ICV with 5 μ g leptin/d or vehicle for 5 days. Each point is the least square mean \pm SEM of six animals. The main effect of leptin was significant: $P < 0.001$.

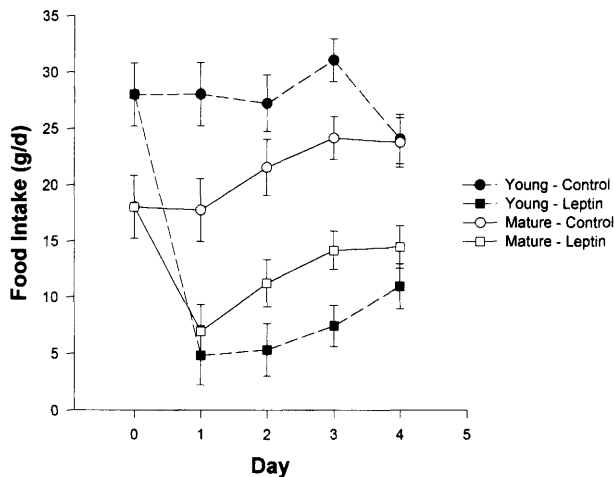


Figure 2. Effect of leptin on food intake in young and mature rats. Six young (3-month-old) and mature (8-month-old) rats were treated icv with 5 μ g leptin/d or vehicle for 5 days. Each point is the least square mean \pm SEM of six animals. The main effect of leptin was significant: $P < 0.001$, and a leptin \times age interaction was observed: $P < 0.01$.

(74% vs. 48%, $P < 0.01$) during the 5 days of treatment; thus, there was an interaction of leptin by age ($P < 0.01$). Water intake was decreased by leptin treatment only during the first 2 days (Fig. 3). After 3 days of the leptin treatment in young rats and 1 day in mature rats, water intake had normalized and was not influenced by further leptin treatment. An interaction of leptin by age was observed ($P < 0.01$) for the reduction of water intake.

Carcass, Organ, and Tissue Weights. Carcass, organ, and tissue weights are shown in Table I. ICV leptin treatment for 5 days decreased carcass weight by 22% and 9%, respectively, for young and mature rats. Leptin treatment did not influence absolute weights of the liver, heart, kidney, or soleus muscle in either age group, although there

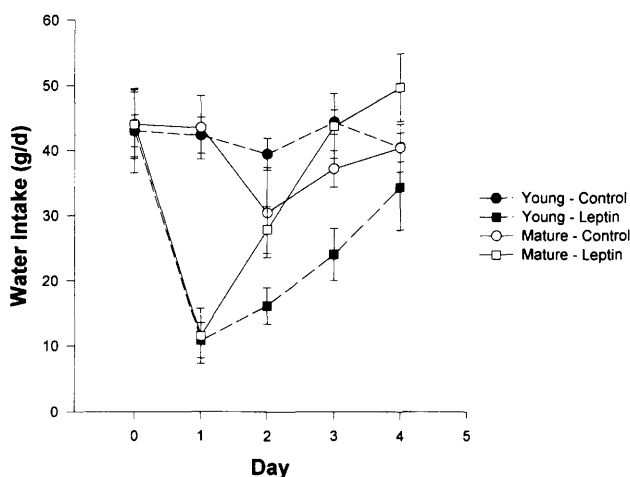


Figure 3. Effect of leptin on water intake in young and mature rats. Six young (3-month-old) and mature (8-month-old) rats were treated icv with 5 mg leptin/d or vehicle for 5 days. Each point is the least square mean \pm SEM of six animals. The main effect of leptin was significant: $P < 0.001$, and a leptin \times age interaction was observed: $P < 0.01$.

was a reduction in the weight of the gastrocnemius muscle in leptin-treated, mature rats ($P < 0.05$). The weights of heart, kidney, and gastrocnemius muscle relative to carcass weights in young rats were increased by leptin treatment ($P < 0.03$), due to a greater loss of carcass weight in these rats. Leptin treatment depleted ($P < 0.005$) retroperitoneal and epididymal fat tissue by 85% and 75%, respectively in young rats, whereas there was no such significant response observed in mature rats.

