Long-term divergent selection on body fatness in mice indicates a regulation system that is independent of leptin production and reception

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ABSTRACT

Divergent selection in mice on fatness over 60 generations produced a fat (F) and a lean (L) line, having about 22% and 4% body fat, respectively. To elucidate the importance of the leptin regulatory feedback loop in the genetic changes produced by this selection, Lep<sup>ob</sup> and Lepr<sup>db</sup> mutations causing leptin production and leptin receptor deficiency, respectively, were introgressed individually into both lines by repeated backcrossing. The fat amount increased significantly in homozygotes for Lep<sup>ob</sup> or Lepr<sup>db</sup> in both lines, for example, in F and L males from 8.5 to 18.8 and 17.2 g (P<0.001) and from 1.25 to 18.0 and 12.7 g (P<0.001), respectively. Line differences were, however, mostly maintained after introgression. Concentrations of circulating leptin were relatively independent of the original lines but heavily dependent on the introgressed genotype. Introgression of leptin production and receptor deficiencies had separate effects from long-term selection, indicating that the genes responsible for the line divergence must act independently of the leptin regulatory system. Energy budget analysis indicated that the major line differences were in the level of energy expended on physical activity, and these differences were preserved following introgression, suggesting that multiple pathways regulate fatness, which may be independently responsive to intervention.

Key words: obesity • mutation • selection • polygenic model • introgression • genetics

Obesity is a common multifactorial disorder and is a major risk for type 2 diabetes, coronary heart disease, sleep apnea, hypertension, and some forms of cancer (1). In the United States, 300,000 premature deaths annually can be directly attributed to obesity.
The financial costs amount to 7% of national healthcare expenditure, and, including indirect costs, the impact on the U.S. economy amounted to about US$70 billion in 1995 (3, 4); in 1997, the World Health Organization declared obesity to be the most serious health threat facing the Western world.

Despite the importance of obesity in predisposing to so many diseases and evidence for its high heritability (5), efforts to identify human genes with major effects on body fatness have been only partially successful (6). However, remarkable progress has been made in the identification and characterization of the genes mutated in monogenic mouse strains. Identification of genes mutated in these strains has facilitated the development of the “lipostatic model” of body fatness regulation from a theory to a well-characterized system, with aspects of its molecular biology now well established. In this model, body fatness is signaled by a hormone produced by fat tissue (leptin), which is perceived by receptors in the hypothalamus. These receptors are co-localized with other systems in the hypothalamus, particularly the neuropeptide Y and melanocortin system (notably the MC4 receptor), which generate efferent control of food intake and energy expenditure via the sympathetic system (7–9). High levels of body fat result in high leptin levels, generating compensatory reductions in food intake and elevated energy expenditure to reduce the level of fatness. In contrast, low levels of body fat result in low leptin concentrations and converse compensatory adjustments. More recently, however, this lipostatic interpretation for the role of leptin has been questioned, and its function as a “starvation signal” emphasized, undermining the lipostatic model and perhaps indicating that other independent regulatory systems may be important in controlling body fatness (10).

We developed polygenic fat and lean lines of mice by long-term selection of over 60 generations. Divergent selection on fatness at 70 and later at 98 days of age was initiated in 1980 (11). This selection gradually generated two highly divergent lines: the fat (F) and the lean (L) line, with 22% and 4% total body fat in males, respectively (12). The heritability for fatness was estimated at ~0.5 (13, 14), near the average of values from selection experiments for body composition in rodents (15) and indicative of a strong genetic component for fatness. A genome-wide quantitative trait locus (QTL) analysis of a cross between the F and L lines revealed four QTLs that contributed substantially to the divergence in obesity: one each on chromosomes 2, 12, 15, and X, explaining 5%, 19%, 14%, and 7%, respectively, of the phenotypic variance in fat percentage of the F2 (16). None of these QTLs mapped to regions of known single-gene obesity mutations (17) but mapped to regions of previously described obesity QTLs and candidate genes (16, 18, 19).

