Corrections

**BIOCHEMISTRY.** For the article “The crystal structure of two macrolide glycosyltransferases provides a blueprint for host cell antibiotic immunity,” by David N. Bolam, Shirley Roberts, Mark R. Proctor, Johan P. Turkenburg, Eleanor J. Dodson, Carlos Martinez-Fleites, Min Yang, Benjamin G. Davis, Gideon J. Davies, and Harry J. Gilbert, which appeared in issue 13, March 27, 2007, of Proc Natl Acad Sci USA (104:5336–5341; first published March 21, 2007; 10.1073/pnas.0607897104), the authors note that, in addition to writing the paper, Benjamin G. Davis should be credited with designing the research and analyzing the data. The corrected author contributions footnote appears below.


**ENVIRONMENTAL SCIENCES.** For the article “Combined climate and carbon-cycle effects of large-scale deforestation,” by G. Bala, K. Caldeira, M. Wickett, T. J. Phillips, D. B. Lobell, C. Delire, and A. Mirin, which appeared in issue 16, April 17, 2007, of Proc Natl Acad Sci USA (104:6550–6555; first published April 9, 2007; 10.1073/pnas.0608998104), the authors note the following. On page 6550, left column, in paragraph 1 of the main text, line 7, the phrase “However, deforestation also exerts a cooling influence by (iv) decreasing the surface albedo” should instead read: “However, deforestation also exerts a cooling influence by (iv) increasing the surface albedo.” Also on page 6550, right column, in Results, paragraph 2, line 1, the phrase “the atmospheric CO₂ concentration is higher by 299, 110, and 5 ppmv” should instead read: “the atmospheric CO₂ concentration is higher by 199, 110, and 5 ppmv.” Lastly, in the Fig. 1 legend, line 8, the phrase “net cooling, near-zero temperature change, and net warming, respectively” should instead read: “net warming, near-zero temperature change, and net cooling, respectively.” These errors do not affect the conclusions of the article.

**GENETICS.** For the article “Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency,” by Fabrice G. Petit, Soazik P. Jamin, Isao Kurihara, Richard R. Behringer, Francesco J. DeMayo, Ming-Jer Tsai, and Sophia Y. Tsai, which appeared in issue 15, April 10, 2007, of Proc Natl Acad Sci USA (104:6293–6298; first published April 2, 2007; 10.1073/pnas.0702039104), the authors note that the e-mail address for corresponding author Fabrice G. Petit appeared incorrectly. The correct address is fabrice.petit@u-psud.fr. The online version has been corrected.

**PHYSIOLOGY.** For the article “Night eating and obesity in the EP3R-deficient mouse,” by Manuel Sanchez-Alavez, Izabella Klein, Sara E. Brownell, Justin V. Tabarean, Christopher N. Davis, Bruno Conti, and Tamas Bartfai, which appeared in issue 8, February 20, 2007, of Proc Natl Acad Sci USA (104:3009–3014; first published February 16, 2007; 10.1073/pnas.0611209104), the authors note that the following acknowledgment was inadvertently omitted from the article: “We thank Professor Shuh Narumiya (Kyoto University, Kyoto, Japan) (4, 5, 21) for the generation and subsequent generous transfer of the EP3R–/– mice.”
Night eating and obesity in the EP3R-deficient mouse

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Adult mice carrying a null mutation of the prostanoid receptor EP3R (EP3R−/− mice) exhibit increased frequency of feeding during the light cycle of the day and develop an obese phenotype under a normal fat diet fed ad libitum. EP3R−/− mice show increased motor activity, which is not sufficient to offset the increased feeding leading to increased body weight. Altered “nocturnal” activity and feeding behavior is present from a very early age and does not seem to require age-dependent factors for the development of obesity. Obesity in EP3R−/− mice is characterized by elevated leptin and insulin levels and >20% higher body weight compared with WT littermates. Abdominal and subcutaneous fat and increased liver weight account for the weight increase in EP3R−/− mice. These observations expand the roles of prostanoid E2 signaling in metabolic regulation beyond the reported stimulation of leptin release from adipose tissue to involve actions mediated by EP3R in the regulation of sleep architecture and feeding behavior. The findings add to the growing literature on links between inflammatory signaling and obesity.