In mature rats, relative weights of liver, heart, kidney, and gastrocnemius muscle were decreased ($P < 0.005$) whereas the relative weights of both retroperitoneal and epididymal fat pads were increased ($P < 0.001$) as compared with those in young rats. Leptin treatment has greater effects in young rats than in mature rats on changes in carcass, organ, and tissue weights. The interactions of leptin \times age ($P < 0.03$) observed for relative epididymal fat and gastrocnemius muscle weights were also consistent with this observation.

Adipose Cellularity and DNA Content. Changes in cell number per unit of tissue in response to leptin treatment and age were accompanied by differences in percentage distribution of cells (Fig. 4). Relative to controls, leptin treatment resulted in a reduction in the number of cells in the 60–80- μ m range, which was associated with an increase in cell numbers in the 30–50- μ m range in the adipose tissue from young rats. However, leptin treatment did not influence the cell distribution in the adipose tissue from mature rats. The total DNA content in the epididymal fat pad from young rats was decreased by leptin treatment ($P < 0.001$) (Fig. 5). Consistent with adipose tissue weight and cell distribution data, the DNA content was not changed by leptin treatment in the epididymal fat pad from mature rats.

Body Composition. In young rats, leptin treatment reduced total lipid, dry matter, and water contents of carcasses ($P < 0.02$, 0.03 and 0.007, respectively) (Table II). Relative to carcass weight, leptin treatment decreased lipid content by 52% ($P < 0.005$), but increased protein content ($P < 0.02$). In mature rats, however, leptin treatment only decreased total ash and water content ($P < 0.05$). Total and relative content of lipid, dry matter, and water in carcasses were all greater in mature rats than in young rats ($P < 0.006$). Young rats displayed more sensitive responses in body composition to leptin treatment than mature rats, especially for lipid contents for which an interaction of leptin \times age was also observed ($P < 0.02$).

Blood Metabolites. Serum insulin and urea nitrogen were not influenced by leptin treatment and age (Table III). Leptin treatment decreased ($P < 0.001$) serum glucose and T4 concentrations in both young and mature rats, whereas the leptin increased serum-free fatty acid concentration only in young rats ($P < 0.05$). Serum concentrations of glucose, T3, and T4 were higher in young rats than in mature rats ($P < 0.03$, 0.02, and 0.05, respectively). There was a significant leptin \times age interaction observed for serum glucose concentrations ($P < 0.04$), which indicated that a greater

Table I. Organ Weights in Young and Mature Rats Following ICV Injection of Leptin^a

Organs	Young Rats			Mature Rats			SEM	Main Effect		
	Control	5 µg Leptin	P-value	Control	5 µg Leptin	P-value		Leptin	Age	Leptin × Age
Carcass, g	265	206	0.001	413	377	0.007	9	0.001	0.001	NS ^b
Liver, g	11.2	9.9	NS	15.4	12.0	0.010	0.9	0.012	0.001	NS
g/100g CW ^c	4.2	4.8	NS	3.7	3.2	NS	0.3	NS	0.001	NS
Heart, g	1.26	1.20	NS	1.64	1.73	NS	0.08	NS	0.001	NS
g/100g CW	0.48	0.58	0.027	0.40	0.46	NS	0.03	0.014	0.005	NS
Kidney, g	2.44	2.36	NS	3.25	3.43	NS	0.09	NS	0.001	NS
g/100g CW	0.92	1.15	0.001	0.79	0.91	0.028	0.04	0.001	0.001	NS
Gastrocnemius muscle, g	1.91	1.70	NS	2.82	2.54	0.045	0.09	0.017	0.001	NS
g/100g CW	0.72	0.83	0.003	0.68	0.68	NS	0.02	0.035	0.001	0.022
Soleus muscle, mg	111	99.8	NS	186	167	NS	11	NS	0.001	NS
mg/g CW	0.42	0.49	NS	0.45	0.44	NS	0.03	NS	NS	NS
Retroperitoneal fat pad, g	0.82	0.12	0.005	2.71	2.14	NS	0.24	0.014	0.001	NS
g/100g CW	0.31	0.06	0.006	0.66	0.56	NS	0.06	0.007	0.001	NS
Epididymal fat pad, g	1.90	0.48	0.003	3.92	3.44	NS	0.29	0.004	0.001	NS
g/100g CW	0.72	0.23	0.001	0.96	0.91	NS	0.09	0.004	0.001	0.017

^a Values are least square means for 6 rats per treatment group.

^b NS, not significant ($P > 0.05$).

^c CW, carcass weight.

