Adipose tissue in offspring of Lepr$^{db/+}$ mice: early-life environment vs. genotype

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Lambin S, van Bree R, Caluwaerts S, Verruysse L, Vergote I, Verhaeghe J. Adipose tissue in offspring of Lepr$^{db/+}$ mice: early-life environment vs. genotype. Am J Physiol Endocrinol Metab 292: E262–E271, 2007. First published September 5, 2006; doi:10.1152/ajpendo.00308.2006.—Gravidas with obesity and diabetes ("diabesity") may transmit this syndrome to their children through genetic and nongenetic mechanisms. Here, we used the Lepr$^{db/+}$ diabese mouse to examine the magnitude of these transmission modes, focusing on adipose tissue (AT). We compared the following six groups: wild-type (+/+) offspring from +-/+ or db/db dams (different early life environment) and db/+ offspring from db/+ dams, fed a standard or high-fat diet. Weight gain (0–8 wk) was higher in +/+ offspring from db/+ vs. +/+ mothers, and even higher in db/+ vs. +/+ offspring from db/+ mothers. In addition, we observed a stepwise increase in AT and adipocyte size in +/+ from +/+ mice, +/+ from db/+ mice, and db/+ mice at 8 wk. Differences in weight and adiposity between +/+ offspring from db/+ vs. +/+ dams were more pronounced in males than in females. Leptin and apelin mRNA levels in white and brown AT were higher in db/db compared with +/+ offspring mice. The high-fat diet amplified AT differences between db/db; however, leptin, apelin, and tumor necrosis factor-α expression were boosted more robustly in db/+ offspring. In conclusion, the genetic transmission of the diabesity phenotype clearly prevailed, but the early-life diabesity environment had discernible effects on postnatal weight gain as well as on adipocyte size and adipokine expression at a postpubertal age. Leptin

There is mounting evidence that the early life (i.e., fetal and neonatal) environment is one of the determinants of the risk of obesity and glucose intolerance or diabetes ("diabesity"). The incidence of diabesity is increased at the low and high end of the birth weight spectrum (reviewed in Ref. 25). Among individuals with high birth weight, the excess risk appears to be attributable only in part to genetic factors. For example, mothers but not fathers of Asian-Indian children with high birth weight were more likely to develop the metabolic syndrome than the parents of children with normal birth weight (35). In Pima Indians, offspring exposed to maternal diabetes remained heavier and showed a fourfold elevated risk of diabetes compared with their siblings born before onset of diabetes (8).

Studies in animal models designed to unravel the early life contribution to diabesity are constrained by the lack of high birth weight in most diabetic models. Streptozotocin-induced diabetes in rodents (widely used as a model of type 1 diabetes) produces small offspring, even when administered at low doses (4). However, an appealing animal model is the C57BL/KsJ-Lepr$^{db/+}$ or Lepr$^{db/db}$ (db/db) mouse, which is heterozygous for a loss-of-function mutation in the leptin receptor gene (Lepr; see Ref. 5) and, unlike its homozygous counterpart, is fertile. In the nongravid state, db/db mice have a comparable food intake, body weight (BW), and fasting glucose and insulin concentrations as wild-type (+/+ ) mice; however, their fat mass is increased, and they display hyperleptinemia and hyperinsulinemia after a glucose challenge (17, 36, 37). During gestation, db/+ dams show hyperphagia, gain more weight, and maintain their relative hyperleptinemia compared with +/+ dams. In addition, both insulin and glucose tolerance tests are abnormal during gestation, and their pups are heavier at birth (17, 19, 21, 36, 37), replicating the macrosomia observed in human gestational diabetes mellitus (GDM). At 24 wk of age, BW of +/+ offspring from db/+ mothers was comparable to that of +/+ offspring from +/+ mice; female but not male offspring showed a higher body fat percentage (measured by dual-energy X-ray absorptiometry) and higher fasting insulin (37).

The aim of the current study was to examine the relative magnitude of the effects on adipose tissue (AT) development conferred by the db/+ early-life environment vs. the db/db genotype. We studied the following parameters: weight gain and AT accumulation until 8 wk; adipocyte area and number, and AT gene expression at 8 wk of age; and insulin sensitivity and glucose tolerance at the same age.

