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Body Composition of Prolactin-, Growth Hormone–, and Thyrotropin-Deficient Ames Dwarf Mice

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Ames dwarf mice have primary deficiency of prolactin (PRL), growth hormone (GH), and thyroid-stimulating hormone (TSH), and live considerably longer than normal animals from the same line. In view of the documented effects of GH, PRL, and thyroid hormones on lean and fat body mass and skeletal growth, and the suspected relationship of body size and composition to life expectancy, it was of interest to examine age-related changes in body composition of Ames dwarf mice. Lean mass, fat mass, bone area, and bone mineral content (BMC) were determined in dwarf and normal mice at the ages of 2, 4.5–6, and 18 mo using dual X-ray absorptiometry. In addition to the expected significant declines in lean mass, bone area, and BMC, dwarf mice exhibited attenuation of the age-related increase in bone mineral density and delayed or attenuated increase in percentage of body fat. Percentage of body fat was lower in adult dwarfs than in the corresponding normal controls. Patterns of age-related changes in body composition in Ames dwarf mice are consistent with the recent report of age-related changes in body composition in PRL receptor knockout mice. We suspect that reduction in relative adiposity may contribute to the previously reported increase in insulin sensitivity of Ames dwarf mice and thus may be a factor in delayed aging and increased longevity of these animals.

Key Words: Fat content; bone mineral density; dual X-ray absorptiometry; growth hormone.

Introduction

Ames dwarf mice, animals with primary deficiency of prolactic (PRL), growth hormone (GH), and thyroid-stimulating hormone (TSH) *(1,2)*, live much longer than their normal siblings *(3,4)*. Hormonal defects in these animals are the result of homozygosity for a recessive mutation at the Prop-1 locus ($Prop1^{df}$, hereafter referred to as df) and consequent failure of differentiation of lactotrophs, somatotrophs, and thyrotrophs during fetal development of the pituitary *(2)*. Ames dwarf (df/df) animals are diminutive in size and hypothyroid *(1,3)*. Females are sterile owing to PRL deficiency and resulting luteal failure, while some males can sire litters *(3,5)*. The extension of life-span of these animals is quite remarkable, approx 50% in males and 60% in females *(4)*, and apparently reflects delayed rather than prolonged aging *(6,7)*. Interestingly, humans with hypopituitarism owing to a mutation of the same gene can live to a very advanced age, apparently longer than normal individuals in the same population *(8)*.

The physiologic mechanisms of prolonged longevity and the metabolic status of Ames dwarf mice are not well understood. Ames dwarfs have reduced body temperature *(9)*, and plasma glucose and insulin levels (*[10]*; Turyn and Bartke, unpublished findings). This resembles findings in animals subjected to caloric restriction, a treatment known to prolong life in mice and other species *(11)*. However, there is considerable evidence that Ames dwarf mice are not caloric restriction mimetics *(12,13)*. Endocrine deficits of Ames dwarf mice can be expected to affect their body composition. Both GH and thyroid hormones are key regulators of the growth of skeleton and soft tissues and are also involved in the regulation of the deposition of fat. The effects of PRL on growth and body composition are not well understood. However, treatment with PRL can stimulate somatic growth in hypopituitary dwarf mice *(3,14)* and PRL-resistant PRL receptor knockout (PRLR-KO) mice exhibit age-related reduction in adiposity (l5). Treatment with PRL was reported to increase food intake and fat deposition in female rats *(16,17)*. However, no information is available on the body composition of Ames dwarf mice or on its alterations in the course of life-span of these animals. It was therefore of interest to compare body composition of Ames dwarf and normal mice at different ages. Using dual X-ray absorptiometry (DXA), we measured lean mass, fat mass, bone mineral content (BMC), and bone mineral density (BMD) in young, adult, and middle-aged Ames dwarf mice and in age- and sex-matched normal animals from the same line. Plasma leptin levels were also determined.

