Diet effects on weight gain and body composition in high growth (hg/hg) mice

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Corva, Pablo M., and Juan F. Medrano. Diet effects on weight gain and body composition in high growth (hg/hg) mice. Physiol Genomics 3: 17–23, 2000.—Nongenetic factors such as nutrition modulate the effects of genes responsible for overgrowth in animals. The goal of this study was to examine the importance of genotype × diet interactions on the effects of a major locus that regulates growth in the mouse. We have examined the phenotype of high growth (hg), a partially recessive autosomal locus that increases growth rate and mature body size. C57BL/6J (C57) and congenic C57BL/6J-hg/hg (HG) mice were fed three experimental diets differing in protein and energy content from 3 to 12 wk of age. HG mice grew faster and were, on average, 51% heavier than C57 at 12 wk of age. Feed intake was higher in HG mice but proportional to the increase in body weight. The magnitude of the differences in body size and composition between lines depended on the interaction between genotype and the protein/energy ratio of the diet. In C57, the diets modified the level of fatness without changing adult lean mass. However, in HG the diets differentially affected both linear growth and body composition. In general, HG had higher plasma levels of insulin-like growth factor I at 3 and 12 wk than C57. Plasma insulin did not differ between lines, but leptin was higher for HG mice. These results show that the effects of hg on growth are modulated by diet composition. Therefore, this mutation could be a valuable model with which to study the genetic and nutritional aspects of overgrowth disorders.

A large number of growth disorders in humans have been classified as overgrowth syndromes (38). Many of these syndromes have genetic origins and may lead to hormonal imbalances that affect growth patterns under different nutritional environments. A typical example is the effect of an increased energy intake leading to obesity in children, as well as an increase in height relative to leaner children (38). The underlying mechanisms of nutrition and linear growth are central to understanding the etiology of many of the interactions leading to overgrowth.

The genetics of growth has extensively used the laboratory mouse as a model of study. However, the majority of mouse mutations affecting growth result in a reduction in body size (24). Increased size mutants have largely been those associated with obesity (24). More recently, some mouse knockouts with increased size have been described (21, 26, 28), but they have specific tissue abnormalities or endocrine dysfunction. In contrast to these models, one natural mutation in the mouse, high growth (hg) (5), has a generalized increase in body size and is not obese (6). Therefore, it constitutes a valuable model with which to study genetic and nutritional aspects of overgrowth.

The hg locus is a partially recessive mutation that dramatically enhances postweaning weight gain and adult body size. Genetic analysis of the mutation has determined that the hg locus is a 500-kb deletion in mouse chromosome 10 (17). The identification and characterization of genes within the hg deletion are in progress, and a positional candidate gene has been identified (18). However, the causality of this particular phenotype is not yet fully understood. Mice homozygous for the deletion show an increased growth rate of up to 50% accompanied by a higher energetic efficiency and/or lower maintenance requirements (1, 7). The effects of the mutation are detected early in development, manifested by delayed muscular cell fusion and an increase in muscle fiber number (41, 42). Interestingly, high growth mice have lower concentrations of growth hormone (GH) but much higher concentrations of insulin-like growth factor I (IGF-I) in plasma than do normal mice (27). Despite substantial changes in growth rate and size, the mice are proportional in the size of body components (12).

Nutrition is the most important nongenetic factor affecting growth and body composition traits in mammals (48). Moreover, complex interactions between genotype and diet composition have been detected in experiments evaluating growth in mice (8, 49, 51). Therefore, the goal of this study was to assess the importance of genotype × diet interactions on the effects of a major locus regulating animal growth. High growth and control mice were fed diets varying in protein and energy content from 3 to 12 wk of age. To describe the effects of hg on important physiological processes related to growth, we measured weight gain, feed intake, body composition and the concentrations of insulin, leptin, IGF-I, and glucose.