Prostaglandin E2 (PGE2) is one of the most important inflammatory mediators in the periphery and in the CNS (1–7). PGE2 is synthesized locally in the brain by neurons and glia expressing COX1, COX2, and PGE synthase (8, 9). The activation of specific Toll-like receptors (TLR) by IL-1 or LPS and the subsequent production of PGE2 mediates different CNS responses such as the febrile response (3, 5, 10, 11), activation of the hypothalamic–pituitary–adrenal axis (12–15), and the central activation of brown fat metabolism/uncoupling (16–18). The central and peripheral actions of PGE2 are mediated by four G protein-coupled receptor-type prostanoid receptors (EP1R–EP4R) (for review, see refs. 19 and 20). Transgenic mice carrying a null mutation for each of the four prostanoid receptors have been generated, and different phenotypes have been described for EP1R−/− (4, 21, 22), EP2R−/− (23–29), EP3R−/− (4, 5, 21), and EP4R−/− (30–32) mice. EP1R−/− mice show a decreased aberrant foci formation to azoxymethane (33). EP2R−/− mice have impaired ovulation and fertilization, salt-sensitive hypertension, impaired vasodepressor response to PGE2, and a loss of bronchodilation (29). EP4R−/− mice have an impaired vasodepressor response to i.v. infusion of PGE2 and decreased inflammation and bone resorption (34). EP3R−/− mice have an impaired febrile response (5), impaired duodenal bicarbonate secretion (35), enhanced vasodepressor response to PGE2 (36), and increased bleeding tendency (37). Effects of the loss of EP3R were also observed on tumor-induced angiogenesis (38), colon cancer development (39), allergic inflammation (40), and inflammatory pain (41). PGE2 levels in these mice were not found to be altered in these studies.

Although EP3R−/− mice have been well characterized, there have not been any reports on obesity or altered feeding patterns in these mice. PGE2 has been shown to have an effect on adipocytes by inhibiting lipolysis and stimulating the secretion of leptin, but the specific prostanoid receptor subtype important for mediating these effects has remained unknown (42). The heterozygous COX2+/− mice but not the COX1−/− or COX2−/− transgenic mice have been shown to develop obesity (43), but no mechanistic explanations involving altered PGE2 signaling have been developed.

Effects of prostaglandin D (PGD) and PGE2 on sleep architecture have been studied extensively (cf. ref. 44), and sleep promotion by PGE2 applied in the subarachnoid space was reported by Ram et al. (45), but the repeated night activity described in this report has not been observed.

EP3R is a G protein-coupled receptor (46) with several splice variants (47), but the tissue-specific distribution of these variants is unknown. The EP3R-like immunoreactivity is richly expressed in the rodent brain, with the highest density of EP3R receptors in the different hypothalamic nuclei involved in thermoregulation and sleep regulation (48–50). We have shown that EP3R mediates the effects of PGE2, a potent pyrogen, on the thermosensitivity of anterior hypothalamic neurons (51). EP3R-like immunoreactivity is also abundant in monoaminergic nuclei such as the raphe nuclei and locus ceruleus (48), where this prostanoid receptor may affect appetite and feeding through modulation of the serotonergic and noradrenergic signaling.

We show here that EP3R−/− mice exhibit an obese phenotype with a “night” eating component that is demonstrated by increased feeding during the light cycle of the day. The obese phenotype in EP3R−/− mice is congruent with previous observations that PGE2 stimulated leptin release, affected lipolysis, and had an effect on the overall endocrine state of the organism (43, 52). More broadly, this finding contributes to the increased understanding of the coupling between obesity and inflammatory signaling (53–55).

Results

EP3R−/− Mice Showed Increased Body Weight Caused by Increased Fat Storage. Male EP3R−/− mice fed ad libitum on an 11% fat diet exhibited an increase in body weight that resulted in an obese phenotype (Fig. L4). EP3R−/− body weights showed a gradual increase, with continuous accumulation up to 20% above that of WT littermate controls by week 20 (t = 4.41; P = 0.001) and continuing thereafter up to a recorded 30% increase by week 40 (t = 4.7; P = 0.006) (Fig. 1B). Body weight gain was primarily attributable to increased adipose tissue deposition (Fig. 1C). Comparison of the body composition of 3- and 6-month-old mice revealed that the early deposition of intraabdominal fat pads (gonadal, retroperitoneal, and mesenteric) in EP3R−/− mice at ≈3 months was followed by significant deposition of fat in adjacent tissues that include the liver and subcutaneous fat pads (inguinal and the groin) in EP3R−/− mice at ≈6 months (Fig. 1D). At 3 and 6 months of age, abdominal fat accounted for 6.62 ± 0.25% of the total body weight in EP3R−/− mice compared with the 3.37 ± 0.69% and 3.46 ± 0.16% in the WT mice.

