

Combined Interleukin-6 and Interleukin-1 Deficiency Causes Obesity in Young Mice

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Proinflammatory cytokines including interleukin (IL)-1 and IL-6 exert pleiotropic effects on the neuro-immuno-endocrine system. Previously, we showed that IL-1 receptor antagonist-deficient (IL-1Ra^{-/-}) mice show a lean phenotype due to an abnormal lipid metabolism. On the contrary, it was reported that IL-6^{-/-} mice exhibit obesity after 6 months of age. This study sought to assess the roles of IL-1 and IL-6 in body weight homeostasis. We generated mice deficient in IL-6 and IL-1Ra (IL-6^{-/-} IL-1Ra^{-/-}) and IL-6, IL-1 α , and IL-1 β (IL-6^{-/-} IL-1^{-/-}). IL-6^{-/-} IL-1Ra^{-/-} mice exhibited a lean phenotype, similar to IL-1Ra^{-/-} mice. On the other hand, IL-6^{-/-} IL-1^{-/-} mice became obese as early as 10 weeks of age, while IL-1^{-/-} mice and IL-6^{-/-} mice were normal at this age. The daily food intake was significantly higher in IL-6^{-/-} IL-1^{-/-} mice than in IL-6^{-/-} IL-1^{+/+} mice, while energy expenditure was comparable in these two strains. Acute anorexia induced by peripheral administration of IL-1 was significantly suppressed in IL-6^{-/-} IL-1^{-/-} mice, but not in IL-1^{-/-} mice or IL-6^{-/-} mice compared with wild-type mice. These results indicate that IL-1 and IL-6 are both involved in the regulation of body fat in a redundant manner in young mice. *Diabetes* 55:971–977, 2006

Interleukin (IL)-1, a major mediator of inflammation, also performs numerous functions related to host defense mechanisms by regulating not only the immune system but also the neural and endocrine systems. IL-1 is produced by a wide variety of cells, including monocytes, macrophages, epithelial cells, endothelial cells, and glial cells. IL-1 receptors are expressed on a wide range of immune, neural, and endocrine cells, reflecting the pleiotropic activities of this molecule (1). Endogenous IL-1 in the brain plays a pivotal role in hypothalamic cytokine expression and the development of anorexia (2). Leptin, released from adipocytes, exerts an inhibitory feedback effect on fat masses by acting on hypothalamic nuclei that express the cognate signal-transducing receptor, ObRb (3). IL-1, which is induced by

leptin, is involved in the leptin-induced suppression of feeding (4). It is interesting to note that serum IL-1 receptor antagonist (IL-1Ra) levels are sevenfold higher in human patients with obesity in comparison with nonobese subjects (5). In addition, a large quantity of IL-1Ra is secreted from adipose tissue, although the biological significance of this phenomenon is not well understood (6). Using IL-1^{-/-} and IL-1Ra^{-/-} mice, we demonstrated the physiological role of IL-1 in feeding behavior and energy metabolism. IL-1Ra^{-/-} mice have a defect in lipid accumulation in adipose tissue, exhibiting leanness (7). Recently, Garcia et al. (8) demonstrated that IL-1R^{-/-} mice developed mature-onset obesity, beginning to deviate from the weight of wild-type mice at 5–6 months of age.

The acute-phase immunoregulatory cytokine IL-6 is secreted from adipose tissue during noninflammatory conditions in humans. Serum IL-6 levels correlate with BMI (9–11), as seen for leptin. Unlike leptin, however, IL-6 is coexpressed with its receptor by neurons of the hypothalamic nuclei that regulate body composition (12,13) and nonneuronal cells, such as astrocytes, microglia, and brain endothelial cells (14). Previous studies have demonstrated that IL-6^{-/-} mice develop mature-onset obesity; IL-6 exerts antiobesity effects centrally by increasing energy expenditure (15,16). Furthermore, the respiratory exchange ratio was higher in young IL-6^{-/-} mice than in wild-type mice, indicating that these mutant animals preferentially oxidize carbohydrates over fat (17).

The fact that IL-6, which is strongly induced by IL-1, shares many biological functions with IL-1 prompted us to investigate the relationship between IL-1 and IL-6 and their roles in body weight homeostasis. To address this issue, we created double knockout mice deficient in both IL-1Ra and IL-6 and triple knockout mice lacking IL-1 α , IL-1 β , and IL-6. We found that IL-6^{-/-} IL-1Ra^{-/-} mice are as lean as IL-1Ra^{-/-} mice. In contrast, IL-6^{-/-} IL-1^{-/-} mice become obese earlier than IL-6^{-/-} mice, beginning to deviate from IL-6^{-/-} mice at 10 weeks of age. These results demonstrate that the effect of IL-1 on body weight homeostasis is independent of IL-6 and that deficiency of IL-1 and IL-6 synergistically induce obesity. We also found that IL-6^{-/-} IL-1^{-/-} mice are hyperphagic in comparison with IL-6^{-/-} animals, despite similar energy expenditures.

