

# The M16 Mouse: An Outbred Animal Model of Early Onset Polygenic Obesity and Diabesity

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## Abstract

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**Objective:** To characterize the phenotypic consequences of long-term selective breeding for rapid weight gain, with an emphasis on obesity and obesity-induced diabetes (diabesity).

**Research Methods and Procedures:** M16 is the result of long-term selection for 3- to 6-week weight gain from an ICR base population. Experiment 1 characterized males from both lines for body weights (3, 6, and 8 weeks), feed (4 to 8 weeks) and H<sub>2</sub>O (6 to 8 weeks) consumption, and heat loss, body composition, and levels of several plasma proteins at 8 weeks of age. Experiment 2 characterized differences between lines for both sexes at three ages (6, 8, and 16 weeks) and fed two diets (high and normal fat). Body weight, composition, blood glucose, and plasma insulin and leptin levels were evaluated after an 8-hour fast.

**Results:** At all ages measured, M16 mice were heavier, fatter, hyperphagic, hyperinsulinemic, and hyperleptinemic relative to ICR. M16 males and females were hyperglycemic relative to ICR, with 56% and 22% higher fasted blood glucose levels at 8 weeks of age.

**Discussion:** M16 mice represent an outbred animal model to facilitate gene discovery and pathway regulation control-

ling early onset polygenic obesity and type 2 diabetic phenotypes. Phenotypes prevalent in the M16 model, with obesity and diabesity exhibited at a young age, closely mirror current trends in human populations.

**Key words:** adiposity, body weight, predisposition, insulin, glucose

## Introduction

Obesity in the United States has been identified as an epidemic for over 2 decades, and yet the number of individuals classified as obese continues to grow (1). The chance of developing type 2 diabetes is greatly increased in people who are overweight or obese (2). Obesity-induced type 2 diabetes, commonly referred to as “diabesity” (3), is not just an adult disease; there have been dramatic increases in the number of obese children plagued by type 2 diabetes as well (4,5).

Unraveling the underlying causes of diabesity has been elusive. Obesity and type 2 diabetes are complex and multifactorial traits, affected by many genes with both direct and indirect effects, and are also strongly influenced by the environment (6,7). Difficulties in using humans as experimental subjects limit progress at both the quantitative and molecular levels. Thus, many researchers have incorporated the use of mouse models for dissecting complex traits (e.g., 8–10).

Previously, selection for 3- to 6-week weight gain for 27 generations created a unique line of mice (M16) from an outbred ICR base population (11). Breeding criterion was within-litter selection for the male and female with the largest weight gain from 3 to 6 weeks of age (11). An ICR control line was maintained in parallel, with random mating from generation to generation but maintaining a similar effective population size (11). Mice from the M16 line are larger at all ages and have increased body fat percentage, fat cell size, fat cell numbers, and organ weights when compared with ICR (12–15). These mice also exhibit hyperpha-

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gia, accompanied by moderate obesity, and are hyperglycemic, hyperinsulinemic, and hypercholesterolemic (16).

The experiments conducted in the present study provide a large phenotypic recharacterization of the M16 and ICR lines of mice to ascertain whether primary phenotypic characteristics acquired as a result of selection have been maintained over the course of many years of potential genetic drift, accrual of inbreeding, or inadvertent selection. In addition, several important phenotypes were measured that had never been evaluated in these lines, including heat loss, bone mineral density (BMD),<sup>1</sup> water consumption, and measurements of levels of multiple plasma hormones and cytokines. The primary objectives were to characterize the phenotypic consequences of long-term selective breeding for rapid weight gain, with an emphasis on obesity and obesity-induced diabetes (diabesity), and to extend such measurements to young ages (6 and 8 weeks) as a model for humans that are still in, or nearing the end of, their growth phases.

## Research Methods and Procedures

### **Mouse Lines**

Mice used in this study originated from outbred stocks of M16 and ICR lines originally housed at North Carolina State University (Raleigh, NC). Colonies established at the University of Nebraska (Lincoln, NE) in 2000 were evaluated.\* All litters were weaned at 3 weeks of age. From each litter, four animals of the same sex were placed in plastic cages with wood chip bedding and ad libitum access to water and feed. Laboratory housing temperatures were maintained at 22 °C, with relative humidity at 35% to 50%, and a light/dark cycle of 12/12 hours starting at 7 AM. All procedures and protocols were approved by The University of Nebraska Institutional Animal Care and Use Committee.

### **Experiment 1: Body Weights, Feed and Water Consumption, Body Composition, Heat Loss, and Blood Analysis**

Eighty male mice per line were fed a standard rodent diet (Teklad 8604 Rodent Chow). Weights were recorded at 3, 6, and 8 weeks of age, with feed intake data collected from 4 to 8 weeks and H<sub>2</sub>O intake from 6 to 8 weeks. Feed and H<sub>2</sub>O intakes were evaluated as raw values and as proportions of 8-week body weight and 8-week metabolic body weight (kilograms<sup>0.75</sup>). Efficiency of weight gain was calculated by dividing weight gain by total intake.