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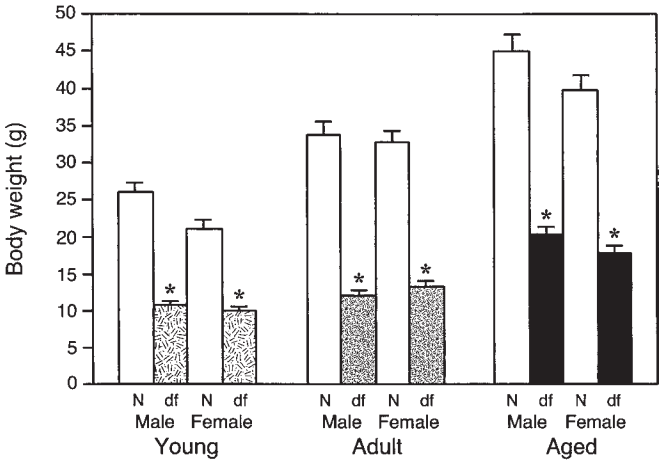

Fig. 1. Body weight in Ames dwarf (shaded or black bars) compared with age- and sex-matched normal animals from same line (open bars). Means ± SEM; 6–10 animals per group; *significantly different from the corresponding normal animals ($p < 0.05$).

Results

Body weight calculated from determinations of lean mass, fat mass, and bone mass was in remarkably close agreement with the results of weighing the animals on an electronic balance prior to freezing ($r = 0.998$; average difference between average "DXA weight" and gravimetric weight of <0.8 g; 22.91 ± 1.29 vs 22.11 ± 1.31 g). Body weight, lean mass, fat mass, percentage of lean, percentage of fat, bone area, BMC, and BMD in normal and Ames dwarf mice at different ages are presented in Figs. 1–3. The remarkable and highly significant decrease in body size (Fig. 1), which is characteristic of Ames dwarf mice *(6,18)* was accompanied by significant decreases in lean mass, bone area, and BMC in both females and males in each age group (Figs. 2 and 3). Fat mass was significantly reduced in dwarf compared with normal mice in each comparison except young females (Fig. 2).

In young dwarf mice, percentage of body fat was numerically higher than in the normal animals, although these apparent differences were not statistically significant (Fig. 2). Percentage of body fat increased significantly with age in normal mice of both sexes and in male dwarfs. The apparent increase in percentage of body fat with age in female dwarfs was not statistically significant. In comparison with normal animals, the age-related increase in percentage of body fat was diminished in female dwarfs and delayed in male dwarfs. In adult and aged dwarf mice, percentage of body fat was numerically lower than in the corresponding normal controls in each of four comparisons. These differences were significant for adult males ($p < 0.02$) and for combined data ($p < 0.04$).

BMD was significantly lower in young male dwarfs, in adult female dwarfs, and in aged dwarfs of both sexes than in the corresponding (age- and sex-matched) normal controls (Fig. 3). In addition, BMD was numerically lower in dwarfs than in normals in the remaining two comparisons (young females and adult males). Comparison of values measured in young and aged animals revealed that BMD significantly increased with age in both dwarf and normal mice of both genders. However, the age-related increase in BMD was much less pronounced in dwarfs than in normal mice.

Plasma leptin levels in young female Ames dwarfs did not differ from those measured in young normal females (Table 1). However, leptin levels significantly increased with age in normal ($p < 0.05$) but not in dwarf females, resulting in a significant difference between genotypes at the age of 11 to 12 mo. There were no significant differences in plasma leptin levels between adult dwarf and normal males.

Discussion

We measured age-related changes in body composition of Ames dwarf mice. These animals are characterized by primary deficiency of PRL, GH, and TSH, diminutive body size, and prolonged longevity. Findings obtained in dwarf mice were compared to the values measured in age- and sex-matched normal animals from the same line.

The key novel finding of our study is that percentage of body fat was significantly lower in adult Ames dwarf mice than in their normal siblings (in data collapsed across genders), and this difference appeared to persist as the animals aged. There is considerable evidence that GH deficiency in the human is associated with increased rather than reduced fat deposition *(19)*. Administration of GH to GH-deficient individuals reduces adipose mass and concomitantly increases lean or fat-free mass *(19)*; that is, it leads to a substantial decrease in percentage of body fat. Conversely, elevation of GH levels above the normal physiologic range is associated with reduced adiposity in acromegalic humans *(20)* and in GH transgenic mice *(21)*. GH treatment increased the weight of muscles, liver, and heart in Snell dwarf mice *(22)*, which, similarly to Ames dwarfs, are GH, PRL, and TSH deficient *(1,23)*. Moreover, hypothyroidism is usually associated with adiposity, and, therefore, the percentage of body fat in Ames dwarf mice, which are GH and TSH deficient, could be expected to be increased rather than reduced. Reduced gonadal activity in female Ames dwarf mice would be another reason to expect increased rather than reduced fat deposition. Estrogen resistance is associated with substantially increased percentage of fat content in estrogen receptor-α knockout mice *(24)*.