RESEARCH DESIGN AND METHODS

Reagents. Recombinant murine IL-1 α was obtained from Pepro Tech EC (London, U.K.). The lyophilized protein was dissolved in pyrogen-free 0.9% NaCl (saline) containing 0.1% BSA (Sigma, A9306).

IL-1Ra^{-/-} mice and mice deficient for both IL-1 α and IL-1 β (IL-1^{-/-} mice) were produced as described (18). IL-6^{-/-} mice were kindly provided by Dr. Manfred Kopf (19). These mice were backcrossed to C57BL/6J mice for eight generations. IL-6^{-/-} IL-1^{-/-} mice were produced by crossing the IL-1^{-/-} and IL-6^{-/-} mice, while IL-6^{-/-} IL-1Ra^{-/-} mice were produced by crossing

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eWAT, epididymal white adipose tissue; FFA, free fatty acid; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; NPY, neuropeptide Y; TAG, triacylglycerol.

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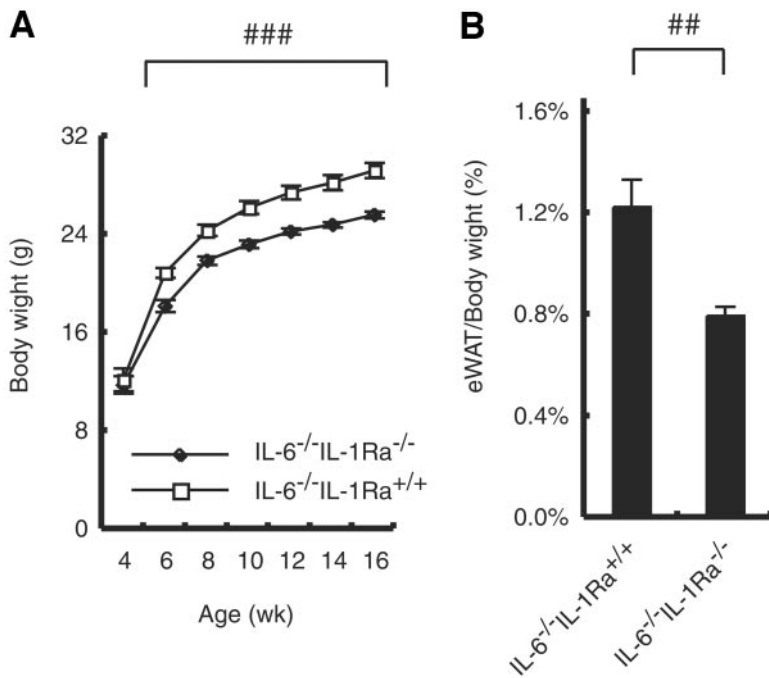


FIG. 1. Growth curves and eWAT volume in IL-6^{-/-} IL-1Ra^{-/-} male mice. **A:** Growth curves of IL-6^{-/-} IL-1Ra^{-/-} ($n = 8$) (\blacklozenge) and IL-6^{-/-} IL-1Ra^{+/+} ($n = 15$) (\square) mice fed by normal diet ad libitum. **B:** eWAT volume in IL-6^{-/-} IL-1Ra^{-/-} and IL-6^{-/-} IL-1Ra^{+/+} mice at 16 weeks. Data are expressed as means \pm SE. Statistical significance was calculated by repeated-measures ANOVA and Student's *t* test (A) or Student's *t* test (B). ## $P < 0.01$; ### $P < 0.001$.

IL-1Ra^{-/-} and IL-6^{-/-} mice. For each experiment, IL-6^{-/-} IL-1^{-/-} mice were produced by crossing IL-6^{-/-} IL-1^{-/-} male mice with IL-6^{-/-} IL-1^{+/-} female mice. IL-6^{-/-} IL-1Ra^{-/-} mice were generated by crossing IL-6^{-/-} IL-1Ra^{+/-} male mice with IL-6^{-/-} IL-1Ra^{+/-} female mice. After weaning, mice were housed individually at 4 weeks of age. Age-matched male littermates or adult (9–16 weeks of age) male mice obtained from a breeder (SLC, Shizuoka, Japan) were used for each experiment. When necessary, mice produced by in vitro fertilization were used to obtain large numbers of mice at one time under the same conditions. Single knockout mice were obtained from homozygous crossings. Mice were kept under specific pathogen-free conditions in environmentally controlled clean rooms at the Center for Experimental Medicine, Institute of Medical Science, University of Tokyo. Animals were housed at an ambient temperature of 24°C under a daily 12-h light (800–2000) and 12-h darkness cycle. All experiments were performed according to the institutional ethical guidelines for animal experimentation and according to safety guidelines for gene manipulation experiments.