Heat loss was measured on 20 male mice per line at ~7 to 8 weeks of age, using 10 direct gradient layer, individual

animal calorimeters (17). Eight-week-old mice, with free access to H<sub>2</sub>O, were withheld from feed for 1.5 hours before processing for analysis of body composition and blood profiling. Mice were decapitated after brief exposure to CO<sub>2</sub> and blood collected and processed for plasma content. The right side scapula brown adipose depot, right epididymal adipose depot, right hind limb subcutaneous adipose depot, and liver were excised, weighed, and immediately snap-frozen in liquid nitrogen. All organ data were evaluated as wet weight and adjusted as a percentage of 8-week body weight. Other tissues snap-frozen in liquid nitrogen for future analyses included hypothalamus, pituitary, kidney (with adrenal gland), heart, spleen, and gastrocnemius muscle. The remainder of each carcass was evaluated for body fat percentage. Lipid weight was determined as the difference between freeze-dried carcass weight before and after a 96-hour ether extraction and was expressed as a percentage of the carcass weight.

Plasma insulin, leptin, and growth hormone (gh) levels were determined using mouse (insulin) and rat (leptin and gh) radioimmunoassay kits (Linco, St. Louis, MO). Tumor necrosis factor-α (TNFα), interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, granulocyte-macrophage colony-stimulating factor, and interferon-γ levels were measured using a Mouse Cytokine Multiplex kit (Linco) run on a Luminex (Austin, TX) analyzer.

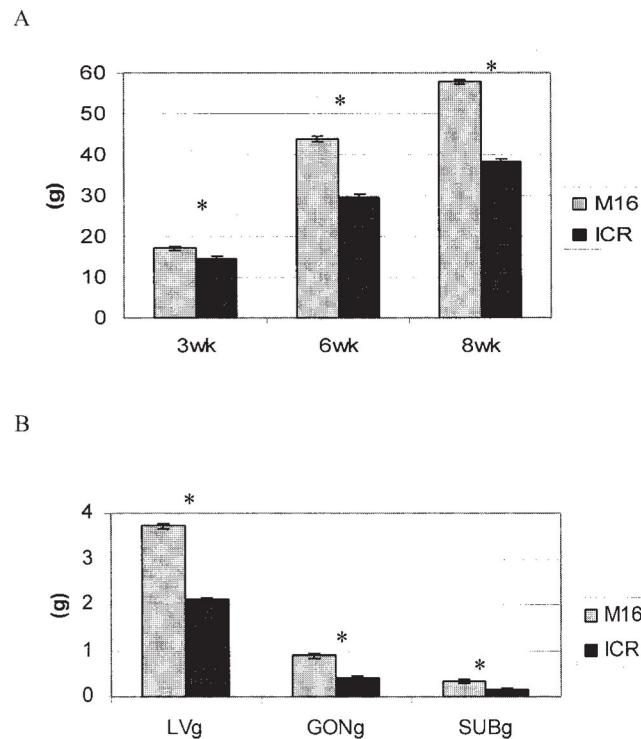
### **Experiment 2: Line, Sex, Age, and Diet Effects on Diabesity**

A total of 240 mice were evaluated in a balanced two × two × two × three factorial design, with line (M16, ICR), sex (male, female), diet [high-fat (FAT) diet, normal (REG) diet], and age (6, 8, 16 weeks) as main effects. Two synthetic diets were fed: REG (10% kcal fat, 20% kcal protein, 70% kcal carbohydrate) and FAT (45% kcal fat, 20% kcal protein, 35% kcal carbohydrate), representing D12450B and D12451 from Research Diets (New Brunswick, NJ). Metabolizable energy values for the REG and FAT diets were 3.85 and 4.73 kcal/g, respectively. Diets were fed from 4 weeks of age until mice were evaluated for body composition. Mice were randomly assigned to cages by sex within line and weighed at 4 weeks of age. Body weights were subsequently recorded at 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, and 16 weeks.

Subsets of mice ( $n = \sim 80$ ) were processed at 6, 8, and 16 weeks of age for evaluation of body composition and blood profiles. Mice were fasted for 8 hours and sacrificed as described in Experiment 1. Measurements of total body fat, lean tissue, and BMD were made using DEX (Lunar Corporation, Madison, WI). Lean tissue and BMD were evaluated only in 8-week-old mice. Right epididymal (males) and perimetrial (females) adipose depots were excised and weighed. Blood glucose levels were measured by glucose oxidase method with the SureStep Blood Glucose

<sup>1</sup> Nonstandard abbreviations: BMD, bone mineral density; gh, growth hormone; TNFα, tumor necrosis factor-α; IL, interleukin; FAT, high fat; REG, normal; BAT, brown adipose tissue; PGON, percentage epididymal fat depot; PSUB, percentage subcutaneous fat depot; PFAT, percentage body fat; QTL, quantitative trait locus.

\*For availability of M16 mice, please query authors.



**Figure 1:** (A) Comparison of body weights at 3, 6, and 8 weeks for M16 and ICR male mice. (B) Comparison of liver weight (LVg), epididymal adipose depot weight (GONg), and subcutaneous adipose depot weight (SUBg) for 8-week-old M16 and ICR male mice. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.001$ ) differences between lines.

Monitoring System (LifeScan Canada Ltd., Burnaby, BC, Canada). Plasma levels of insulin and leptin were determined using radioimmunoassay as described earlier.

