

# IL-1 Plays an Important Role in Lipid Metabolism by Regulating Insulin Levels under Physiological Conditions

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## Abstract

IL-1 is a proinflammatory cytokine that plays important roles in inflammation. However, the role of this cytokine under physiological conditions is not known completely. In this paper, we analyzed the role of IL-1 in maintaining body weight because IL-1 receptor antagonist-deficient (IL-1Ra<sup>-/-</sup>) mice, in which excess IL-1 signaling may be induced, show a lean phenotype. Body fat accumulation was impaired in IL-1Ra<sup>-/-</sup> mice, but feeding behavior, expression of hypothalamic factors involved in feeding control, energy expenditure, and heat production were normal. When IL-1Ra<sup>-/-</sup> mice were treated with monosodium glutamate (MSG), which causes obesity in wild-type mice by ablating cells in the hypothalamic arcuate nucleus, they were resistant to obesity, indicating that excess IL-1 signaling antagonizes the effect of MSG-sensitive neuron deficiency. IL-1Ra<sup>-/-</sup> mice showed decreased weight gain when they were fed the same amount of food as wild-type mice, and lipid accumulation remained impaired even when they were fed a high-fat diet. Interestingly, serum insulin levels and lipase activity were low in IL-1Ra<sup>-/-</sup> mice, and the insulin levels were low in contrast to wild-type mice after MSG treatment. These observations suggest that IL-1 plays an important role in lipid metabolism by regulating insulin levels and lipase activity under physiological conditions.

Key words: IL-1-deficient mouse • IL-1 receptor antagonist-deficient mouse • obese • skinny model • energy homeostasis

## Introduction

IL-1 is a major mediator of inflammation. It also performs numerous functions related to host defense mechanisms, by regulating not only the immune system but also the neuronal and endocrine systems that interface with the immune system (1–3). IL-1 consists of two molecular species, IL-1 $\alpha$  and IL-1 $\beta$ , both of which exert similar, but not completely overlapping, biological functions through the IL-1-type I receptor (RI). Another IL-1 receptor, the IL-1-type II receptor (RII), has also been identified, but this receptor is not considered to be involved in signal transduction; rather, it is thought to play more of a regulatory role, as a “decoy.” Another member of the IL-1 gene family, IL-1 receptor antagonist (IL-1Ra), binds to IL-1 receptors without exerting agonistic activity. This molecule together with IL-1RII and the secretory forms of IL-1RI and IL-1RII are considered to be negative regulators of IL-1 signaling, participating in the complex regulation of IL-1 activity. IL-1 is produced by a large variety of cells, including monocytes/macrophages,

epithelial and endothelial cells, and glial cells. IL-1Rs are also expressed on a wide range of cells of the immune, neural, and endocrine systems, reflecting the pleiotropic activities of this molecule (4).

We have shown that both IL-1 $\alpha$  and IL-1 $\beta$  are induced in the brain of a mouse after injection with turpentine. The stress response against turpentine injection as determined by the development of fever and secretion of glucocorticoid is abolished in IL-1 $\alpha$ / $\beta$  double-deficient (IL-1<sup>-/-</sup>) mice, suggesting that endogenous brain IL-1 plays important roles in the stress response (5). Endogenous brain IL-1 also plays a pivotal role in the development of anorexia and hypothalamic cytokine expression upon administration with LPS (6). Interestingly, IL-1 is constitutively expressed

*Abbreviations used in this paper:* ARH, arcuate nucleus in the hypothalamus; BAT, brown adipose tissue; CRF, corticotropin releasing factor; FFA, free fatty acid; IL-1Ra, IL-1 receptor antagonist; IL-1<sup>-/-</sup>, IL-1 $\alpha$ / $\beta$  double-deficient; IL-1Ra<sup>-/-</sup>, IL-1Ra-deficient; LPL, lipoprotein lipase; MC3/4R, melanocortin-3/4 receptor; MSG, monosodium glutamate; PHP, post-heparin plasma; POMC, pro-opiomelanocortin; RI, type I receptor; RII, type II receptor; TAG, triacylglycerol; TC, total cholesterol; UCP, uncoupling protein; VCO<sub>2</sub>, carbon dioxide production; VO<sub>2</sub>, oxygen consumption; WAT, white adipose tissue.

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in health in specific areas of the brain, including the hypothalamus of humans and rodents (7, 8), and IL-1RI is also expressed in the brain, notably in the endothelial cells of blood vessels in the hippocampus and hypothalamus (9, 10). Because locomotive activity of IL-1 $\alpha$ / $\beta$ <sup>-/-</sup> mice was lower than control mice (11) and sleep regulation was abnormal in IL-1<sup>-/-</sup> mice as well as in IL-1RI<sup>-/-</sup> mice (12, 13), brain IL-1 may play important regulatory roles in maintaining homeostasis of the host under physiological conditions.

We found recently that IL-1Ra gene-deficient (IL-1Ra<sup>-/-</sup>) mice are lean and show growth retardation, suggesting that IL-1 may also be involved in energy homeostasis (5). In this context, IL-1 was reported to be involved in the feeding suppression caused by leptin, which is released from adipocytes and suppresses food intake through actions in the hypothalamus (14–17). IL-1 promotes the release of corticotropin releasing factor (CRF; references 18, 19), melanocortins, and other neuropeptides (4). CRF suppresses feeding behavior when administered intracerebroventricularly (20, 21). However, mice lacking both CRF RI and RII do not show any abnormal feeding behavior, indicating that the absence of CRF signaling does not accelerate feeding (22). On the other hand, excess IL-1 signaling induced by exogenously administered IL-1 or by leptin suppresses feeding behavior through a mechanism involving central melanocortin-3/4 receptors (MC3/4Rs; reference 23). Furthermore, mice with knockouts of MC3/4Rs develop mature-onset obesity (24–26), and likewise, mice lacking the pro-opiomelanocortin (POMC) gene, which encodes the precursor of an endogenous agonist for MC3/4Rs ( $\alpha$ -melanocyte stimulating hormone), also develop obesity (27). Thus, these receptor signals are crucial for the feeding regulation under physiological conditions. However, it is not known whether IL-1, which is detected in the brain under physiological conditions, plays any role in the regulation of feeding behavior through these receptors.

IL-1 has also been suggested to be involved in peripheral energy homeostasis through endocrine mechanisms. IL-1 $\beta$  selectively destroys the insulin-producing  $\beta$  cells, but not the  $\alpha$  cells, in vitro (28). This cytotoxic effect of IL-1 is suggested to be mediated by the induction of inducible nitric oxide synthase or prostaglandins (29–31). On the other hand, it was reported that IL-1 acts as a hypoglycemic agent not only in normal animals but also in alloxan-induced diabetic and genetically diabetic mice (32) and increases insulin and glucagon levels, suggesting that IL-1 has antidiabetic effects (33). Thus, the effects of IL-1 on glucose and insulin metabolism are somewhat conflicting, probably reflecting differences in experimental conditions.

Moreover, IL-1 has been suggested to directly modulate lipid metabolism by suppressing the activity of lipoprotein lipase (LPL), the enzyme regulating the disposal of lipid fuels in the body (34, 35). IL-1 may also regulate adipocyte function, as IL-1 inhibits adipocyte maturation and the synthesis of fatty acid transport proteins in adipose tissue in vitro (36, 37). These findings indicate that the IL-1/IL-

1Ra system may control lipid and lipoprotein metabolism through direct actions on adipose tissues. However, it remains unclear whether IL-1 is involved in energy homeostasis under physiological conditions and, if so, the mechanism involved is unknown.