Body weight and food intake measurement. Body weight and food intake of IL-6^{-/-} IL-1Ra^{+/+} and IL-6^{-/-} IL-1Ra^{-/-} pups, derived from the crosses of IL-6^{-/-} IL-1Ra^{+/+} males and IL-6^{-/-} IL-1Ra^{+/+} females, were measured once a week in the afternoon, beginning from the day of weaning at 4 weeks of age. Body weight and food intake of IL-6^{-/-} IL-1^{-/-} and IL-6^{-/-} IL-1^{+/-} pups, derived from the crosses of IL-6^{-/-} IL-1^{-/-} males with IL-6^{-/-} IL-1^{+/-} females, were measured once a week in the afternoon, beginning from the day of weaning at 4 weeks of age. The obese phenotype in IL-6^{-/-} IL-1^{-/-} mice was independently confirmed in mice derived from in vitro fertilization using IL-6^{-/-} IL-1^{-/-} sperm with IL-6^{-/-} IL-1^{-/-}, ^{+/-}, or ^{+/+} eggs. Epididymal white adipose tissue (eWAT) and the soleus muscle were dissected and weighed at the indicated times. Male mice at 16 weeks of age were subjected to food restriction for 5 days by administration of 5% of their body weight of normal diet. Male mice at 12–14 weeks of age were subjected to IL-1 treatment. Mice were housed individually 1 week before the experiment, and peripheral IL-1 (1 μ g/kg body wt i.p.) injection was performed at 1700. Food intake was measured 24 h after injection.

Measurement of body temperature. The intraperitoneal body temperatures of mice were measured using a telemetry system (ELAMS system; BioMedic Data System, Maywood, NJ), as described previously (20). Mice were moved to a cold room (4°C) at 1000. Body temperature was then recorded at regular intervals over the next 24 h.

Indirect calorimetry. Whole-body O₂ consumption and CO₂ production were measured in a respiration chamber, measuring 140 \times 80 \times 90 mm in size, ventilated with fresh air at a rate of 200 ml/min. The difference in concentrations of O₂ and CO₂ between inflow and outflow air was measured with a differential O₂ analyzer (LC700E; Toray, Tokyo, Japan) and two CO₂ sensors (GMW22D; Vaisala, Helsinki, Finland), respectively. Each mouse was placed in the chamber for 23 h. To avoid the influence of emotional thermogenic responses to cage-exchange stress, the data recorded during the 1st h were not analyzed. The results were then corrected for metabolic body mass (g^{0.75}).

Blood constituents. To analyze blood constituents, 16-week-old IL-6^{-/-} IL-1Ra^{-/-}, IL-6^{-/-}, IL-1Ra^{-/-}, and wild-type mice and 13-week-old IL-6^{-/-} IL-1^{-/-}, IL-6^{-/-}, IL-1^{-/-}, and wild-type mice were examined. Blood glucose levels were measured by the glucose oxidase method (Terumo), while serum triacylglycerol (TAG) and free fatty acid (FFA) levels were examined by colorimetric assays (triglyceride-E and NEFA-C tests, respectively; Wako Pure Chemical Industries). Serum insulin and leptin levels were both measured by enzyme-linked immunosorbent assay (Seikagaku, Tokyo, Japan) and radioimmunoassay (Eiken, Tokyo, Japan).

Statistical analysis. All values were calculated as means \pm SE. Differences among body weight curves, food intake, and body temperatures were evaluated by a repeated-measures ANOVA, in which factor 1 was the between-groups factor and factor 2 was the within-subject factor (the different ages). Comparisons of the two groups were analyzed by the Student's *t* test. To compare more than two groups, ANOVA was performed followed by Scheffé's tests. In all analyses, a two-tailed probability of <5% (i.e., $P < 0.05$) was considered to be statistically significant.

RESULTS

IL-1Ra^{-/-} mice are lean, independent of IL-6 action. If excess IL-1 signaling in the IL-1Ra^{-/-} mice causes leanness solely by enhancing IL-6 signaling, then IL-6 deficiency should cancel the lean phenotype seen in IL-1Ra^{-/-} mice. By 6 weeks of age, IL-6^{-/-} IL-1Ra^{-/-} male mice exhibited leanness in comparison to IL-6^{-/-} male mice (18.3 \pm 0.5 g IL-6^{-/-} IL-1Ra^{-/-} [$n = 8$] vs. 20.9 \pm 0.4 g IL-6^{-/-} IL-1Ra^{+/+} [$n = 15$], $P < 0.01$) (Fig. 1A). eWAT weight per body weight at 16 weeks of age was significantly reduced in male IL-6^{-/-} IL-1Ra^{-/-} mice in comparison to male IL-6^{-/-} (Fig. 1B; Table 1), while skeletal muscle weight was not significantly different (data not shown). These results demonstrate that excess IL-1 signaling promotes leanness independent of IL-6 signaling.