#### Statistical Analyses

Data were analyzed using the generalized linear models procedure of SAS (SAS Institute, 1990). The model used in Experiment 1 contained the fixed effect of line. The model

used for Experiment 2 contained fixed effects of line, sex, diet, and age, and all two-way interactions. Results are presented as least-square means, representing estimators of the class or subclass marginal means that are expected for a balanced design. Least-square estimates of model parameters are those values that minimize the sum of squares of residuals from the model fitted. Significance was accepted as  $p < 0.05$ , with specific  $p$  values listed in tables and figures or in text when no table or figure was used. Phenotypic correlations among dependent variables, adjusted for fixed effects, were analyzed using multivariate ANOVA.

## Results

### Experiment 1: Body Weights, Feed and Water Consumption, Body Composition, Heat Loss, and Blood Analysis

Mice from the M16 line were 15%, 48%, and 52% heavier than ICR mice at 6, 8, and 16 weeks of age, respectively (Figure 1A). All organ weights measured except for brown adipose tissue (BAT) were significantly greater in M16 (Figure 1B). Differences between lines for organ weights expressed as a percentage of body weight followed the same trend. M16 mice had increased epididymal adipose (0.89 vs. 0.41 grams) and subcutaneous adipose (0.34 vs. 0.16 grams) depot weights. Expressed as a percentage of body weight, M16 mice had a percentage epididymal fat depot (PGON) of 1.53%, percentage subcutaneous fat depot (PSUB) of 0.59%, and overall percentage body fat (PFAT) of 15.8%, relative to ICR with PGON of 1.07%, PSUB of 0.42%, and PFAT of 9.19%. Relative differences between lines observed for PGON, PSUB, and PFAT were 43%, 40%, and 72%, respectively. BAT was greater in the ICR line expressed as wet weight (12%;  $p < 0.01$ ) or as a percentage of body weight (29%;  $p < 0.001$ ).

Significant differences between lines were observed for unadjusted 4- to 8-week feed intake, unadjusted 6- to 8-week H<sub>2</sub>O intake, H<sub>2</sub>O intake adjusted for 8-week body

**Table 1.** Least-square means ( $\pm$ SE) for feed intake, H<sub>2</sub>O intake, and heat loss in M16 and ICR male mice

Line	FI*	H <sub>2</sub> O Int d†	H <sub>2</sub> O Int adj‡	Heat loss§
M16	8.78 $\pm$ 0.17	18.40 $\pm$ 0.34	32.85 $\pm$ 0.70	132.45 $\pm$ 2.45
ICR	6.14 $\pm$ 0.17	9.84 $\pm$ 0.35	25.90 $\pm$ 0.71	150.94 $\pm$ 2.51
<i>p</i> value	0.0001	0.0001	0.0001	0.0001

\* Unadjusted feed intake per day (g).

† Unadjusted H<sub>2</sub>O intake per day (mL).

‡ H<sub>2</sub>O intake adjusted for 8-week body weight (mL/8-week weight  $\times$  100).

§ Heat loss (kcal/kg<sup>0.75</sup> per day).

**Table 2.** Least-square means ( $\pm$  SE) for insulin, leptin, TNF $\alpha$ , IL-6, and GH in 8-week-old M16 and ICR male mice

Line	Ins*	Lep†	TNF $\alpha$ ‡	IL-6§	GH**
M16	2.49 $\pm$ 0.29	6.52 $\pm$ 0.60	12.30 $\pm$ 0.90	19.14 $\pm$ 0.61	3.02 $\pm$ 1.6
ICR	0.98 $\pm$ 0.28	2.76 $\pm$ 0.61	7.77 $\pm$ 0.91	14.60 $\pm$ 0.59	4.92 $\pm$ 1.5
p value	0.0007	0.0001	0.0012	0.001	0.25

\* Insulin (pmol/mL).

† Leptin (pmol/mL).

‡ Tumor necrosis factor alpha (pg/mL).

§ Interleukin-6 (pg/mL).

\*\* Growth hormone (ng/mL).

weight, and heat loss (Table 1). The M16 line exhibited a higher level of feed efficiency measured from 4 to 8 weeks of age ( $p < 0.01$ ). No significant differences were noted for feed intake when adjusted for body weight or metabolic body weight (data not shown).

Least-square means for plasma hormone/cytokine levels are presented in Table 2. The M16 line had significantly higher levels of insulin, leptin, TNF $\alpha$ , and IL-6 (154%, 136%, 58%, and 31%, respectively) than ICR. Plasma gh levels were not significantly different between lines. No significant differences were observed between lines for the remainder of cytokines measured at 8 weeks of age. Correlations among most traits were low to moderate, whereas those among measurements of fat were moderate to high (Table 3). Feed intake was not significantly correlated with any of the other traits in this analysis.

### Experiment 2: Line, Sex, Age, and Diet Effects on Diabesity

M16 mice were heavier than ICR mice at all ages measured. Males were significantly heavier than females at all ages ( $p < 0.001$ ). Growth curves for line-by-sex-by-diet subclasses are presented in Figure 2. Line-by-sex interactions were observed from 6 to 10 weeks. Differences between lines in males plateaued from 10 to 14 weeks, whereas differences between lines for females continued on a positive plane. Effect of diet on body weight was observed from ages 5 to 16 weeks, with the FAT diet resulting in heavier mice, but no line-by-diet or sex-by-diet interactions were observed.