MATERIALS AND METHODS

Animals and diets. The hg locus has been introgressed into the C57BL/6J (C57) background by nine backcrosses to cre-
ate the congenic line C57BL/6J-hg/hg (HG). In the present experiment, animals from the twenty-second generation of inbreeding C57BL/6J-hg/hg were used. Male mice were chosen for this study because of availability and the larger difference in size of this sex with respect to controls (5). Twelve mice from each line, C57 control and HG congenic were allocated to one of three diets. The diets (Dyets, Bethlehem, PA) were formulated to vary the quantity of crude protein and fat using the same ingredients. The control diet (C), considered appropriate to support normal growth, had 22.5% crude protein and 3.4 kcal/g, with 11% of the energy coming from fat. The fat content was modified in a high-energy diet (HE) to increase the energy level to 3.9 kcal/g (23% of the energy from fat) while maintaining a constant protein concentration. A high-energy, high-protein diet (HEP) contained the same fat and energy content as the HE diet, but had a higher protein content (26.1%) to maintain the same protein/energy ratio as in diet C. Diet pellets were of similar size and hardness. Mice were housed in Plexiglas cages bedded with wood shavings under controlled conditions of temperature (21°C ± 2°C), humidity (40–70%) and lighting (14 h light, 10 h dark, lights on at 6 AM), and managed according to the guidelines of the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Experimental procedures. Mice were weighed to the nearest 0.1 g at 2 wk of age and at weaning at 3 wk of age. After weaning, mice were allocated to a specific diet (2 or 3 animals per cage) and weighed every 4 days during the experimental period (3–12 wk of age). The feed intake of each cage was estimated as the difference between the offered feed and the remnant at 4-day intervals without considering spillage. Water was offered ad libitum throughout the experiment. Five mice of each line were killed at 3 wk of age to measure IGF-I concentration in plasma.

At 12 wk of age, a blood sample was collected through the retro-orbital sinus in plasma separator tubes (Microtainer; Becton-Dickinson, Franklin Lakes, NJ) under isoflurane (AErrane; Ohmeda, Liberty Corner, NJ) anesthesia. Mice were weighed, their naso-anal length was measured to the nearest millimeter, and then the mice were killed by cervical dislocation. Liver, spleen, kidneys, gastrocnemius muscle, testes, and skin were removed from the carcass and individually weighed. Three intra-abdominal fat pads (gonadal, mesenteric, and retroperitoneal) and one subcutaneous fat pad (femoral) were dissected, weighed and returned to the carcass.

Body water content was determined by freeze-drying the carcasses to a constant weight. Lipid content was estimated by extraction with ether for 7 days and then with acetone for 5 days in a Soxhlet apparatus. Body ash content was determined by incinerating the carcass in a muffle furnace at 575°C for 16 h. One femur bone was removed from the partially ashed carcass and measured to 0.1 mm. Plasma glucose was measured by the glucose oxidase method with a glucose analyzer (model 2700, Yellow Spring Instruments) (16). Leptin levels were determined using a mouse immunoassay (Linco, St. Louis, MO) (16). Commercial rat immunoassays were used for the measurements of insulin (Linco) (16) and IGF-I (Diagnostic Systems Laboratories, Webster, TX). IGF-I samples were extracted with acid-ethanol following instructions from the kit manufacturer and diluted to allow use of the kit standard curve. This kit was validated based on the parallelism of dose-response curves of rat and mouse samples.

Statistical analyses. All data were analyzed with linear models that included the fixed effects of line, diet, litter, number of mice per cage and two-way interactions, using SAS (36). A Duncan multiple range test was used to compare differences among line and diet means (α = 0.05).

RESULTS

Live weight gain and feed intake. Live weight changes between 2 and 12 wk of age for mice from each line-diet combination are presented in Fig. 1. A higher growth rate for HG mice in the 3- to 7-wk interval produced most of the difference in mature body size between lines independently of the diet. Adult HG mice were, on average, 51% heavier than C57 mice.

The larger HG mice had a higher feed intake throughout the experiment (Fig. 2A). However, voluntary intakes were not different between lines when they were expressed as a function of metabolic weight (W^{0.75}, Fig. 2B). Voluntary intake was similar for C57 mice on the HE and HEP diets and significantly lower than the group on the C diet. Such an effect was not appreciable in HG when intake was compared on a per animal basis.
basis, but this difference was observed per unit of metabolic weight. These results suggest that the energy content of the diet set the limit for voluntary intake and that the same mechanism operated in both lines.