To elucidate the role of the IL-1 system in energy homeostasis in this paper, we examined feeding behavior and peripheral metabolic changes of IL-1<sup>-/-</sup> and IL-1Ra<sup>-/-</sup> mice under physiological conditions and in response to manipulating food intake. The results suggest that IL-1 plays an important role in energy homeostasis under physiological conditions, acting via a peripheral mechanism.

## Materials and Methods

**Animals and Diets.** IL-1Ra<sup>-/-</sup> and IL-1<sup>-/-</sup> mice were produced by homologous recombination as described previously (5). These mice were backcrossed to the C57BL/6J strain mice for eight generations. Mice were housed individually from weaning at 3 wk old, and were allowed free access to chow and water, except when described separately. Mice were kept under specific pathogen-free conditions in an environmentally controlled clean room at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. They were housed at an ambient temperature of 24°C and a daily cycle of 12 h light and darkness (8:00–20:00). All experiments were performed according to the institutional ethical guidelines for animal experiments and according to the safety guidelines for gene manipulation experiments.

Body weight and food intake were measured in the morning, at least twice a week, beginning from the day of weaning. 20-wk-old mice were killed, and white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) pads were dissected and weighed. Intraperitoneal body temperature was measured as described previously (5). Food restriction of male mice at 8 wk old was performed for 18 d by feeding mice 0.9 g of normal chow each day. In food shift experiments, mice were fed a normal chow diet (fat, 5.1%; total energy, 4.18 kcal/g) or a high-fat normocaloric diet (fat, 23.6%; total energy, 4.55 kcal/g) from 9 to 17 wk old.

For hypothalamic lesion-induced obesity, newborn mice were administered either saline or monosodium glutamate (MSG) (4 mg/g body weight/day) i.p., from postnatal days 1 to 7 (38, 39). Body weight and food intake were measured in the morning once a week up to 20 wk old, the mice were killed, and serum and WAT were collected for measuring insulin level and WAT weight.

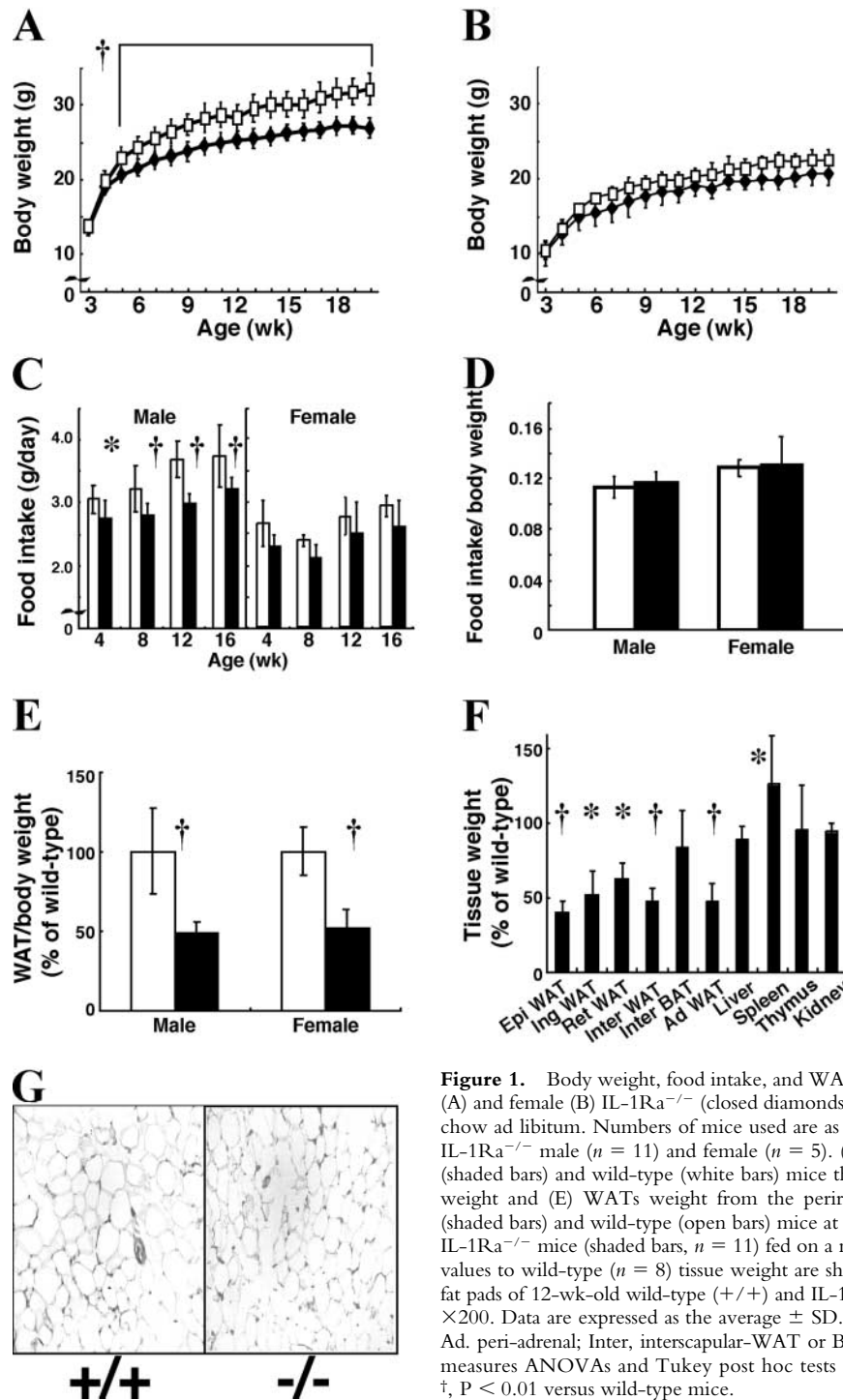
**Indirect Calorimetry.** Amount of oxygen consumption (VO<sub>2</sub>) and carbon dioxide production (VCO<sub>2</sub>) were simultaneously determined by indirect calorimetry using Respina (IH26; NEC Sanei Instruments Ltd.). Male mice at 15 wk old were housed in separate chambers for 1 h before the experiment. 10-min measurements were performed three times every 60 min during the middle of the light cycle (11:00–17:00) under a constant air-flow rate (200 ml/min). VO<sub>2</sub> and VCO<sub>2</sub> were calculated from the oxygen consumption and carbon dioxide production curves using the constant regions corresponding to resting period.

**Fasting Experiments.** To examine expression levels of neuropeptides and uncoupling proteins (UCPs), age-matched mice (6–8 wk old) were divided into three groups ( $n = 4$  per group). The fed group was allowed to feed ad libitum, the fasted group was fasted for 48 h, and the refed group was allowed to feed freely for 4 h after a 48-h fast. Mice were allowed free access to water during these experiments. Mice were killed, and brain and

peripheral tissues were dissected. Northern blot analyses for total RNA and poly (A)<sup>+</sup> RNA were performed as described previously (5).  $\beta$ -Actin was used as an internal control.