Differences in body composition measured as fat, lean, and BMD were observed for 8-week-old M16 and ICR mice (Table 4). Both male and female M16 mice had increased fat and lean tissue weight compared with ICR. Adjusted for body weight, the M16 mice had increased percentage fat and decreased percentage lean. BMD differences followed the same trend, with both sexes of M16 exceeding ICR

controls. M16 mice were fatter for all ages measured (Figure 3). Significant sex differences were observed for PFAT at 16 weeks, with females being 27% fatter. A line-by-sex interaction was detected only at 16 weeks, when male M16 mice were 20% fatter than ICR males, and female M16 mice were 60% fatter than ICR females. Both male and female M16 mice were significantly different from their ICR counterparts for PGON (Figure 4). Effect of the FAT diet was significant for PGON at all ages, although mice on the FAT diet had greater PFAT only at 16 weeks of age. No significant interactions were found for line-by-diet for any adipose-related traits in this study (data not shown).

A significant effect of line was observed for fasted blood glucose levels at 6 and 8 weeks ( $p < 0.001$ ; Figure 5). Effects of diet and sex were also significant at these ages (data not shown); the FAT diet resulted in increased ( $p < 0.001$ ) blood glucose levels of 26% and 15% at 6 and 8 weeks, respectively, but males had 31% (6 weeks) and 50% (8 weeks) higher ( $p < 0.0001$ ) levels than females. Significant line-by-sex interaction effects were seen at 8 and 16 weeks (Figure 5). For example, although M16 males had 56% greater fasted blood glucose levels than ICR males at 8 weeks, the difference between lines for females was 22%. A significant diet-by-sex interaction effect was also observed at 6 and 8 weeks ( $p < 0.001$ ; data not shown). This interaction was represented by the FAT diet increasing fasted blood glucose by 26% in males, with no observable difference in females. No line-by-diet interactions were observed for blood glucose levels.

Line effects for males were significant for plasma levels of insulin, with M16 mice having greater levels than ICR (Figure 6). Leptin levels were significantly different for both males and females between lines at all ages measured (Figure 7). As an example of these line differences, M16 male mice had levels of insulin and leptin that were 288% and 229% greater than ICR male mice, respectively, at 8 weeks of age.

**Table 3.** Correlation matrix adjusted by fixed effects for line in M16 and ICR male mice

	8wk	GONG	PGON	SUBg	PSUB	LVg	PLV	BATg	PBAT	H <sub>2</sub> O**	FI††
8wk	1.0	0.29*	0.25*	0.19†	-0.11	0.63*	-0.20‡	0.22†	-0.25†	0.11	0.14
GONG		1.0	0.94*	0.73*	0.67*	0.30*	0.07	0.41*	0.25†	-0.14	-0.06
PGON			1.0	0.64*	0.69*	0.12	0.13	0.34*	0.31*	-0.15	-0.09
SUBg				1.0	0.94*	0.23†	0.07	0.38*	0.25†	-0.32*	-0.01
PSUB					1.0	0.03	0.13	0.32*	0.34*	-0.33*	-0.06
LVg						1.0	0.61*	0.27†	-0.04	0.20‡	0.11
PLV							1.0	0.09	0.18‡	0.12	0.05
BATg								1.0	0.86*	-0.13	-0.08
PBAT									1.0	-0.12	-0.16
H <sub>2</sub> O										1.0	-0.02
FI											1.0

\*  $p < 0.001$ .†  $p < 0.01$ .‡  $p < 0.05$ .

|| 8-Week body weight.

GONG, Epididymal adipose depot weight; PGON, Epididymal adipose depot expressed as a percentage of body weight; SUBg, Subcutaneous adipose depot weight; PSUB, Subcutaneous adipose depot expressed as a percentage of body weight; LVg, Liver weight; PLV, Liver expressed as a percentage of body weight; BATg, BAT weight; PBAT, BAT expressed as a percentage of body weight.

\*\* H<sub>2</sub>O intake 6 to 8 weeks.

†† Feed intake 4 to 8 weeks.

Table 5 contains the correlation matrix for insulin, leptin, glucose, PFAT, PGON, and body weight for mice at 8 weeks of age. There are moderate to strong positive correlations among most traits except for those involving glucose, which had low but significant correlations with insulin, leptin, and PFAT.

