

Mature-Onset Obesity in Interleukin-1 Receptor I Knockout Mice

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Interleukin-1 (IL-1) is a major mediator of inflammation that exerts its biological activities through the IL-1 type I receptor (IL-1RI). The body weights of IL-1RI^{-/-} mice of both sexes started to deviate from those of wild-type mice at 5–6 months of age and were 20% higher at 9 months of age. Visceral and subcutaneous fat mass, measured by dual-energy X-ray absorptiometry and magnetic resonance imaging, was markedly (1.5- to 2.5-fold) increased. Lean body mass and crown-rump length were also slightly (11 and 5%, respectively) increased, as was serum IGF-I. Obese IL-1RI^{-/-} mice were insulin resistant, as evidenced by hyperinsulinemia, decreased glucose tolerance, and insulin sensitivity. To elucidate the mechanisms for the development of obesity, young preobese IL-1RI^{-/-} mice were investigated. They showed decreased suppression of body weight and food intake in response to systemic leptin treatment. The decreased leptin responsiveness was even more pronounced in older obese animals. Moreover, spontaneous locomotor activity and fat utilization, as measured by respiratory quotient, were decreased in preobese IL-1RI^{-/-} mice. In conclusion, lack of IL-1RI-mediated biological activity causes mature-onset obesity. This obese phenotype is preceded by decreased leptin sensitivity, fat utilization, and locomotor activity. *Diabetes* 55:1205–1213, 2006

The prevalence of obesity is growing rapidly in many parts of the world and reaching epidemic proportions in several developed countries (1). Overweight and obesity are associated with increased risk of metabolic disorders, such as type 2 diabetes and hyperlipidemia, and thereby increased risk of cardiovascular mortality. Although the regulation of body

weight and body composition involves input from lifestyle and environment, compelling scientific evidence indicates that propensity to develop obesity is in large part attributable to genetic factors. Over the last decade, the study of different transgenic and knockout mouse models has contributed to the identification of new factors involved in the complex mechanisms regulating energy balance and to the clarification of the contribution of genetics to obesity (2,3). Moreover, a number of human genes have been identified in which major missense or nonsense mutations as well as genetic variations are associated with obesity-related phenotypes. Many of these genetic variants have occurred in molecules identical or similar to those identified as a cause of obesity in rodents, supporting their involvement in the regulation of body weight homeostasis also in humans (4).

Interleukin-1 (IL-1) is a major mediator of inflammation and exerts effects on the neuro-immuno-endocrine system (5). Infection, injury, and inflammation are associated with negative energy balance, characterized by reduced food intake, weight loss, increased thermogenesis, and fever. IL-1 could be of importance for these effects, as peripheral or central injection of IL-1 induces a marked rise in body temperature (fever) and suppression of appetite (6). Conversely, a decreased IL-1 receptor activation attenuates inflammation and affects mortality in mice with bacterial infection (7–9). The IL-1 system is composed of two agonist ligands, IL-1 α and - β . These molecules exert similar but not completely overlapping biological activities through the IL-1 type I receptor (IL-1RI), while IL-1 type II receptor is a decoy receptor (5). Another member of IL-1 family is the IL-1 receptor antagonist (IL-1Ra) that also binds to IL-1 receptors without inducing a cellular response, thereby antagonizing the effects of IL-1 α and - β (5).

There are indications of an interaction between the IL-1 system and fat metabolism. IL-1Ra is produced by human white adipose tissue (WAT) and is upregulated by obesity and lipopolysaccharide administration in mouse WAT, suggesting that WAT is an important source of IL-1Ra in obesity and possibly also inflammation (10). Moreover, the serum levels of IL-1Ra are markedly higher in obese compared with nonobese subjects, as IL-1Ra has a positive correlation with leptin concentrations and BMI (11). It has also been reported that IL-1Ra treatment can counteract the effects of leptin (6). On the basis of these findings, it has been speculated that upregulation of endogenous IL-1Ra and decreased activation of IL-1RI by IL-1 might result in a partial blockade of hypothalamic actions of leptin in obesity, contributing to an acquired resistance to

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DEXA, dual-energy X-ray absorptiometry; IL-1, interleukin-1; IL-1Ra, IL-1 receptor antagonist; IL-1RI, IL-1 type I receptor; LBM, lean body mass; MC4-R, melanocortin-4 receptor; MRI, magnetic resonance imaging; RER, respiratory exchange ratio; WAT, white adipose tissue.

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TABLE 1

Body weight and body composition (measured by DEXA) in 5-month-old littermate IL-1RI^{+/+}, IL-1RI^{+/-}, and IL-1RI^{-/-} mice

	IL-1RI ^{+/+}	IL-1RI ^{+/-}	IL-1RI ^{-/-}	<i>P</i>
Body weight (g)	23.23 ± 1.25	24.67 ± 0.41	26.21 ± 0.54	0.004
Body fat (g)	4.21 ± 0.83	4.92 ± 0.41	5.86 ± 0.32	0.03
Lean body mass (g)	19.02 ± 0.64	19.75 ± 0.32	20.35 ± 0.36	0.07

Data are means ± SEM, *n* = 4–16. Statistical significance was calculated by linear regression.

leptin. However, to date, it has not been investigated whether depletion of IL-1RI activation results in obesity.

IL-1 could be of importance in peripheral energy homeostasis by affecting carbohydrate metabolism. The results of *in vitro* studies suggest that IL-1 can counteract insulin at the level of the hepatocyte (12). On the other hand, IL-1 has been suggested to act as an antidiabetic agent, as treatment with IL-1 decreases blood glucose in both normal and genetically diabetic animals (13,14). However, there are few studies exploiting mouse knockout models to investigate the effects of endogenous IL-1 on glucose metabolism (15,16).

The aim of the present study was to investigate whether IL-1 receptor stimulation by endogenous ligands plays a role for regulation of body fat mass in healthy individuals, as has previously been observed during inflammation and other illnesses. Therefore, the effect of IL-1RI knockout on body fat accumulation was investigated in mice. These studies were done on mice of different ages, as regulation of body fat is age dependent.