RESEARCH DESIGN AND METHODS

Animals, experimental design, and sample collection. The study protocol was approved by the local ethical committee for animal research. Seven-week-old C57BL/KsJ$^{db/+}$ (abbreviated hereafter as db/+ ) and C57BL/KsJ$^{+/+}$ (+/+ ) mice were purchased from the BKS.Cg-m $^+/+$ Lepr$^{+/-}$/J strain at the Jackson Laboratory (Bar Harbor, ME). The mice were housed in a temperature-, humidity- and light-controlled environment and had ad libitum access to tap water and a standard laboratory chow containing 51% (wt/wt) carbohydrate, 3% (wt/wt) fat, and 21% (wt/wt) protein (Trouw, Gent, Belgium). A colony was established with female db/+ mice as breeders. From 8 wk of age, db/+ and +/+ female offspring (F1 generation) of breeder mothers were mated overnight with +/+ males; successful mating was confirmed by a copulatory plug and defined as day (d) 0.5 of gestation. The F1 dams were weighed on d0.5 and d18.5, and they were allowed to deliver. The morning following delivery, the BW of all pups (F2 generation) was recorded to the nearest 0.01 g.

We studied the following three groups of F2 mice: db/+ offspring from db/+ mothers (abbreviated hereafter as db/+ < db/+ ), +/+ offspring from db/+ mothers (+/+ < db/+ ), and +/+ offspring from +/+ mothers (+/+ < +/+ ); they were weighed weekly from d7 to d56. The genotype was registered on d7 from the coat color, with +/+ .
mice being gray rather than black because of concurrent homozygosity for the misty mutation (m/m; see Ref. 30); sex was registered between d10 and d14. The following two diets were examined: the standard chow and a high-fat (“western”) chow containing 49% (wt/wt) carbohydrate from sucrose and corn starch, 21% (wt/wt) fat from anhydrous milk fat, and 17% (wt/wt) protein (TD 88137; Harlan, Zeist, the Netherlands; see Ref. 32). The standard diet contained 8.5% kcal as fat with a calorific value of 12.7 KJ/g, with corresponding values of 42% and 18.8 KJ/g for the high-fat diet. The latter diet was started on d10 postpartum in the F1 dams to avoid transient exposure by the F2 pups to the standard diet. The F2 mice were weaned at 4 wk of age.

The experiments were performed in both female and male F2 offspring, unless indicated otherwise. Blood and tissue samples were obtained after an overnight fast. Blood samples were collected by tail snipping in heparinized tubes (plasma) or allowed to clot (serum), obtained after an overnight fast. Blood samples were collected by tail snipping before and 15, 30, 45, and 60 min after glucose administration. Blood glucose concentrations were measured using a glucometer (Glucocard Memory 2; Menarini, Florence, Italy).

The experiments were performed in both female and male F2 offspring, unless indicated otherwise. Blood and tissue samples were obtained after an overnight fast. Blood samples were collected by tail snipping in heparinized tubes (plasma) or allowed to clot (serum), centrifuged, and stored at −20°C. After the mice were killed by spinal cord elongation, all bilaterally available intra-abdominal (mesenteric, perigonadal, and perirenal) white adipose tissue (WAT) was carefully dissected, as well as the subcutaneous inguinal WAT, the interscapular brown adipose tissue (BAT), and the liver. Tissue samples were either snap-frozen in liquid nitrogen (quantitative RT-PCR) or immersed in Bouin’s solution (histology).

Insulin tolerance tests and glucose tolerance tests. Insulin tolerance tests (ITTs) were performed in normally fed mice injected with insulin (1 U/kg ip, Humulin Regular; Eli Lily, Indianapolis, IN); blood samples were obtained by tail snipping before and 15, 30, 45, and 60 min after insulin administration. Blood glucose concentrations were measured using a glucometer (Glucocard Memory 2; Menarini, Florence, Italy).

Glucose tolerance tests (GTTs) were performed after an overnight fast (standard diet only). We injected 2 g glucose/kg ip and obtained blood samples for glucose measurement before and 20, 30, 60, 90, and 120 min after glucose administration. Blood samples were also collected in heparinized tubes for insulin measurement.

Biochemical assays. Plasma insulin was measured by a mouse-specific enzyme-linked immunoassay (Mercodia, Uppsala, Sweden) and plasma leptin by a mouse-specific RIA (Linco, St. Charles, MO). The level of free fatty acids (FFA) in serum was determined enzymatically using the nonesterified fatty acid C assay (Wako, Neuss, Germany) adapted to 96-well microtiter plates (18). The triglyceride content of the liver was measured after chloroform/methanol extraction (9) with a combined enzymatic-colorimetric method (GPO-PAP, Roche, Mannheim, Germany).