IL-1^{-/-} mice exhibit normal weight gain until 8 months. Considering the fact that IL-1Ra^{-/-} mice show leanness (7) and IL-1R^{-/-} mice develop obesity (8), we asked whether IL-1^{-/-} mice develop obesity. To exclude the effect of maternal effect, we analyzed pups delivered from ICR mice transferred with eggs made by in vitro fertilization. IL-1^{-/-} and IL-1^{+/-} pups were analyzed at 8 months. As demonstrated in Table 2, they were not significantly different in body weight, eWAT volume, glucose,

TABLE 1
Body weight, eWAT weight, and serum parameters in IL-6^{-/-} IL-1Ra^{-/-} mice at 16 weeks of age

	Body weight (g)	WAT (g)	WAT/body weight (%)	Insulin (pg/ml)	Leptin (pg/ml)	Nonesterified fatty acids (mEq/l)
Wild type	27.2 ± 0.2	0.30 ± 0.01	1.10 ± 0.03	1,419 ± 40	919 ± 26	ND
IL-1Ra ^{-/-}	24.3 ± 0.3*	0.21 ± 0.01*	0.87 ± 0.02*	972 ± 31†	636 ± 38†	ND
IL-6 ^{-/-}	27.9 ± 0.1	0.34 ± 0.01	1.22 ± 0.03	1,631 ± 59	859 ± 28	1.19 ± 0.02
IL-6 ^{-/-} IL-1Ra ^{-/-}	24.5 ± 0.2*	0.21 ± 0.01*	0.85 ± 0.02*	931 ± 70†	533 ± 33†	1.11 ± 0.04

Data are means ± SE. Statistical significance was calculated by ANOVA followed by either Scheffe's test (body weight, WAT, WAT/body weight, insulin, and leptin) or the Student's *t* test (nonesterified fatty acid). **P* < 0.01; †*P* < 0.05. ND, not determined.

and insulin level. These results indicate that IL-1^{-/-} mice show normal body weight homeostasis under our experimental conditions until 8 months.

Deficiency of IL-1 and IL-6 synergistically induces obesity. To examine the possible compensatory effect of IL-6 signaling on body weight homeostasis in IL-1^{-/-} mice, we next analyzed the effect of IL-1 deficiencies on IL-6^{-/-} mice. By 10 weeks of age, a significant increase in body weight was evident in the male IL-6^{-/-} IL-1^{-/-} mice in comparison to IL-6^{-/-} IL-1^{+/+} mice (Fig. 2A). At 13 weeks, body weight, eWAT weight, and eWAT weight/body weight in male IL-6^{-/-} IL-1^{-/-} mice increased significantly in comparison to IL-6^{-/-} IL-1^{+/+} animals (Fig. 2B–D). Again, skeletal muscle weight was not significantly different (data not shown). Body weight and eWAT volume of IL-1^{-/-} and IL-6^{-/-} mice were not significantly different from wild-type mice at 13 weeks (Fig. 2B–D), consistent with the previous reports demonstrating that IL-6^{-/-} mice develop mature-onset obesity (15) and that IL-1^{-/-} mice exhibit normal weight gain until 20 weeks (7). These results indicate that there is mutual redundancy for the suppression of body fat gain by IL-6 and IL-1 in young mice. The lean and obese phenotypes seen in IL-6^{-/-} IL-1Ra^{-/-} and IL-6^{-/-} IL-1^{-/-} mice, respectively, were only obvious in male mice, as previously seen with IL-1Ra^{-/-} female mice, whose body weight was indistinguishable from wild-type mice (7).

We measured basal levels of glucose, insulin, leptin, TAG, and FFAs in IL-6^{-/-} IL-1Ra^{-/-} and IL-6^{-/-} IL-1^{-/-} mice. Serum insulin and leptin levels in IL-6^{-/-} IL-1Ra^{-/-} male mice were significantly lower than those measured in IL-6^{-/-} IL-1Ra^{+/+} mice under fasting conditions, while glucose, TAG, and FFA levels were not significantly different (Table 1; data not shown). IL-6^{-/-} IL-1Ra^{-/-} mice did not differ significantly from IL-1Ra^{-/-} mice in any of the parameters we examined (Table 1). Serum leptin levels in IL-6^{-/-} IL-1^{-/-} mice, however, were significantly higher than those observed in IL-6^{-/-} IL-1^{+/+} mice under fasting conditions (Fig. 2F), despite comparable levels of other serum constituents, including glucose (153 ± 9 mg/dl IL-6^{-/-} IL-1^{-/-} vs. 150 ± 10 mg/dl IL-6^{-/-} IL-1^{+/+}; NS), insulin (Fig. 2E), TAG (138 ± 14 mg/dl IL-6^{-/-} IL-1^{-/-} vs. 175 ± 14 mg/dl IL-6^{-/-} IL-1^{+/+}; NS), and FFAs (1.14 ± 0.05