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respectively (Fig. 1C). Subcutaneous fat pads at both ages accounted for 2.49 ± 0.33% and 5.2 ± 0.28% of the total body weight in EP3R−/− mice compared with the 1.99 ± 0.32% and 1.47 ± 0.12% in the WT mice, respectively (Fig. 1D). No significant differences in the brown adipose tissue percentage of body weight were seen between EP3R−/− and WT mice.

**EP3R−/− Mice Showed High Serum Levels of Insulin and Leptin at 3 and 6 Months of Age.** Measurements of circulating levels of insulin and leptin demonstrated that EP3R−/− mice had significantly elevated levels of both hormones. At 3 and 6 months of age, insulin levels accounted for 24.9 ± 0.33% and 5.2 ± 0.28% of the total body weight in EP3R−/− mice compared with the 1.99 ± 0.32% and 1.47 ± 0.12% in the WT mice, respectively (Fig. 1C). Subcutaneous fat pads at both ages accounted for 2.49 ± 0.33% and 5.2 ± 0.28% of the total body weight in EP3R−/− mice compared with the 1.99 ± 0.32% and 1.47 ± 0.12% in the WT mice, respectively (Fig. 1D). No significant differences in the brown adipose tissue percentage of body weight were seen between EP3R−/− and WT mice.

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**EP3R−/− Mice Showed Impaired Glucose Tolerance and Insulin Resistance.** At 3 months of age, the increased adiposity in EP3R−/− mice was accompanied by abnormalities in glucose metabolism. Even though mice were fasted for 24 h before the glucose-tolerance test, basal levels of glucose in EP3R−/− mice were significantly higher compared with WT mice (EP3R−/−, 149.25 ± 15.91 mg/dl; WT, 103.01 ± 10.1 mg/dl; t = 2.45; P = 0.04) (Fig. 3A). After the mice were challenged with a 1.5 mg of glucose per gram of body weight, serum glucose concentrations were similarly elevated at 15 min in EP3R−/− and WT mice, but serum glucose concentrations reached higher levels in EP3R−/− mice at 30 min (445 ± 21.08 mg/dl) than in the WT mice (381 ± 22.54 mg/dl) (t = 2.08; P = 0.04). At 60 min after the glucose
challenge, glucose levels were similar in both EP3R−/− and WT mice, but higher levels in EP3R−/− mice were reached again at 120 min (EP3R−/−, 242.25 ± 19.55 mg/dl; WT, 136.25 ± 20.42 mg/dl; t = 3.78; P = 0.0091) and 180 min (EP3R−/−, 197.6 ± 6.38 mg/dl; WT, 119.24 ± 3.72 mg/dl; t = 5.17; P = 0.0021) (Fig. 3A).

At 6 months of age, abnormalities in glucose metabolism were significantly higher in EP3R−/− mice than at 3 months of age (Fig. 3B). Basal levels of glucose in EP3R−/− mice were significantly higher compared with WT mice (EP3R−/−, 196.4 ± 22.84 mg/dl; WT, 105.82 ± 6.17 mg/dl; t = 4.16; P = 0.0024). After a glucose challenge, EP3R−/− mice showed higher glucose levels at 15 min (465.2 ± 22.05 mg/dl) than did WT mice (382.5 ± 22.31 mg/dl; t = 2.6; P = 0.0283). The same was true at 30 min (EP3R−/−, 487.8 ± 11.7 mg/dl; WT, 404.83 ± 33.55 mg/dl; t = 2.26; P = 0.049), 60 min (EP3R−/−, 420.6 ± 23.54 mg/dl; WT, 282 ± 22.15 mg/dl; t = 4.27; P = 0.0021), 120 min (EP3R−/−, 286.1 ± 25.93 mg/dl; WT, 233.65 ± 16.75 mg/dl; t = 1.7; P = 0.1135), and 180 min (EP3R−/−, 184.6 ± 15.14 mg/dl; WT, 146.5 ± 9.21 mg/dl; t = 2.24; P = 0.048) (Fig. 3B).