**Probes.** Probes for Northern blot hybridization were amplified by PCR with the following specific primers, using mouse hypothalamus and WAT cDNA as templates: agouti gene-related protein sense: 5'-ATGCTGACTGCAATGTTGCTG-3', antisense: 5'-CTAGGTGCGACTACAGAGGT-3'; cocaine-amphetamine-regulated transcript sense: 5'-ATCGAAGCGTTGCAAGAAGT-3', antisense: 5'-GGAATATGGGAACCGAAGGT-3'; melanin-

concentrating hormone sense: 5'-ATGGCAAAGATGACTCTCTCT-3', antisense: 5'-GACTTGCCAACATGGTCGGTA-3'; POMC sense: 5'-GCTTGCATCCGGGCTTGCAA-3', antisense: 5'-TCACTGGCCCTTCTTGTGCG-3'; UCP1 sense: 5'-ATGGTGAACCCGACAACCTTC-3', antisense: 5'-TTATGTGTACAATCCACTG-3'; UCP2 sense: 5'-ATGGTTGGTTTCAAGGCCAC-3', antisense: 5'-TCAGAAAGGTGCCTCCCGAG-3'; UCP3 sense: 5'-ATGGTTGGACTTCAGCCCTC-3', antisense: 5'-TCAAAAACGGAGATTCCCGCA-3'; resistin sense: 5'-AGCTGTGGGACAGGAGCTAA-3', antisense: 5'-CCTG-



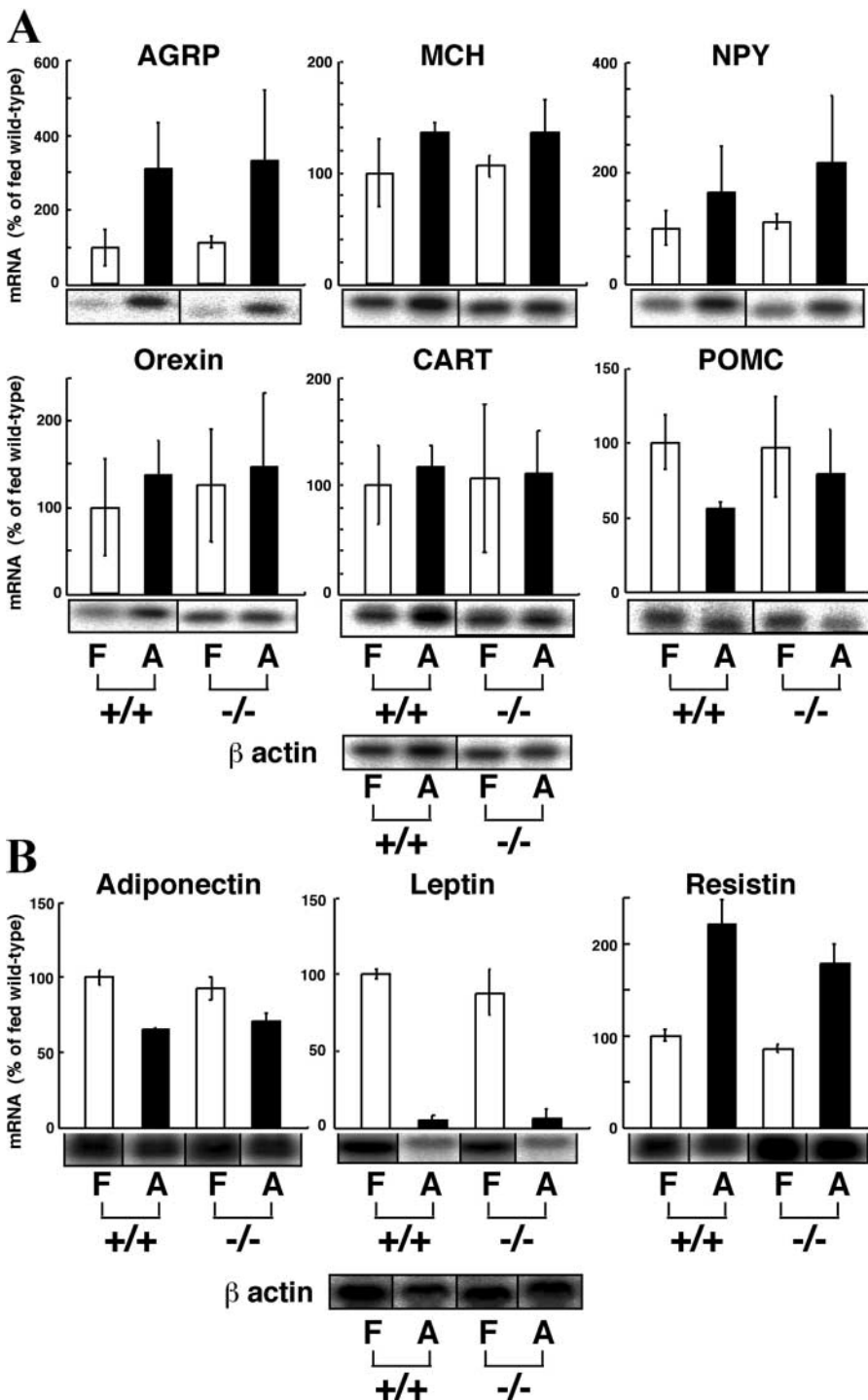
**Figure 1.** Body weight, food intake, and WAT weight in IL-1Ra<sup>-/-</sup> mice. Growth curves of male (A) and female (B) IL-1Ra<sup>-/-</sup> (closed diamonds) and wild-type mice (open squares), fed on a normal chow ad libitum. Numbers of mice used are as follows: wild-type male (*n* = 8) and female (*n* = 5); IL-1Ra<sup>-/-</sup> male (*n* = 11) and female (*n* = 5). (C) Daily food intake of male and female IL-1Ra<sup>-/-</sup> (shaded bars) and wild-type (white bars) mice that are shown in A and B. (D) Food intake per body weight and (E) WATs weight from the perireproductive tissues per body weight of IL-1Ra<sup>-/-</sup> (shaded bars) and wild-type (open bars) mice at 20 wk old. (F) Fat pads and various tissues from male IL-1Ra<sup>-/-</sup> mice (shaded bars, *n* = 11) fed on a normal chow were weighed at 20 wk old, and relative values to wild-type (*n* = 8) tissue weight are shown. (G) Paraffin sections of WAT from epididymal fat pads of 12-wk-old wild-type (+/+) and IL-1Ra<sup>-/-</sup> (-/-) mice. Hematoxylin and eosin staining.  $\times 200$ . Data are expressed as the average  $\pm$  SD. Epi, epididymal; Ing, inguinal; Ret, retroperitoneal; Ad, peri-adrenal; Inter, interscapular-WAT or BAT. Statistical significance was calculated by repeated measures ANOVAs and Tukey post hoc tests (A–C), and by Student's *t* tests (D–F). \*, *P* < 0.05, †, *P* < 0.01 versus wild-type mice.

TAGAGACCGGAGGACA-3'; adiponectin sense: 5'-GCACG-AGGGATGCTACTGTT-3', antisense: 5'-CCATACACCTG-GAGCCAGAC-3'. The probe for mouse leptin was a generous gift from K. Nakao (Kyoto University, Kyoto, Japan), and those for rat neuropeptide Y and rat pro-orexin were from T. Sakurai (Tsukuba University, Tsukuba, Japan).

**Blood Constituents.** To analyze blood constituents, 9–10-wk-old mice were used. Blood samples were collected by retro-orbital puncture under anesthesia at indicated times. Blood glucose levels and serum triacylglycerol (TAG), free fatty acid (FFA),

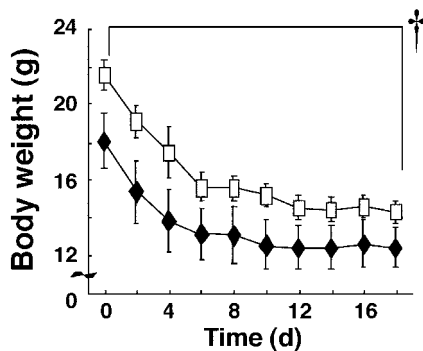
and total cholesterol (TC) levels were measured by the glucose oxidase method (Terumo Co.) and by colorimetric assays (tri-glyceride-L test, NEFA-C test [Wako Pure Chemical Industries Ltd.]; Determiner TC 555 [Kyowa Medex Co.]), respectively. Serum insulin and leptin levels were both measured by ELISA (Seikagaku Co.).