mEq/l IL-6^{-/-} IL-1^{-/-} vs. 1.14 ± 0.06 mEq/l IL-6^{-/-} IL-1^{+/+}; NS), between IL-6^{-/-} IL-1^{-/-} mice and IL-6^{-/-} IL-1^{+/+} mice.

Increase in food intake in IL-6^{-/-} IL-1^{-/-} mice. As obesity develops when energy intake (feeding) chronically exceeds total body energy expenditure (21), we measured food intake (Fig. 3A and B) and energy expenditure in IL-6^{-/-} IL-1^{-/-} and IL-6^{-/-} IL-1^{+/+} mice (Fig. 3C). IL-6^{-/-} IL-1^{-/-} mice exhibited modest hyperphagia, beginning at 8 weeks of age, a time at which body weight did not differ significantly from IL-6^{-/-} IL-1^{+/+} mice (Fig. 3A). Both total food intake over 7–10 weeks and total food intake per body weight at 7 weeks were significantly increased in IL-6^{-/-} IL-1^{-/-} mice in comparison to IL-6^{-/-} IL-1^{+/+} mice (Fig. 3B). Food intake in IL-6^{-/-} IL-1^{-/-} mice was also significantly increased from the quantities observed for IL-6^{-/-} IL-1^{+/+} mice after 10 weeks, and food intake/body weight in IL-6^{-/-} IL-1^{-/-} mice older than 10 weeks was also higher than the controls, although it was not significant (data not shown). These results indicate that hyperphagia contributes to increase in food intake and obesity in IL-6^{-/-} IL-1^{-/-} mice.

We then examined the possibility that energy expenditure may be decreased in IL-6^{-/-} IL-1^{-/-} mice in comparison with control mice. We first analyzed the rate of weight loss following food restriction in IL-6^{-/-} IL-1^{-/-} mice. At 16 weeks of age, a point at which obesity was obvious in IL-6^{-/-} IL-1^{-/-} mice, animals were fed with a 5% body weight standard laboratory diet. Weight reduction over 5 days in all animals was not significantly different (*P* = 0.330, *F* = 4.600) (data not shown). We then assessed possible effects on cold sensitivity in IL-6^{-/-} IL-1^{-/-} mice. After exposure to 4°C, the rectal temperatures of IL-6^{-/-} IL-1^{-/-} mice were not significantly different from those observed in wild-type mice (*P* = 0.783, *F* = 5.591) (data not shown), suggesting that adaptive thermogenesis in IL-6^{-/-} IL-1^{-/-} mice did not differ significantly. Finally, using indirect calorimetry, we measured the metabolic rates of IL-6^{-/-} IL-1^{-/-} and IL-6^{-/-} IL-1^{+/+} mice at 8–10 weeks of age, a time at which the body weights of these animals were not significantly different. Oxygen consumption in IL-6^{-/-} IL-1^{-/-} mice show a tendency to decrease in comparison to IL-6^{-/-} IL-1^{+/+} mice (Fig. 3C). The 24-h

TABLE 2
Body weight, eWAT weight, and serum parameters in IL-1^{-/-} mice at 8 months of age

	Body weight (g)	WAT (g)	WAT/body weight (%)	Glucose (mg/dl)	Insulin (pg/ml)	Nonesterified fatty acids (mEq/l)
IL-1 ^{+/+}	38.8 ± 3.2	1.23 ± 0.17	3.14 ± 0.30	168 ± 12	2,140 ± 500	1.11 ± 0.07
IL-1 ^{-/-}	39.7 ± 2.7	1.65 ± 0.36	4.06 ± 0.74	149 ± 5	3,010 ± 670	0.94 ± 0.10

Data are means ± SE. Statistical significance was calculated by Student's *t* test.