The choice of candidate genes for the present study was based on earlier experiments on sensitivity to leptin in the F line. Leptin was administered to males from the F line and a control (C) line derived from the same base population (20). Treated males in both the F and C lines had significantly \((P<0.05)\) lower mean body weight (BW), food intake, and fatness than did untreated animals, but the effects of treatment did not differ significantly between the F and C lines. These results indicate that sensitivity to leptin remained in the F line after almost 60 generations of selection and suggest that the selection had disrupted a separate pathway for regulatory control over body fatness. To further investigate this possibility, we repeatedly backcrossed the recessive mutations \(\text{Lep}^{ob}\) and \(\text{Lepr}^{db}\) (formerly named \(ob\) and \(db\)) separately into both the F and L lines and then made inter se matings to generate families segregating for all three genotypes. Thus, we
had available F-line mice, designated F $ob/ob$, F $ob/+$, F $db/db$, and F $db/+$, which were homozygous or heterozygous for the respective mutation, and wild-type F$++$ mice. The same groups were available for the L line. We assessed body fatness, circulating leptin, and energy budgets in these segregating litters.

**MATERIALS AND METHODS**

**Origins and derivation of the lines divergently selected on fatness**

Animal procedures were in accordance with United Kingdom Home Office regulations.

The outbred selection lines were initiated in Edinburgh from a three-way cross (two inbred [CBA, JU] and one outbred [CFLP]) base (11). One line was selected for increased fat content and the other for decreased fat content. Selection for the first 20 generations was on the ratio of gonadal fat pad weight to BW of 10-wk-old males and subsequently on dry matter content of males at 14 wk, both indicators being strongly correlated with fat content (21). Inbred lines (Fi and Li) were initiated from a single family of each of the selection lines in generation 47 and maintained by full sib mating (12). At the time of the last backcross, the inbred lines Fi and Li had been maintained by full sib matings for 22 and 21 generations, respectively, with the inbreeding coefficient $F > 0.99$.

**Introgression of the Lep$^{ob}$ and Lepr$^{db}$ mutations**

Male mice (n=1–4 per recurrent line) heterozygous for the Lep$^{ob}$ mutation (C57BL/6OlaHsd-Lep$^{ob}$) or Lepr$^{db}$ mutation (C57BL/6OlaHsd-Lepr$^{+}$) (both from Harlan OLAC, Oxon, UK) were used to initiate seven generations of repeated marker assisted backcrossing to each selection line (F and L). Four backcross lines segregating for either Lep$^{ob}$ or Lepr$^{db}$ were produced. Genotypes for the Lep$^{ob}$ and Lepr$^{db}$ mutations were determined using specific polymerase chain reaction-restriction fragment length polymorphism assays (22, 23). Animals from the outbred selection lines F and L (generations 64–69) and from the inbred lines Fi and Li were used as the recurrent partner for the first five and last two generations, respectively, with the final backcross to mice of generations 22 and 21 from lines Fi and Li, respectively. Heterozygous animals of those lines (designated here as F$ob/+$, F$db/+$, L$ob/+$, and L$db/+$) were mated *inter se* in generation eight to give wild-type, heterozygous, and homozygous animals (Table 1). These animals have an expected proportion of 99.2% of the background genotype from the corresponding selection lines F or L.

**Rectal temperature**

The rectal temperature of 93 male mice was measured between 12.00 and 16.00 h at the age of 82 ± 5.5 (SD) days and on 79 of them twice (repeatability 0.95) using a rectal probe (K-type thermocouple with a HI-92801, Hanna Instruments, Woonsocket, RI) inserted 2.5 cm into the rectum. The average temperature from both measurements was used. The body temperature of 34 females (at an average age of 181 ± 22 (SD) days was obtained similarly, using a Digitron 2751-K (0.1°C) (SIFAM Instruments, Devon, UK) (24).
Resting metabolic rate (RMR)

We measured the RMR of these 34 individual female mice (average age of ∼181 days). Immediately before a measurement of RMR, BW (0.01 g) was recorded using a Sartorius balance (Sartorius AG, Goettingen, Germany) and rectal body temperature was measured. Mice were not denied access to food or water before respirometry measurements, but most food intake (>90%) in these mouse lines occurs nocturnally (C.C. Velten and L. Bünger, unpublished observations) and mice were measured at least 4 h after lights on. RMR was quantified as oxygen consumption at 30°C (within the thermoneutral zone) during the light phase (between 10.00 and 17.00 h), using an open-flow respirometry system and the protocol previously described (24).