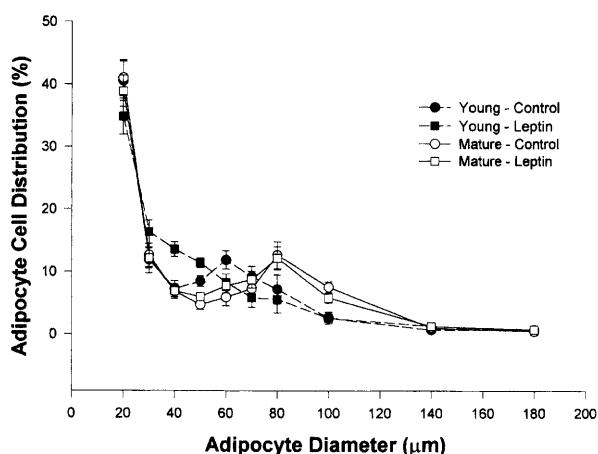


Figure 4. Effect of leptin on adipocyte size distribution in epididymal fat tissue from young and mature rats. Adipocyte size distribution is expressed as a percentage of the total number of cells in epididymal fat tissue. Samples from young and mature rats, which were treated icv with 5 µg leptin/d or vehicle for 5 days, were fixed in osmium tetroxide prior to determination of cell number and size distribution using a Coulter counter and channelizer (Model ZB, Coulter Electronics).

response of serum glucose concentrations to leptin treatment occurred in young rats than in mature rats.

Discussion

Recent studies have demonstrated that leptin negatively regulates the mass of the adipose tissue by reducing food intake and increasing energy expenditure (5). The biochemical mechanisms involved in adipose tissue include an increase in lipolysis rate (19, 20) and an activation of apoptotic process (15). Leptin insensitivity or resistance occurred in obese humans and rodents. High leptin levels have

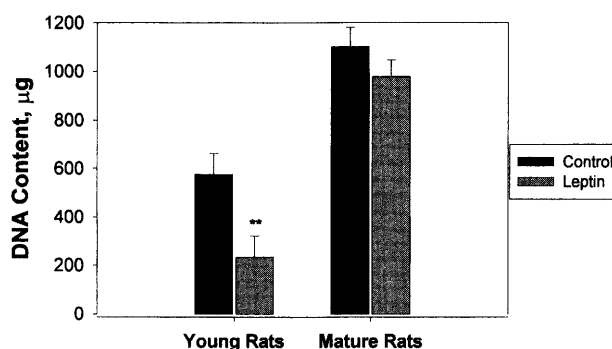


Figure 5. Effect of leptin on total DNA content in the epididymal fat pad from young and mature rats. The 50-mg samples from young and mature rats, which were treated icv with 5 µg leptin/d or vehicle for 5 days, were homogenized with lysis buffer, and the DNA content was quantitated using a bisbenzimidazole fluorescence assay (17). The main effect of leptin ($P < 0.007$) and age ($P < 0.001$) was significant. **: $P < 0.01$.

been observed in obese individuals (3, 10, 21) that are apparently relatively insensitive to endogenous leptin, and obesity likely occurs as a consequence of this condition (3, 21). No evidence was found for an association of blood leptin concentration and age (22). Obese strains of mice also indicated a variability of leptin insensitivity to central and peripheral recombinant leptin administration (5). An increase in dietary fat was also reported to induce resistance to leptin action in normal FVB mice (10).

Growth and maturity are complex phenomena that have many metabolic consequences, including a change in the rates of protein and lipid accretion (11), associated with a change in endocrine hormone release and function (12–14). Obesity, which was induced by high fat diets, and which shortened life span and was associated with growth, matu-

Table II. Body Composition in Young and Mature Rats Following ICV Injection of Leptin^a