Body composition. Results from the analysis of body components were consistent with those obtained for 12-wk body weight (Table 1). The carcass and organs were heavier in HG than in C57. HG mice also had longer bodies and bones, as well as a disproportionate increase in skin weight. Reported skin weights do not include subcutaneous fat removed from the carcass and, hence, are fat free. The weight of skin fat was added to the carcass fat for the statistical analyses. When comparisons of empty body composition were made within a line, the diet effects differed for HG but not for C57 mice. HG mice grew to be the largest mice in the experiment. These mice had longer as well as

Table 1. Size of body components of 12-wk-old high growth and control male mice fed three different diets (n = 12)

<table>
<thead>
<tr>
<th>Line</th>
<th>Diet</th>
<th>Body, g</th>
<th>Carcass, g</th>
<th>Fat-free Skin, g</th>
<th>Body Length, cm</th>
<th>Femur Length, mm</th>
<th>Liver, g</th>
<th>Spleen, g</th>
<th>Testes, g</th>
<th>Kidneys, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>C</td>
<td>25.6(0.8)</td>
<td>17.0(0.4)</td>
<td>2.87(0.21)</td>
<td>15.4(0.09)</td>
<td>1.05(0.05)</td>
<td>0.083(0.005)</td>
<td>0.212(0.005)</td>
<td>0.337(0.012)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>27.3(0.7)</td>
<td>17.8(0.4)</td>
<td>2.87(0.19)</td>
<td>15.3(0.08)</td>
<td>1.13(0.04)</td>
<td>0.081(0.005)</td>
<td>0.213(0.004)</td>
<td>0.337(0.011)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>26.3(0.7)</td>
<td>17.0(0.4)</td>
<td>2.85(0.19)</td>
<td>15.3(0.08)</td>
<td>1.11(0.04)</td>
<td>0.083(0.005)</td>
<td>0.214(0.005)</td>
<td>0.334(0.011)</td>
<td></td>
</tr>
<tr>
<td>HG</td>
<td>C</td>
<td>37.1(0.8)</td>
<td>23.9(0.4)</td>
<td>5.04(0.22)</td>
<td>16.6(0.09)</td>
<td>1.55(0.05)</td>
<td>0.116(0.006)</td>
<td>0.261(0.005)</td>
<td>0.427(0.013)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>42.7(0.9)</td>
<td>27.6(0.5)</td>
<td>6.32(0.23)</td>
<td>16.9(0.10)</td>
<td>1.65(0.05)</td>
<td>0.122(0.006)</td>
<td>0.275(0.006)</td>
<td>0.500(0.014)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>39.9(0.7)</td>
<td>25.6(0.4)</td>
<td>5.75(0.19)</td>
<td>16.7(0.08)</td>
<td>1.63(0.05)</td>
<td>0.126(0.005)</td>
<td>0.264(0.005)</td>
<td>0.467(0.011)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means, with SE in parentheses; n = 12. HG, High Growth; C57, control mice; C, control diet; HE, high-energy diet; HEP, high-energy high-protein diet. Diet means within line sharing a common superscript (a, b, c) are not significantly different (P > 0.05). Line means within diet were significantly different for all traits (P < 0.05).
heavier bodies, a greater femur length, heavier carcasses, greater skin weights, and heavier kidneys than mice fed the C and HEP diets.

The weights of the organs were also analyzed as a percentage of body weight. Line was a significant source of variation for the relative weight of kidneys (C57, 1.27 ± 0.02%; HG, 1.16 ± 0.01%) and testes (C57, 0.81 ± 0.01%; HG, 0.67 ± 0.02%).

HG mice had larger fat pads than did C57 mice, but no difference was detected when the weights of single fat pads were expressed as a percentage of body weight, except for gonadal fat in animals fed the HE diet (Table 2).

Carcass chemical composition. Diet composition had very distinctive effects on the chemical composition of carcasses from the two lines (Table 3). C57 mice had similar amounts of water, ash, and lean matter, independently of which diet they were fed. However, C57 mice on the HE diet had a larger amount of fat in the carcass than did the other two groups. This result indicates that the protein/energy ratio of the diet had a stronger effect on fat accretion than the absolute protein or energy levels (Table 3). The higher fat percentage of the HE group was compensated for by a decrease in the percentage of water, lean matter, and ash.