Food and water intake and scoring cages for dirtiness

Using special food hoppers constructed to avoid spillage, food intake of males was measured weekly between 78 and 98 days of age. Water intake was measured during about the same age period by weighing the water bottle every other day. After they were cleaned, the cages of mature males (aged between 70 and 120 days) were assigned a “dirtiness score” (0 to 4) over 3 days by two independent individuals, with high values indicating very dirty.

Carcass dissection, blood glucose measurements, and analysis of body composition

In total, 291 animals (Table 1) were killed by cervical dislocation at 106 ± 7 (SD) days, and plasma was collected from trunk blood and stored at −20°C for the leptin assays. Immediately after the sacrifice, one drop of this blood was used to measure blood glucose by the glucose oxidase method, using a One Touch Profile Blood Glucose Meter (LifeScan, Milpitas, CA). Mice were not denied access to either food or water preceding glucose measurements, but most food intake occurs nocturnally and hence measurements were initiated at least 5 h after lights were switched on.

BW was recorded, and the interscapular brown adipose tissue, the gonadal fat pads, and s.c. fat were taken, weighed, and prepared for related analyses. Their weights were later added together to obtain total body fat. The dry matter weight of the remaining carcass was determined by freeze drying. Fat percentage was predicted from the regression equation of fat percentage on dry matter derived previously on wild-type males at 70 days (21), a similar equation to that obtained on 100-day-old mice of various genotypes (25). The amount of body fat and fat-free body weight (ffBW) were estimated from BW and estimated fat percentage.

Leptin assay

The plasma leptin values assayed in laboratory 1 were somewhat unexpected, particularly for heterozygotes. Therefore, 173 out of the 184 samples (insufficient plasma in 11 samples for a second analysis) were analyzed blind in a second independent laboratory, using different assays. There was a relatively strong correlation (r=0.68) between data generated in the two laboratories, and the overall means were only slightly different, 3.93 vs. 4.36 ng/ml. Laboratory 1: Blood plasma samples were assayed for leptin by Linco Bioanalytical Services, (LINCO Research, St.
Charles, MO) using the radioimmunoassay, double antibody system Linco mouse leptin kit (catalog # ML-82K; http://www.lincores.com/protocols/ml-82k.html). A few values (n=9 of 184, mostly L animals and ob/ob or +/+ animals) were assayed as very low (<0.25 ng/ml) and could not be repeated because of low sample volume, so 0.25 ng/ml was used in the analysis (X was in the range from 0.2 to 0.8), which is assumed to have a very minor effect on the means only. Laboratory 2: Circulating leptin concentrations were determined using an ELISA with a sensitivity threshold of 0.025 ng/ml. Forty-five animals (out of 173) of the L+/+, Fob/ob, and Lob/ob animals were found to be below this threshold, which is not surprising given that L+/+ animals have very little fat and that introgression of Lepob mutation eliminates leptin production. For them, the threshold value was used in the calculated means. Substituting 0 instead of 0.025 had only minor effects on the estimated means.

Statistical data analysis

Data obtained from dissection were analyzed using the following model: 

\[ Y = M + L + G + T(G) + S + F(L,G) + LS + GS + LG + LGS + TS + LT + LTS + e \]

where fitted effects were M, overall mean; L(1-2), line (F, L); G(1-2), introgressed mutation (Lepob, Leprdb); T(G), genotype (1-3) within gene; S(1-2), sex (if data on both sexes); F(L,G), family within line and gene; e, residual error, and specified interactions. All effects were fitted as fixed except F(L,G) and e, which were fitted as random. The line effect was tested against F(L,G), and all other effects were tested against e. The model for RMR included BW and temperature as random covariates, but the family effect was ignored. ANOVA was undertaken using the GLM procedure of the SAS System for Windows Release 6.08 (SAS Institute, Cary, NC). Before the final analysis, data on wild-type and heterozygous animals were analyzed separately. Because these genotypes differed significantly for several traits, including fat content, heterozygotes and wild types were generally not combined (but see Table 2 legend).