RESEARCH DESIGN AND METHODS

The targeting strategy for IL-1RI gene disruption has been previously described in detail (8). IL-1RI-deficient mice (B6.129S7-Il1r1^{tm1Imx}) that had undergone five backcrosses to the C57BL/6 background and their wild-type C57BL/6J controls were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were kept and bred at the animal facility of Göteborg University (Göteborg, Sweden). The animals were maintained under standardized nonbarrier conditions, with *ad libitum* access to water and pelleted food (Lactamin, Stockholm, Sweden). These low-fat pellets (R34) have a nutrient content of 4% fat, 16.5% protein, and 58.0% carbohydrate (total calorific content 3 Kcal/g). To obtain littermate controls for the confirmatory experiment presented in Table 1, age-matched male and female IL-1RI^{-/-} and wild-type mice obtained by homozygous crossing were mated. Thereafter, their offspring were used for heterozygous crossing to obtain IL-1RI^{+/+}, IL-1RI^{+/-}, and IL-1RI^{-/-} mice to confirm the previously observed obese phenotype. All procedures concerning the mice were conducted in accordance with protocols approved by the local ethics committee on animal care at Göteborg University.

Analysis of body composition. Analysis of body fat and lean body mass (LBM) in IL-1RI^{-/-} and wild-type controls was performed by dual-energy X-ray absorptiometry (DEXA) with the Norland (Fort Atkinson, WI) pDEXA Sabre and the Sabre Research software (version 3.9.2), as described (17,18). The crown-rump length was measured on the same DEXA scans as the body fat and LBM and was defined as the distance between the crown of the skull to a point located in the middle line of the two caput femoris. Analysis of body composition in 5-month-old female littermate control mice was performed by DEXA using the Lunar PIXImus Mouse Densitometer (Wipro GE Healthcare, Madison, WI) (19) with the mice under inhalation anesthesia with Isoflurane (Forene; Abbot Scandinavia, Solna, Sweden).

Examination of body fat distribution was performed by magnetic resonance imaging (MRI). Mice were anesthetized and placed in the center of a 7 Tesla MR system (Bruker BioSpin, Ettlingen, Germany). A volume coil with 72-mm inner diameter and a homogeneous RF field of 100 mm along the axis of the magnetic field was used for radiofrequency pulses. A multislice spin-echo sequence (TR 1,000 ms, TE 10.2 ms) was used to acquire 46 axial slices with 1-mm slice thickness and 1.75-mm interslice distance. Total package extension was 91 mm along the magnetic field axis, i.e., all regions of the body were covered. The field of view was selected with 4.5 × 4 cm², and matrix size was 256 × 192 (in plane resolution 176 × 208 μm). Total measurement time of the spin-echo sequence was 3 min and 12 s. Next, spin-echo measurements were repeated; however, fat signals were suppressed

by a gauss pulse followed by a crusher gradient in slice orientation. Thus, only signal arising from water content of the tissue was acquired.

Next, 18 coronal slices with a field of view of 10 × 4.5 cm² and a matrix of 400 × 200 were acquired (measurement time 3 min and 20 s). During data processing, the coronal slices were used for the determination of the axial slices' position relative to the location of organs or tissue structures within the animals. Gray scale of MRI images expands over a range of 256 steps (16 bit). Multiplication of the gray scale with a factor of 0.33 limited the gray scale to 85 steps. Next, the gray scale of the fat-suppressed images was shifted by adding 170, i.e., to the region 170–255 within the full gray scale. Corresponding slices of the images were added. Thus, pixels where a signal was present in normal images but not in fat-suppressed images get a gray value between 86 and 170. Pixels with a signal in both normal and fat-suppressed images are calculated to have a gray value >170. A specific color lock-up table was constructed that only reflected signals in the gray scale between 86 and 170, while signals above or below were set to black. The calculated images reflected fat only.

Axial sections for calculation of subcutaneous and abdominal fat were selected from the section that visualized the most caudal part of the gonadal fat, adjacent to the very cranial pole of the testes, to the most cranial part of the ventricular fundus. In average, 18 sections were used per animal for calculations. Regions of interest for each of the fat depots were manually defined in each slice of the fat images (Paravision 3.0.2 software; Bruker BioSpin, Ettlingen, Germany), and pixel areas were measured. The subcutaneous and abdominal areas per slice were multiplied with slice distance to yield the corresponding fat volume. Fat weight was then calculated with a specific mass of 0.87 for fat tissue (20). Contribution of the skin to the MRI signal was negligible and assumed to <2%.

To confirm DEXA and MRI results regarding fat mass, 14- and 19-month-old male and 12-month-old female mice were killed and three intra-abdominal fat pads (gonadal, retroperitoneal, and mesenteric) and one subcutaneous fat pad (inguinal, in the groin) were dissected and weighed.

Food intake measurements. Food intake and body weights were monitored daily for a period of 4 days in single-housed wild-type and IL-1RI^{-/-} mice at the indicated ages (Table 2). After a week of acclimatization to the new environment, the amount of diet ingested was calculated as the difference between the weight of the food remaining in the food bin and the amount of preweighed food added the day before. Values were corrected taking into account the food spillage, considered as food pellets on the bottom of the mice cages.

Serum parameters analysis. To analyze serum parameters, blood samples were collected from the tail vein of mice at the indicated ages. Serum insulin and leptin were determined by radioimmunoassay (Linco Research, St. Charles, MO), as was IGF-I (Mediagnost, Reutlingen, Germany). Levels of cholesterol and triglycerides were quantified by enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany).

Glucose and insulin tolerance tests. Intravenous glucose tolerance tests were performed in both 7-month-old male and female mice and repeated later with older animals (9–10 months old). The experiments were carried out essentially as described earlier (21,22).

The glucose-reducing effect of insulin injection was assessed in nonfasted awake 8- to 9-month-old male mice in a similar way as described previously (23,24). Blood was withdrawn from the tail without anesthesia before a load of human insulin was administered (1 unit/kg *i.p.*; Sigma-Aldrich, Stockholm, Sweden). Further samples were collected 15, 30, and 60 min after the insulin challenge. Blood glucose levels were determined by an ABL 700 series analyzer (Radiometer, Copenhagen, Denmark).