Adipocyte number and area. Adipocytes were isolated from 50- to 100-mg perigonadal and inguinal WAT by a slightly modified version of Rodbell’s (27) method, with all procedures at 37°C. WAT was washed in saline, cut into small pieces, and transferred to a vial with 3 ml Krebs-Ringer bicarbonate (KRB) buffer with 2% BSA, 3 mM glucose (pH 7.4), and 1 mg/ml collagenase. The vial was incubated for 1 h in a shaking water bath, manually shaken to liberate adipocytes, and centrifuged (1 min at 400 g), and the top layer was washed with 5 ml KRBA; this procedure was repeated two times. The adipocytes were resuspended in 1 ml KRBA, stained with trypan blue, and counted in a Fuchs-Rosenthal chamber.

Adipocyte area was assessed in paraffin-embedded 3-μm-thick sections from perigonadal and inguinal WAT stained with hematoxylin-eosin. The area of all adipocytes was measured by computer-assisted image analysis (KS400, Zeiss, Germany) in 7.5 ± 0.1 (SE) fields in female mice and in 8.1 ± 0.1 fields in male mice, corresponding to 1,937 ± 137 adipocytes in females and 1,447 ± 85 in males.
Quantitative RT-PCR. RT-PCR analyses were performed in female offspring only. Total RNA was extracted from 50–100 mg homogenized WAT (mesenteric, perigonadal, perirenal, and inguinal) and BAT using Tripure Reagent (Roche). The RNA concentration was determined spectrophotometrically, and RNA was stored at -80°C. Reverse transcription reactions were performed from 100 ng RNA using Taqman Reverse Transcription Reagents with 2.5 μM random hexamers (all RT-PCR materials were obtained from Applied Biosystems, Lennik, Belgium).

Oligonucleotide primers and probes were obtained for the following eight mRNA species: Peroxisome proliferator-activated receptor-γ2, sterol regulatory element-binding factor 1 (Srebf1), leptin, resistin, glycerolkinase, adiponectin (Acrp30), apelin, and tumor necrosis factor (TNF)-α. Several of these mRNA species (Srebf1, leptin, glycerolkinase, adiponectin, and TNF-α) were altered in mice with WAT-specific reduced Lepr expression (16). Primers and probe sets for PPARγ2 were as follows: sense primer 5'-CGCTGATGGCAGCTGCTATGA-3', antisense primer 5'-AATGGCATCTCTGTGTCAACCA-3', probe 5'-FAM-CACCTGCAAAGAAATTACGTGTCAACCA-3'. Predesigned Taqman gene expression assays were purchased for Srebf1 (Mm00550338_m1), leptin (Mm00434759_m1), resistin (Mm00443562_m1), glycerolkinase (Mm00433907_m1), adiponectin (Mm00445641_m1), apelin (Mm00443258_m1), and TNF-α (Mm00550338_m1). Quantitative RT-PCR was performed using the ABI 7000 sequence detector. All mixtures consisted of a 20× concentration mix of unlabeled sense and antisense primers and Taqman MGB probe (FAM-dye labeled). 18S rRNA was used as the housekeeping gene, and primers and Vic-Tamra probe were obtained as Predeveloped Assay Reagents. For each target gene, we ran a validation experiment by creating a standard curve with six cDNA dilutions (1:2 to 1:64). The ΔC_T (C_T target gene – C_T 18S rRNA, where C_T is threshold cycle) for each dilution was plotted vs. the log amount of cDNA. The absolute value of the slope of these graphs did not exceed 0.1 for any of the target genes, indicating equal PCR efficiency.

PCR amplifications were performed in duplicate wells using 1:8 sample dilutions of cDNA and 2× TaqMan PCR Master Mix, with a reaction volume of 10 μl. Thermal cycling conditions were as follows: 2 min at 50°C (uracil-N-glycosylase incubation) and 10 min at 95°C followed by 50 two-temperature cycles (15 s at 95°C for melting and 1 min at 60°C for annealing and extension). Samples were analyzed using Sequence Detection Software 1.1. The data were obtained as C_T values and normalized to the expression levels of 18S rRNA. To compare differences related to background, diet, or fat pad, results were expressed as the relative expression (ΔΔC_T = ΔC_T target group – ΔC_T control group). The final values were expressed as 2^-ΔΔC_T (mean ± SE) with the resultant numerical values representing the n-fold increase or decrease of the target mRNA with respect to the calibrator, and the SE calculated as follows: SE = [2^-ΔΔC_T ln2]SD(ΔΔC_T)/(no. of samples/group)^{1/2}.