Responses	Young Rats			Mature Rats			SEM	Main Effect		
	Control	5 µg Leptin	<i>P</i> -value	Control	5 µg Leptin	<i>P</i> -value		Leptin	Age	Leptin × Age
Lipid, g	14.7	5.84	0.02	29.3	28.2	NS ^b	2.2	0.05	0.001	NS
g/100g CW ^c	5.52	2.63	0.005	7.15	7.44	NS	0.66	0.05	0.001	0.02
Protein, g	28.0	28.4	NS	48.3	44.8	NS	2.3	NS	0.001	NS
g/100g CW	10.7	13.5	0.02	11.8	12.0	NS	0.80	NS	NS	NS
Dry matter, g	48.7	39.7	0.03	87.4	80.0	NS	2.5	0.007	0.001	NS
g/100g CW	18.5	18.6	NS	21.4	21.4	NS	0.96	NS	0.006	NS
Ash, g	6.01	5.14	NS	9.90	7.09	0.02	0.83	0.03	0.001	NS
g/100g CW	2.29	2.41	NS	2.41	1.89	NS	0.24	NS	NS	NS
Water, g	217	173	0.007	325	296	0.05	10	0.002	0.001	NS
g/100g CW	81.5	81.4	NS	78.6	78.7	NS	1.0	NS	0.006	NS

^a Values are least square means for 6 rats per treatment group.

^b NS, not significant (*P* > 0.05).

^c CW, carcass weight.

Table III. Serum Metabolites in Young and Mature Rats Following ICV Injection of Leptin^a

Responses	Young Rats			Mature Rats			SEM	Main Effect		
	Control	5 µg Leptin	<i>P</i> -value	Control	5 µg Leptin	<i>P</i> -value		Leptin	Age	Leptin × Age
Insulin, µU/ml	19.3	20.4	NS ^b	18.9	18.1	NS	2.4	NS	NS	NS
Urea nitrogen, mg/dl	5.12	3.84	NS	5.42	4.37	NS	0.97	NS	NS	NS
Glucose, mg/dl	281	115	0.001	304	231	0.02	21	0.001	0.003	0.04
Fatty acids, µEqiv./L	269	409	0.05	286	327	NS	41	0.05	NS	NS
T ₃ , ng/dl	65.1	65.6	NS	76.6	89.1	NS	6.8	NS	0.02	NS
T ₄ , µg/dl	2.89	1.53	0.001	3.17	2.19	0.005	0.23	0.001	0.05	NS

^a Values are least square means for 6 rats per treatment group.

^b NS, not significant (*P* > 0.05).

urity, and age (12), was recently reported to be positively correlated with a high level of blood leptin concentration (10). These observations support the possibility of age-related leptin insensitivity. The present study for the first time demonstrates a decrease in leptin sensitivity as animals age. Leptin treatment resulted in reduced food intake and body weight in both young immature and mature rats. The reduction of body weights was greater in young rats than in mature rats (19% vs. 9%). In the case of food intake, the magnitude of the change was also different, with younger rats showing a greater response (significant leptin × age interaction).

Other changes in response to leptin treatment were commensurate with the effects on intake and body weight. In the young rats, there was a 60-g difference in carcass weight whereas a 36-g difference was observed in mature rats. Based on selected organ weights, this was not accounted for by changes in liver, heart, kidney, or muscle weights. The lack of effect on lean tissue mass is supported by the chemical composition results that showed no change in protein and water content. In contrast, weights of two specific fat pads and chemically determined carcass lipid content were reduced with leptin treatment, a result observed in young rats but not in mature rats. Mature rats treated with leptin only showed a nonsignificant trend for a reduction in fat pad weight and in carcass lipid.

The reduced fat pad mass is accounted for by both changes in cell size and number. The adipocyte size distribution comparing control and leptin-treated rats showed a shift toward a greater proportion of small cells in the leptin-treated group. In addition, there was a change in cell number as determined by two indices. First, total cell number from the Coulter counter indicated a reduction in number. The drawback to this method is that cells smaller than 20 µm in diameter are not detected. A reduction in number can be interpreted as a reduction in detectable cells. The other index was DNA per pad, which was also reduced in the leptin treated group. Whereas other cell types besides adipocytes contribute to the total DNA in the pad, this is further support for loss of cells. These findings are further confirmed by our recent report of apoptosis in adipose tissue of leptin-treated rats (15). There was no significant change in cell-size distribution and DNA content in mature rats.

In young rats, the weights of heart, kidney, and gastrocnemius muscle relative to carcass were increased by leptin treatment. In contrast to the young rats, liver and gastrocnemius muscle weights were reduced in the older mature rats treated with leptin. These changes by leptin treatment were accounted for by a reduction of lipid content in those organs (7).