**Post-Heparin Plasma (PHP) Lipase Activity.** Male mice at 9–11 wk old were fasted from one day before the experiment, i.v. injected with 100 U/kg body weight of heparin, and plasma were collected 5 min later. Total and hepatic lipase activities



**Figure 2.** Normal expression of hypothalamic peptides and adipo-cytokines after fasting in  $IL-1Ra^{-/-}$  mice. (A) 5  $\mu$ g poly (A)<sup>+</sup> RNAs from the hypothalamus of fed or fasted mice (four mice for each lane) were purified, and Northern blot hybridization was performed with indicated probes. (B) 20  $\mu$ g total RNAs from epididymal WAT were prepared, and hybridized with indicated probes. F, fed ad libitum (white bars), A, fasted for 48 h (shaded bars). +/+, wild-type mice, -/-,  $IL-1Ra^{-/-}$  mice. Autoradiographs are representative in three independent experiments using at least four animals in one group. Data are expressed as the average  $\pm$  SD of all the experiments.





**Figure 3.** Impairment of energy storage in IL-1Ra<sup>-/-</sup> mice. Body weight changes of age-matched wild-type (open squares,  $n = 5$ ) and IL-1Ra<sup>-/-</sup> (closed diamonds,  $n = 5$ ) male mice under food restriction (0.9 g/day of a normal chow). Data are expressed as the average  $\pm$  SD. †,  $P < 0.01$  versus wild-type mice

were measured using lipase activity assay kit (Progen Biotech).

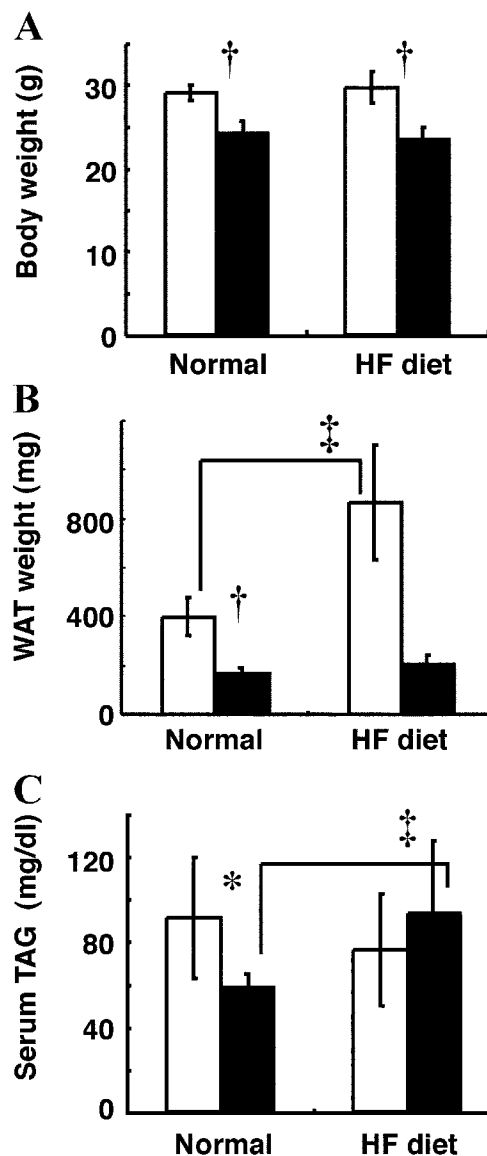
**Glucose Tolerance Test and Insulin Tolerance Test.** For the glucose tolerance test, 8–12-wk-old mice were fasted from the previous day and injected i.p. with 1.5 mg/g body weight glucose. For the insulin tolerance test, mice were fasted for a day and injected i.p. with 0.75 mU/g body weight human recombinant insulin (Sigma-Aldrich). Blood samples were collected by retro-orbital puncture.

**Histological Examination.** Adipose tissue and pancreas of 9–10-wk-old mice were fixed by 10% phosphate-buffered formalin and embedded in paraffin. 10- $\mu$ m sections were stained with hematoxylin/eosin.

**Statistical Analysis.** All values were calculated as average  $\pm$  SD. Comparisons were made using the Student's  $t$  test, one-way, or repeated measures ANOVA, Fisher's PLSD, or the Tukey post hoc test, when appropriate.

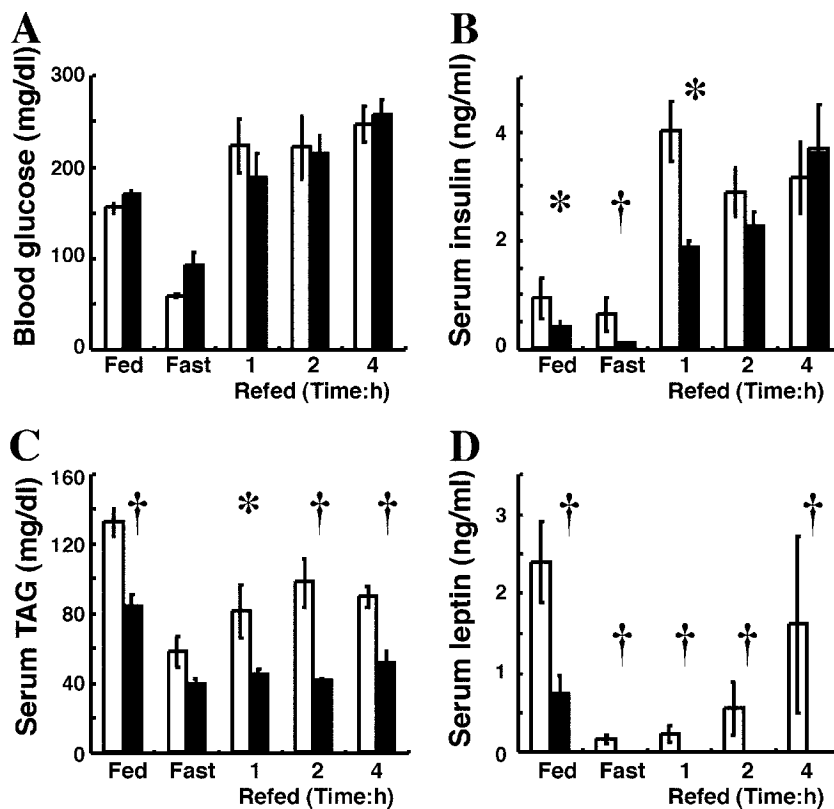
## Results

**Growth, Food Intake, and Body Weight of IL-1Ra<sup>-/-</sup> Mice.** To elucidate the role of IL-1 in body weight and energy homeostasis, we examined the growth of IL-1Ra<sup>-/-</sup>, IL-1<sup>-/-</sup>, and wild-type littermate mice. IL-1<sup>-/-</sup> mice showed normal weight gain until 20 wk old, in both males and females (reference 5 and unpublished data). Growth of IL-1Ra<sup>+/-</sup> (heterozygous) mice was also similar to wild-type mice (unpublished data). On the other hand, the body weight and daily food intake of IL-1Ra<sup>-/-</sup> male mice were lower than those of wild-type mice at 4 wk old, and the difference in body weight increased with age (Fig. 1, A and C). The body weight and daily food intake of IL-1Ra<sup>-/-</sup> female mice were also lower than those of wild-type mice, although the difference was not statistically significant (Fig. 1, B and C). However, food intake per gram body weight was similar between IL-1Ra<sup>-/-</sup> and wild-type mice, suggesting that the reduced food intake in mutant animals may reflect the difference in body weight (Fig. 1 D). These observations indicate that a lack of IL-1 signaling is dispensable in the maintenance of body weight, but that excess IL-1 signaling, due to IL-1Ra deficiency, suppresses weight gain under physiological conditions.



