

Leptin Resistance During Aging Is Independent of Fat Mass

Ilan Gabriely,^{1,2} Xiao Hui Ma,^{1,2} Xiao Man Yang,^{1,2} Luciano Rossetti,¹ and Nir Barzilai^{1,2}

Increased fat mass, abdominal adiposity, and insulin resistance are typical findings in aging mammals and are frequently associated with leptin resistance and increased plasma leptin levels. To examine whether leptin's failure in aging is due to aging per se or to changes in body fat mass or distribution, we studied aging rats that underwent caloric restriction throughout their lives, maintaining their youthful body fat pattern and metabolic profile. Leptin's action was assessed by measuring its ability to regulate food intake, fat mass and its distribution, peripheral and hepatic insulin action, and its own gene expression in fat. Our results show that leptin's action is markedly diminished in aging rats, independently of their body fat pattern. Leptin's failure in this model suggests its causative role in the metabolic decline seen with aging. *Diabetes* 51:1016–1021, 2002

Aging is associated with a metabolic decline characterized by the development of changes in fat distribution, obesity, and insulin resistance (1–3). All these metabolic alterations are associated with a variety of age-related diseases that subsequently result in increased mortality (4–9). It has been recently demonstrated that leptin, a 16-kDa fat-derived peptide, can modulate many of the metabolic alterations characteristic of aging (10–12). Chronic administration of leptin decreases food intake and induces reduction in fat mass (FM) and visceral fat (VF), with a parallel significant improvement in hepatic and peripheral insulin action (10–14). This finding suggests that alterations in leptin action may play a role in the metabolic phenotype of aging. Indeed, the dramatic increase in plasma leptin levels in aging animal models and in humans suggests a leptin-resistant state (15–18). Although the increase in plasma leptin concentration in aging may be partially attributed to the development of obesity (which is associated with leptin resistance [19]), the increase in plasma leptin level during aging is often disproportionate to the increase in the amount of fat (16–19). We therefore hypothesize that aging per se is associated with a failure in leptin's action,

independent of obesity or changes in body fat distribution. Thus, leptin resistance of aging may represent a perpetuating factor in developing and maintaining obesity and its clinical consequences.

Because aging is frequently associated with obesity, it is difficult to identify whether leptin failure is due to obesity, the process of aging per se, or both. To overcome this difficulty, we used caloric restriction throughout aging in a rodent model and prevented the typical age-related changes in body composition. We reasoned that aging rats would remain leptin resistant even when kept relatively lean and “metabolically young” by caloric restriction.

RESEARCH DESIGN AND METHODS

Animals. A total of 36 male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were housed in individual cages and subjected to a standard light (6:00 A.M. to 6:00 P.M.) to dark (6:00 P.M. to 6:00 A.M.) cycle. Rats were assigned to caloric restriction (to 55% of the calories consumed by ad libitum-fed rats) at the postpuberty age of ~12 weeks and were studied at 3 months of age ($n = 18$) before caloric restriction or at 20 months of age ($n = 18$). The diet consisted of 64% carbohydrate, 30% protein, and 6% fat, with a physiological fuel value of 3.3 kcal/g food. One week before the *in vivo* studies, rats were anesthetized by inhalation of methoxyflurane, and indwelling catheters were inserted in the right internal jugular vein and in the left carotid artery. The venous catheter extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch. This method of anesthesia allows fast recovery and normal food consumption after 1 day (12–14). In addition, all rats were implanted with subcutaneous minipumps that delivered either recombinant mouse leptin ($\sim 0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) or a similar volume of normal saline (NS). Thus, the young and aging animals were subdivided into three groups: 1) receiving NS and allowed to eat ad libitum ($n = 6$), 2) receiving leptin and fed ad libitum ($n = 6$), and 3) receiving NS and pair-fed (PF) to match food intake in the leptin group ($n = 6$). All rats were studied after ~6 h of fasting while awake and unstressed. This experimental protocol was used on rats weighing 300–320 g before the experiment and was designed to achieve a similar percentage of FM in the leptin and PF animals.

Body composition. Lean body mass and FM were calculated as described elsewhere (12–14). Briefly, rats received an intra-arterial bolus injection of 20 μCi tritiated-labeled water ($^3\text{H}_2\text{O}$; New England Nuclear, Boston, MA), and plasma samples were obtained at 30-min intervals. Steady-state conditions for plasma $^3\text{H}_2\text{O}$ specific activity were achieved within 45 min in all studies. Five plasma samples obtained between 1 and 3 h were used to calculate the total body distribution of water. Epididymal, perinephric, and mesenteric fat depots were dissected and weighed at the end of each experiment. Hepatic triglyceride content was measured from liver homogenate by a triglyceride (GPO-Trinder) kit (Sigma, St. Louis, MO) (20–22).

Hyperinsulinemic-euglycemic clamp. All rats received a primed continuous (15–40 μCi bolus, 0.4 $\mu\text{Ci}/\text{min}$) infusion of high-performance liquid chromatography-purified [^3H -3]glucose (New England Nuclear) throughout the study. After 120 min, a primed continuous infusion of insulin ($3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and a variable infusion of a 25% glucose solution were started and periodically adjusted to clamp the plasma glucose concentration at the basal level for an additional 120 min. Somatostatin ($1.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused to suppress endogenous insulin secretion (12–14).