Leptin treatment. The responsiveness to leptin reduction of cumulative food intake and body weight was examined in both preobese (4-month-old) and obese (10-month-old) wild-type and knockout mice. For this purpose, recombinant mouse leptin (kindly provided by Dr. Parlow, National Hormone Pituitary Program-NIDDK) was dissolved in 0.01 mol/l PBS, 0.1% BSA. The animals were injected twice daily with leptin (at doses of 10, 25, or 120 μg/day *s.c.*) over a period of 3 days preceded by a run-in period of vehicle treatment for 3 days to customize the animals to daily injections. Finally, after the leptin

TABLE 2
Characteristics of IL-1RI^{-/-} male mice

Variable	Age (months)	Wild type	IL-1RI ^{-/-}
Organ relative weights (% body wt)			
Liver	14	4.83 ± 0.05%	6.10 ± 0.25%*
Kidneys	14	1.59 ± 0.03%	1.47 ± 0.05%
Spleen	14	0.51 ± 0.03%	0.50 ± 0.02%
Crown-rump length (mm)			
	4	75.85 ± 0.64	76.57 ± 0.89
	9	76.94 ± 1.05	80.48 ± 0.79†
BMI (g/cm ²)			
	4	0.55 ± 0.01	0.57 ± 0.01
	9	0.63 ± 0.01	0.70 ± 0.02†
Food intake (g/day)			
	4	3.87 ± 0.15	4.17 ± 0.15
	10	4.55 ± 0.19	5.27 ± 0.09‡
Food intake (g/day/g body wt)			
	4	0.136 ± 0.004	0.142 ± 0.004
	10	0.120 ± 0.011	0.110 ± 0.005
Serum parameters			
Leptin (μg/l)			
	4	2.52 ± 0.45	2.91 ± 1.18
	9	12.50 ± 2.88	20.64 ± 2.09‡
	11	16.53 ± 1.88	26.71 ± 0.62*
Triglycerides (mmol/l)			
	14	1.34 ± 0.13	1.07 ± 0.10
Cholesterol (mmol/l)			
	14	2.27 ± 0.11	2.34 ± 0.11

Data are means ± SEM, $n = 4-10$. * $P < 0.001$, † $P < 0.05$, ‡ $P < 0.01$ vs. corresponding wild type. Statistical significance was calculated by Student's t test.

treatment all of the animals received vehicle injections for a period of 3 days. Food intake and body weight were recorded every morning during treatment. **Indirect calorimetry and locomotor activity measurements.** Energy expenditure was measured by indirect calorimetry as previously described (18) using an indirect open-circuit calorimeter (Oxymax; Columbus Instruments, Columbus, OH). After the system was calibrated against standard gas mixtures, 4-month-old wild-type and knockout mice were placed in individual acrylic calorimeter chambers (21.4 × 11.5 × 13.2 cm) with free access to food

and water. Energy expenditure, defined as oxygen consumption (V_{O_2}), and carbon dioxide production (V_{CO_2}) were measured during a period of 23 h (9:00 A.M. till 8:00 A.M.) at room temperature. Measurements were done pairwise, and expired air was analyzed for a 60-s period every 16 min using an electrochemical oxygen analyzer and a carbon dioxide sensor (Oxymax). The respiratory exchange ratio (RER) was calculated as V_{CO_2}/V_{O_2} (volume of CO_2 produced per volume of O_2 consumed [milliliters per kilogram per minute]). Total and ambulatory activities were measured simultaneously with an

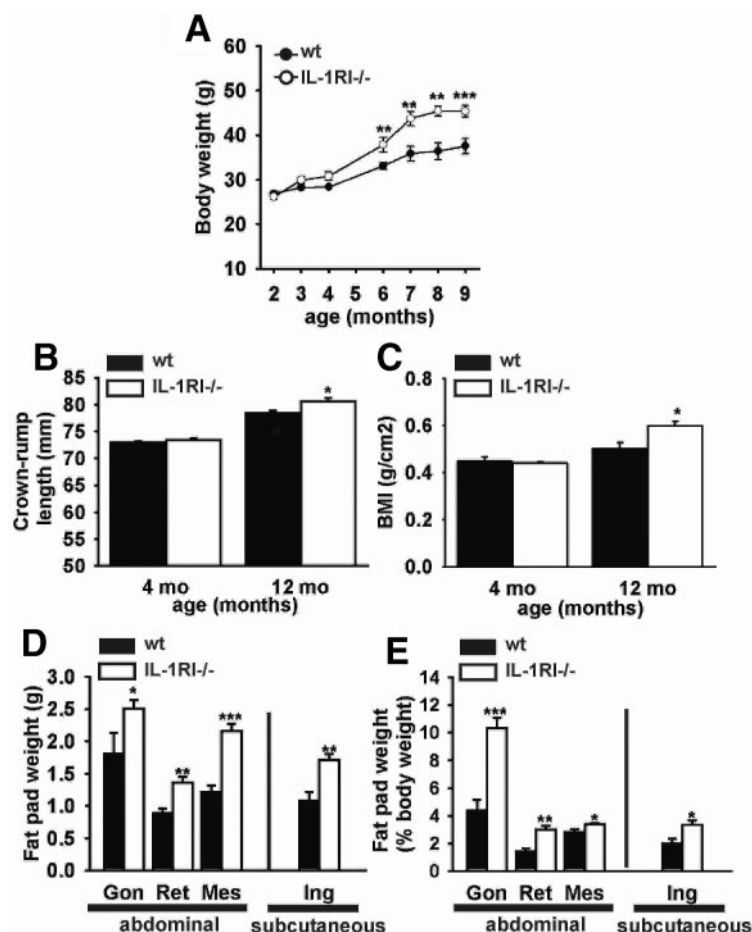


FIG. 1. Increased body weight, body growth, BMI, and dissected fat pad weight in older, but not younger, IL-1RI^{-/-} mice. **A:** Growth curves of male IL-1RI^{-/-} and wild-type (wt) mice fed on normal diet ad libitum; $n = 13-14$. **B and C:** Crown-rump length, measured by DEXA, and BMI, calculated as the square of body weight/square of crown-rump length in 4-month-old preobese and 12-month-old obese wild-type and IL-1RI^{-/-} female mice. **D and E:** Weights of dissected fat pads in wild-type and IL-1RI^{-/-} mice. Intra-abdominal fat pads (gonadal, Gon; retroperitoneal, Ret; and mesenteric, Mes) and the inguinal (Ing) (a subcutaneous fat pad in the groin) in 14-month-old male (**D**) and 12-month-old female (**E**) mice; $n = 7-8$. Values expressed as means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t test for IL-1RI^{-/-} vs. corresponding wild-type mice.