Unlike the C57 mice, diet had a greater effect on the carcass composition in the HG line of mice. The HE diet resulted in mice with significantly higher water, lean mass, and ash content in their carcasses than those on the HEP and C treatments, with the C diet having the least amounts. Fat content, as a percentage of the carcass weight, was no different between mice on the C and HE diets; however, mice on the HEP diet were leaner than those on the C diet. The increase in fat content was compensated for by the decrease in relative water content. There were no significant differences among diets with respect to lean matter and ash percentages in the HG mice.

**Hormone levels.** IGF-I concentration in plasma at 3 wk of age, before the mice were allocated to the experimental diets, was 35% higher in HG than in C57 mice (488.9 ± 47.0 and 360.4 ± 85.0 ng/ml, respectively). At 12 wk of age, IGF-I concentration was higher for HG mice on the C and HEP diets (Table 4). A genotype × diet interaction was detected also for leptin and glucose concentrations (Table 4), the two lines differing only on the HE diet. When compared within lines, leptin, IGF-I, and glucose were at higher concentrations in C57 mice on the HE diet. However, for HG mice, IGF-I and glucose concentrations were higher in those on the C diet. The concentration of leptin in plasma was closely associated with carcass fat percentage in individual animals ($r_p = 0.91$). Insulin levels in plasma were not significantly affected by either line or diet.

**DISCUSSION**

HG mice had a noticeably higher growth rate after weaning (3 wk of age), as well as an extended period of growth, regardless of the diet (Fig. 1). Similar changes in weight gain patterns have been detected between control and transgenic mice for the growth hormone (GH) gene (20). Also, selection experiments for growth rate in mice showed that selection at early ages

<table>
<thead>
<tr>
<th>Line</th>
<th>Diet</th>
<th>Gonadal</th>
<th>Mesenteric</th>
<th>Retroperitoneal</th>
<th>Femoral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>C</td>
<td>1.44(0.11)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81(0.04)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32(0.05)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97(0.07)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.55(0.24)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>1.99(0.10)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.99(0.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55(0.04)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15(0.07)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.68(0.21)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>1.54(0.11)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.82(0.07)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37(0.05)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96(0.06)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.68(0.23)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HG</td>
<td>C</td>
<td>1.53(0.12)&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.78(0.05)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38(0.05)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00(0.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.71(0.26)&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>1.65(0.13)&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.86(0.09)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49(0.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03(0.07)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04(0.27)&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>1.37(0.11)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71(0.07)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53(0.05)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84(0.06)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.25(0.22)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means, with SE in parentheses; $n = 12$. Diet means within line sharing a common superscript (a, b, c) are not significantly different ($P > 0.05$). *Line means within diet that are significantly different ($P < 0.05$).

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changed the mature body size without affecting the shape of the growth curve (2). Considered together, these results suggest that growth rate and mature body size are regulated by many common genes, and the generalized effect of \( hg \) on weight gain is achieved through an interaction with those genes.

The differences in growth rates between the lines were accompanied by changes in feed intake (Fig. 2). In a growing animal, feed intake is regulated by the interaction between protein and energy requirements and the composition of the diet (19). Slight departures from optimum ratios can be compensated for by the animal through changes in feed intake. Therefore, the protein/energy ratio of the diet becomes an important determinant of body composition. In this experiment, the C and HEP diets had the same protein/energy ratio; therefore, C57 mice on those diets ate different amounts of feed but at the end of the experimental period had the same body composition (Table 3). The HE diet had a higher energy/protein ratio, and C57 mice on this diet were 30 and 24% fatter than mice on the C and HEP diets, respectively (Table 3). It is important to note that the three diets altered the body composition but did not affect mature lean mass, organ weight, or bone length of the C57 control mice.