**Figure 4.** Impairment of lipid accumulation into WATs in IL-1Ra<sup>-/-</sup> mice. Mice were fed with a normal chow (Normal) or a high-fat diet (HF diet) for 8 wk. Body weight (A), epididymal WAT mass (B), and serum TAG level (C) were compared between normal chow and high-fat diet groups. IL-1Ra<sup>-/-</sup> (shaded bars,  $n = 5$ ) and wild-type (white bars,  $n = 5$ ) mice at the age of 17 wk were used. Data are expressed as the average  $\pm$  SD. Statistical significance was determined by one-way ANOVAs and Fischer's PLSDs. \*,  $P < 0.05$ , †,  $P < 0.01$  versus wild-type mice fed with the same chow. ‡,  $P < 0.01$  versus the same genotype mice fed with a normal chow.

The fat mass per body weight of male IL-1Ra<sup>-/-</sup> mice was less than half that of wild-type mice (Fig. 1 E). Histological analysis of adipose tissue, however, revealed that adipocytes of IL-1Ra<sup>-/-</sup> mice under fed conditions exhibited normal morphology and cell volume compared with those of wild-type mice (Fig. 1 G), suggesting that adipocyte differentiation was not altered in IL-1Ra<sup>-/-</sup> mice. Female IL-1Ra<sup>-/-</sup> mice also had significantly less fat mass than wild-type mice (Fig. 1 E), although their body weights were similar (Fig. 1 B). These results suggest that fat storage is



**Figure 5.** Decreased serum levels of insulin, TAG, and leptin in IL-1Ra<sup>-/-</sup> mice. Blood glucose (A), serum insulin (B), TAG (C), and leptin (D) levels in body weight matched wild-type (8 wk old, white bars) and IL-1Ra<sup>-/-</sup> (15 wk old, shaded bars) mice were measured. “Fed” samples were collected from mice fed ad libitum 2 d before fasting. “Fasted” samples were collected after 48 h of fasting, and refeeding started (0 h). Data are expressed as the average  $\pm$  SD, and are reproducible for three independent experiments using at least four mice in each genotype. Statistical significance was determined by Student’s *t* tests. \*,  $P < 0.05$ , †,  $P < 0.01$  versus wild-type mice.

impaired in IL-1Ra<sup>-/-</sup> mice. No difference was observed in the weights of other tissues between IL-1Ra<sup>-/-</sup> and wild-type mice, except for the spleen, which was increased in size in IL-1Ra<sup>-/-</sup> mice (Fig. 1 F).

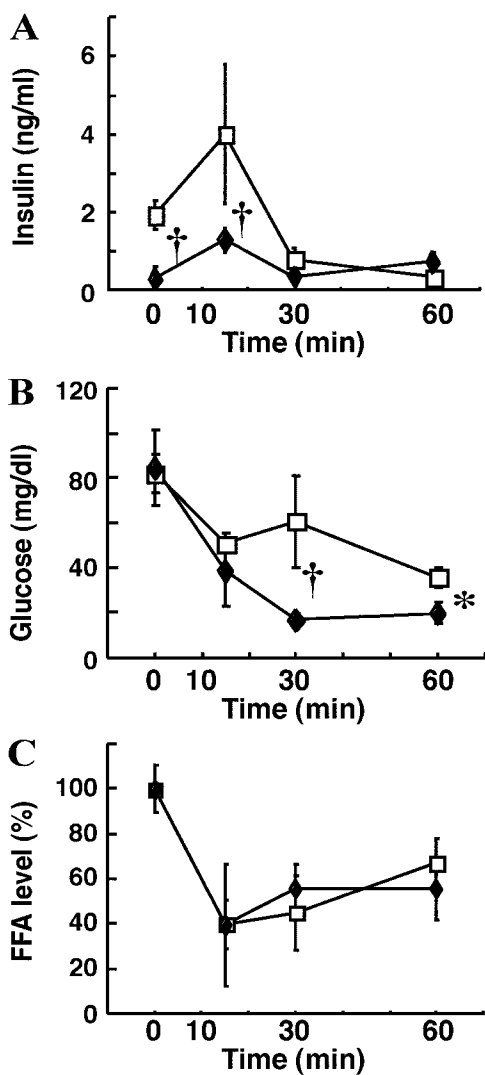
**Expression of Energy Regulatory Factors in Peripheral Tissues.** We assessed the regulation of energy expenditure in the periphery. Expression levels of UCPs (UCP1, UCP2, and UCP3), which are involved in heat production via the uncoupling of oxidative metabolism from ATP generation in BAT, WAT, and skeletal muscles, were compared. We found that UCP expression levels were normal in IL-1Ra<sup>-/-</sup> mice (unpublished data). Moreover, basal body temperature of IL-1Ra<sup>-/-</sup> mice was similar to wild-type mice (wild-type mice:  $37.8 \pm 0.6^\circ\text{C}$ ,  $n = 9$ ; IL-1Ra<sup>-/-</sup> mice:  $37.5 \pm 0.7^\circ\text{C}$ ,  $n = 9$ ,  $P = 0.29$ ). Changes in body temperature in response to fasting or cold stress (at  $4^\circ\text{C}$  for 6 h) were also similar to those of wild-type mice (unpublished data). Although locomotive activity in IL-1Ra<sup>-/-</sup> mice, as assessed by the open-field test, was slightly lower than that of wild-type mice, motor function, as evaluated by the rota-rod test, was similar between IL-1Ra<sup>-/-</sup> and wild-type mice (unpublished data).

Furthermore, we examined energy expenditure using indirect calorimetry.  $\text{VO}_2$  and  $\text{VCO}_2$  of IL-1Ra<sup>-/-</sup> mice were somewhat lower compared with wild-type mice, although the difference was not statistically significant ( $\text{VO}_2$  wild-type mice:  $60.5 \pm 8.2$  ml/kg/min,  $n = 13$ ; IL-1Ra<sup>-/-</sup> mice:  $52.2 \pm 11.8$  ml/kg/min,  $n = 8$ ,  $P = 0.053$ ;  $\text{VCO}_2$  wild-type mice:  $53.2 \pm 12.3$  ml/kg/min,  $n = 13$ ; IL-1Ra<sup>-/-</sup> mice:  $44.9 \pm 15.9$  ml/kg/min,  $n = 8$ ,  $P = 0.094$

average  $\pm$  SD). However, respiratory exchange ratio was normal (wild-type mice:  $0.845 \pm 0.094$ ,  $n = 13$ ; IL-1Ra<sup>-/-</sup> mice:  $0.853 \pm 0.148$ ,  $n = 8$ ,  $P = 0.56$ ). These data suggested that the energy expenditure in IL-1Ra<sup>-/-</sup> mice is not elevated and energy expenditure mechanisms are normal in IL-1Ra<sup>-/-</sup> mice under physiological conditions.