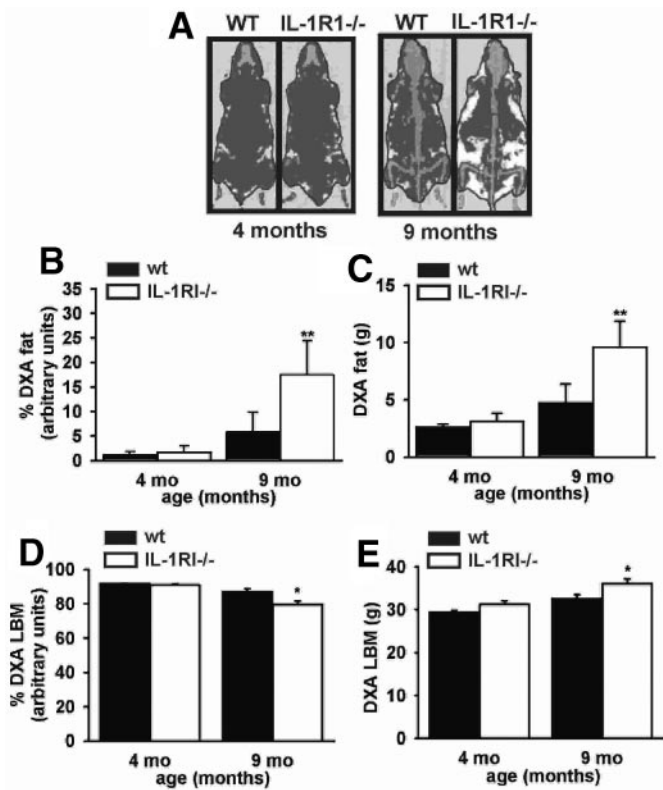


FIG. 2. Enhanced body fat content and LBM in older, but not younger, IL-1RI^{-/-} mice. **A:** Representative DEXA image analyses of fat mass (white) and LBM (black) in 4-month-old preobese and 9-month-old obese male wild-type (wt) and IL-1RI^{-/-} mice. **B–E:** Percentage and total body fat and LBM in wild-type and IL-1RI^{-/-} male mice; *n* = 5–9. Values expressed as means ± SEM. **P* < 0.05, ***P* < 0.01, Student's *t* test for IL-1RI^{-/-} vs. corresponding wild-type mice.

Optovarimex System (Columbus Instruments). The number of beam breaks was recorded in intervals of 1 min during the overall experimental period and data subsequently calculated as average number of beam breaks every 30 min. **Statistical analysis.** All analyses were performed using the SPSS statistical software package (version 11.5.1; SPSS, Chicago, IL). All values were calculated as average ± SEM. Comparisons between two groups were performed

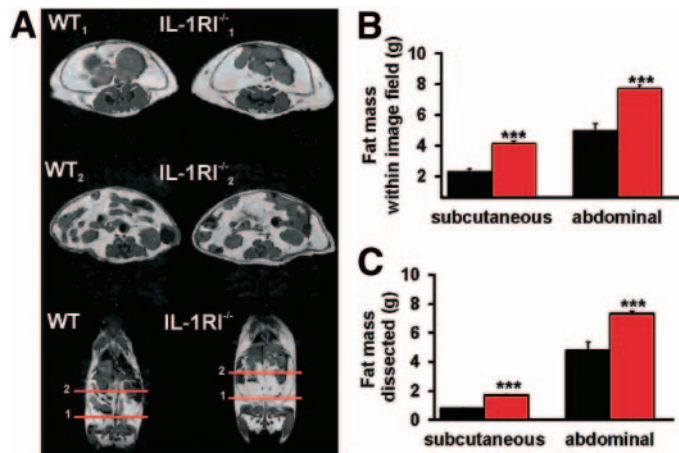


FIG. 3. Increased visceral and subcutaneous fat mass, as measured by MRI analysis, in IL-1RI^{-/-} mice. **A:** Representative axial (four upper) and coronal (two lower) slices obtained by MRI in 19-month-old wild-type (wt) and IL-1RI^{-/-} male mice. Red lines on the coronal slices depict the position where the representative axial slices were selected (1,2). **B:** Fat mass in male wild-type (black bars) and IL-1RI^{-/-} (red bars) mice. **C:** Weights of dissected fat pads in the corresponding animals; *n* = 4–7. Values expressed as means ± SEM. ****P* < 0.001, Student's *t* test for IL-1RI^{-/-} vs. corresponding wild-type mice.

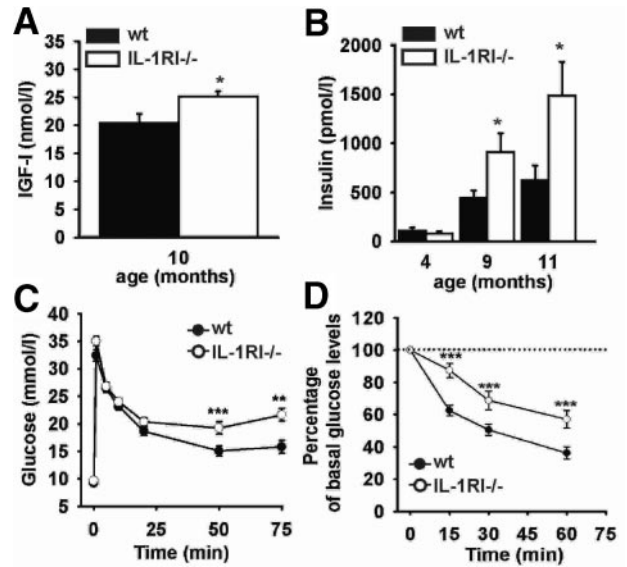


FIG. 4. Increased basal serum IGF-I and insulin levels and decreased glucose tolerance and insulin sensitivity in older IL-1RI^{-/-} mice. **A:** Serum IGF-I levels in obese 10-month-old male wild-type (wt) and IL-1RI^{-/-} mice; *n* = 10. **B:** Serum insulin levels in preobese 4-month-old and obese 9- and 11-month-old male wild-type and IL-1RI^{-/-} mice; *n* = 6–13. **C:** Glucose tolerance test. Glucose (1 g/kg i.v.) was given to 7-month-old anesthetized male and female mice. The test was repeated twice with similar results; *n* = 12. **D:** Insulin tolerance test. Obese (8- and 9-month-old) male wild-type and IL-1RI^{-/-} mice received 1 unit/kg insulin immediately after time 0. Glucose levels are normalized to the value at time 0; *n* = 10. Values expressed as means ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, Student's *t* test for IL-1RI^{-/-} vs. corresponding wild-type mice.