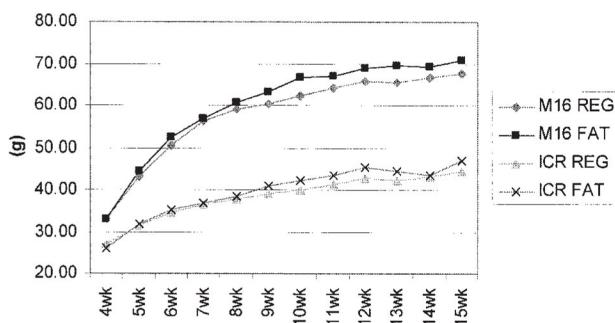
## Discussion

Model animals with accelerated growth curves often have a genetic predisposition for obesity. Selection for body weight in mice has resulted in correlated responses of increased fatness in many experiments (10,18). Selection for increased 3- to 6-week postweaning gain in the M16 line of mice was first reported by Hanrahan et al. (11). Since then, numerous publications have described various phenotypic consequences between M16 and its unselected ICR base population resulting from the selection process (e.g., 12–14). However, ~90 generations have passed since selection ended. Although the M16 and ICR lines have been maintained with an outbreeding mating structure, factors such as random drift and gradual accumulation of inbreeding may have had significant effects on phenotypic differences between the lines. Furthermore, new phenotypic measurements are available that can better define the consequences of selection for growth rate on traits related to obesity and type 2 diabetes.

Differences in body weights observed in these experiments were as expected, with M16 mice being heavier than ICR at all ages. Differences are slightly lower than reported from earlier experiments (13,14,16,19). This may be due to drift or inbreeding, but these influences are confounded with potential effects of location and diet (Figure 8). However, despite being a closed population for ~90 generations and accumulating an estimated coefficient of inbreeding of between 0.5 and 0.75, few deleterious effects of inbreeding on weight and body composition phenotypes have surfaced. In addition, both M16 and ICR lines maintain excellent reproductive fitness and maternal characteristics (data not shown). In comparison with M16i (20), a fully inbred line derived from M16 by full-sib mating, M16 animals are heavier at all ages on a normal diet until 16 weeks of age (Figure 8), but the lines have similar phenotypes for heat loss and plasma levels of insulin and leptin (data not shown). Females from the M16i line also have significantly decreased reproductive fitness when compared with the M16 and ICR lines (data not shown).

Hyperinsulinemia, insulin resistance, fasting hyperglycemia, and  $\beta$ -cell dysfunction later in life are characteristics of type 2 diabetes in humans (21). Eighty to ninety percent of type 2 diabetics are found to be overweight, with increased proportions of abdominal adipose distribution (3). The M16 line shows an accelerated growth curve resulting in pheno-

A



B

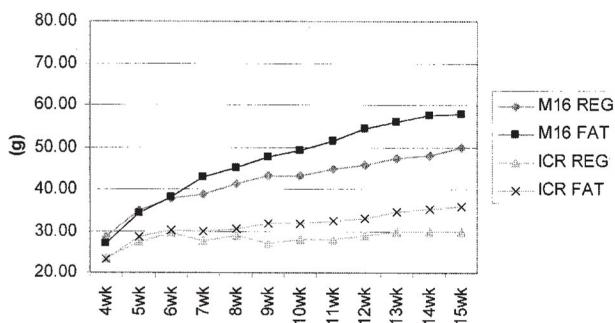
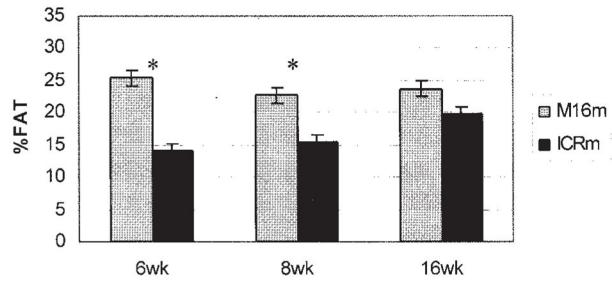


Figure 2: (A) Least-square means for body weights of M16 and ICR male mice from 4 to 15 weeks of age fed REG and FAT diets. (B) Least-square means for body weights of M16 and ICR female mice from 4 to 15 weeks of age fed REG and FAT diets.

A



B

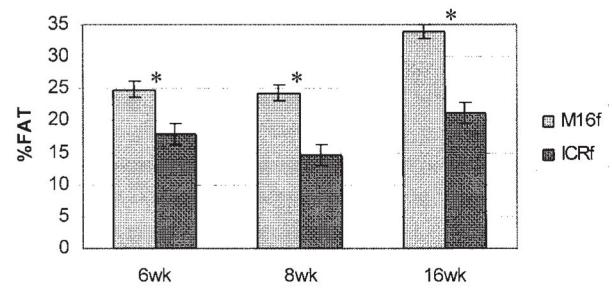


Figure 3: (A) Comparison of percentage total body fat between M16 and ICR male mice at 6, 8, and 16 weeks. (B) Comparison of percentage total body fat between M16 and ICR female mice at 6, 8, and 16 weeks. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.05$ ) differences between lines.

**Table 4.** Least-square means ( $\pm$ SE) for total grams of fat, total grams of lean, %fat, %lean, and BMD for 8-week-old male and female M16 and ICR mice

Line	Fat <sup>g</sup>	Lean <sup>†</sup>	%Fat <sup>‡</sup>	%Lean <sup>§</sup>	BMD <sup>**</sup>
<b>Males</b>					
M16	13.44 $\pm$ 0.78	38.28 $\pm$ 0.56	22.76 $\pm$ 1.16	67.14 $\pm$ 1.39	0.070 $\pm$ 0.001
ICR	5.37 $\pm$ 0.64	28.45 $\pm$ 0.59	15.43 $\pm$ 1.15	73.78 $\pm$ 1.15	0.064 $\pm$ 0.001
<i>p</i> value	0.001	0.001	0.01	0.01	0.01
<b>Females</b>					
M16	9.92 $\pm$ 0.64	29.75 $\pm$ 0.47	24.29 $\pm$ 1.26	66.98 $\pm$ 1.15	0.064 $\pm$ 0.001
ICR	3.78 $\pm$ 0.81	21.81 $\pm$ 0.59	14.62 $\pm$ 1.59	74.01 $\pm$ 1.45	0.061 $\pm$ 0.001
<i>p</i> value	0.001	0.001	0.01	0.01	0.01

\* Total grams of fat.