In contrast to the results obtained with C57 mice, HG mice on the C diet were smaller than mice on the HE and HEP diets and fatter than those on the HEP diet (Tables 1–3). Moreover, HG mice on the HE diet grew larger in lean mass and tended to be fatter than those on the HEP diet. This association between increased energy intake, obesity, and increased linear growth has also been described in children (38); however, the factors responsible are still obscure. In our experiment, the effect of diet on linear growth was seen only in HG mice. Furthermore, when the lines were compared across diets, C57 and HG mice had similar body fat percentages when animals were fed the C diet, but HG was significantly leaner than C57 when the animals were fed the HE and HEP diets. The differential response in linear growth and body composition of the lines to the different diets is indicative of the interactions between nutrition and genotype. Nutritional studies in humans have shown that genotype-environment interactions are an important factor determining differences in weight change, in response to treatments such as long-term overfeeding (32). Therefore, \( hg \) could serve as a good model for the study of such interactions.

Body fat distribution is considered a better predictor of cardiovascular diseases and diabetes than obesity per se, with the amount of abdominal fat being associated with higher risks of disease (22). Since fat distribution is under genetic control (4), it is of interest to establish whether this trait is differentially affected by \( hg \). Despite the difference in fatness among mice from the different line-diet groups, no major differences were detected in the relative size of fat pads, indicating that the interactions between genotype and diet affected the total amount of energy stored as fat but not its distribution in the body (Table 2).

Oberbauer and colleagues (29) proposed that differences in maturity could be responsible for the lower level of fatness detected in HG mice in previous experiments. Scaling can account for most of the differences in body composition when lines or breeds of different adult sizes are compared at a fixed age (44). However, this explanation can be ruled out, because in the present experiment body compositions were compared at 12 wk of age, when all mice were at or very close to maturity. The alteration in nutrient partitioning could alternatively be the result of metabolic changes elicited by high levels of IGF-I. Similar effects have been identified in transgenic mice with extra copies of the GH gene (47).

Closely associated with the amount of fat in the carcass, leptin concentrations in plasma differed between lines only when animals were fed the HE diet (Table 4). The mean leptin concentration for C57 mice on the HE diet was almost 77% higher than for C57 mice on the other two diets and 56% higher than HG mice on the same diet. This effect resulted from the exponential relationship between leptin levels and fatness. This kind of response, detected both in mice (14) and humans (23), is indicative of a condition known as leptin resistance. It is worth noting that line C57 has a particular predisposition to obesity and alterations in leptin and insulin metabolism when fed high-fat diets (43, 46). Mice developing leptin resistance maintain a high level of intake, storing the extra energy as fat, despite the high concentration of leptin. Some C57 mice on the HE diet reached very high levels of fatness that were not seen in their HG counterparts. The extremely high levels of leptin in 4 of 12 C57 mice

<table>
<thead>
<tr>
<th>Line</th>
<th>Diet</th>
<th>Leptin (ng/ml)</th>
<th>Insulin, (ng/ml)</th>
<th>Glucose, mg/dl</th>
<th>IGF-I, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57</td>
<td>C</td>
<td>3.42(0.79)</td>
<td>0.402(0.067)*</td>
<td>189.0(13.4)b</td>
<td>279.3(23.7)*</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>5.50(0.65)**</td>
<td>0.392(0.049)*</td>
<td>232.8(9.9)**</td>
<td>346.4(21.3)</td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>2.80(0.65)b</td>
<td>0.312(0.055)*</td>
<td>198.3(10.3)b</td>
<td>286.8(24.7)*</td>
</tr>
<tr>
<td>HG</td>
<td>C</td>
<td>3.64(0.65)b</td>
<td>0.433(0.061)*</td>
<td>201.7(12.2)b</td>
<td>422.2(31.6)*</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>3.52(0.65)**</td>
<td>0.335(0.063)*</td>
<td>172.3(12.7)**</td>
<td>313.4(31.8)</td>
</tr>
<tr>
<td></td>
<td>HEP</td>
<td>3.03(0.62)a</td>
<td>0.312(0.053)*</td>
<td>179.9(10.6)b</td>
<td>351.5(24.0)*</td>
</tr>
</tbody>
</table>

Values are means, with SE in parentheses; \( n = 12 \). IGF-I, insulin-like growth factor I. Diet means within line sharing a common superscript (a, b, c) are not significantly different (\( P > 0.05 \)). *Line means within diet that are significantly different (\( P < 0.05 \)).
inflated the mean for that treatment. In fact, mice with the same fat percentage in their carcasses had similar levels of leptin independent of the line. Leptin metabolism was not affected by the high concentrations of IGF-I in HG mice on the C and HEP diets. This result is supported by experiments conducted with adipocytes growing in vitro (15).