RESULTS

Body composition

The female wild-type animals from the F and L lines differed in body fatness at 106 days by an average 3.9 g (6.3 g fat for F+/+ and 2.4 g for L+/+) and the males by 7.2 g (8.5 g for F+/+ and 1.3 g for L+/+) (Table 1). In leptin receptor-deficient mice (F db/db and L db/db), the body fat contents of both lines increased significantly (P<0.05), but the difference between the lines was preserved--in females, the fat amount was 5.4 g higher in F db/db than in L db/db, and in males, the fat amount was 4.5 g higher in F db/db than in L db/db. Leptin production-deficient mice (F ob/ob and L ob/ob) were also fatter than the wild-type mice. The difference in fat amount between the lines was preserved in females (F ob/ob 4.1 g higher than L ob/ob) but not in males (F ob/ob 0.8 g higher than L ob/ob). Heterozygous mice (F ob/+, F db/+, L ob/+, and L db/+) had body fat contents that were not significantly different (P>0.05) from the wild-type mice, and the differences between the lines were also preserved in each of these cases. The differences between F ob/+ and L ob/+ were 4.9 g (females) and 5.8 g (males), and the differences between F db/+ and L db/+ were 5.4 g (females) and 4.5 g (males).
Similar results to this analysis, using uncorrected fat amounts, were also obtained with the amount of fat, when ffBW was used as a covariate (Fig. 1). This analysis accounts for differences in BW and avoids the automatic “part-whole regression” of fat weight on total BW. The regression of fat (g) on ffBW (g) was significantly ($P<0.05$) affected by line, being much higher for F than L, but not by degree of fatness ($b±se$; combined F $ob/ob$ and F $db/db$ vs. combined L $ob/ob$ and L $db/db$: F $0.37±0.17$, L $−0.07±0.14$; combined F $ob/+$, F $db/+$, and $F+/+$ vs. combined L $ob/+$, L $db/+$, and $L+/+$: F $0.36±0.05$, L $0.05±0.02$) even though the L-line homozygotes had similar high fatness levels to those in the F line (Fig. 2). Consequently, there is a clear difference between the lines in the relation between the amount of fat and ffBW.

**Plasma glucose and leptin**

Blood glucose values rose significantly ($P>0.05$) in both lines and sexes when leptin or leptin receptor deficiencies were introgressed, although there was no significant difference between the $F+/+$ and $L+/+$ (Table 1). Glucose values increased much more in the F- than in the L-line background. For example, in females, $Fdb/db$ glucose values increased by 21.7 mmol/l from $F+/+$, whereas in $Ldb/db$ mice, glucose values increased only by 8.3 mmol/l from $L+/+$. Similar trends can be observed also in males and in comparisons of $ob/ob$ and $+/+$. The diabetes in $Fob/ob$, $Lob/ob$, $Fdb/db$, and $Ldb/db$ animals was accompanied by polydipsia, resulting also in a higher “dirty cage score” (Table 2). But again, the effects in the L-line background (genotypes $L ob/ob$ and $L db/db$) were less severe than in the F-line background (genotypes $Fob/ob$ and $Fdb/db$). This suggests that in the L-line genetic background, glucose values of $ob/ob$ or $db/db$ genotypes cannot be increased to the extent allowed in the F-line genetic background.

Leptin was assayed in two independent laboratories, using different methods of analysis, but because they gave concordant results, data presented here are only from the laboratory in which all animals were assayed. If expressed as circulating concentrations per gram of body fat tissue, the lowest leptin concentrations (0.05–0.09 ng/ml/g fat) were observed in the leptin-deficient mice ($F ob/ob$ and $L ob/ob$) of both sexes as expected. $F+/+$ and $L+/+$ mice produced leptin at a concentration that was $≈10–20$ times greater than that produced by $ob/ob$ animals, $≈0.4–0.8$ ng/ml/g fat.

Absolute circulating concentrations were higher in the F than in L animals (Table 1) as observed previously (26), but here differences did not reach statistical significance ($P>0.05$). For example, in males, absolute leptin concentrations in $F+/+$ were significantly increased (2.5 ng/ml) compared with $L+/+$ males (1.0 ng/ml), as observed previously (20). However, when expressed as leptin concentrations per gram of body fat, the reverse is true in that $L+/+$ males produce significantly more leptin than $F+/+$ males per gram of fat (0.84 vs. 0.46 ng/ml/g fat, $P>0.05$). The difference between the F and L males was preserved in leptin-receptor-deficient animals whereby $L db/db$ males had significantly higher values than $F db/db$ males (0.7 vs. 0.4 ng/ml/g fat). The comparisons of these genotypic groups were not significantly different in females, however. Animals heterozygous for the $Lep^{ob}$ mutation ($F ob/+)$ and $L ob/+)$ produced a lower amount of leptin per gram of body fat than the wild-type animals (49–60% of wild-type in F and 80% in L), although their body fatness was similar (see previous discussion on body composition).
Energy budget analysis and body temperature