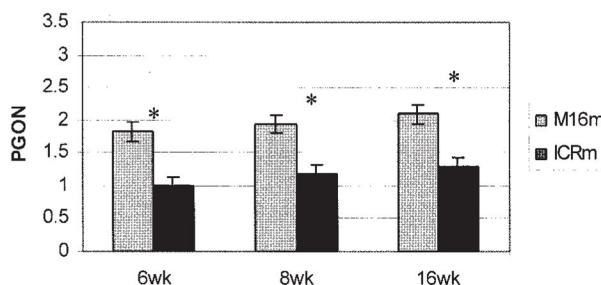
† Total grams of lean.

‡ Grams of fat divided by total tissue mass expressed as a percentage.

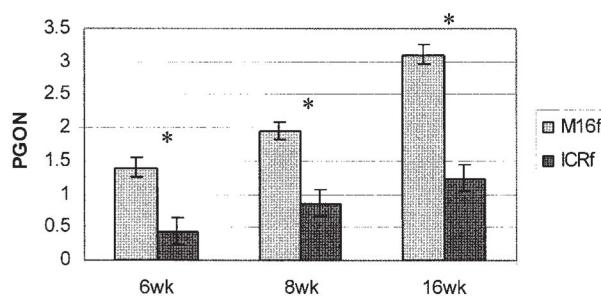
§ Grams of lean divided by total tissue mass expressed as a percentage.

\*\* BMD ( $\text{g}/\text{cm}^2$ ).

A



B

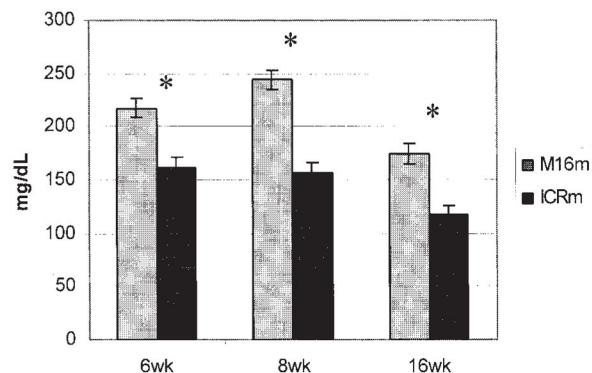


**Figure 4:** (A) Comparison of right epididymal adipose depot expressed as a percentage of body weight (PGON) between M16 and ICR male mice at 6, 8, and 16 weeks. (B) Comparison of right perimetrial adipose depot expressed as a percentage of body weight (PGON) between M16 and ICR female mice at 6, 8, and 16 weeks. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.05$ ) differences between lines.

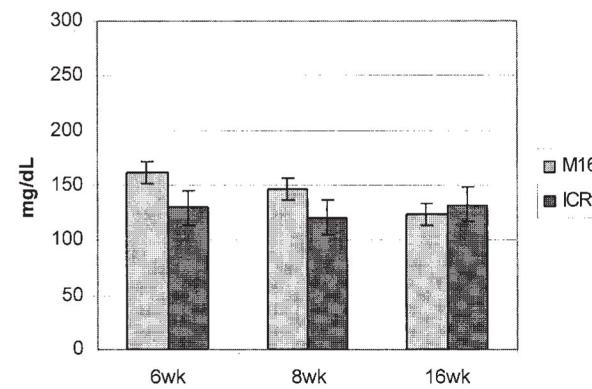
types consistent with a model for both obesity and type 2 diabetes. Given that M16 mice are outbred and that their obesity and glucose/insulin phenotypes are polygenic in nature, the M16 line may be an excellent model for study of diabesity in humans. Studies in humans show an association of abdominal adipose accumulation and an increase in the prevalence of type 2 diabetes (3,22,23). Samaras et al. (24) showed the genetic correlation in humans between insulin resistance and abdominal adipose to be moderate ( $r = 0.41$ ), whereas the correlation with total fat was slightly less ( $r = 0.24$ ). M16 mice are fatter at a younger age, and individuals display a greater PGON. Correlations of PGON with insulin ( $r = 0.42$ ) and with overall PFAT ( $r = 0.32$ ) were found in the present study. The lack of significant correlation between feed intake and other traits is somewhat puzzling. A possible explanation may be that feed intake was measured on a per cage basis, using the average of four animals per cage. The result may have been an inflated error of measurement and a downward bias in correlation estimates.