We determined the ability of insulin to acutely stimulate glucose disposal or clearance by performing an acute insulinchallenge in EP3R−/− and WT mice at both 3 and 6 months of age. At 3 months of age, the ability of insulin to acutely stimulate glucose disposal in EP3R−/− mice was significantly blunted at 15 min, indicating a short-term decrease in insulin sensitivity (EP3R−/−, 158.3 ± 10.59 mg/dl; WT, 125.8 ± 11.79 mg/dl, which corresponded to a 84.36% and 63.63% reduction in baseline values of EP3R−/− and WT, respectively; P = 0.0246) (Fig. 3C and D). At 6 months of age, basal levels of glucose were higher in EP3R−/− mice (EP3R−/−, 287.25 ± 48.32 mg/dl; WT, 173.02 ± 17.16 mg/dl), and acute insulin challenge was unable to stimulate glucose disposal in EP3R−/− mice at 15 min (EP3R−/−, 265 ± 53.71 mg/dl; WT, 125.3 ± 16.04 mg/dl, which corresponded to a 90.45% and 73.01% reduction in baseline values of EP3R−/− and WT, respectively; P = 0.046). Even though values at 30 min (EP3R−/−, 280.12 ± 48.5 mg/dl; WT, 148.02 ± 13.21 mg/dl), 60 min (EP3R−/−, 274.07 ± 41.69 mg/dl; WT, 156.23 ± 22.43 mg/dl), or 120 min (EP3R−/−, 290.94 ± 44.14 mg/dl; WT, 185.16 ± 28.89 mg/dl) were significantly higher in EP3R−/− mice, comparisons of the percentage of change from their own baseline were not significant (Fig. 3D and D').

EP3R−/− Mice Exhibited Increased Motor Activity and Core Body Temperature. Radiotelemetric evaluation of motor activity indicates that EP3R−/− mice displayed peaks of increased motor activity during the light part of the day, when mice usually spend most of their time sleeping. Increased nocturnal activity was recorded continuously over 21 days in six EP3R−/− mice and six WT littermates. Continuous motor activity profile during 21 days is shown (Fig. 4A), and this profile demonstrates that the peaks of nocturnal activity in EP3R−/− mice are not episodic but instead are recurrent daily events occurring with a frequency of between two and four episodes per night (Fig. 4A and C). The increase in motor activity during the light cycle in EP3R−/− mice compared with WT mice was punctuated and separated by phases in which the motor activity of the EP3R−/− mice was indistinguishable from that of the WT mice (Fig. 4B and C). However, cumulative analysis demonstrated that these peaks contributed to an overall 60.3% increased motor activity in EP3R−/− mice. Increased motor activity in EP3R−/− mice was also observed during the dark cycle, and this increase accounted for 24.73% of the motor activity (Fig. 4B). Core body temperature of EP3R−/− mice was slightly elevated during the corresponding peaks of increased motor activity (see Fig. 4D).

EP3R−/− Mice Showed Increased Food Consumption. Measurement of food consumption in EP3R−/− and WT mice demonstrated that EP3R−/− mice ate significantly more food than the WT littermates at both ages. At 3 months of age, hourly food intake was similar between EP3R−/− and WT mice during the light cycle. However, EP3R−/− mice during the dark period showed an increase in food intake characterized by a continuous feeding (monophasic), whereas WT littermate controls showed an increase in feeding followed by a period of temporary decrease and then by a second increase in feeding, making the pattern biphasic (Fig. 5A). As a consequence, statistical differences are only observed in the dark period at 0:00 and 1:00 a.m. (0:00: EP3R−/−, 0.338 ± 0.02 g; WT, 0.236 ± 0.03 g; t = 2.35; P = 0.0466; 1:00: EP3R−/−, 0.298 ± 0.03 g; WT, 0.184 ± 0.03 g; t = 2.33; P = 0.0481). At 6 months of age, the increase in feeding strongly correlated with the peaks of activity and elevated temperature during both the light/inactive and the dark/active part of the day (Fig. 5B). Cumulative food intake was higher in EP3R−/− mice in both periods. During the light phase, significant differences were at 7:00 a.m. (EP3R−/−, 0.19 ± 0.03 g; WT, 0.09 ± 0.02 g; t = 2.25; P = 0.0378), 9:00 a.m. (EP3R−/−, 0.17 ± 0.04 g; WT, 0.05 ± 0.01 g; t = 2.51; P = 0.022), 15:00 p.m. (EP3R−/−, 0.16 ± 0.02 g; WT, 0.04 ± 0.02 g; t = 3.27; P = 0.004), and 17:00 p.m. (EP3R−/−, 0.17 ± 0.06 g; WT, 0.03 ± 0.01 g; t = 2.14; P = 0.047).