Plasma samples for determination of [^3H]glucose specific activity were obtained at 10-min intervals throughout the clamp. Samples were also obtained for determination of plasma insulin, leptin, and free fatty acid (FFA) concentrations at 30-min intervals throughout the study. The total volume of blood withdrawn was ~3.0 ml per study. To prevent volume depletion and

From the ¹Department of Medicine, Division of Endocrinology, Diabetes Research and Training Center, ²Institute for Aging Research, Albert Einstein College of Medicine, Bronx, New York.

Address correspondence and reprint requests to Nir Barzilai, Institute for Aging Research, 1300 Morris Park Ave., Belfer Bldg. #701, Bronx, NY 10461. E-mail: barzilai@aecom.yu.edu.

Received for publication 13 September 2001 and accepted in revised form 17 December 2001.

G.I. and X.H.M. contributed equally to this work.

ds, double-stranded; EGP, endogenous glucose production; FFA, free fatty acid; FM, fat mass; NS, normal saline; PF, pair-fed; SOCS-3, suppressor of cytokine signaling 3; TG, triglyceride; VF, visceral fat.

TABLE 1
Effects of leptin on food intake and body composition after 7 days of subcutaneous leptin administration

	3 months old			20 months old		
	NS	Leptin	PF	NS	Leptin	PF
Leptin (ng/ml)	1.2 ± 0.4*	23.2 ± 5.2	1.1 ± 0.3*	3.4 ± 0.2*‡	23.7 ± 3.5	2.9 ± 0.3*‡
Food intake (kcal/day)	76 ± 4*	28 ± 7	28 ± 3	80 ± 17*	46 ± 9‡	46 ± 5‡
Body weight (g)	310 ± 12	296 ± 7	281 ± 15	341 ± 16	319 ± 15	317 ± 9
Change in body weight (g)	20 ± 2†	-33 ± 2	-30 ± 1	15 ± 3†	-21 ± 4	-28 ± 3
% Fat	10 ± 1†	6 ± 1	6 ± 1	12 ± 1†	6 ± 1	6 ± 1
Total VF (g)	7.8 ± 1.5*	2.4 ± 0.1§	6.7 ± 0.7	6.3 ± 1.2	5.4 ± 0.7	5.2 ± 0.8
Lean body mass (g)	280 ± 15	255 ± 11	247 ± 15	307 ± 21	302 ± 12	298 ± 15
Hepatic triglyceride (mg/g)	2.4 ± 0.3	0.5 ± 0.2§	1.3 ± 0.3	1.3 ± 0.3	1.0 ± 0.3	1.1 ± 0.2

Data are means ± SE. Data for food intake and body weight, change in body weight from basal, percent body fat (% fat), total VF, lean body mass, and hepatic triglyceride content in 3- and 20-month-old calorie-restricted rats at the end of the study are shown. Osmotic minipumps containing either leptin or vehicle were implanted subcutaneously in the three groups of either 3- or 20-month-old calorie-restricted rats 1) receiving vehicle and ad libitum fed for 7 days (NS), 2) receiving leptin for 7 days, or 3) receiving vehicle for 7 days and PF to match the food intake of the leptin group (PF). * $P < 0.001$ vs. leptin; † $P < 0.001$ vs. leptin and PF; ‡ $P < 0.01$ vs. 3 months old; § $P < 0.01$ vs. 3-month-old NS, PF, and 20-month-old leptin.

anemia, a solution (1:1 vol/vol) of ~3.0 ml fresh blood (obtained by heart puncture from a littermate of the test animal) and heparinized saline (10 units/ml) were infused in a constant rate throughout the study. At the end of the insulin infusion, rats were anesthetized (60 mg/kg body wt pentobarbital i.v.) and epididymal, mesenteric, and perinephric fat pads were dissected and weighed at the end of each experiment. All tissue samples were stored at -80°C for subsequent analysis (12–14). The study protocol was reviewed and approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine.

Whole-body glycolysis and glycogen synthesis. The rate of glycolysis was estimated from the rate of conversion of [³H-3]glucose to ³H₂O as previously described (12,13). Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present either in ³H water or in glucose. Plasma-tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Whole-body glycogen synthesis was estimated by subtracting whole-body glycolysis from whole-body glucose uptake (R_d).

Leptin gene expression. Total RNA from fat depots was prepared following Clontech's protocol with some modifications as previously described (23,24). The total RNA was analyzed in a 1% agarose gel containing 2.2 mol/l formaldehyde before use. The first-strand cDNA was synthesized from 3 µg total RNA in 20-µl final incubation volume using the SuperScript Preamplification System for First Strand cDNA Synthesis (Life Technologies, Carlsbad, CA) with a random primer. PCR was performed in a 50-µl reaction mixture containing 4 µl of the above first-strand cDNA, 5 µl of 10× PCR buffer (Mg+2 plus; Boehringer, Mannheim, Germany), 1 µl of 10 mmol/l dNTP mix, 4 pmol of each primer, and 2.5 units *Taq* DNA polymerase (Life Technologies). For leptin, the sequence of upstream primer is TCC TAT CTG TCC TAT GTT CAA GCT GTG, downstream primer is CAA CTG TTG AAG AAT GTC CTG CAG AGA, and the expected RT-PCR product was 454 bp. The conditions for PCR were 94°C for 45 s and 69°C for 2 min (42 cycles) using a GeneAmp PCR System 9600 (Perkin-Elmer, Boston, MA). Each assay was repeated for 10, 20, and 30 cycles to establish linearity. Each experiment was repeated three times for each individual animal. As a control, we used β-actin gene expression, described in detail elsewhere (23). To correct for loading irregularities, quantification of the leptin signal was performed by scanning densitometry, normalized for β-actin signal, which is not typically affected by insulin.