The heavier skin of the HG mice contributed to the difference in live weight between the two lines (Table 1). Reiser and colleagues (35) detected differences in the biochemical properties of skin collagen between these two lines and attributed the differences to the altered level of IGF-I. However, the weight of the skin was not reported. Targeted expression of the Igf-I gene to the skin has been shown to enhance skin growth in transgenic mice (3). Because hair was not removed from the skin, we were not able to establish to what extent the weight of the hair mass could have contributed to variations in skin weight. However, our empirical observations when handling the animals suggest that HG mice have a larger hair mass than C57 mice. There is extensive data indicating that IGF-I has a stimulatory effect on the activity of the hair follicle. For example, overexpression of Igf-I in the skin of transgenic mice resulted in increased vibrissa growth (40), and a similar effect was elicited for wool growth in transgenic sheep (10, 11). In humans, IGF-I has been shown to regulate the growth of hair follicles in vitro (34). IGF-I concentrations were higher in HG mice than in C57 mice at 3 wk of age and also at 12 wk of age with the exception of the HE diet. Higher levels of Igf-I have been reported for HG mice at several ages up to 6 mo (27, 35). The level of IGF-I in plasma at 12 wk of age appeared to be associated with the nutritional status. Glucose concentrations paralleled those of IGF-I, but the nature of this association in relation to diet composition was line specific (Table 4). The higher level of Igf-I observed in C57 mice when fed the HE diet (diet with a higher energy/protein ratio) is consistent with results obtained in obese children (31) and in adult female patients during overfeeding (13). However, this association between diet composition and IGF-I was distorted in HG mice, where the animals on the C diet had the highest IGF-I levels but did not differ in fatness from mice on the HE diet. Hyperinsulinemia has been involved in some of the metabolic effects elicited by overfeeding and obesity (38). Also, insulin is known to regulate Igf-I expression and IGF-I levels in plasma (33, 45). However, we were not able to detect a clear association among insulin levels, lines, and diets.

Given the known effects of IGF-I on cell proliferation, differentiation, and survival (39), the changes detected in growth and body composition point to IGF-I as one of the factors responsible for establishing the HG phenotype. Transgenic mice producing high levels of systemic IGF-I exhibited enhanced cell proliferation and increased organ and body size (25). Similar effects were elicited when Igf-I expression was targeted to muscle (9), skin (3), and intestine (30). Although HG mice also exhibited increased organ and body size, none of those transgenic models exactly resembles the HG phenotype. Recently, two independent groups (37, 50) demonstrated that systemic IGF-I from the liver is not necessary to achieve normal postnatal growth in the mouse, reinforcing the importance of IGF-I synthesis in nonhepatic tissues to promote growth. These results challenge the assumption that an increased IGF-I concentration in plasma is responsible for the HG phenotype. However, a preliminary experiment conducted in our laboratory to compare liver and muscle Igf-I expression between HG and control mice on the same diets as the present experiment indicated that HG mice have higher Igf-I expression than C57 in the muscle, but not in the liver. This result is relevant because, even though IGF-I is subjected to posttranscriptional regulation, regulation of gene expression seems to be the principal level of control (33). Experiments are currently in progress to rigorously test the importance of Igf-I expression on IGF-I concentration in plasma and on growth rate between the C57 and HG lines.

In summary, HG mice grew larger than controls in all experimental diets and tended to be leaner at maturity. The hg locus prevented some of the deleterious effects of a high-fat diet on C57 mice, such as excessive fatness and development of leptin resistance. We confirmed the existence of important interactions between genotype and nutrition in an animal model of enhanced growth. To learn how hg integrates with other loci to trigger the dramatic changes in growth that we describe here, we are currently in the process of mapping genetic modifiers of hg for weight gain and body composition. The integration of knowledge about genetic and nutritional factors that modulate the effects of a major locus on growth can contribute to a better understanding of the complex etiology of overgrowth disorders.

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