Because the observed alterations in body composition of Ames dwarf mice are not consistent with reduced function of the somatotropic axis and the pituitary-thyroid axis in these animals, it is tempting to speculate that they may be related to PRL deficiency. PRL exerts complex effects on lipid metabolism *(25–27)*, presumably including direct, PRLR-mediated effects on the adipocyte *(28)*. Reports on the effects of PRL on the mass of adipose tissue are contro-

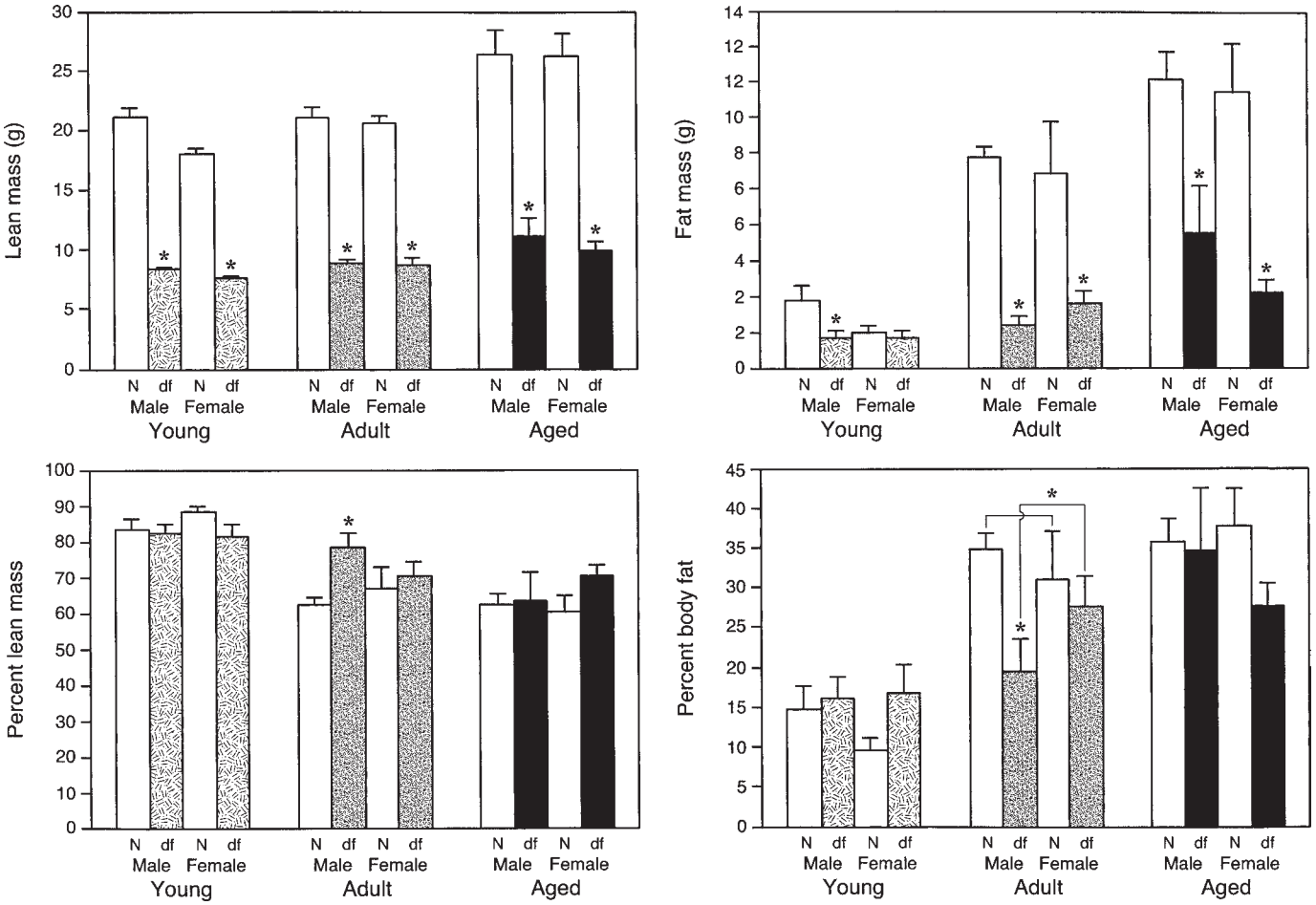

Fig. 2. Lean mass and fat mass (**top**) and percentage of lean mass and fat mass (**bottom**) in Ames dwarf (shaded or black bars) compared with age- and sex-matched normal animals from same line (open bars). Means ± SEM; 6–10 animals per group; *significantly different from the corresponding normal animals ($p < 0.05$); other statistical comparisons in the text.

versial. Adipose tissue weight was reduced in hyperprolactinemic male mice *(29)*, and the amount of retroperitoneal fat was diminished in transgenic female mice overexpressing PRL *(28)*. In female rats, PRL treatment stimulated food intake and fat deposition *(16,17)*. Particularly relevant to interpretation of the present findings is the recent report of progressive reduction in fat mass in mice that were PRL resistant as a consequence of targeted disruption of PRLR *(15)*. In adult female PRLR-KO mice, plasma leptin levels were also significantly reduced *(15)*. Findings in PRL-KO animals closely resemble results obtained in Ames dwarf mice in the present study. Thus, it can be suspected that PRL deficiency may have caused, or at least contributed to, the age-related decline in the relative (vs wild-type controls) percentage of body fat and plasma leptin levels in Ames dwarfs.