In parallel, we also used real-time PCR for quantification. Real-time PCR was performed by a light cycle (Roche, Mannheim, Germany) using the one-step RT-PCR system. The reactions were done using the Light Cycler-Fast start DNA Master SYBR green kit. The components for PCR in a final volume of 20 µl included 2 µl of a commercial ready-to-use reaction mix for PCR (Light Cycler -DNA master SYBR Green -I) of *Taq* DNA polymerase, reaction buffer, dNTP mix, and SYBR Green -I dye; 10 mmol/l MgCl₂ SYBR green I dye binds to the minor groove of double-stranded (ds)-DNA, and the fluorescence is greatly enhanced by binding. The magnesium chloride final concentration was 4 mmol/l, the primers final concentration was 1.0 µmol/l, and 2 µl of 1:10-diluted template DNA was added. The reactions were cycled 50 times with a 95°C denaturation for 10 s, a 62°C annealing for 7 s, and 72°C for 12 s with slopes of 20°C/s, 20°C/s, and 20°C/s, respectively. Fluorescence was acquired after heating at 20°C/s to a temperature 2°C below the product melting temperature and holding for 60 s. During the various stages of PCR, different intensities of fluorescence signals can be detected depending on the

amount of dsDNA present. The fluorescence is recorded at the end of each cycle and monitored from cycle to cycle. Serial dilution of the peptide's plasmid DNA was used to create the standard curve. The crossing points were identified by the intersection of the best-fit line with the log linear portion of the standards amplification curve. The standard curve is the plot of the crossing point versus the log of copy number. The concentration of the products in the sample were calculated by extrapolation to the standard curve by using light cycler analysis software (Roche). mRNA obtained from all rats and real-time PCRs were repeated three times on each sample.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA). Plasma insulin and corticosterone were measured by radioimmunoassay (14,24). Plasma [³H]glucose radioactivity was measured in duplicate in the supernatants of Ba(OH)₂ and ZnSO₄ precipitates of plasma samples (20 µl), which were evaporated to dryness to eliminate tritiated water.

Statistical analysis. All values shown are expressed as means ± SE. Statistical analyses were performed using analysis of variance in multiple comparisons. Where *F* ratios were significant, further comparisons were made using Student's *t* tests.

A *P* value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows (SPSS, Chicago).

RESULTS

Effects of leptin on food intake. During the study, plasma leptin levels were similar in aging and young leptin-treated groups. These levels (~20 ng/ml) were similar to the basal leptin concentrations in aging ad libitum-fed rats and were within the physiological range of leptin's action (21). Under similar leptin concentrations, young rats decreased their food intake by ~70%, whereas the aging rats decreased their food intake by only ~40% compared with the respective ad libitum-fed (NS) control animals (Table 1, Fig. 1).

Effect of leptin on body composition. To demonstrate the effects of leptin on changes in fat distribution, independent of food intake, these studies compared animals treated with leptin to a PF control. Young and aging PF animals had similar body weight, percentage fat, and total VF. The decrease in body weight in all leptin-treated animals was similar to that in the PF group. However, leptin and pair-feeding decreased body weight in young rats by ~25% more than it did in aging rats. As planned, all leptin-treated rats and PF rats had a similar percentage FM when studied. A marked difference in the amount of VF was noted in animals after leptin administration, resulting in an approximately twofold higher amount of VF in the aging, as compared with the young, leptin-treated animals.

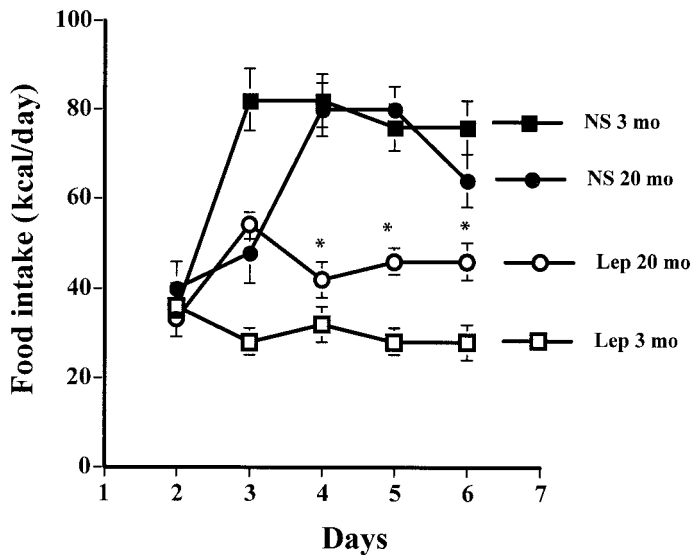


FIG. 1. Effect on food intake of chronic physiological increases in plasma leptin levels. Young (3 months, $n = 12$) and aging (20 months, $n = 12$) caloric-restricted rats were implanted with mini-osmotic pumps to infuse either leptin (Lep) or saline (NS) and allowed ad libitum feeding for 7 days (see RESEARCH DESIGN AND METHODS). The figure represents food intake (kcal/day) from day 2 (after recovery). Values of caloric intake are presented in Table 1. * $P < 0.005$ vs. all others. Leptin at 3 months was significantly ($P < 0.001$) different versus young and aging NS.