with unpaired Student's *t* test and one-way ANOVA, followed by the Bonferroni post hoc test when differences between more than two experimental groups were analyzed. Data derived from the same animal at several times were analyzed with ANOVA for repeated samples to evaluate differences between experimental groups. *P* values of <0.05 were considered significant. When appropriate, values were normalized by logarithmic transformation. Linear regression analysis was performed with fat mass calculated by MRI as the independent variable and adipose tissue dissected as the dependent variable. Pearson's correlation coefficient (*r*) was calculated. The gene dosage effect of the IL-1RI allele in IL-1RI^{+/+}, IL-1RI^{+/-}, and IL-1RI^{-/-} sibling controls was also evaluated with linear regression.

RESULTS

Increased body weight, body growth, and adiposity in older IL-1RI^{-/-} mice. As previously described (8), there was no significant difference in body weight between wild-type and IL-1RI^{-/-} mice at 4 months of age. The body weights of IL-1RI^{-/-} mice started to deviate from those of wild-type mice at 5–6 months of age and were increased by 20% at 9 months of age (Fig. 1A; *P* < 0.01 with ANOVA for repeated measures at months 2–9). The effects of IL-1RI gene knockout on longitudinal bone growth were evaluated with DEXA. The results showed that body length, measured as crown-rump length, was slightly increased in older IL-1RI^{-/-} female (4% higher for IL-1RI^{-/-} mice, Fig. 1B) and male (5% higher for IL-1RI^{-/-} mice, Table 2) mice compared with corresponding wild-type mice (*P* < 0.05). We did not observe any significant difference in crown-rump length between wild-type and IL-1RI^{-/-} mice at 4 months of age (Fig. 1B and Table 2). BMI was significantly increased in older IL-1RI^{-/-} female (Fig. 1C) and male (Table 2) mice, indicating that the increased body weight accumulation is associated with development of obesity and not only growth. Analysis of body composition was performed by dissection of fat pads, DEXA, and

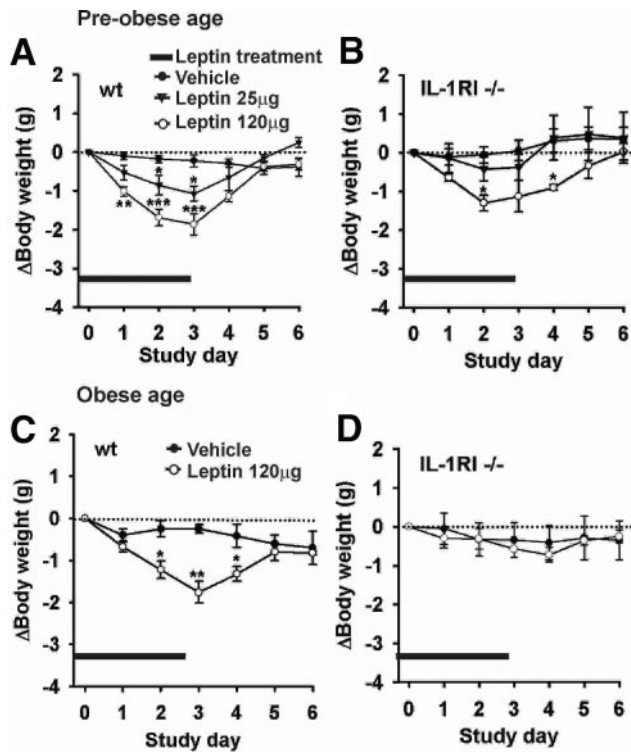


FIG. 5. Decreased suppression of body weight by leptin in preobese (A and B) and older obese (C and D) IL-1RI^{-/-} mice. A and B: Effect of leptin on body weight in 4-month-old preobese male wild-type (wt) (A) and IL-1RI^{-/-} (B) mice. C and D: Effect of leptin on body weight in 10-month-old obese male wild-type (C) and IL-1RI^{-/-} (D) mice. Male mice were given two daily injections of leptin (25 µg/day s.c. or 120 µg/day s.c.) or vehicle; $n = 4-12$. Black bars represent the 3-day period of treatment. Values expressed as means \pm SEM * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. corresponding wild-type mice, one-way ANOVA followed by a Bonferroni post hoc test (A and B) and Student's t test (C and D).

MRI. Dissection of fat pads in the older male mice (14 months old; Fig. 1D) and female mice (12 months old; Fig. 1E) showed that the absolute and relative weights of the three abdominal fat depots (gonadal, retroperitoneal, and mesenteric), as well as the subcutaneous inguinal fat depot, were increased in the older IL-1RI^{-/-} mice (Fig. 1D-E). In female IL-1RI^{-/-} mice, abdominal fat weight per mouse was increased by 37–77% and subcutaneous fat by 58% (not shown) compared with that in wild-type mice. In male IL-1RI^{-/-} mice, abdominal fat pad weights in relation to body weight were increased by 20–135% and subcutaneous fat by 67% (not shown).