Plasma corticosterone levels are normal in old Ames dwarfs of both sexes but are reduced in young female dwarfs and elevated in young dwarf males *(10)*. While pathologic excess of glucorticoids leads to obesity, chronic mild elevation of corticosterone is associated with reduced fat deposition in calorically restricted mice and rats *(11,30)* and in transgenic mice overexpressing GH *(21)*. It seems likely that reduced percentage of body fat in adult and aged Ames dwarf mice is related to significant reductions in plasma glucose and insulin levels (*[10]*; Turyn and Bartke, unpublished findings) and may contribute to increased insulin sensitivity (*[3,6]*; unpublished data) in these animals. Age-related increase in adiposity is associated with insulin resistance *(31)*.

Comparison of body composition in young, adult, and aged mice in the present study (Fig. 2) indicates that the age-related increase in percentage of body fat was attenuated in female Ames dwarfs and delayed in male Ames dwarfs. This can be interpreted as additional evidence for delayed aging in these animals *(3,4,6,7)*. Thus, the observed differences between Ames dwarf and normal mice in percentage of body fat may reflect the fact that "adult" and "aged" dwarfs are biologically younger than normal animals of the same chronological age. One could also speculate that attenuation of the age-related increase in adiposity in Ames dwarfs contributes to their improved sensitivity to insulin and prolonged longevity. Additional studies will be necessary to