However, subcutaneous FM was comparable in the young PF rats and in the aging PF rats (10.1 ± 2.1 and 14.1 ± 2.8 g, respectively [$P = 0.892$]). Because leptin commits FFA from hepatic triglyceride (TG) stores to β -oxidation, we measured hepatic TG levels as markers of fat depletion. In the young rats, hepatic triglycerides decreased significantly with leptin, whereas in the aging rats, leptin was less effective in decreasing hepatic triglycerides, indicating its biological failure during the study (Table 1, Fig. 2).

Effects of leptin on hepatic and peripheral insulin action. Young and aging PF animals had similar plasma glucose and insulin levels and similar insulin-mediated glucose uptake. In all groups, plasma insulin and glucose levels were maintained at similar levels during the insulin clamp period. Plasma corticosterone concentrations were higher in all caloric-restricted animals compared with the NS ad libitum-fed rats (186 ± 25 , 168 ± 21 , and 125 ± 18 ng/ml in the 3-month and 20-month caloric-restricted animals and the NS ad libitum-fed rats, respectively; $P = \text{NS}$). Plasma FFA concentration seemed to be increased at basal and was not suppressed by insulin in the aging rats to the same extent as in the young rats. However, in each age-group, FFAs were similar in the leptin and PF groups. This physiological hyperinsulinemia decreased endogenous glucose production (EGP) by $\sim 90\%$ in young rats treated with leptin compared with $\sim 60\%$ in the young PF animals. In contrast, the decrease in EGP in aging animals was $\sim 50\%$ in both leptin and PF aging animals. Thus, aging totally neutralized the ability of leptin to modulate EGP. Finally, peripheral insulin action was improved in young animals treated with leptin to a greater degree than it was in aging rats. The 35% increase in glucose uptake in young animals treated with leptin was accounted for by an ~ 6 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ increase in the rate of glycogen synthesis