We constructed energy budgets for the different genetic groups of mice by using the recorded food intakes and RMRs. Food intake of the wild-type and heterozygote mice did not differ between the F and L lines (averaging 5.53 and 5.26 g/day, respectively; Table 2). In F and L mice with leptin production and receptor deficiencies, the daily food intake increased by 2.5–3 g and by 1.3–1.4 g, respectively, but there were no differences between the L and F lines in the extent of increase following introgression of \( \text{Lep}^{ob} \) or \( \text{Lepr}^{db} \) mutations. We converted these estimates of food intake into total daily energy demands, using an estimated metabolizable energy content of 9.8 kJ/g for their diet (27). In thermoneutrality, RMR \((\text{O}_2 \text{ consumption ml/min})\) was strongly dependent on body mass \((F=13, P<0.05)\). We converted these measured RMRs to daily energy expended on RMR, assuming a respiratory quotient of 0.8 (Table 2). Mice from the F line were heavier on average in all conditions than mice from the L line (Table 1 and 2), so the total energy expenditure at rest was much higher in F-line animals than in L-line animals. Combining data obtained on females and males allows the preliminary calculation of the difference between the energy expended at rest and the total daily energy expenditure. This reflects the combined energy expended on physical activity and thermoregulation, and the ratio between these two is called the physical activity level. Total energy expenditure on physical activity and thermoregulation was much greater in the L-line mice than in the F-line mice, independent of the introgression (Table 2), and the physical activity level was also much greater in the L line (3.2–3.6) when compared with the F line (1.6–2.5).

Consistent with this energy data, the body temperature for L+/+ animals was higher than for F+/+ by about 0.85°C (standard error of the difference=0.87, \( P>0.05 \)) in females and 1.32°C (s.e.d. = 0.63, \( P<0.05 \)) in males (Table 1). In F and L lines with leptin or leptin receptor deficiencies, body temperature dropped in both lines by ~2.5°C in females and by ~0.9°C in males (Table 1) but did not change significantly in heterozygotes. The differences between the L and F lines were preserved in all pairwise comparisons across genotype and sexes apart from F \( \text{db}/+ \) vs. L \( \text{db}/+ \), where the difference was negligible (Table 1).

DISCUSSION

The present and related experiments show that long-term selection can easily split a common base population into highly diverging lines, confirming that body fatness has a strong genetic basis. The F line derived here resembles in several respects typical Western human populations in which the genetic basis of obesity is predominantly polygenic. Humans homozygous for loss-of-function mutation in genes associated with monogenic rodent obesity are relatively rare (6). These, at least \( \text{Lep}^{ob} \) and \( \text{Lepr}^{db} \), seem not to segregate in the F line either. This can be deducted from the following findings. First, the outbred F line is approaching a selection plateau with body fat percentage values of ~20–23%. The introgression of defects in leptin receptor and leptin production increased body fatness to between 34 and 38%, indicating that introgression of both mutations brought in genetic variability that was not present before. Second, the effects of introgressing leptin production and receptor deficiencies were approximately additive to the effects generated by the selection lines. Third, the four QTL found in an F2 cross between F and L did not map to regions of known single-gene mutations for obesity (16). Selection over 60 generations therefore has changed the frequency of “lean” and “fat” alleles in both lines, and
further experiments are needed to identify the alleles that are responsible for the phenotypic difference between the F and L lines.

Here, we provide evidence that the causal genetic differences between the F and L lines are in genes that work independently of the leptin regulatory system. In the analysis of the fat amount of eight comparisons in both sexes between the F and L genetic backgrounds with introgressed Lepto or Leprdb mutation, the difference in fat amount observed between F+/+ and L+/+ was preserved in seven cases. Similar conclusions can be drawn from the analysis of fat amount by using frBW as a covariate, which corrects for weight differences. The reasons for the line x Lepto x sex interaction, causing the nonstatistically significant difference in fat amount between Fob/ob and Lob/ob males, remain unknown and need further investigation. Overall, this suggests that body fatness in F and L lines is controlled by a regulation system that is independent of leptin production and reception.