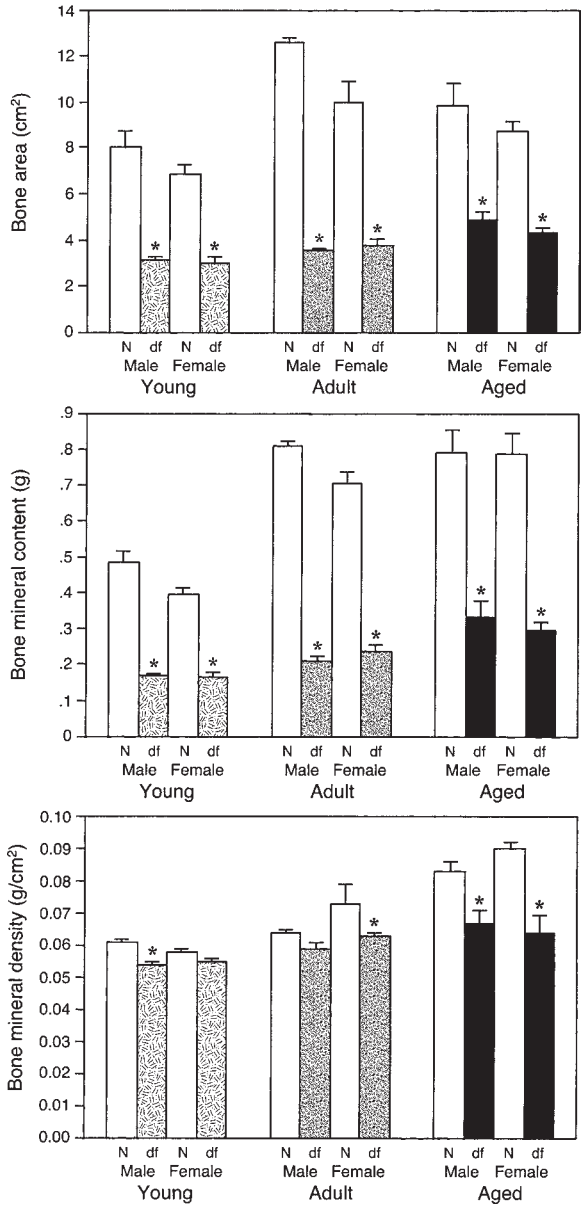

Fig. 3. Bone area (**top**), BMC (**middle**) and BMD (**bottom**) in Ames dwarf (shaded or black bars) compared with age- and sex-matched normal animals from same line (open bars). Means ± SEM; 6–10 animals per group; *significantly different from the corresponding normal animals ($p < 0.05$); other statistical comparisons in the text.

characterize regional (e.g., visceral vs subcutaneous) distribution of adipose tissue in dwarf mice and to relate this information to insulin signaling in these animals. Significance of visceral fat in this regard *(32)* is of particular interest.

Measurements of plasma leptin levels revealed a significant age-related increase in normal females from this line that paralleled a striking increase in percentage of body fat. This increase was entirely absent in female dwarfs. Moreover, plasma leptin levels remained low in 2-yr-old female dwarf mice. Absence of significant differences in plasma leptin levels in male Ames dwarfs compared with normal mice at the age of 9 to 10 mo corresponds to lack of differences in body fat measured in 11- to 12-mo-old males.

A reduction in BMD in Ames dwarf compared with normal mice was not unexpected. Reduced BMD was reported in GH-deficient patients along with evidence that it increases in response to GH replacement *(19)*. A decline in BMD in the elderly is believed to be owing in part to falling GH levels and can be reduced or partially reversed by administration of GH *(33,34)*. Sjogren et al. *(35)* recently reported reduced BMD in GH receptor KO mice. These animals are GH resistant and, similarly to Ames dwarfs, have extremely low plasma insulin-like growth factor-1 (IGF-1) levels *(36, 37)*. The role of IGF-1 in the control of bone growth and composition is well documented *(39)*. Our preliminary studies in transgenic mice overexpressing human GH suggest that chronic excess of signaling via GH and/or PRL receptors is associated with increased BMD (0.063 ± 0.003 vs 0.057 ± 0.006; $p < 0.0532$ based on five animals per group). It is of interest that the difference between Ames dwarf and normal mice in BMD increased with age. This difference is attributed to dwarfs exhibiting severe attenuation of the increase in BMD that occurred in normal animals between the ages of 2 and 18 mo. It is unclear whether this difference may be related to the delayed aging of dwarf animals. In an interesting contrast to these findings in mice and in GH-deficient patients, osteopenia was reported in hyperprolactinemic women, and there is some evidence that it cannot be explained solely in terms of reduced estrogen levels in these patients *(39)*.

In summary, we have presented evidence that an age-related increase in adiposity is delayed and/or attenuated in Ames dwarfs compared with normal mice. The resulting reduction in percentage of body fat in middle-aged dwarfs resembles findings in PRLR-KO mice and correlates with the previously reported enhanced sensitivity to insulin and delayed aging in these remarkably long-living mutants. BMD in middle-aged dwarfs is lower than in normal animals of the same chronological age and resembles values measured in young normal mice.

Materials and Methods

Animals

Ames dwarf and normal animals were derived from a closed breeding colony maintained since 1984 at Southern Illinois University at Carbondale. Previously published data on longevity *(4)*, various physiologic parameters related to aging *(4,9,10,40)*, and effects of caloric restriction *(12,13)* in Ames dwarf mice were obtained using animals from the same line.