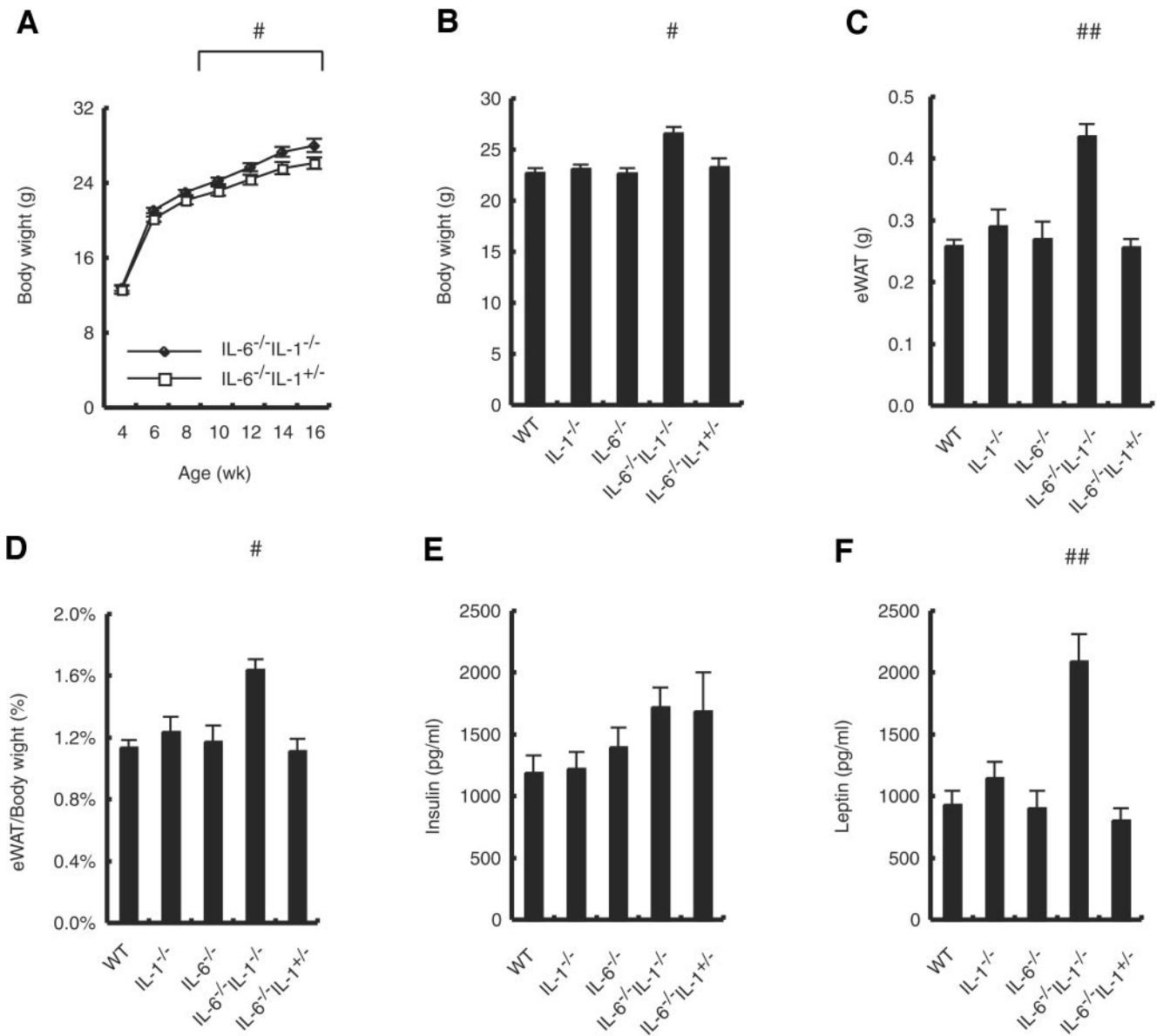


FIG. 2. Growth curves, eWAT volume, serum insulin, and leptin levels in IL-6^{-/-} IL-1^{-/-} male mice. **A:** Growth curves of IL-6^{-/-} IL-1^{-/-} (n = 14) (◆) and IL-6^{-/-} IL-1^{+/-} (n = 12) (□) mice, fed by normal diet ad libitum. **B:** Body weight in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice at 13 weeks. **C:** eWAT volume in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice at 13 weeks. **D:** eWAT volume/body weight in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice at 13 weeks. **E:** Serum insulin levels in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice at 13 weeks. **F:** Serum leptin levels in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice at 13 weeks. Data are expressed as means ± SE. Statistical significance was calculated by repeated-measures ANOVA and Student's *t* test (A) or by one-way ANOVA followed by Scheffe's test (B–F). #*P* < 0.05; ##*P* < 0.01.

average respiratory exchange ratio also did not differ significantly (IL-6^{-/-} IL-1^{-/-} mice: 0.87 ± 0.01; IL-6^{-/-} IL-1^{+/-} mice: 0.87 ± 0.01). Collectively, we failed to reveal any significant differences in energy expenditure in IL-6^{-/-} IL-1^{-/-} mice.