Relative to ICR, M16 mice had increased water consumption after adjustment for body weight. This 2-fold difference

A



B

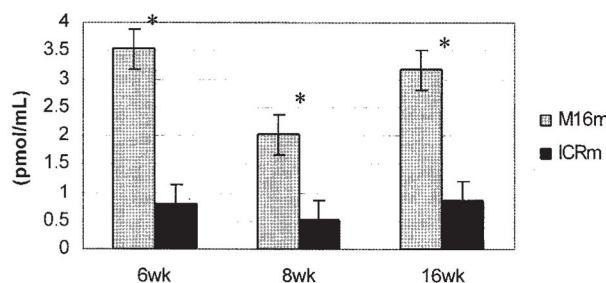


**Figure 5:** (A) Comparison of blood glucose levels (mg/dL) in M16 and ICR male mice at 6, 8, and 16 weeks. (B) Comparison of blood glucose levels (mg/dL) in M16 and ICR female mice at 6, 8, and 16 weeks. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.05$ ) differences between lines.

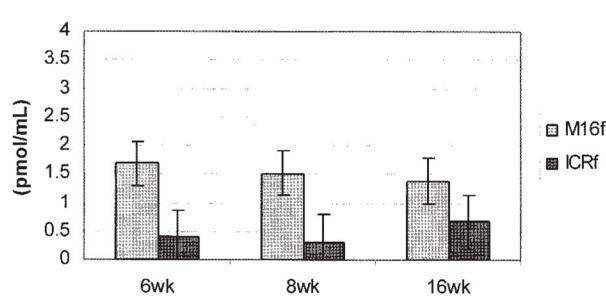
may be a result of increased feed intake coupled with enhanced growth rate. Water intake differences were also shown in the Tsumura, Suzuki, Obese, Diabetes (TSOD) polygenic obesity mouse model (25). However, the large difference in water intake between TSOD and control mice was not present at 8 weeks of age, but a 2- to 3-fold difference existed by 16 weeks. Any relationship between water intake and type 2 diabetes phenotype in the M16 model is not clear and warrants further investigation.

Results from this study demonstrate that the M16 line has increased liver weight relative to body weight and has greater amounts of regional and overall body fat when compared with ICR, confirming earlier studies (14,15). Weight of BAT was lower in the M16 line, similar to results found earlier by Saxton et al. (26). Although activity in BAT was not measured, it is possible that lower BAT mass may be contributing to the reduced heat loss observed in the

A



B



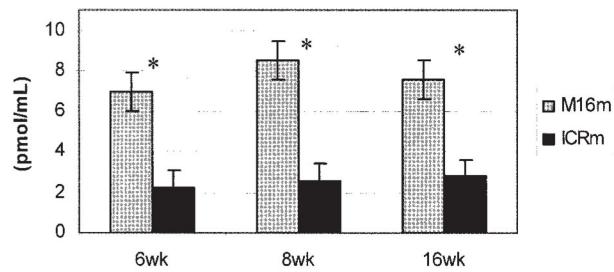
**Figure 6:** (A) Comparison of insulin plasma levels (pmol/mL) in 6-, 8-, and 16-week-old M16 and ICR male mice. (B) Comparison of insulin plasma levels (pmol/mL) in 6-, 8-, and 16-week-old M16 and ICR female mice. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.01$ ) differences between lines.

M16 line. Rodent BAT is a highly specialized tissue, with extensive sympathetic innervations, increased blood supply, extremely high mitochondria content, and a high degree of expression of uncoupling proteins (27,28). Moody et al. (29) have found evidence for quantitative trait loci (QTLs) in mice for BAT weight in close proximity to QTLs for heat loss, suggesting evidence of pleiotropic gene action. This difference in heat loss may also be a result of the decreased percentage of lean tissue in M16 relative to ICR.

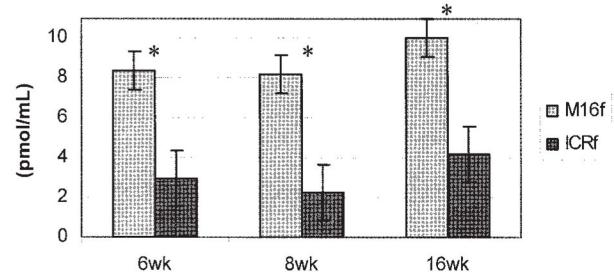
Male M16 mice were previously shown (16) to be hyperinsulinemic and hyperglycemic. Data from the present study confirmed these differences and, in addition, demonstrated the presence of hyperleptinemia in M16 mice. Differences between lines for levels of glucose and insulin seemed to be greater in this experiment when compared with previous data. This may be a result of differences in experimental design, diet, or assay used, or a result of potential random drift and/or accumulation of inbreeding.