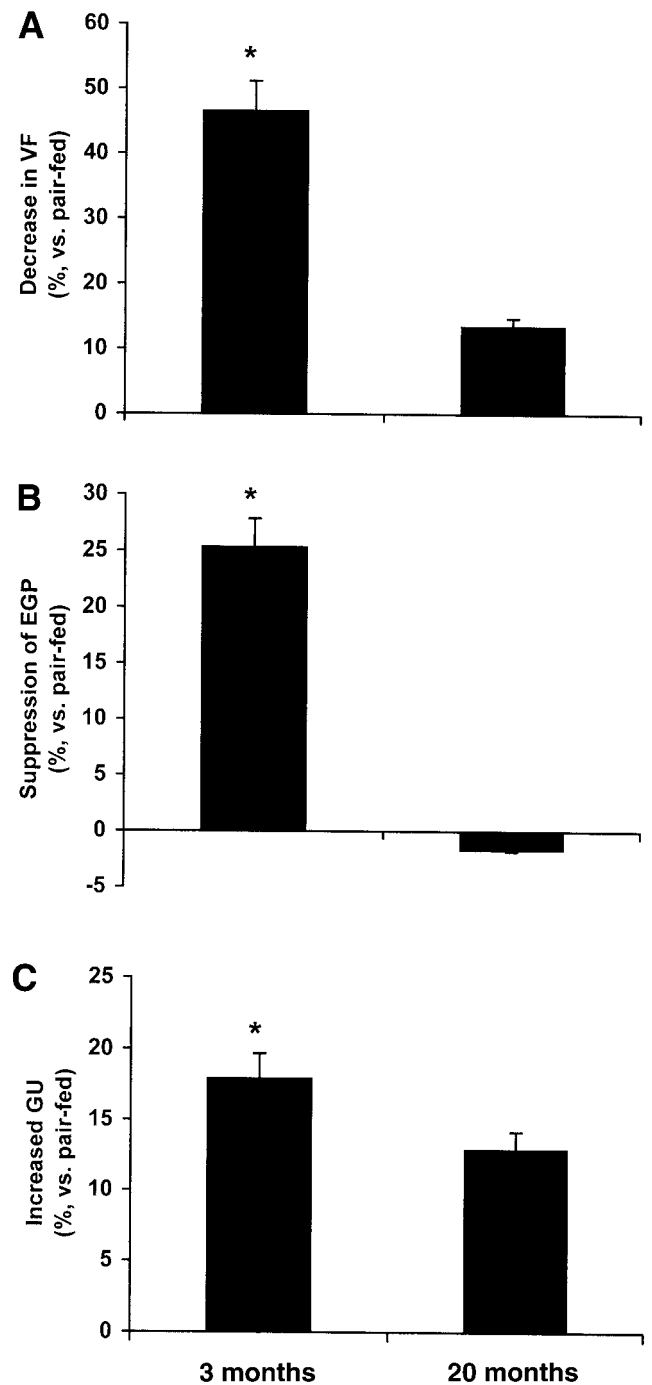


FIG. 2. Effect of chronic physiological increases in plasma leptin levels on VF (A). Young (3 months, $n = 12$) and aging (20 months, $n = 12$) caloric-restricted rats were implanted with mini-osmotic pumps to infuse either leptin or saline and were PF for 7 days (see RESEARCH DESIGN AND METHODS). The figure represents the percent decrease in total VF (i.e., perinephric, mesenteric, and epididymal VF depots) by leptin infusion in young and aging animals compared with PF animals. * $P < 0.001$ vs. 20 months. B: The suppression of EGP. The figure represents the ability of insulin to suppress EGP in leptin-treated animals compared with PF animals. EGP was approximately threefold higher in young PF animals than in the leptin group (Table 3). Leptin did not significantly change insulin's ability to suppress EGP in aging rats. * $P < 0.001$ vs. 20 months. C: Increased insulin-mediated glucose uptake (GU). * $P < 0.01$ vs. 20 months.

and only an ~ 3 mg \cdot kg $^{-1}$ \cdot min $^{-1}$ increase in rates of glycolysis, supporting the role of leptin in preserving carbohydrate stores. With aging, the effect of leptin on

TABLE 2
Biochemical characteristics during an insulin clamp in leptin-treated aging rats

	3 months old		20 months old	
	Leptin	PF	Leptin	PF
Insulin (pmol/l)				
Basal	27 ± 6	34 ± 8	36 ± 9	37 ± 9
Clamp	517 ± 36	549 ± 68	544 ± 30	513 ± 70
Glucose (mmol/l)				
Basal	6.4 ± 0.2	6.5 ± 0.3	6.6 ± 0.2	6.3 ± 0.1
Clamp	6.6 ± 0.3	6.6 ± 0.3	6.1 ± 0.2	6.4 ± 0.1
FFA (mmol/l)				
Basal	0.58 ± 0.08	0.58 ± 0.08	0.71 ± 0.10	0.66 ± 0.05
Clamp	0.23 ± 0.03	0.25 ± 0.03	0.45 ± 0.11*	0.57 ± 0.17*

Data are means ± SE. Plasma concentrations of insulin, glucose, and FFA at basal (postabsorptive) and during the insulin clamp studies (clamp) in 3- and 20-month-old calorie-restricted rats ($n = 6$ in each group are shown). The values represent steady-state levels obtained by averaging five plasma samples during each experimental period. * $P < 0.001$ vs. 3 months old.

glucose uptake was diminished, and the glycogen synthesis rate was not stimulated significantly with leptin (Tables 2 and 3, Fig. 2).

Leptin gene expression. Leptin has been shown to regulate its own gene expression (22). Here we demonstrate that leptin's ability to suppress its own expression after prolonged infusion is diminished with aging. These studies compared leptin's gene expression in animals treated with leptin with a PF control, demonstrating an approximately twofold higher leptin message in the aging leptin-treated rats (Fig. 3).

DISCUSSION

Although aging rats were manipulated to be metabolically similar to young rats, they exhibited a marked decrease in leptin's ability to regulate food intake, body fat distribution and stores, and peripheral and hepatic insulin action and exhibited a marked decrease in leptin's effect on its own gene expression in fat.

Ad libitum-fed caged rats closely mimic the metabolic phenotype of aging people in the Western world. An unrestricted diet and sedentary lifestyle result in increased FM, abdominal obesity, and the development of insulin resistance throughout aging. The high levels of leptin

usually observed in obese subjects suggest relative resistance to this peptide. However, two other observations have suggested that aging is a leptin-resistant state independent of obesity. First, although plasma leptin levels increase parallel to the increase in fat, they continued to increase as the animal gets older, disproportionately to the increase in FM (16,25). We have previously demonstrated that leptin levels in our ad libitum rat model increased with obesity from ~1 to ~11 ng/ml between 2 and 8 months (25). However, whereas body weight did not further increase much at 20 months, leptin levels doubled to an averaged ~20 ng/ml. Second, it was previously demonstrated that leptin is a regulator of body fat distribution, inducing a specific decrease in VF (12). This regulation fails with aging, and, in our ad libitum rat model, visceral FM increased with obesity from ~5 to ~16 g between 2 and 8 months, and it further increased to an average of ~26 g, although body weight did not increase much with further aging (25). Thus, we hypothesized that a failure in leptin's pathway may play a role, even when accounting for body fat and its distribution.