Food intake after IL-1α treatment is higher in IL-6^{-/-} IL-1^{-/-} mice. IL-1 plays a central role in central nervous system-mediated host defense mechanisms, including fever development, anorexia, and body weight loss (22–24). As previous studies demonstrated that IL-6^{-/-} mice are resistant to IL-1-induced fever development (20,25), we examined the possibility that IL-6^{-/-} mice are resistant to IL-1-induced anorexia (Fig. 4). Anorexia was assessed in IL-6^{-/-} IL-1^{-/-}, IL-1^{-/-}, IL-6^{-/-}, and wild-type mice following a peripheral injection of mouse IL-1α. IL-6^{-/-}

IL-1^{-/-} mice were resistant to IL-1α-induced reduction in food intake/body weight in comparison with IL-1^{-/-} and wild-type mice. Food intake/body weight after IL-1α treatment was higher in IL-6^{-/-} IL-1^{-/-} mice than in IL-1^{-/-} or wild-type mice (Fig. 4), while food intake/body weight after vehicle treatment was not significantly different (data not shown). The responses of IL-6^{-/-} and IL-6^{-/-} IL-1^{-/-} mice were not significantly different each other (Fig. 4). These results indicate that endogenous expression of IL-6 is involved in IL-1-induced anorexia and also suggest the possibility that expression of IL-1 under physiological conditions in IL-6^{-/-} mice reduces food intake and that the absence of endogenous IL-1 expression in IL-6^{-/-} IL-1^{-/-} mice contributes to increase in food intake and development of an obese phenotype.

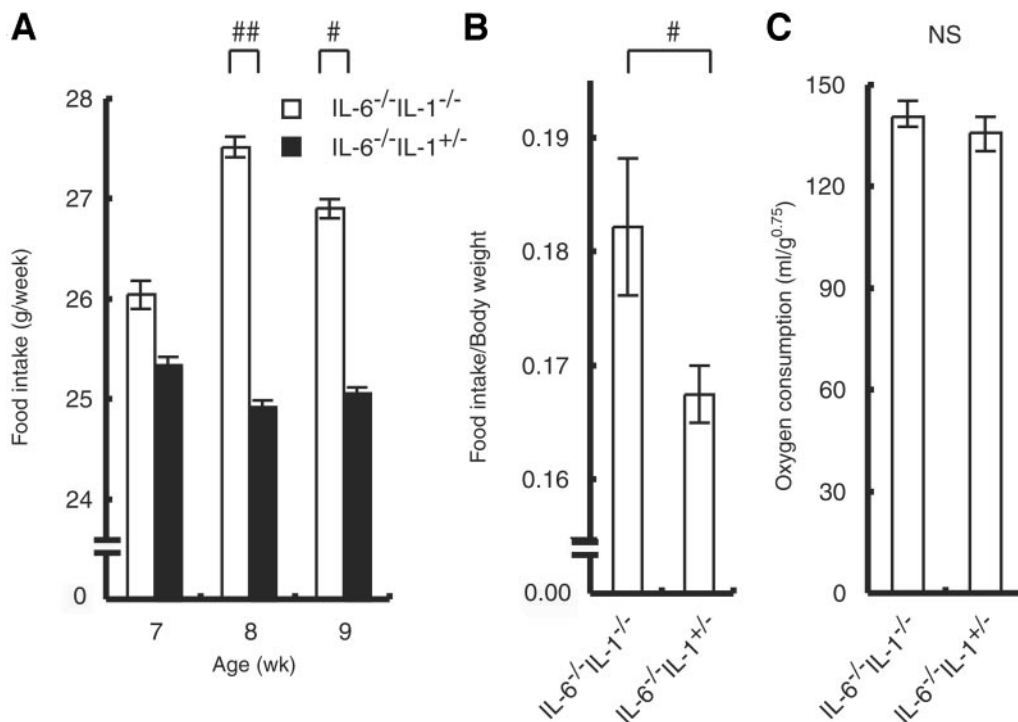


FIG. 3. Food intake and energy expenditure in IL-6^{-/-} IL-1^{-/-} male mice. **A:** Weekly food intake from 7 to 9 weeks of age in IL-6^{-/-} IL-1^{-/-} ($n = 6$) and IL-6^{-/-} IL-1^{+/-} mice ($n = 8$). **B:** Daily food intake per body weight from 7 to 9 weeks in IL-6^{-/-} IL-1^{-/-} and IL-6^{-/-} IL-1^{+/-} mice. Daily food intake per body weight was calculated by dividing the total food intake by the body weight at 7 weeks of age. **C:** Metabolic rate in IL-6^{-/-} IL-1^{-/-} ($n = 5$) and IL-6^{-/-} IL-1^{+/-} ($n = 6$) mice. Data are expressed as means \pm SE. Statistical significance was calculated by repeated-measures ANOVA and Student's *t* test (**A**) by Student's *t* test (**B** and **C**). # $P < 0.05$; ## $P < 0.01$; NS, not significant.