The energy budget analysis for the L and F lines shows that the major differences between the lines may reside in the energy devoted to physical activity, and not in food intake or resting energy demands. Total energy expenditure on physical activity and thermoregulation was much greater in the L-line mice than in the F-line mice independent of the introgression of Lepto or Leprdb. Also the physical activity level defined as the ratio between the daily energy intake and the daily energy expended on rest was much greater in the L line than in the F line. Because the dominant effect of leptin is on regulation of food intake, with secondary effects on resting energy expenditure, the independence of the line effects from the introgressions is consistent with the energy budgets. Therefore, the energetics data strongly suggest that the major difference between the L and F lines resides in differences in their physical activity level and expenditure on thermoregulation rather than differences in food intake and resting metabolic rate and that these differences are preserved when leptin production and reception deficiencies are introduced.

The body temperature for L+/+ animals was higher than for F+/+ animals, and the introduction of leptin or leptin receptor deficiencies caused a significant drop in body temperature in both lines. The differences between the L and F lines were preserved in seven of eight pairwise comparisons except in F db/+ vs. L db/+, in which there was no significant difference. Because introgression resulted in decreased body temperature but the energy budgets were essentially similar, the indication is that the major difference between the F and L lines could be in physical activity rather than thermogenesis, a hypothesis that needs to be tested in the future.

Leptin measurements showed the expected drop of leptin levels close to zero in ob/ob animals. However, in males, the differences in leptin concentrations per gram of fat observed between F and L were preserved upon introgression of Leprdb. Comparisons in females were not significantly different, but the female data should not be overemphasised because the sample size was very small. Therefore, from comparisons of leptin levels in F db/db vs. L db/db, a similar conclusion can be reached for fat amount, frBW, energy budget data, and body temperature in that the line differences are preserved after introgression, confirming the major conclusion of this study that the F- and L-line differences may be due to pathways regulated independently from the leptin pathway.
The effect of reduced leptin production per gram of body fat in heterozygotes has been observed previously in both mice (26) and humans (27), although in these studies, compensatory changes in body fatness were also apparent to normalize total circulating levels, an effect that we did not observe. In addition, db/+ animals had similar leptin concentrations as db/db homozygotes, notwithstanding their normalization of body fatness. Despite these heterozygote effects, the overall patterns of variation in leptin production occurred independently of the F and L lines, again pointing to an independent regulatory system controlling fatness in these lines.

Our study supports the notion that regulation of body fatness involves multiple regulatory systems, which may act independently. In particular, our data indicate a genetic basis for the differences in body fatness could be in the levels of energy expenditure spent on physical activity. Characterization of causal genes and pathways controlling the fatness in the F and L lines might uncover independent regulatory systems. Such systems may present novel targets for intervention-based therapies.

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REFERENCES


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\(^a\)SD averaged over groups.
\(^b\)One mouse less for fat (%), fat (g), and (fat free body weight) (fBW).
\(^c\)Gonadal fat pad weight (GF PW) expressed as percentage of total body fat.
\(^d\)Sample sizes for three groups for leptin were higher or lower by the value given as exponent of the glucose sample sizes.
\(^e\)Leptin per gram of body fat.