To support this hypothesis, we subjected our aging rats to a lifetime of caloric restriction. This method allowed us to study a model that, while chronologically aged, had fat depots similar to the young. Indeed FM, fat distribution, and peripheral insulin action were all similar in the PF young and aging groups. In the aging rats, these metabolic advantages were achieved not only by calorie restriction but also because leptin levels were increased approximately threefold in the aging NS and PF group. Other differences between the young and aging PF animals were increases in food intake (matching for the failure of leptin to decrease food intake with aging) and less suppression of plasma FFA and glucose production during the insulin clamp. Although these changes indicate a relative decreased insulin action on the liver and adipose tissue, they are minor compared with the severalfold differences observed between young and aging ad libitum-fed animals (25). Because most metabolic aspects were similar in this PF aging model, we focused on analyzing the differences between PF and leptin-treated animals within each age-group rather than between ages. Our data support the findings by Scarpace et al. (26,27) of leptin resistance in aged rats characterized by impaired leptin signal transduc-

TABLE 3
Hepatic and peripheral insulin sensitivity in leptin-treated aging rats

	3 months old		20 months old	
	Leptin	PF	Leptin	PF
EGP ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)				
Basal	9.8 ± 0.5	8.2 ± 0.2	9.0 ± 0.3	8.9 ± 0.2
Clamp	0.9 ± 0.3	2.9 ± 0.3*	4.6 ± 0.8†	4.4 ± 0.1†
Glucose uptake ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)				
Clamp	18.7 ± 1.0*	13.9 ± 1.2	14.0 ± 0.6*	12.4 ± 0.7
Glycolysis ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)				
Clamp	7.0 ± 0.2*	5.3 ± 0.1	6.2 ± 0.2	5.2 ± 0.9
Glycose-synthesis ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)				
Clamp	13.3 ± 0.3*	8.6 ± 0.2	7.8 ± 0.2	7.2 ± 0.1

Data are means ± SE. Data for EGP before (basal) and after (clamp) the initiation of a hyperinsulinemic-euglycemic clamp are shown. Glucose uptake, glycolysis, and glycogen synthesis in 3- and 20-month-old calorie-restricted rats ($n = 5$ in each group are given). The values represent steady-state levels ($[3\text{-}^3\text{H}]\text{glucose}$) obtained by averaging the last four plasma samples during each experimental period. * $P < 0.01$ leptin vs. PF; † $P < 0.01$ vs. 3 months old.

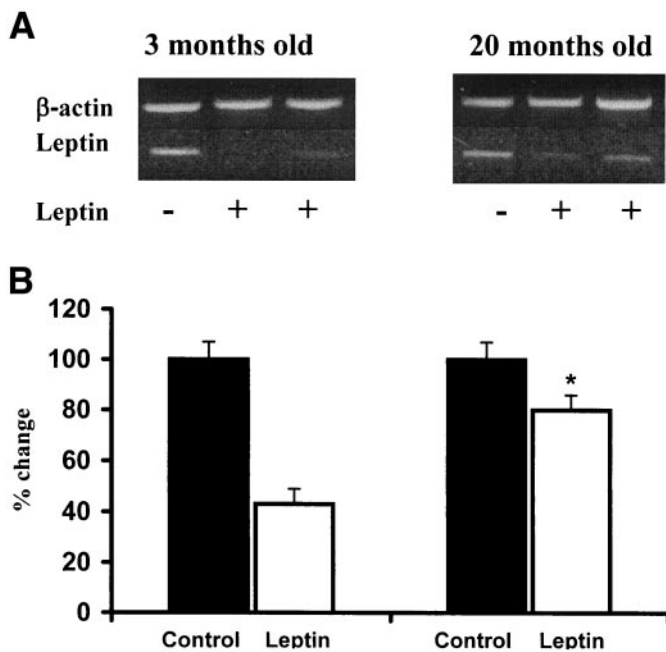


FIG. 3. Effect on leptin gene expression of chronic physiological increases in plasma leptin levels. Young and aging rats were studied with either leptin or saline and PF as previously described. Leptin's gene expression was assayed qualitatively by RT-PCR of subcutaneous white tissue, followed by quantitative real-time PCR. The figure represents RT-PCR (A) and real-time PCR quantification of leptin's percent gene expression compared with the appropriate control (B). * $P < 0.01$ vs. PF.

tion with age-related obesity (26) and diminished decrease in food intake and no increase in energy expenditure in leptin-treated aged rats compared with young rats (27). In these studies, the impairments in leptin action may have been due to either elevated obesity and serum leptin with age, due to age itself, or both. However, our experimental model was designed to match the body weight in aging and young rats to exclude obesity, which normally accompanies aging, as a factor implicated in leptin resistance of aging. Furthermore, our studies have looked at other effects of leptin in this aging model that may play fundamental roles in aging-related diseases.

The mechanisms for leptin resistance are important and under intensive investigation. An early suggestion was that the biological properties of the blood-brain barrier limit the transfer of leptin to its receptors in the arcuate nucleus beyond certain plasma levels (28). However, with gradual increasing levels, leptin initially should be able to cross this barrier, and resistance at the leptin transfer could be explained only when higher levels are achieved. An attractive hypothesis is the paradoxical induction of the suppressor of cytokine signaling 3 (SOCS-3) (29,30). When SOCS-3 expression was compared in the hypothalamus and in white adipocytes of young and aging ad libitum-fed Zucker (+/+) rats before and after induction of hyperleptinemia, hypothalamic SOCS-3 mRNA was approximately three times higher in aging rats (19). Because these studies were done in an obese model with defective leptin action, the effects noted may have been secondary to both factors. However, the observation that our aging leptin-treated rats have failed to decrease their own gene expression is in line with such explanation. This result may have contributed to increasing levels of leptin, inducing further suppression of