We also examined the expression levels of the adipocyte-derived cytokines (adipo-cytokines), adiponectin, leptin, and resistin, which are involved in lipid and glucose metabolism and insulin resistance (Fig. 2 B). No differences in expression levels of these adipo-cytokines were detected between IL-1Ra<sup>-/-</sup> and wild-type mice, suggesting that these adipo-cytokines are not involved in the decreased energy storage in IL-1Ra<sup>-/-</sup> mice.

**Expression of Energy Regulatory Factors in the Central Nervous System.** To elucidate whether or not hypothalamic feeding suppression mechanisms are involved in the lean phenotype of IL-1Ra<sup>-/-</sup> mice, we analyzed the expression levels of energy regulatory factors in the brain. We examined the expression levels of agouti gene-related protein (AGRP), melanin-concentrating hormone (MCH), neuropeptide Y (NPY), and orexin mRNAs as orexigenic peptides, and cocaine-amphetamine-regulated transcript (CART) and POMC mRNAs as anorexigenic factors using hypothalami from either ad libitum-fed mice or from 48-h fasted mice (Fig. 2 A). No significant differences between wild-type and IL-1Ra<sup>-/-</sup> mRNA levels were observed, however, under either fed or fasted conditions. These results show that the expression of major hypothalamic factors regulating feeding behavior is normal in IL-1Ra<sup>-/-</sup> mice.



**Figure 6.** Reduction of insulin levels in IL-1Ra<sup>-/-</sup> mice. Insulin levels after glucose tolerance test (A), and glucose (B) and FFA (C) levels after insulin tolerance test in wild-type (open squares) and IL-1Ra<sup>-/-</sup> (closed diamonds) mice. Relative FFA levels are expressed as the percentage relative to the value at time 0 (before insulin administration). Data are expressed as the average  $\pm$  SD. The results are reproducible in three independent experiments using at least four mice for each genotype. Statistical significance was determined by Student's *t* tests. \*,  $P < 0.05$ , †,  $P < 0.01$  versus wild-type mice.

**Effect of Limited Diet on Body Weight Maintenance.** We analyzed the energy storage efficiency of these mutant mice by measuring body weight changes in response to food restriction. When daily food was restricted to 0.9 g of a normal chow, the body weight of wild-type mice decreased to two-thirds of the initial weight after 14 d and maintained that level until 18 d (Fig. 3). Under ad libitum feeding conditions, wild-type mice eat  $3.2 \pm 0.4$  g/day, whereas mutant mice eat  $2.8 \pm 0.2$  g/day. Interestingly, similar body weight loss was also observed in food-restricted IL-1Ra<sup>-/-</sup> mice, and the body weight of IL-1Ra<sup>-/-</sup> mice still remained significantly lower than that of wild-type mice. These results clearly demonstrate that IL-1Ra<sup>-/-</sup> mice

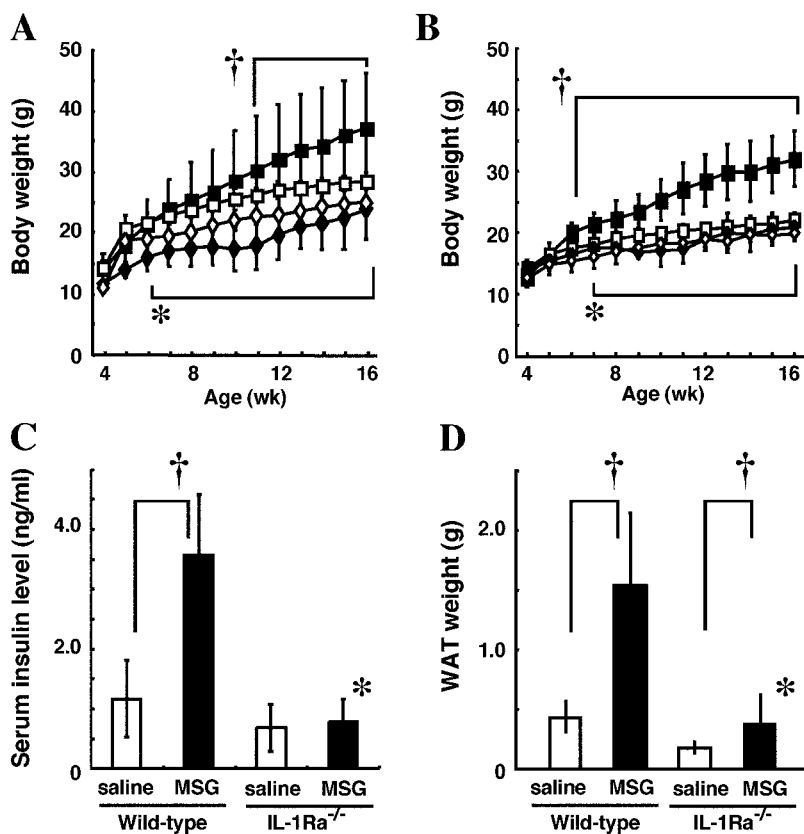
have defects in lipid storage even when they consume the same amount of food as wild-type mice, which is inconsistent with the notion that the lean phenotype of this mutant mouse is not caused by a central mechanism but by peripheral mechanisms.

**Lipid Utilization in IL-1Ra<sup>-/-</sup> Mice.** Next, we studied mice that were fed a high-fat, normocaloric diet, or a normal chow for 8 wk. Body weight did not differ between the high-fat diet group and the normal chow group, suggesting that mice from both groups obtained equivalent amounts of energy from these diets (Fig. 4 A). However, epididymal WAT mass was significantly increased in wild-type mice fed the high-fat diet (Fig. 4 B). In contrast, IL-1Ra<sup>-/-</sup> mice fed the high-fat diet did not gain additional adipose mass (Fig. 4 B). Serum TAG levels in these mice reached the same levels as in wild-type mice, although the levels in mice fed normal diet were significantly low (Fig. 4 C). These results suggest that lipid uptake into adipose tissues is impaired in IL-1Ra<sup>-/-</sup> mice.

**Blood Constituents in IL-1Ra<sup>-/-</sup> Mice under Physiological Conditions and after Refeeding.** To examine possible involvement of the endocrine system in the deficiency of lipid uptake seen in IL-1Ra<sup>-/-</sup> mice, we measured basal levels of glucose, insulin, leptin, TAG, TC, and FFA. Serum insulin, leptin, and TAG levels in IL-1Ra<sup>-/-</sup> male mice were significantly lower than those of wild-type mice under fed conditions (Fig. 5). On the other hand, levels of other blood constituents, including glucose, TC, and FFA, were comparable between wild-type and IL-1Ra<sup>-/-</sup> male and female mice (TC wild-type mice: male  $[92 \pm 19$  mg/dl],  $n = 5$  [female,  $68 \pm 13$  mg/dl,  $n = 4$ ]; IL-1Ra<sup>-/-</sup> mice: male  $[85 \pm 11$  mg/dl,  $n = 5$ ], female  $[72 \pm 19$  mg/dl,  $n = 6$ ]; FFA wild-type mice: male  $[1.04 \pm 0.12$  mEq/l,  $n = 5$ ], female  $[0.91 \pm 0.03$  mEq/l,  $n = 4$ ]; IL-1Ra<sup>-/-</sup> mice: male  $[0.91 \pm 0.14$  mEq/l,  $n = 5$ ], female  $[1.18 \pm 0.04$  mEq/l,  $n = 6$ ]; average  $\pm$  SD).