During the dark phase, significant differences were at 0:00 a.m. (EP3R−/−, 0.36 ± 0.03 g; WT, 0.17 ± 0.04 g; t = 3.08; P = 0.006) and 1:00 a.m. (EP3R−/−, 0.27 ± 0.03 g; WT, 0.15 ± 0.03 g; t = 2.2; P = 0.041).

Both EP3R−/− and WT mice underwent food deprivation for 24 h, and subsequent food intake was measured every 12 h. Baseline measurement for 3 days confirmed differences in food intake. Food deprivation increased food intake in the next dark and light period in both strains, and full recovery was reached on day 4 (Fig. 5B).


Discussion

The observations reported here that EP3R−/− mice have an obese phenotype include measurements of body weight, as well as endocrine parameters known to increase with obesity, such as insulin and leptin plasma levels. Although it has been reported that PGE2 stimulates leptin release from adipocytes (52), we show that the lack of EP3R signaling in EP3R−/− mice does not prevent the large increase in leptin levels (Fig. 2 Lower), which is commensurate with the increased fat deposits (Fig. 1 C). Thus, PGE2 stimulation of leptin release does not exclusively depend on signaling through the EP3 prostanoid receptor subtype.

The levels of PGE2 in obese humans are elevated (56). It will be interesting to test the hypothesis that such elevation could be attributable to disruption of PGE2-negative feedback through EP3R.

EP3R receptors are broadly expressed in the periphery and in the brain. Our study did not determine the site(s) of action that contributes to the obese phenotype of EP3R−/− mice, because both peripheral and central expression of EP3R is absent in the null mouse. The exact localization of the prostanoid receptor(s)-mediated febrile effects by transgenic and lentiviral techniques is currently being studied by Lazarus (57), and similar techniques will be needed to precisely determine the site(s) at which the lack of PGE2 signaling via EP3R contributes to this obese phenotype. Although it is possible to speculate that obesity in EP3R−/− mice may be caused in part by the lack of hypothalamic PGE2 signaling, numerous peripheral actions of PGE2 are also mediated through this prostanoid receptor subtype (34).

The finding of increased feeding coupled with nocturnal motor activity in EP3R−/− mice compared with WT littermates suggests that EP3R−/− mice do not stabilize sleep and may wake up more easily. The role of PGE2 as a somnogenic agent alongside prostaglandin D in sleep has been reported (45). Our data suggest that EP3R may actually mediate a part of this response.

The most likely brain–EP3R-linked response is the febrile response. It has been shown that EP3R−/− mice do not mount fever in response to the pyrogens IL-1β and LPS, suggesting that the PGE2 action in the anterior hypothalamus, raphe pallidus, and other sites involved in the generation of the fever response involves EP3R (5). The basal temperature regulation may also involve EP3R-mediated effects of PGE2, and we observed a slightly elevated core body temperature in freely moving ad libitum-fed EP3R−/− mice. The increased core body temperature and motor activity would be predicted to lead to a decrease in body weight because energy demands are increased when higher body temperature needs to be maintained, but it appears as though the increase in food consumption/energy intake has a more pronounced effect, thus resulting in body weight gain.

The onset of obesity does not occur late in development in EP3R−/− mice and does not seem to require additional age-dependent factors to come into play. It is important to note that
the obesity in EP3R−/− mice does not result from the high-fat diet that is now commonly used to achieve an obese state in experimental animals; rather, this obesity in EP3R−/− mice occurs on standard chow and results from increased feeding in the absence of a commensurate increase in energy expenditure. This increase in energy intake relative to energy expenditure is often deemed to be an important factor in the etiology of common forms of human obesity. In addition, it is worth noting that EP3R−/− mice show another common feature of weight-gain scenarios in humans: night eating.

It is important to note that the lack of the full spectrum of inflammatory signaling has been shown recently to lead to obesity in a multitude of transgenic models including IL-1R1−/− (58), IL-1β−/−/IL-6−/− double knockout (59), and IL-18−/− (60) mice. These observations, together with those in the EP3R−/− mice described here, suggest that inflammatory molecules and/or signaling may be required for keeping body weight homeostasis. The exact mechanism leading to obesity in these models is not known, although effects of cytokines on insulin resistance and insulin receptor substrate expression (61) and phosphorylation have been proposed (62). It will be important to determine whether those mechanisms are distinct or similar to those leading to obesity in EP3R−/− mice. Full pharmacological characterization of the contribution of EP3 mediated-signaling in obesity and other phenotypes awaits the introduction of an EP3 prostanoid receptor-specific antagonist, in the same manner that EP1 antagonist-mediated impulsive behavior served to verify the involvement of that receptor subtype in the behavioral effects (63).