their action. Alternatively, this failure may be important in the central nervous system at the P-signal transducers and activators of transcription-3 (P-STAT-3) receptor level (26) or neuropeptide Y (31), as described in obese aging rats, or possibly downstream to the leptin receptor pathway. A special insight into the failure of leptin's action with aging comes from a transgenic mice model that overexpresses leptin (32). In this model, although increased leptin levels prevented increases in FM in young animals, it failed to prevent fat accretion with aging. It has been previously demonstrated that glucocorticoids may modulate leptin's secretion and activity in different animal models (33,34). To analyze the possibility of a glucocorticoid increase due to caloric restriction as an indirect mechanism of leptin resistance, we measured plasma corticosterone levels in all groups. Whereas plasma corticosterone levels were increased in the caloric-restricted animals (the difference, however, did not reach statistical significance), the same pattern was observed in both the young and aging animals, suggesting that this variation in glucocorticoid levels did not play a significant role in the leptin resistance observed in our model.

From an evolutionary perspective, any postreproduction phenotype should be neutral in its effect on evolution. Thus, it is questionable whether aging healthily has an evolutionary role. In accordance with this hypothesis, we believe that although leptin has a role in pubertal development and reproduction (in addition to its role in the neuroendocrine adaptation to starvation), it is limited in its ability to function during aging. Thus, age-related failure of the leptin pathway may have a causative role in many of the lipotoxic/metabolic effects of aging (35).

From a comparative point of view, most people in the Western world are overweight when they reach middle-age. Leptin's failure may be an important biological initiator of events leading to obesity and to further decrease leptin's action. With aging, many people exhibit abdominal obesity, insulin resistance, and increased leptin levels in proportion to their body and fat weights (36,37), which may be modulated when aging is associated with losses of lean body mass and subcutaneous fat (38,39). The consequence of obesity in humans is decreased life span due to an increase in all causes of death (40,41). Caloric restriction retards many of the features of aging and extends life span dramatically in a variety of animal models (42,43). Taken together, these data suggest that longevity is enhanced in caloric-restricted rats and lean people who are protected from many of the lipotoxic effects of aging and obesity (40–43).

Our study did not identify the underlying mechanism responsible for leptin resistance in aging animals. Similarly, our data cannot exclude that, although the attenuation of leptin-induced anorexia in aging rats may be due to leptin resistance of aging, it may also be due to leptin-independent mechanisms activated by chronic caloric restriction. As previously discussed, a major unanswered question is whether leptin resistance induced by aging occurred within or outside the brain. Future studies analyzing the effects of aging on leptin action using central (intracerebroventricular) infusions of leptin or its antagonists could further clarify the underlying mechanisms of leptin resistance.

Increased plasma leptin levels with aging suggest resistance to leptin action and may explain why elderly subjects have abdominal obesity and insulin resistance. However, this study also supports the notion that leptin failure leads to the initiation of the metabolic characteristics of aging.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (RO1-AG18381 to N.B. and RO1-DK 45024 and RO1-DK 48321 to L.R.), the American Diabetes Association, and the Core Laboratories of the Albert Einstein Diabetes Research and Training Center (DK 20541).

The authors thank Rachel S. Berger for her significant input.