As shown by representative DEXA images in Fig. 2A, the white areas (containing >50% fat) were larger in 9-month-old IL-1RI^{-/-} mice than in wild-type mice, while there was no difference at 4 months of age (Fig. 2A). Total fat mass, as evaluated by DEXA, in a group of 9-month-old IL-1RI^{-/-} male mice was about twofold higher than in their corresponding wild-type controls, while no obvious differences between genotypes were found at 4 months of age. Similar results were seen for percentage of body fat (Fig. 2B and C). The percentage of LBM in relation to body weight was significantly lower in obese older IL-1RI^{-/-} mice than in corresponding wild-type mice (Fig. 2D), but the absolute LBM was instead increased in older IL-1RI^{-/-} mice (11% higher for IL-1RI^{-/-} mice; Fig. 2E). In general, the effects of IL-1RI knockout on body fat accumulation and other metabolic parameters did not differ between the sexes.

To exclude that the effect of IL-1RI knockout on body

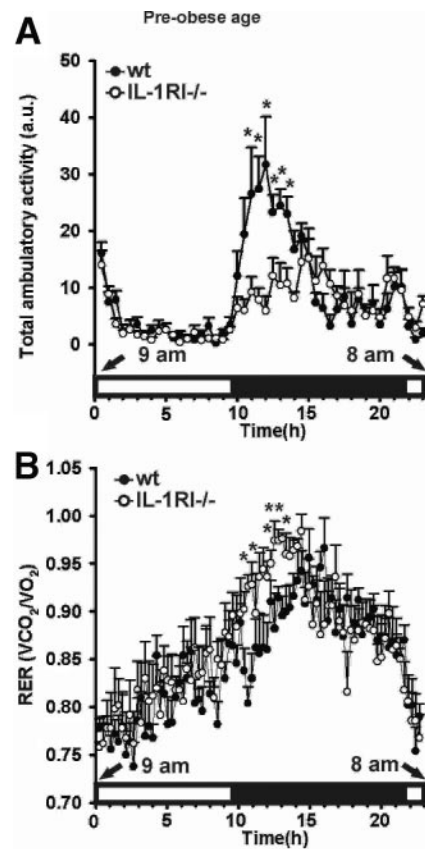


FIG. 6. Locomotor activity is decreased and RER is increased in preobese IL-1RI^{-/-} mice. Total ambulatory activity (A) and RER (B) measured in young (4-month-old) IL-1RI^{-/-} and wild-type (wt) male mice at room temperature. Black horizontal bars depict the dark period in a 12:12-h light-dark cycle. Data were collected during a 23-h period (9:00 A.M. till 8:00 A.M.). Values expressed as means \pm SEM; $n = 5-6$. * $P < 0.05$, ** $P < 0.01$, Student's t test for IL-1RI^{-/-} vs. corresponding wild-type mice.

weight and fat mass was merely due to strain differences, the effect was confirmed in littermate controls derived from heterozygous crossing. In 5-month-old female mice, body weight was higher in IL-1RI^{-/-} than in IL-1RI^{+/-} mice, which both had higher levels than IL-1RI^{+/+} mice. In a similar way, the total body fat content in IL-1RI^{+/-} and IL-1RI^{-/-} female mice, as evaluated by DEXA, was increased by 17 and 39%, respectively, in relation to IL-1RI^{+/+} mice. These gene dosage effects were significant, as calculated by linear regression (Table 1). The LBM also tended to be higher in IL-1RI^{-/-} and IL-1RI^{+/-} mice than in IL-1RI^{+/+} mice, but this difference did not reach significance (Table 1).

To further confirm the contribution of intra-abdominal and subcutaneous fat depots to this enhanced fat mass, a more detailed analysis of body composition was performed by MRI in 19-month-old wild-type and IL-1RI^{-/-} mice. Calculations of fat content performed in axial slices (examples in Fig. 3A, upper and middle panels), selected to cover the inguinal fat depot as well as the complete abdominal cavity, revealed that both subcutaneous and intra-abdominal fat mass contents in IL-1RI^{-/-} mice were on average 1.5–1.7 times higher than those in wild-type mice (Fig. 3B). Dissection of fat pads in the same animals yielded similar results (Fig. 3C). The minor differences between the methods may be accounted for MRI covering a larger fraction of the subcutaneous fat depot than fat

dissection. For both subcutaneous and abdominal fat there were good correlations between the mass of dissected fat pads and fat mass as measured by MRI (subcutaneous fat $r = 0.942$, $P < 0.01$; abdominal fat $r = 0.932$, $P < 0.01$).

Leptin levels have been shown to correlate with fat mass in humans and rodents (25,26). Reflecting the increase in adiposity in older IL-1RI^{-/-} mice, circulating leptin levels were almost twofold higher in male IL-1RI^{-/-} mice at 9–11 months of age (Table 2) and in female IL-1RI^{-/-} mice at 11 months of age (IL-1RI^{-/-} 26.71 ± 2.34 $\mu\text{g/l}$ and wild-type 15.72 ± 2.34 $\mu\text{g/l}$, $P < 0.01$). However, no differences were found between knockout and wild-type mice at 4 months of age (Table 2). The relative weights of dissected nonfat organs were also calculated. The results showed that the relative liver weight was significantly increased in older IL-1RI^{-/-} mice, while the relative weight of the kidneys and spleen were unchanged (Table 2). Serum triglycerides and cholesterol levels in older IL-1RI^{-/-} mice were comparable with those of wild-type mice (Table 2).

Increased IGF-I, insulin levels, and insulin resistance in older IL-1RI^{-/-} mice. To investigate possible underlying mechanisms to the enhanced LBM and body length in IL-1RI^{-/-} mice, we measured the serum IGF-I. The levels were increased by ~20% in 10-month-old IL-1RI^{-/-} mice compared with wild-type controls (Fig. 4A). The increased adiposity in older adult IL-1RI^{-/-} mice was accompanied by abnormalities in glucose metabolism. Basal insulin levels were found to be about twofold elevated in obese 9-month-old IL-1RI^{-/-} mice ($207 \pm 40\%$ of wild-type, $P < 0.05$) and at 11 months of age ($188 \pm 46\%$ of wild-type, $P < 0.05$), while in younger (4-month-old) preobese IL-1RI^{-/-} mice insulin levels were similar to those observed in wild-type mice (Fig. 4B). Basal hyperinsulinemia was seen in association with normal basal glucose levels in IL-1RI^{-/-} mice compared with wild-type mice (wild-type 9.2 ± 0.4 mmol/l and IL-1RI^{-/-} 9.7 ± 0.6 mmol/l, $P > 0.05$). After an intravenous injection of glucose (1 mg/g), plasma glucose concentrations were similarly elevated in IL-1RI^{-/-} and wild-type mice for the first 20 min but then became higher in the knockouts at 50 min (+28%) and 70 min (+37%) postinjection, indicating a mild impairment of plasma glucose elimination (Fig. 4B; $P < 0.01$ with ANOVA for repeated measures at min 0–70). The glucose elimination rate, as determined between 5 and 50 min after glucose injection was $0.93 \pm 0.10\%/min$ in wild-type vs. $0.61 \pm 0.10\%/min$ in IL-1RI^{-/-} mice ($P < 0.05$).