Increases in serum fatty acids by leptin treatment in young rats were accounted for by an increase in the lipolysis

rate in adipose tissue (19, 20). However, only a trend for the increase in serum fatty acids was observed in mature rats by leptin treatment ($P > 0.1$). An interaction of leptin by age that was observed for the reduction of serum glucose levels by leptin treatment once again indicated that 8-month-old mature rats showed a leptin insensitivity or resistance. The present study also demonstrated that a decrease in T4 concentration is associated with leptin treatment, which may partially explain why leptin can reduce food intake but also cause an associated increase in energy expenditure.

Apparently, older mature rats showed lower responses in body weight loss, feeding behavior, fat depot loss, carcass lipid content, and blood hormone profile to ICV leptin administration. There are two possible explanations for this observation. First, the dose of leptin used was not adjusted for body weight; thus, both ages received the same dose. The rationale for this approach was that brain size and thus, cerebroventricular volume are similar (within 5%) at the two ages (23). Second, the present experiment demonstrated that older rats are less sensitive to leptin than younger animals. The mechanism involved in the increased leptin resistance as animals grow to maturity may be accounted for by a decreased sensitivity in the neural pathway activated by leptin, or a reduced function of the leptin receptor in the brain associated with age. This hypothesis requires further investigation. Whereas our study does not address the issue, it is possible that the leptin/leptin receptor messages change with age in rats. In summary, rats develop an increased leptin resistance as they age.

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1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**:425–432, 1994.
2. Lee G, Proenca R, Montez J, Carroll K, Darvishzadah J, Lee J, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**:632–635, 1996.
3. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human rodent: Measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med* **1**:1155–1161, 1995.
4. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse *ob* protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**:546–549, 1995.
5. Halaas JL, Boozer C, Blair-West J, Fidathusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci USA* **94**:8878–8883, 1997.
6. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier JS. Role of leptin in the neuroendocrine response to fasting. *Nature* **382**:250–252, 1996.
7. Harris RBS, Zhou J, Redmann SM, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A leptin dose-response in obese (*ob/ob*) and lean (*+/?*) mice. *Endocrinology* **139**:8–19, 1998.
8. Fredrik L, Arner P, Nordfors L, Schalling M. Overexpression of the obese (*ob*) gene in adipose tissue of human obese subjects. *Nat Med* **1**:950–953, 1995.
9. Zhongmin M, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M. Radioimmunoassay of leptin in human plasma. *Clin Chem* **42**:924–946, 1996.
10. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. *Nat Med* **1**:1311–1314, 1995.
11. Hartsook E, Mitchell H. The effect of age on the protein and methionine requirement of the rat. *J Nutr* **60**:173–195, 1965.
12. Stunkard AJ. Nutrition, aging and obesity: A critical review of complex relationship. *Intern J Obesity* **7**:201–220, 1983.
13. Sano T, Kovacs KT, Scheithauer BW, Young WF. Aging and the human pituitary gland. *Mayo Clin Proc* **68**:971–977, 1993.
14. Copeland KC, Colletti RB, Devlin JT, McAuliffe TL. The relationship between insulin-like growth factor-I, adiposity, and aging. *Metabolism* **39**:584–587, 1990.
15. Qian H, Azain MJ, Compton MM, Hartzell DL, Hausman GJ, Baile CA. Brain administration of leptin causes deletion of adipocytes by apoptosis. *Endocrinology* **139**:791–794, 1998.
16. Hersmann HJ, MacNeil MD. Variables in estimation of adipocyte size and number with a particle counter. *J Anim Sci* **62**:980–990, 1986.
17. Labarca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* **102**:344–352, 1980.
18. Harris RBS, Martin RJ. Specific depletion of body fat in parabiotic partners of tube-fed obese rats. *Am J Physiol* **247**:R380–R386, 1984.
19. Chen G, Kazunori K, Xue Y, Lee Y, Zhou Y-T, O'Doherty R, Newgard CB, Unger RH. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. *Proc Natl Acad Sci USA* **93**:14795–14799, 1996.
20. Shimabukuro M, Loyama K, Chen G, Wang M-Y, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci USA* **94**:4637–4641, 1997.
21. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334**:292–295, 1996.
22. Zhongmin M, Gingerich RL, Santiago JV, Klein S, Smith CH, Landt M. Radioimmunoassay of leptin in human plasma. *Clin Chem* **42**:942–946, 1996.
23. Donaldson HH, Hatai S. On the weight of the parts of the brain and on the percentage of water in them according to brain weight and to age, in albino and in wild norway rats. *J Comp Neurol* **53**:263–307, 1931.