After a 48-h fast, blood constituents were periodically measured after refeeding, using weight-matched wild-type and IL-1Ra<sup>-/-</sup> mice, to avoid a possible confounding influence of body weight on recovery. In this experiment, body weight and food intake were measured under fed, fasted, and refeed conditions. IL-1Ra<sup>-/-</sup> mice consumed as much food as wild-type mice during refeeding, reconfirming that appetite was not reduced in these mice (unpublished data). Although glucose levels were normal, insulin, TAG, and leptin levels in response to refeeding were altered in IL-1Ra<sup>-/-</sup> mice (Fig. 5). Decreased levels of serum TAG and leptin in IL-1Ra<sup>-/-</sup> mice persisted for 4 h after refeeding, even though mutant mice consumed the same amount of food as wild-type mice (Fig. 5, C and D).

Interestingly, insulin levels were significantly low in IL-1Ra<sup>-/-</sup> male mice under physiological conditions. This trend was more clearly apparent under fasted conditions. Upon refeeding, insulin levels in IL-1Ra<sup>-/-</sup> mice were also significantly lower than in control mice, with a delayed peak in insulin secretion after refeeding (Fig. 5 B). Similar results were obtained when age-matched mice were used



**Figure 7.** Resistance of IL-1Ra<sup>-/-</sup> mice to MSG-induced obesity. Total body weights of male (A) and female (B) control (wild-type and IL-1Ra<sup>+/-</sup>) mice (squares) and IL-1Ra<sup>-/-</sup> mice (diamonds) after MSG (closed symbols) or saline (open symbols) treatment were measured (MSG-treated control mice: male,  $n = 6$ , female,  $n = 8$ ; saline-treated control mice: male,  $n = 13$ , female,  $n = 10$ ; MSG-treated IL-1Ra<sup>-/-</sup> mice: male,  $n = 9$ , female,  $n = 5$ ; saline-treated IL-1Ra<sup>-/-</sup> mice: male,  $n = 13$ , female,  $n = 5$ ). Serum insulin levels (C) and WAT weight from epididymis (D) of male control mice and IL-1Ra<sup>-/-</sup> mice at 20 wk old after MSG (shaded bars) or saline (white bars) treatment. Data are expressed as the average  $\pm$  SD. Statistical significance was calculated by repeated measures ANOVAs and Tukey post hoc tests. \*,  $P < 0.05$  MSG-treated IL-1Ra<sup>-/-</sup> mice versus MSG-treated wild-type mice. †,  $P < 0.05$  MSG-treated versus saline-treated mice.

for the experiments (unpublished data). These observations indicate that IL-1Ra deficiency affects insulin levels in the circulation. Although we do not know whether this is due to suppression of the insulin production or secretion, or even acceleration of the clearance at this moment, we use the phrase “insulin secretion” as it represents the sum of these processes in the following sections.

The effects of IL-1Ra deficiency on insulin secretion and insulin sensitivity were examined by glucose tolerance test and insulin tolerance test, respectively. After administration of glucose, the insulin response was significantly reduced in IL-1Ra<sup>-/-</sup> mice relative to wild-type mice (Fig. 6 A), although blood glucose levels were not significantly different between IL-1Ra<sup>-/-</sup> and wild-type mice (unpublished data). Furthermore, the sensitivity of blood glucose levels to insulin was significantly increased in IL-1Ra<sup>-/-</sup> mice (Fig. 6 B), although the effect of insulin on FFA release was similar between IL-1Ra<sup>-/-</sup> and wild-type mice (Fig. 6 C). The defect in insulin secretion in mutant mice was not due to developmental defects, because the  $\beta$  cells appeared normal by hematoxylin/eosin and  $\beta$  cell-specific staining (unpublished data). These results indicate that IL-1Ra deficiency affects lipid metabolism and insulin secretion independently from feeding behavior.

**MSG-induced Obesity and Metabolic Disorders in IL-1Ra<sup>-/-</sup> Mice.** MSG treatment ablates cells in the arcuate nucleus in the hypothalamus (ARH), which are involved in the metabolic regulation and leptin signaling (40), and this treatment induces maturity-onset obesity in wild-type

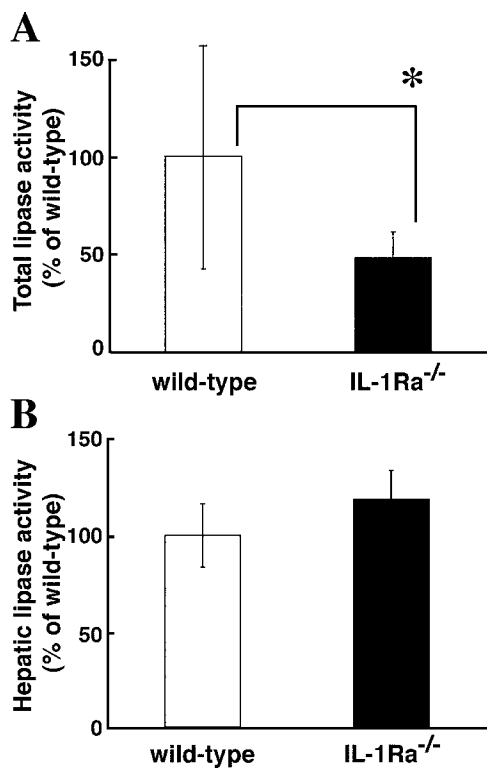
mice without affecting appetite (36, 39, 41). Because IL-1RI mRNA is expressed in the ARH (9, 42), we examined the possibility that the action of IL-1 may be mediated by the neurons in the ARH. As shown in Fig. 7 (A and B), both male and female IL-1Ra<sup>-/-</sup> mice showed complete resistance to obesity in contrast to wild-type mice. WAT weight was increased, although much less than MSG-treated wild-type mice (Fig. 7 C). Interestingly, serum insulin level was not elevated in IL-1Ra<sup>-/-</sup> mice in clear contrast to wild-type mice (Fig. 7 D). These results indicate that excess IL-1 signaling generated in IL-1Ra<sup>-/-</sup> mice antagonizes the effect of ARH lesion.

**Effect of IL-1Ra Deficiency on Lipase Activity.** To further demonstrate the effects of IL-1Ra deficiency on lipid metabolism, we studied lipase activity in IL-1Ra<sup>-/-</sup> mice. In the plasma of IL-1Ra<sup>-/-</sup> mice after heparin administration, total lipase activity, but not hepatic lipase activity, was significantly reduced compared with wild-type mice (Fig. 8). These observations suggest that IL-1 is also involved in the regulation of lipase activity under physiological conditions.

## Discussion

In this paper, we examined the physiological role of IL-1 on feeding behavior and energy metabolism using IL-1<sup>-/-</sup> and IL-1Ra<sup>-/-</sup> mice. We found that IL-1Ra<sup>-/-</sup> mice have a defect in lipid accumulation in adipose tissue, although IL-1<sup>-/-</sup> mice do not show any apparent abnormalities. Thus, IL-1 signal is not necessarily required for the lipid





**Figure 8.** Reduced PHP lipase activity in IL-1Ra<sup>-/-</sup> mice. Mice were administered 100 U/kg heparin intravenously, and plasma were collected after 5 min (A) total lipase activity and (B) hepatic lipase activity of the PHP from age-matched wild-type (white bars,  $n = 8$ ) and IL-1Ra<sup>-/-</sup> (shaded bars,  $n = 5$ ) male mice. Data are expressed as the average  $\pm$  SD. Statistical significance was determined by Student's  $t$  tests. \*,  $P < 0.05$  versus wild-type mice.

metabolism, but its excess signaling is harmful for the energy homeostasis of the body. The defect was more severe in males than in females, probably reflecting hormonal differences.