Note: Means sharing a common letter (a–i) are not significantly different (\(P>0.05\)). Because body temperature (Tb) was taken at different ages in females and males, the group comparisons were done within sexes.
### TABLE 2. Food intake (FI) and water intake (WI) and resting metabolic rate (RMR)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Fob/ob</th>
<th>Fob/+</th>
<th>Fdb/db</th>
<th>F db/+</th>
<th>Lob/ob</th>
<th>Lob/+</th>
<th>Ldb/db</th>
<th>L db/+</th>
<th>SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI 78–105 (g)</td>
<td>29.5a</td>
<td>25.2ab</td>
<td>7.10d</td>
<td>19.8b</td>
<td>6.4d</td>
<td>14.3c</td>
<td>9.3cd</td>
<td>5.64</td>
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</tr>
<tr>
<td>WI 78–105 (g/g)</td>
<td>0.568a</td>
<td>0.525ab</td>
<td>0.181d</td>
<td>0.409bc</td>
<td>0.200d</td>
<td>0.396bc</td>
<td>0.345c</td>
<td>0.15</td>
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</tr>
<tr>
<td>n</td>
<td>25</td>
<td>32</td>
<td>70</td>
<td>20</td>
<td>20</td>
<td>25</td>
<td>45</td>
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<tr>
<td>Cage score</td>
<td>2.90a</td>
<td>2.83a</td>
<td>2.55a</td>
<td>2.55a</td>
<td>1.67b</td>
<td>1.54b</td>
<td>1.55b</td>
<td>0.74</td>
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<tr>
<td>n</td>
<td>9</td>
<td>17</td>
<td>19</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>8</td>
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<tr>
<td>FI 78–98 (g/d)</td>
<td>8.04a</td>
<td>6.95ab</td>
<td>5.65c</td>
<td>8.35a</td>
<td>6.59b</td>
<td>5.30c</td>
<td>1.06</td>
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<tr>
<td>DEI kJ/day</td>
<td>78.8</td>
<td>68.1</td>
<td>55.4</td>
<td>81.8</td>
<td>64.6</td>
<td>51.9</td>
<td>10.8</td>
<td></td>
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<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
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<tr>
<td>RMR 181 (ml O₂/min)</td>
<td>1.28a</td>
<td>1.47a</td>
<td>0.76cd</td>
<td>0.89bc</td>
<td>0.53de</td>
<td>0.68de</td>
<td>0.50e</td>
<td>0.37</td>
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<tr>
<td>BW 181 (g)</td>
<td>71.7a</td>
<td>61.3abc</td>
<td>45.7d</td>
<td>64.1ab</td>
<td>28.6e</td>
<td>52.2cd</td>
<td>26.0e</td>
<td>6.4</td>
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<tr>
<td>DER (kJ/day)</td>
<td>37.0</td>
<td>42.5</td>
<td>22.0</td>
<td>25.7</td>
<td>15.3</td>
<td>19.7</td>
<td>14.5</td>
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<tr>
<td>PA (kJ/day)</td>
<td>41.8</td>
<td>25.8</td>
<td>33.5</td>
<td>56.1</td>
<td>35.7</td>
<td>44.9</td>
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<td>PAL</td>
<td>2.12</td>
<td>1.60</td>
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<td>3.33</td>
<td>3.28</td>
<td>3.59</td>
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</tr>
</tbody>
</table>

*SD averaged over groups. Numbers after the trait abbreviation indicate age of the animals.

Note: Means sharing a common letter (a–e) are not significantly different ($P>0.05$). WI and FI were obtained on males and RMR and the corresponding body weight (BW 181) on females. Because FI and WI were mostly measured on groups of two males comprising heterozygous and wild-type or homozygous animals, no analysis between wild-type and heterozygous animals was possible. The weekly measured FI of male mice is more variable than the body weight ($CV_{FI}=15–20\%$, $CV_{BW}=\sim10\%$). Because it did not show any obvious age trend when animals were nearly matured, the FI was averaged over the ages of 77–98 days. Daily energy intake (DEI) is the metabolizable energy intake obtained by multiplying the food intake by the metabolizable energy content of the diet (9.8 kJ/g). Daily energy expended on rest (DER) is the oxygen consumption converted to energy expenditure assuming a respiratory quotient (RQ) of 0.8. Physical activity (PA) is the difference between the DEI and DER and includes some energy expended on thermoregulation at room temperature. The physical activity level (PAL) is the ratio of DEI to DER.
Figure 1. Fat amount corrected for fat-free body weight. Data shown are the least square means ±SE. For $n$, see Table 1. Columns sharing a common letter are not significantly different ($P>0.05$).
Figure 2. Linear regression between fat-free body weight and fat amount. All data were designated to four groups, pooled over sexes, differing substantially in fat, to obtain reasonable numbers in each group. 1) Combined F ob/ob and F db/db (n=48, b$_{(1)}$=0.37, s$_{(b)}$=0.17); 2) combined L ob/ob and L db/db (n=44, b$_{(2)}$=–0.07, s$_{(b)}$=0.14); 3) combined F ob/+, F db/+, and F +/+ (n=118, b$_{(3)}$=0.36, s$_{(b)}$=0.05); and 4) combined L ob/+, L db/+, and L +/+ (n=80, b$_{(4)}$=0.05, s$_{(b)}$=0.02). The difference b$_{(1)}$ vs. b$_{(2)}$ did not reach significance ($P>0.05$), but b$_{(3)}$ was significantly higher than b$_{(4)}$. 