Ames dwarf mice were produced by mating heterozygous carriers of the df (Prop-1^{df}) mutation (df/+) or homozygous dwarf males (df/df) with heterozygous females (df/+). Normal siblings of Ames dwarfs (df/+ or +/+) served as controls. After weaning, the animals were housed in groups of

Table 1
Leptin Levels and Body Weight in Ames Dwarf and Normal Mice (means ± SE)

	Normal		Ames dwarf	
	Body wt (g)	Leptin (ng/mL)	Body wt (g)	Leptin (ng/mL)
Females				
2–3 mo old	24.5 ± 1.02	9.1 ± 1.74	12.3 ± 0.72[a]	10.7 ± 3.10
11–12 mo old	36.4 ± 0.67	19.2 ± 1.20	14.2 ± 1.17[a]	10.4 ± 3.11[a]
24–26 mo old	—	—	16.9 ± 1.72	8.8 ± 1.74
Males				
9–10 mo old	40.9 ± 1.85	16.6 ± 2.12	20.7 ± 0.82[a]	20.2 ± 1.90

[a]Significantly different from normal controls; $p < 0.05$.

five in shoe-box type plastic microisolator units; dwarfs were housed with other dwarfs or with normal females. The animals had free access to 5008f Mouse Diet (minimum 23% protein and 6.5% fat; PMI, Brentwood, MO) and tap water; light cycle was 12 h light:12 h dark, and room temperature was set at 22ºC. Monitoring of sentinel animals housed in the same room in cages without microisolator tops indicated that the animals were seronegative for all common mouse pathogens.

Young (8-wk), adult (4.5–6 mo), and middle-aged (18 mo) mice were killed via CO_2 asphyxiation, weighed, and frozen with hind feet extended and tail deflected to be parallel to the trunk, i.e., in a body configuration suitable for DXA studies. The age of 18 mo corresponds to approximately ¾ of the average life-span of normal animals from this line and approximately ½ of the average life-span of dwarf animals *(4,13)*. For brevity, animals in this age group are hereafter referred to as "aged." Each age group consisted of Ames dwarf and normal animals of both genders, 6–10 mice of every genotype/gender combination. During 4 d preceding euthanasia, all animals in the young group were fed Teklad LM-485 Mouse/Rat (minimum 19% protein and 5% fat) diet containing fenbendazole (150 ppm), which was used as part of the 9-wk program to eliminate pinworms from the Vivarium. Additional animals fed standard (unmedicated) diet were bled by orbital sinus puncture under ether anesthesia for measurements of plasma leptin levels. The protocols for these studies were approved by an institutional animal care committee.

DXA Measurements

Frozen carcasses were shipped to Eli Lilly. Body composition was measured by DXA using a Norland pDEXA *(19)*. The system provides a noninvasive method for quantification of whole-body composition and is based on the differential attenuation of high- and low-energy X-rays by the tissues in the scan area. Soft tissues attenuate the energy beam less than bone; of the soft tissue mass, fat tissue attenuates the beam less than lean tissue. Fat mass consists primarily of adipose tissue, but lean mass includes organs, tendons, cartilage, blood, and body water in addition to skeletal muscle. In the present study, fat mass, lean mass, and BMC (bone mass) were measured and are reported. Mice were placed on the instrument platform in the ventral position. Measurements were performed at a speed of 7 mm/s and a resolution of 0.5 × 0.5 mm. Bone surface area was monitored and set at constant values by adjusting the histogram averaging width. Quality controls included phantom ID2232 and Calibration Standard. In several series of replicate measurements, coefficients of variation (CVs) for lean body mass determined by DXA were 2.4–7.4%, average 5.8%, and for fat measured by DXA in the same mice were 2.9–13.0%, average 8.0%, depending on the size of the animal. Moreover, we compared results obtained by DXA with results obtained by nuclear magnetic resonance, or by extraction and chemical analysis, and observed excellent agreement over a wide range of mouse body weights and body composition.

Statistical Analyses

Plasma leptin levels were measured by radioimmunoassay (Linco, St. Charles, MO). This assay was linear from 0.2 to 20 ng/mL and intra- and interassay CVs were 4.7 and 13.7%, respectively.

The data were analyzed statistically by analysis of variance followed by protected least significant differences test for individual comparisons, using $p < 0.05$ as the criterion for significance.

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