In summary, mice that are null for the prostanoid receptor EP3 display increased feeding throughout the day along with additional feeding activity peaks during the night (or light period), resulting in obesity. These mice may represent an important obesity model.

Materials and Methods

Animals. All procedures were approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute and were carried out on male EP3R−/− mice backcrossed to C57BL/6 background over more than eight generations and on WT littermates.

Food-Intake Measurements. Mice were fed ad libitum with mouse breeder diet (S-2335 Mouse Breeder, gross energy kcal (1 kcal = 4.18 kJ)/g 4.39, protein % 17.50, fat % 11.72, fiber % 3.36; Harlan Teklad, Madison, WI) and separated in two groups on each age (n = 10 each group). Food intake was monitored every hour during 24 h. For daily consumption, food and body weight were monitored twice per day at the onset of the dark and light period (6:00 a.m. and 6:00 p.m.) for 9 days. Mice were deprived of food for 24 h on day 4. Observation of food consumption was evaluated for 5 additional days after the food deprivation. Body weight was normalized for metabolic demands of body mass according to Kleiber’s function (g weight loss/g baseline weight0.75).

Body Weight and Fat Distribution. At the end of this period, mice were anesthetized, and intraabdominal fat pads (gonadal, retroperitoneal, and mesenteric), liver, subcutaneous fat pad (inguinal and the groin), and brown adipose tissue were dissected and weighed.

Glucose-Tolerance Tests. A glucose-tolerance test was performed at the onset of the light cycle (6:00 a.m.). Mice were weighed and fasted for 24 h before the glucose-tolerance test. Access to drinking water was allowed during this period. On the day of the test, baseline glucose levels and body weight were determined before challenge with a glucose load of 1.5 mg of glucose per gram of body weight (D-glucose, anhydrous; Sigma–Aldrich, St. Louis, MO) dissolved in sterile distilled water (0.75 g of D-glucose, anhydrous in 10 ml of sterile water). The mouse was restrained by holding the excess skin at the base of the neck between the technician’s thumb and forefinger. The mouse’s tail was left hanging out and placed on a glass slide, and a segment of ≈1 mm in length was cut off the tip of the tail by using a sharp razor blade. A small drop (≈5 μl) of blood was placed on the
mice were returned, and food and water was provided ad libitum.

Plasma Levels of Leptin and Insulin. In the morning, the mouse was injected i.p. by using a 1-ml syringe and a 1-ml needle. A blood sample (n = 6 for each group) was collected at 6:00 a.m. and 6:00 p.m., at the onset of the light dark cycle, for the determination of leptin, and values were collected and averaged ± SEM. Mouse insulin was determined by RIA using a Rat Insulin RIA kit (250 tubes; catalog no. RI-13K; Millipore) according to manufacturer’s instructions (n = 6 for each group).

Telemetry Device Implant. EP3R–/- and WT littermate male mice were anesthetized with isoflurane (induction, 3–5%; maintenance, 0.9–1.5%) and implanted with radio telemetry devices (TA10TA-F20; Data Sciences, Inc., St. Paul, MN) into the peritoneal cavity for core body temperature measurement. Animals were allowed to recover for 2 weeks before being submitted for freely moving recording (n = 10 for each group). Mice were maintained in a temperature-controlled room (25°C) on a 12-h light–dark cycle (light on at 6 a.m.). Core body temperature and motor activity sensors were located in the transmitter implant. The cages were positioned onto the receiver plates. Radio signals from the core body temperature and motor activity of each animal (number of horizontal movements) were continuously monitored with a fully automated data-acquisition system (Dataquest A.R.T.; Data Sciences, Inc.).

Data Analysis. Data were grouped and analyzed by using the paired t test or ANOVA with repeated measures followed by post hoc Newman–Keuls test. All results are expressed as means ± SE. Metabolic efficiency was calculated as the energy intake divided by the body weight gain over a certain period. Linear relationships were estimated by using Pearson’s moment correlation coefficient.

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