Data analysis. Data analysis was performed with NCSS 2004 software (Kaysville, UT). We used the two-sample t-test for comparisons of data from two groups. For comparisons of data from more than two groups, we used one-way ANOVA followed, if P < 0.05, by Fisher’s least significant difference (LSD) post hoc multiple-comparison test to detect intergroup differences. We used general linear

![Figure 2](https://www.ajpendo.org/images/2007/01/ajpendo070133f02.jpg)

A. Sum of 4 WAT depots per animal (g)

B. Weight BAT (g)

Data were shown as means ± SE.

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model (2-factor) ANOVA for comparison of data from the six studied groups with different genetic background and postnatal diet; through this analysis, we discerned the main effects of background and diet, as well as the interaction between both. If the ANOVA indicated an overall difference at $P < 0.05$, within-background and/or within-diet differences were examined further by Fisher's LSD test. The interaction between background and diet was considered relevant if $P < 0.15$. All data are presented as means ± SE.

RESULTS

**BW of F1 dams and birth weight of F2 offspring.** Although we detected no significant difference in BW of $db/+$ and $+/+$ mice on $d0.5$ of gestation (21.9 ± 0.6 and 20.6 ± 0.5 g, respectively; $P = 0.13$), $db/+$ dams weighed more than $+/+$ dams on $d18.5$ (35.5 ± 0.75 vs. 32.0 ± 0.9 g, $P = 0.005$); thus, gestational BW gain was higher in $db/+$ dams (13.8 ± 0.4 vs.
12.0 ± 0.7 g, \( P = 0.016 \)). Fasting glucose levels on \( d18.5 \) were not different between \( \text{db}+/+ \) and \(+/+\) dams (5.7 ± 0.2 vs. 5.6 ± 0.1 mmol/l, \( P = 0.57 \)). Total litter weight was increased in \( \text{db}+/+ \) pregnancies [8.42 ± 0.37 \((n = 13)\) vs. 7.04 ± 0.56 g \((n = 8)\), \( P = 0.046 \); individual fetal weight 1.34 ± 0.01 \((n = 78)\) vs. 1.28 ± 0.01 g \((n = 44)\), \( P = 0.0003 \), but litter size was comparable (6.3 ± 0.3 in \( \text{db}+/+ \) and 5.5 ± 0.4 g in +/+ pregnancies; \( P = 0.11 \)).

Weight gain of \( F2 \) offspring. BW at \( d7 \) (before separation by sex) was 2.68 ± 0.06 g in +/+ < +/+ offspring, 3.24 ± 0.07 g in +/+ < \text{db}+/+ offspring, and 3.52 ± 0.09 g in \( \text{db}+/+ < \text{db}+/+ \) offspring (ANOVA: \( P < 0.001 \), all groups different from one another by post hoc test). Figure 1 shows the BW curves in male and female offspring between \( d10–14 \) and \( d56 \). There was a clear difference in BW of males according to their background throughout the experimental period; the differences were attenuated in females, especially between +/+ < +/+ and +/+ < \text{db}+/+ offspring and more so in the 21% fat group. Compared with the 3% fat diet, the 21% fat diet increased BW to a comparable extent in the three background groups (data not shown).