Most hyperinsulinemic mouse models are hyperleptinemic (30). An increased probability for type 2 diabetes coupled with obesity is seen when animals become hyperinsulinemic and hyperleptinemic (31). The molecular

A



B



**Figure 7:** (A) Comparison of leptin plasma levels (pmol/mL) in 6-, 8-, and 16-week-old M16 and ICR male mice. (B) Comparison of leptin plasma levels (pmol/mL) in 6-, 8-, and 16-week-old M16 and ICR female mice. Values are expressed as least-square means ( $\pm$ SE). Asterisks denote significant ( $p < 0.01$ ) differences between lines.

mechanisms for the relationship between insulin and leptin that define their roles in various metabolic processes are extremely well studied (32,33). Yet, the underlying genetic mechanisms contributing to type 2 diabetes phenotypes still remain largely unknown (31,34). It is thought that leptin resistance is similar in nature to the pathogenesis of insulin resistance that occurs in obese and type 2 diabetic individuals (35). This hypothesis is based on studies in obese rodents and humans that showed no or little response to exogenous levels of leptin (36). Nisender and Schwartz (37) have proposed a hypothesis whereby leptin and insulin have some level of cross-talk in hypothalamic signal transduction pathways because both have been shown to activate phosphatidylinositol 3-kinase signaling. This idea was based on overlapping effects on energy homeostasis in the hypothalamus by both proteins, even though the hormones and receptors themselves are unrelated.

Fasted levels of blood glucose are often used as an indicator of type 2 diabetes. At a relatively young 8 weeks of age, M16 males had average glucose measures well over 220 mg/dL. M16 females also had significantly higher blood glucose levels at 8 weeks than their ICR counterparts but at relatively lower levels than in males. Both the TSOD and Tallyho mouse are also hyperglycemic for males only (25,38). Gender-specific QTLs have been identified show-

**Table 5.** Correlation matrix adjusted by fixed effects for line, sex, diet, and age among growth and fat traits in M16 and ICR mice

	8wk§	PFAT	PGON††	Insulin	Glucose	Leptin
8 weeks	1.0	0.54*	0.57*	0.39*	0.10	0.49*
PFAT		1.0	0.89*	0.32*	0.16‡	0.67*
GONG			0.95*	0.46*	0.13	0.70*
PGON				1.0	0.13	0.72*
Insulin					1.0	0.52*
Glucose						0.22*
Leptin						1.0

\*  $p < 0.001$ .†  $p < 0.01$ .‡  $p < 0.05$ .

§ 8-week weight.

|| Percentage body fat determined by DXA.

†† Epididymal/perimetrial adipose depot expressed as a percentage of body weight.

GONG, Epididymal adipose depot weight.

ing sex-specific adipose pad and total fat regulation (39). Studies in humans have identified sex differences for lipolysis, lipogenesis, glucose regulation, insulin sensitivity, and blood pressure (40,41). Gender differences in steroids are thought to be contributing to these differences involving major pathways of metabolism (42).

There was an increase in BMD in M16 mice relative to ICR mice. This is in agreement with studies comparing humans with type 2 diabetes to nondiabetics (43–45), which have shown a relationship between increased BMD and type 2 diabetes.

Differences between lines for TNF $\alpha$  and IL-6 are not surprising. TNF $\alpha$  has been shown to be highly expressed in

adipose tissue of obese individuals and is thought to be involved in insulin resistance in obesity and type 2 diabetes (46). A high percentage of obese individuals show elevated plasma levels of TNF $\alpha$  and IL-6 (47–49). The elevated levels of TNF $\alpha$  and IL-6 in M16 mice potentially represent markers of early age onset of obesity in this animal model.

Selection for rapid postweaning weight gain in the M16 line of mice has created an excellent polygenic model to study the genetics of growth, obesity, and type 2 diabetes. Relaxed selection over many generations has resulted in minimal reduction in phenotypic differences between the M16 and ICR lines. Relative to many monogenic models of obesity (e.g., *ob/ob* and *db/db*), the M16 mouse does not exhibit the same degree of adiposity. However, when compared with many other polygenic models of obesity (e.g., 18,50), the increased fatness expressed by the M16 line is extremely robust. Although a FAT diet does increase adiposity and blood glucose levels in these mice, early onset obesity and type 2 diabetes phenotypes expressed in the M16 line are not dependent on high dietary fat intake levels, as demonstrated by the lack of line  $\times$  diet interaction effects for these traits. Given the outbred status of M16, and the polygenic nature of the phenotypes, it may represent a highly relevant model for human populations. Furthermore, because M16 mice exhibit obesity and are hyperglycemic and hyperinsulinemic at a young age, they seem to fit trends in diabesity that are being observed worldwide (51,52). The M16 model may, thus, be a relevant tool to dissect the genomic, proteomic, and metabolomic underpinnings of these complex traits.

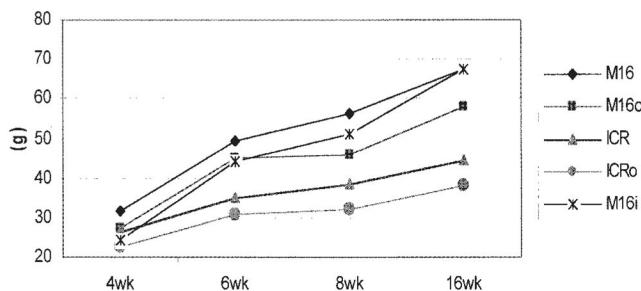


Figure 8: Least-square means for body weights of M16 and ICR male mice at 4, 6, 8, and 16 weeks for current generations (M16 and ICR) and at the time period immediately postselection (M16o and ICRO). Data for M16i (representing a fully inbred derivative of M16) were collected at approximately the same time as those for M16 and ICR.

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