REFERENCES

- Tollefsbol TO, Cohen HJ: Role of protein molecular and metabolic aberrations in aging, in the physiologic decline of the aged, and in age-associated diseases. *J Am Geriatr Soc* 34:282-294, 1986
- Rinn WE: Mental decline in normal aging: a review. *J Geriatr Psychiatry Neurol* 1:144-158, 1988
- Fraze E, Chlou M, Chen Y, Reaven GM: Age related changes in postprandial plasma glucose, insulin, and FFA concentrations in non-diabetic individuals. *J Am Geriatr Soc* 35:224-228, 1987
- Hazzard WR, Ettinger WH Jr: Aging and atherosclerosis: changing considerations in cardiovascular disease prevention as the barrier to immortality is approached in old age. *Am J Geriatr Cardiol* 4:16-36, 1995
- Ferrannini E, Vichi S, Beck-Nielsen H, Laakso M, Paolisso G, Smith U: European Group for the Study of Insulin Resistance (EGIR): insulin action and age. *Diabetes* 45:947-953, 1996
- Barzilai N, Gupta G: Interaction between aging and syndrome X: new insights on the pathophysiology of fat distribution. *Ann N Y Acad Sci* 892:58-72, 1999
- Shimokata H, Tobin JD, Muller DC, Elahi D, Coon PJ, Andres R: Studies in the distribution of body fat. I. Effects of age, sex, and obesity. *J Gerontol* 44:M66-M73, 1989
- Kotz CM, Billington CJ, Levine AS: Obesity and aging. *Clin Geriatr Med* 15:391-412, 1999
- Reaven GM: Banting Lecture: Role of insulin resistance in human disease. *Diabetes* 37:1595-1607, 1988
- Rentsch J, Levens N, Chiesi M: Recombinant ob-gene product reduces food intake in fasted mice. *Biochem Biophys Res Commun* 214:131-136, 1995
- Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543-546, 1995
- Barzilai N, Wang J, Massillon D, Vuguin P, Hawkins M, Rossetti L: Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* 100:3105-3110, 1997
- Barzilai N, She L, Liu L, Wang J, Hu M, Vuguin P, Rossetti L: Decreased visceral adiposity accounts for leptin's effect on hepatic but not peripheral insulin action. *Am J Physiol* 277:E291-E298, 1999
- Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J: Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J Biol Chem* 272:22758-22763, 1997
- Ahren B, Mansson S, Gingerich R, Havel PJ: Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. *Am J Physiol* 273:R113-R120, 1997
- Li H, Matheny M, Nicolson M, Tumer N, Scarpace PJ: Leptin gene expression increases with age independent of increasing adiposity in rats. *Diabetes* 46:2035-2039, 1997
- Sanchez-Rodriguez M, Garcia-Sanchez A, Retana-Ugalde R, Mendoza-Nunez VM: Serum leptin levels and blood pressure in the overweight elderly. *Arch Med Res* 31:425-428, 2000
- Rossetti L, Faridi N, Atluri R, Chaiken RL, Lebovitz HE: Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 84:137-144, 1999
- Wang ZW, Pan WT, Lee Y, Kakuma T, Zhou YT, Unger RH: The role of leptin resistance in the lipid abnormalities of aging. *FASEB J* 15:108-114, 2001
- Barzilai N, Banerjee S, Hawkins M, Chen W, Rossetti L: Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. *J Clin Invest* 101:1353-1361, 1998
- Carr TP, Andresen CJ, Rudel LL: Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. *Clin Biochem* 26:39-42, 1993
- Barzilai N, She L, Liu B-Q, Hu M, Rossetti L: Surgical removal of visceral fat in rats reverses hepatic insulin resistance. *Diabetes* 48:94-98, 1999
- Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L: A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 393:684-688, 1998
- Sainio EL, Lehtola T, Roininen P: Radioimmunoassay of total and free corticosterone in rat plasma: measurement of the effect of different doses of corticosterone. *Steroids* 5:609-622, 1988
- Gupta G, She L, Ma X-H, Yang X-M, Hu M, Rossetti L, Barzilai N: Ability of insulin to modulate hepatic glucose production with aging rats is impaired by fat accumulation. *Am J Physiol* 278:E985-E991, 2000
- Scarpace PJ, Matheny M, Shek EW: Impaired leptin signal transduction with age-related obesity. *Neuropharmacology* 39:1872-1879, 2000
- Scarpace PJ, Matheny M, Moore RL, Tumer N: Impaired leptin responsiveness in aged rats. *Diabetes* 49:431-435, 2000
- Maresh GA, Maness LM, Zadina JE, Kastin AJ: In vitro demonstration of a saturable transport system for leptin across the blood-brain barrier. *Life Sci* 69:67-73, 2001
- Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS: The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem* 274:30059-30065, 1999
- Wang Z, Zhou YT, Kakuma T, Lee Y, Kalra SP, Kalra PS, Pan W, Unger RH: Leptin resistance of adipocytes in obesity: role of suppressors of cytokine signaling. *Biochem Biophys Res Commun* 277:20-26, 2000
- Li H, Matheny M, Tumer N, Scarpace PJ: Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. *Am J Physiol* 275:E405-E411, 1998
- Qiu J, Ogas S, Lu R, Chehab FF: Transgenic mice overexpressing leptin accumulate adipose mass at an older, but not younger, age. *Endocrinology* 142:348-358, 2001
- Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J, Stephens TW: Regulation of expression of ob mRNA and protein by glucocorticoids and cAMP. *J Biol Chem* 271:5301-5304, 1996
- Zakrzewska KE, Cusin I, Sainsbury A, Rohner-Jeanrenaud F, Jeanrenaud B: Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. *Diabetes* 46:717-719, 1997
- Unger RH, Zhou YT: Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 50 (Suppl. 1):S118-S121, 2001
- Van Den Saffele JK, Goemaere S, De Bacquer D, Kaufman JM: Serum leptin levels in healthy aging men: are decreased serum testosterone and increased adiposity in elderly men the consequence of leptin deficiency? *Clin Endocrinol (Oxf)* 51:81-88, 1999
- Baumgartner RN, Ross RR, Waters DL, Brooks WM, Morley JE, Montoya GD, Garry PJ: Serum leptin in elderly people: associations with sex hormones, insulin, and adipose tissue volumes. *Obes Res* 7:141-149, 1999
- Moller N, O'Brien, Nair KS: Disruption of the relationship between fat content and leptin levels with aging in humans. *J Clin Endocrinol Metab* 83:931-934, 1998
- Isidori AM, Strollo F, More M, Caprio M, Aversa A, Moretti C, Frajese G, Riondino G, Fabbri A: Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. *J Clin Endocrinol Metab* 85:1954-1962, 2000
- Calle EE, Thun JM, Petrelli JM, Rodriguez C: Body-mass index and mortality in a prospective cohort. *N Engl J Med* 341:1097-1002, 1999
- Williamson DF, Pamuk E, Thun M, Flanders D, Byers T, Heath C: Prospective study of intentional weight loss and mortality in never-smoking overweight US white women aged 40-64 years. *Am J Epidemiol* 141:1128-1141, 1995
- Barzilai N, Gupta G: Revisiting the role of fat mass in the life extension induced by caloric restriction. *J Gerontol A Biol Sci Med Sci* 54:B89-B96, 1999
- Masoro EJ: Caloric restriction and aging: an update. *Exp Gerontol* 35:299-305, 2000