AT accumulation and function in \( F2 \) offspring. Comparing +/+ < \text{db}+/+ with +/+ < +/+ male offspring (i.e., examining effects of early life \( \text{db}+/+ \) environment), we found that the weight of the individual WAT depots (4/animal) was slightly but significantly higher in +/+ < \text{db}+/+ mice (data not shown), and the same was true for BAT weight (Fig. 2) and liver weight (data not shown). In addition, their adipocytes were larger and the number of adipocytes expressed per fat pad were lower (Fig. 3). However, the weight of the sum of four WAT depots per animal (Fig. 2), the number of adipocytes expressed per gram fat (Fig. 3), circulating leptin and FFA (Fig. 4), and liver triglyceride content (36.0 ± 7.6 mg/g liver in +/+ < +/+ offspring and 39.1 ± 4.7 mg/g in +/+ < \text{db}+/+ offspring fed the standard diet; \( P = 0.73 \)) were not significantly different. In female offspring (data not shown) there was a significant difference for individual WAT weight (\( P < 0.001 \)) and adipocyte area (\( P < 0.001 \)). Comparing \( \text{db}+/+ < \text{db}+/+ \) and +/+ < \text{db}+/+ male offspring (i.e., examining effects of \text{db}+/+ genotype with similar early life environment), we found a robust increase in WAT and BAT weight (Fig. 2), adipocyte area (Fig. 3), and plasma leptin but not FFA (Fig. 4). Adipocyte cellularity was lower when expressed per gram fat, but not when expressed per fat pad (Fig. 3). Liver weight was increased in \( \text{db}+/+ \) mice (data not shown), but liver triglyceride content was comparable (35.1 ± 4.6 mg/g in males fed the standard diet; \( P = 0.54 \) compared with +/+ < \text{db}+/+ mice). These genotype-related differences were comparable in female offspring (data not shown). Finally, comparing male \( F2 \) mice on the high-fat diet with those on the standard diet, we found higher WAT and...
BAT weight (Fig. 2) but lower liver weight (data not shown), as well as adipocyte hypertrophy (Fig. 3). The diet-induced effects were clearly greater in db/+ compared to +/+ mice (Figs. 2–4), but were comparable in females compared with males (data not shown).

Adipose gene expression in female F2 offspring. We found no difference (ANOVA: P > 0.05) according to background in the expression of adiponectin, glycerokinase, and Srebf in any fat depot of mice fed the standard diet (data not shown; mRNA levels not measured in offspring fed the high-fat diet). Resistin mRNA levels in BAT were lower in db/+ compared with +/+ offspring, but there was no difference in any WAT depot (data not shown).

However, we found robust differences in the mRNA levels of leptin, apelin, TNF-α, and PPARγ2 (Figs. 5 and 6). Comparing +/+ < db/+ with +/+ < +/+ offspring, the expression of leptin and apelin was higher in both WAT and BAT, whereas TNF-α and PPARγ2 expression were unchanged. Comparing db/+ with +/+ offspring from db/+ dams, we found a markedly elevated expression of leptin in WAT, as expected; in addition, apelin and TNF-α expression were increased. Offspring raised on the high-fat diet had higher leptin, apelin, TNF-α, and PPARγ2 mRNA levels in BAT than those raised on the standard diet and were more pronounced in db/+ than in +/+ mice. In BAT, the expression of TNF-α and PPARγ2 was also augmented by high-fat feeding (data not shown), but leptin expression was unchanged and apelin expression was reduced.

Insulin sensitivity and glucose tolerance. Both male and female db/+ F2 offspring were less sensitive to exogenous insulin than +/+ offspring, but there was no difference between the +/+ groups according to their early life environment (Fig. 7). Increasing the amount of dietary fat (data in male offspring only) did not change the sensitivity to insulin (P = 0.80).

During the ip GTTs (standard diet only), male but not female +/+ offspring from db/+ dams had an increased glucose excursion after an ip glucose challenge than +/+ offspring from +/+ dams; their insulin output during the GTT was comparable, however (Fig. 8). Comparing db/+ offspring with +/+ offspring from db/+ dams, glucose levels were similar, but db/+ offspring had a higher insulin output, significantly so in females.

DISCUSSION

We documented that +/+ mice exposed to a db/+ environment during perinatal life exhibit a weight gain pattern and AT characteristics (8 wk postnatal age) that are intermediate between those of +/+ offspring from +/+ mothers and db/+ offspring from db/+ mothers. In particular, we found larger adipocytes and elevated leptin and apelin expression in +/+ mice exposed to the db/+ early-life environment. However, these effects were not amplified by feeding the +/+ < db/+
Yamashita et al. (37) found that, although BW was comparable in male than in female offspring (Fig. 1), but it was not increased in db/db offspring. Overall, the alteration in AT phenotype conferred by the early-life db/db environment was smaller than that conferred by the db/db genotype or even the high-fat diet. Although some rodent studies have previously examined the relationship between maternal obesity or diabesity and glucose tolerance in rodents (1), we found no changes in adiponectin, glycerolkinase, or sterol regulatory element-binding factor-1 mRNA levels in WAT (16). In contrast to mice with WAT-specific Lepr knockout in mice, although both models were associated with a decrease in WAT of db/db mice in the body fat fraction (16), as well as by the high-fat diet. Our data extend results obtained in db/db mice (14) and mice with a reduction of Lepr in BAT (16), as well as in rats fed a high-fat diet (1). The macrophage infiltration that accompanies AT expansion is the main source of TNF-α (7, 33). TNF-α overexpression was observed in pronounced obesity only (14), which may explain why TNF-α mRNA was normal in +/+ < db/db offspring, showing mild AT expansion. 4) PPARγ2 expression was reduced in db/db mice compared with +/+ mice when fed the standard diet (ANOVA for this diet; P > 0.001) but was substantially increased when fed the high-fat diet. Because the AT phenotype of selective PPARγ2 knockout in mice is unclear at this time (22, 38), it is difficult to speculate on the mechanism of our results; however, upregulation of PPARγ2 mRNA in WAT of mice fed a high-fat diet for an extended period is well documented (31).