We also determined the ability of insulin to acutely stimulate glucose disposal by performing an acute insulin challenge test in older obese (8- to 9-month-old) male mice (Fig. 4C). The IL-1RI^{-/-} mice showed 40–58% ($P = 0.001$, ANOVA for repeated measures) higher blood glucose levels, indicative of decreased insulin sensitivity, compared with wild-type mice 15–60 min after insulin injection.

Effects of leptin administration on food intake and body weight in IL-1RI^{-/-} mice. It has been suggested that leptin resistance is a contributing factor to the development of obesity (27), and IL-1 has been proposed to mediate leptin effects in the central nervous system (6,11,28). To test whether lack of IL-1RI activation can cause leptin resistance that precedes the development of obesity, we studied the effect of leptin treatment on body weight and food intake in young (4-month-old) wild-type and IL-1RI^{-/-} mice. Leptin was given for 3 days by injections at doses of 10, 25, and 120 $\mu\text{g}/\text{mouse}$ s.c. Basal

food intake was not significantly different between 4-month-old IL-1RI^{-/-} mice and wild-type mice (Table 2). Neither wild-type nor IL-1RI^{-/-} mice responded to the lower dose of 10 μg leptin (data not shown). However, only wild-type mice, not IL-1RI^{-/-} mice, responded to the intermediate dose of 25 $\mu\text{g}/\text{mouse}$ by decreased food intake (-22% for leptin- vs. vehicle-treated wild-type mice, $P = 0.01$, and -13% for leptin- vs. vehicle-treated IL-1RI^{-/-} mice, $P = 0.23$) and body weight (-3.5% for leptin-treated wild-type mice; Fig. 5A and B). Food intake tended to be lower in leptin-treated wild-type mice than in leptin-treated IL-1RI^{-/-} mice but did not reach significance ($P = 0.11$). Both IL-1RI^{-/-} and wild-type mice responded to the higher dose of leptin (120 μg) by decreased food intake (-28 to -30% for leptin-treated wild-type and IL-1RI^{-/-} vs. vehicle-treated controls, respectively, $P < 0.01$) and body weight (-6.6 and -3.8% for leptin-treated wild-type and IL-1RI^{-/-} mice, respectively; Fig. 5A and B). All of the effects by leptin on body weight were confirmed by ANOVA for repeated measures, except for the effect of 120 μg leptin in preobese IL-1RI^{-/-} mice (Fig. 5B), supporting the conclusion that preobese IL-1RI^{-/-} mice are partly leptin resistant.

Increased leptin levels and increased leptin resistance are common obesity-associated features (27). In line with this, there was no decrease in food intake or body weight in response to leptin given in a high dose (120 $\mu\text{g}/\text{mouse}$) in older (10-month-old) obese IL-1RI^{-/-} mice. Older wild-type mice showed a decrease in both food intake (-28% for leptin-treated wild-type vs. vehicle-treated controls, not shown, $P < 0.05$) and body weight (-4.9% for leptin-treated wild-type mice; Fig. 5C and D) in response to this dose of leptin. At 10 months of age, the absolute amount of food consumed per day was higher in IL-1RI^{-/-} than in wild-type mice of the same age (Table 2). However, the food intake in relation to body weight was the same in wild-type and IL-1RI^{-/-} mice (Table 2).

Reduced locomotor activity and increased RER in IL-1RI^{-/-}. Because physical activity-related energy expenditure is a main contributor to total energy expenditure and energy balance, we examined locomotor activity as well as energy expenditure by indirect calorimetry in young (4-month-old) preobese IL-1RI^{-/-} and wild-type mice. IL-1RI^{-/-} showed a decreased ambulatory activity in comparison with wild-type mice at six consecutive time points during the first hours of the dark period ($P < 0.05$, ANOVA for repeated measures for the first 6 h of the dark period), whereas there was no difference in other parts of the light cycle (Fig. 6A). No significant differences were detected in total energy expenditure (calculated as oxygen consumption in milliliters per minute per animal, data not shown). However, the RER was higher during several measured time points during the first half of the dark cycle ($P < 0.05$, ANOVA for repeated measures for the first 6 h of the dark period), indicating that the IL-1RI^{-/-} mice then oxidize carbohydrates rather than fat for energy utilization (Fig. 6B).

DISCUSSION

The results of the present study indicate that knockout of the gene coding for IL-1RI, the only known IL-1 receptor with signaling activity, causes a specific increase in fat mass in mice. In a recent article, Matsuki et al. (16) reported that excess IL-1 activity due to gene knockout of the IL-1 receptor antagonist in mice caused leanness and

resistance to diet-induced obesity, without any overt signs of illness. Taken together, these two studies clearly demonstrate that IL-1RI activation can regulate body fat *in vivo* at both sub- and supra-physiological levels. Our results are the first to indicate a tonic fat-suppressing effect by IL-1RI activation in healthy animals.

It is assumed that inflammation in general decreases insulin sensitivity (29). Some *in vitro* results have suggested that IL-1 also decreases insulin response (12). On the other hand, the IL-1RI knockout mice investigated in the present study had increased basal insulin secretion in the absence of hypoglycemia and decreased insulin sensitivity and glucose tolerance, suggesting that IL-1 activity is associated with increased sensitivity to insulin. Conversely, Matsuki et al. (16) found that IL-1R antagonist knockout mice had increased insulin sensitivity, and IL-1 treatment has previously been reported to increase insulin sensitivity in rodents (14,30). Although it cannot be excluded that IL-1 can decrease insulin sensitivity at high pathological levels and/or in conjunction with other cytokines and inflammation, most data so far indicate that IL-1 increases insulin sensitivity *in vivo* over a range of doses. Our finding adds to this by indicating that endogenous IL-1 tonically enhances insulin sensitivity in older individuals, either by decreasing obesity or by direct obesity-independent mechanisms.