It is well-known that IL-1 is involved in fever, anorexia (loss of appetite), and cachexia that develop during infection, inflammation, cancer, or physical stress (4, 43). Fever and feeding suppression caused by leptin are also mediated by hypothalamic IL-1 (44). IL-1 can activate POMC neurons in the ARH where IL-1RI mRNA is expressed (9, 42), and the anorexic, but not pyrogenic, actions of IL-1 are mediated by central MC3/4Rs (23). Recently, two groups reported that cachexia was ameliorated by central MC3/4R blockade, indicating cancer anorexia is also mediated by central melanocortin pathway (45, 46). Furthermore, it was reported that leptin was induced by LPS through induction of IL-1 (47). Thus, it seemed likely that leanness of IL-1Ra<sup>-/-</sup> mice might be resulted from feeding suppression mediated by the ARH–melanocortin pathway. However, our findings suggest that the lean phenotype of IL-1Ra<sup>-/-</sup> mice is not caused by feeding suppression. This is because food intake per gram body weight is normal, and mutant mice showed a lean phenotype even when they were fed the same amount as wild-type mice. IL-1Ra<sup>-/-</sup> mice show normal energy expenditure and heat production. Furthermore, expression lev-

els of major hypothalamic factors involved in the melanocortin system are normal. Thus, we conclude that leanness of IL-1Ra<sup>-/-</sup> mice results not from feeding suppression but from metabolic disorder in the periphery that is caused directly by excess IL-1 signaling or indirectly through central mechanisms. IL-1 signaling under physiological conditions may be too weak to evoke feeding suppression, and only a large excess IL-1 signaling, such as that produced under pathological conditions, may suppress appetite through a hypothalamic mechanism.

We have shown that IL-1<sup>-/-</sup> mice do not show any apparent abnormality in feeding behavior or body temperature under physiological conditions. Consistent with our observations, it was reported that mice doubly deficient for the CRF RI and RII, which function down-stream of IL-1 in the hypothalamus, do not show any abnormalities under physiological conditions (22). Furthermore, IL-1Ra transgenic mice, with either the endogenous IL-1Ra promoter or the glial fibrillary acidic protein promoter, did not show any alterations in body weight (48, 49). Chronic central administration of IL-1Ra also did not affect food intake and weight gain in rats (50). Collectively, these observations indicate that IL-1 is not necessarily required for the control of appetite or body temperature under physiological conditions, although it plays most important roles under pathological conditions.

IL-1 reportedly suppresses intestinal lipid absorption and lipid accumulation in vivo, although the mechanism has not been completely elucidated (51). Although serum TAG levels are low in IL-1Ra<sup>-/-</sup> mice, this is not a result of defects in intestinal lipid absorption, because TAG levels in the chylomicron fraction of serum lipoproteins in IL-1Ra<sup>-/-</sup> mice are similar to those in wild-type mice (unpublished data). On the other hand, lipid accumulation is inhibited in mutant mice, because these mice show decreased fat accumulation in adipose tissue even when fed a high-fat diet, which leads to serum TAG levels similar to wild-type mice. The ability of embryonic fibroblasts to differentiate into mature adipocytes in vitro, however, is normal in IL-1Ra<sup>-/-</sup> mice, indicating that the ability of adipocyte progenitor cells to differentiate into mature adipocytes is normal in mutant mice (unpublished data). Furthermore, fatty acid uptake by in vitro-differentiated adipocytes from IL-1Ra<sup>-/-</sup> mice is also normal. Therefore, these observations suggest that IL-1 does not directly affect the differentiation or function of adipocytes, but rather affects adipocyte function by an indirect mechanism.

In this context, it is noteworthy that serum insulin levels in IL-1Ra<sup>-/-</sup> mice are significantly low under free-fed conditions and during recovery from starvation. Impaired insulin secretion is also observed after glucose administration. Furthermore, insulin secretion upon glucose administration is suppressed by the administration of IL-1 in wild-type mice (unpublished data), in agreement with previous works (52, 53). These findings indicate that excess IL-1 signaling suppresses insulin secretion from the pancreas.

Insulin is a major regulator of lipid metabolism in adipocytes, and it promotes adipocyte TAG store by fostering

the differentiation of preadipocytes, stimulating glucose transport and TAG synthesis, and inhibiting lipolysis (54). Insulin also increases the uptake of fatty acids derived from circulating lipoproteins by stimulating LPL activity (55–57) and promoting the trafficking of fatty acid transporters in adipose tissue (58). Actually, we showed that PHP lipase activity is reduced in IL-1Ra<sup>-/-</sup> mice. Thus, decreased insulin may cause reduced fat accumulation in adipose tissue of IL-1Ra<sup>-/-</sup> mice. It is also possible that excess IL-1 signaling affects LPL activity resulting in the suppression of fat accumulation.

However, normal serum glucose levels are maintained in IL-1Ra<sup>-/-</sup> mice under physiological conditions, despite decreased insulin levels. This is because insulin sensitivity is increased in IL-1Ra<sup>-/-</sup> mice as monitored by insulin tolerance tests. In contrast, the sensitivity of serum FFA to insulin is not increased in IL-1Ra<sup>-/-</sup> mice, and the expression of adiponectin and resistin, which are involved in the insulin sensitivity of adipose tissue (59–61), are not changed. These results indicate that the sensitivities of serum glucose and FFAs to insulin are different, and only lipid metabolism may be affected by the deficiency of insulin levels in IL-1Ra<sup>-/-</sup> mice.

It is known that MSG-sensitive neurons are involved in the peripheral lipid metabolism because disruption of these neurons causes obesity in wild-type mice, probably by activating the vagus nerves without affecting food intake (62). It is also known that MSG treatment activates insulin secretion in wild-type mice (63). In contrast, disruption of the ARH neurons by MSG treatment did not cause obesity in IL-1Ra<sup>-/-</sup> mice. Serum insulin levels were also not increased in these MSG-treated mutant mice, indicating that excess IL-1 signaling antagonizes the effects of the ARH neuron damage. However, it remains to be elucidated whether IL-1 acts on the pancreas so as to antagonize the effect of vagus nerve activation or directly suppresses vagus nerve activation. Nonetheless, these observations support the notion that IL-1 suppresses lipid accumulation in peripheral tissues by reducing blood insulin levels.

We reported previously that IL-1Ra<sup>-/-</sup> mice on a BALB/cA background spontaneously develop chronic inflammatory arthropathy resembling rheumatoid arthritis, after weaning (64). On the C57BL/6J background, these mutant mice, however, scarcely develop arthritis even at an older age (>24 wk old; reference 64). Because no IL-1Ra<sup>-/-</sup> mice on this genetic background develop arthritis at 5 wk old, whereas the lean phenotype develops as early as 5 wk old, leanness is not likely to be caused by autoimmunity or inflammation.

In summary, we have shown that the lean phenotype of IL-1Ra<sup>-/-</sup> mice is not caused by feeding suppression, but rather by impaired lipid accumulation. We showed that IL-1Ra<sup>-/-</sup> mice exhibit defects in postprandial insulin secretion and lipid metabolism. These results indicate that the IL-1 system plays a pivotal role in maintaining insulin homeostasis under physiological conditions. The IL-1Ra<sup>-/-</sup> mouse is a unique model for leanness and should be of use to further investigate obesity, diabetes, and lipid metabolism disorders.

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