In contrast to mice with WAT-specific Lepr reduction (16), we found no changes in adiponectin, glyceraldehyde, or sterol regulatory element-binding factor-1 mRNA levels in WAT of db/db mice, although both models were associated with a reduced hypoglycemic effect of insulin (Fig. 7). WAT characteristics common to both models that may translate
into insulin resistance include adipocyte hypertrophy (26), TNF-α overexpression (15), and reduced PPAR expression (22, 38). Of note, we found no change in serum FFA in db/mice.

We found that male offspring from mothers had a better glucose tolerance at 8 wk than +/+ or db/+ mice from db/+ mothers (Fig. 8). However, we did not replicate this observation in females, and we therefore think it is premature to propose a mechanism for this finding. Given the importance of adipocyte size in the development of insulin resistance and glucose intolerance (26, 34), it would be interesting to reexamine insulin and glucose tolerance in db/db mice at a later age. Compared with +/+ offspring, db/+ offspring were hyperinsulinemic during the GTT, confirming previous data (37), but they maintained normal glucose tolerance. Indeed, several studies have shown that glucose tolerance is normal in female adult nongravid db/mice, whereas it is clearly impaired in late gestation (6, 17, 19, 36, 37), rendering the gravid db/mouse an attractive model of human GDM. Similarly to gravid db/+ mice, GDM women are insulin resistant and show increased fat mass and plasma leptin concentrations (20).

Evidence from U.S. and European populations indicates that the progeny of women with GDM have a higher prevalence of obesity and glucose intolerance, at least until early adulthood (8, 11, 29). However, the challenge here is to dissect out the relative contributions of the intrauterine milieu, the genetic constitution, and the postnatal environment. In effect, the association between GDM and the body mass index of the progeny interacted with breastfeeding duration (28) and was much attenuated after controlling for current maternal or parental body mass index (11, 29).

A caveat of this study is that the misty (m) color mutation may explain part of the difference in postnatal growth between +/+ (m/m) and db/+ (M/m) mice (30); however, db/+ mice without coat color mutation also exhibited a diabesity phenotype (6). Another limitation is that we have no reliable data on food intake (when placed in metabolic cages, many animals refrained from normal eating). However, because food intake was comparable in nonpregnant db/+ mice and +/+ controls (17, 37), it is unlikely that food intake was increased in 3- to 8-wk-old +/+ < db/+ offspring. Finally, cross-fostering experiments are needed to delineate the role of the neonatal vs. the intrauterine environment in the altered AT phenotype in +/+ < db/+ offspring.

Collectively, our data have demonstrated that an early-life db/+ environment contributes in small part to the transmission of the AT and metabolic db/+ phenotype from the maternal mouse to her offspring but that this contribution is not amplified by a high-fat diet.

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Fig. 8. Glucose tolerance test (GTT) in male and female F2 offspring (standard diet only) at 8 wk postnatal age. Blood glucose and plasma insulin concentrations during an ip GTT in +/+ < +/+ +/+ mice, +/+ < +/+ db/+ mice, and db/+ < +/+ db/+ mice; the AUC is shown on right. Statistical analysis (for the glucose and insulin concentrations at each time point, and the AUC values) was performed by one-way ANOVA followed (if P < 0.05) by Fisher’s LSD multiple-comparison test. In the male offspring, one statistical outlier was excluded. The data are shown as means ± SE; groups not sharing the same letter are significantly different from one another (P < 0.05).

GRANTS

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