In the present study, we found that preobese IL-1RI knockout mice were less sensitive to the suppression of body weight and food intake by peripheral leptin treatment given over 3 days. This finding is in line with previous results by Luheshi et al. (6) indicating that IL-1 activity is of importance for the effects of single injections of leptin. These and other authors (6,28) also demonstrated that leptin treatment increases hypothalamic levels of IL-1 β . Leptin resistance appears likely to contribute to development of obesity, as originally hypothesized by Ahima et al. (31). Based on the present results, it can be postulated that the enhanced leptin resistance observed by us and others (6) in young preobese mice with defective IL-1 signaling contributes to the development of mature-onset obesity.

We observed that preobese IL-1RI knockout mice had a decreased fat-to-carbohydrate oxidation ratio, measured as increased RER. Similar observations have been made in other obese mouse models, such as in Ghrelin-treated mice (32) and IL-6 knockout mice (18). In addition, the IL-1RI knockout mice had suppressed locomotor activity during the early dark period, in line with results from IL-1 β knockout mice (33). Differences in spontaneous locomotor activity and posture allocation have recently attracted interest in relation to human obesity (34,35). Interestingly, Zurlo and coworkers (36,37) showed in longitudinal studies that both high RER and low spontaneous locomotor activity are predictors of obesity in Pima Indians. In summary, there is ample evidence in the literature that both decreased fat oxidation and decreased spontaneous locomotor activity could contribute to subsequent development of obesity in the IL-1RI knockout mice.

We have been unable to consistently measure any increase in feeding or decrease in energy expenditure in IL-1RI knockout mice that could explain their development of obesity. However, the 5-g difference in fat mass that occurred during 5 months (between months 4 and 9; Fig. 2C), equals ~ 0.03 g fat or 0.3 Kcal energy in fat/day. The daily energy expenditure, calculated as described (32), was ~ 10 Kcal/day per mouse. Consequently, a 3% difference in daily energy expenditure and/or food intake

would be enough to get the difference in fat mass observed in this study, although it is difficult to measure with available techniques. Similar problems have been encountered in other mouse models of obesity (32). Alternatively, the experimental paradigms may influence the measurements of energy metabolism, as shown in mice with decreased melanocortin-4 receptor (MC4-R) activation (38,39). On the other hand, the decreases in fat oxidation and locomotor activity in preobese IL-1RI knockout mice provide logical mechanisms for the development of obesity in IL-1RI knockout mice. As discussed above, these mechanisms would be in line with those described in several other murine and clinical models of obesity.

The present results suggest that IL-1RI activation by endogenous ligands may exert a mild catabolic effect in healthy animals. Besides the effect on fat mass, IL-1RI knockout also resulted in increased absolute LBM and longitudinal growth measured as crown-rump length. It seems likely that the increased serum IGF-I levels that we observed in IL-1RI knockout mice play a role for this effect. A possible mechanism could be IL-1 effects at the level of the hypothalamus (28) that influence the neuroendocrine regulation of growth hormone secretion (40). It cannot be ruled out that the increase in serum IGF-I also contributes to the increased obesity (41) or that the obesity affects the serum IGF-I levels. The increase in fat mass was larger than the increase in LBM, causing a decrease in relative LBM. The IL-1RI knockout mice have several similarities with mice lacking MC4-Rs, which display obesity, although this occurs earlier and is more severe than in the IL-1RI knockout mice. Like IL-1RI knockout mice, the MC4-R knockout mice have increased longitudinal growth and enhanced serum insulin levels (42). Mice ectopically expressing the MC4-R antagonist agouti have enhanced serum IGF-I levels (43). The MC4-R knockout mice also display decreased leptin sensitivity, and it is believed that MC4-R stimulation partly mediates the effect of leptin, although there are few studies of leptin sensitivity in preobese MC4-R knockout mice. Defects of the MC4-R gene are the most common known causes of severe obesity in humans, and, like the MC4-R knockout mice, these patients have increases in longitudinal bone growth LBM, and serum insulin levels (44). In relation to the similarities between IL-1RI and MC4-R deficiencies, it could be noted that these two systems have been suggested to interact with each other (45). The increases in longitudinal growth and LBM in IL-1RI knockout mice are not seen in IL-6 knockout mice, although both strains develop mature-onset obesity, and IL-6 and -1 have many biological effects in common (18,22).

We previously reported that IL-6-deficient (IL-6 $^{-/-}$) mice develop mature-onset obesity, which could partly be reversed by IL-6 replacement, suggesting a role for IL-6 in long-term regulation of adipose tissue mass in mice (22,46), although this effect has not been observed during all experimental conditions (47). In humans, a single nucleotide polymorphism in the IL-6 gene promoter, which is associated with reduced IL-6 production *in vitro* (48) and *in vivo* (49), has also been found to be associated with overweight and decreased basal metabolic rate in several recent studies (50,51). IL-6 is a pleiotropic cytokine known to exert effects outside the immune system (52); therefore, the effect on metabolism could be specific for IL-6. However, the results of the present study and another recent one (53) indicate that three different cytokines, IL-1, IL-6, and GM-CSF (granulocyte/macrophage-colony stim-

ulating factor) all suppress fat mass, probably via a central nervous system effect. Moreover, knockout of IL-RI, IL-6, and GM-CSF all result in mature-onset obesity, indicating that age-related cofactors are needed to obtain the obese phenotype (22,53). It has recently been reported that IL-1 receptor modulation also affects body fat in IL-6 knockout mice (54), but else there is little knowledge about the interactions between these three cytokines. Taken together, these results indicate interactions between several components of the immune system and body fat regulation. It is intriguing that several immune factors also seem to exert antiobesity effects in healthy individuals with no overt immune activation, and more studies are needed to clarify the underlying mechanisms for this.

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