DISCUSSION

In this study, we examined the effect of IL-1 deficiency on body weight and found that increases in body weight were only observed in mice of the IL-6-deficient background. No differences were observed in single IL-1^{-/-} until 8 months or IL-6^{-/-} mice until 4 months. In contrast, the body weight of IL-6^{-/-} IL-1Ra^{-/-} mice was similar to that of IL-1Ra^{-/-} mice, indicating that excess IL-1 signaling promotes leanness independent of IL-6 signaling. These results indicate that both IL-1 and IL-6 are involved in the regulation of body weight homeostasis, playing compensatory roles through independent pathways. Food intake increased only in IL-6^{-/-} IL-1^{-/-} mice, while IL-1^{-/-} and IL-6^{-/-} mice exhibit normal feeding behavior, suggesting that the activities of these cytokines on body weight in young adult animals are primarily by their effects on feeding behavior. As the acute decreases in food intake induced by IL-1 treatment were significantly suppressed in IL-6^{-/-} IL-1^{-/-} mice in comparison to IL-1^{-/-} and wild-type mice, endogenous IL-1 and IL-6 comprise compensatory mechanisms influencing IL-1-induced decreases in food intake.

Our observations that the leanness in IL-1Ra^{-/-} mice is not dependent on IL-6 and that the obesity in IL-1^{-/-} mice is dependent on IL-6 seem apparently paradoxical. However, these observations could be explained by mutual redundancy of the suppression mechanisms of body fat gain by IL-6 and IL-1 in young mice. Absence of both IL-1 and IL-6 is needed to cancel the suppression, at least in young mice. We have previously demonstrated that food intake in IL-1Ra^{-/-} mice was not significantly different from wild-type mice and that hypoinsulinemia accompanied with increased insulin sensitivity is responsible for leanness in IL-1Ra^{-/-} mice (7). Thus, our observation that the leanness in IL-1Ra^{-/-} mice is not dependent on IL-6 indicates that IL-6 is not responsible for hypoinsulinemia in IL-1Ra^{-/-} mice. In contrast to IL-1Ra^{-/-} mice, we found that food intake in IL-6^{-/-} IL-1^{-/-} mice were increased compared with IL-6^{-/-} IL-1^{+/-} mice. Thus, our observa-

tion that the obesity in IL-1^{-/-} mice is dependent on IL-6 indicates that IL-6 is responsible for IL-1-induced suppression of food intake. These effects are only evident in IL-6^{-/-} IL-1^{-/-} mice due to compensatory mechanisms such as local increase of IL-6 or increased sensitivity in IL-6 signaling pathway in IL-1^{-/-} mice. Although we have examined the basal levels of IL-6 in IL-1 knockout mice and of IL-1 in IL-6 knockout mice, we could not find a significant difference in the expression levels in the brain (20), adipose tissue, and skeletal muscle compared with wild-type mice (data not shown), and we could not detect basal levels for serum IL-1 or IL-6 in knockout mice in our enzyme-linked immunosorbent assay system (26) (data not shown). However, it is possible that basal levels of IL-6 and IL-1 could be differently regulated in IL-6^{-/-} and IL-1^{-/-} mice in tissues not examined. The fact that IL-1Ra^{-/-} mice show normal food intake suggests that regulation of IL-1 signaling by IL-1Ra to regulate appetite in hypothalamus have small significance.

The roles of IL-6 on body weight regulation were established by series of studies by Dr. Jansson's group (15,16). They proposed that IL-6 acts on the central nervous system to regulate energy expenditure, because intracerebroventricular injection of IL-6 increased energy expenditure, although basal energy expenditure in IL-6^{-/-} mice did not change compared with wild-type mice. As leptin induces hypothalamic IL-1 β expression and intracerebroventricular injection of IL-1Ra suppresses leptin-induced appetite loss (4), it is likely that IL-1 in the brain is responsible for obesity in IL-6^{-/-} IL-1^{-/-} mice (in this study) and leanness in IL-1Ra^{-/-} mice (7). As we found that IL-1-induced suppression of appetite is partially dependent on IL-6 (Fig. 4), IL-1 signaling and IL-6 signaling leading to appetite loss are likely to cross talk within the brain. Although, downstream mediators responsible for increased food intake in IL-6^{-/-} IL-1^{-/-} mice are not clear at present. One possible explanation is leptin resistance. We found that serum leptin levels were significantly increased in IL-6^{-/-} IL-1^{-/-} mice (Fig. 2F), indicating poten-

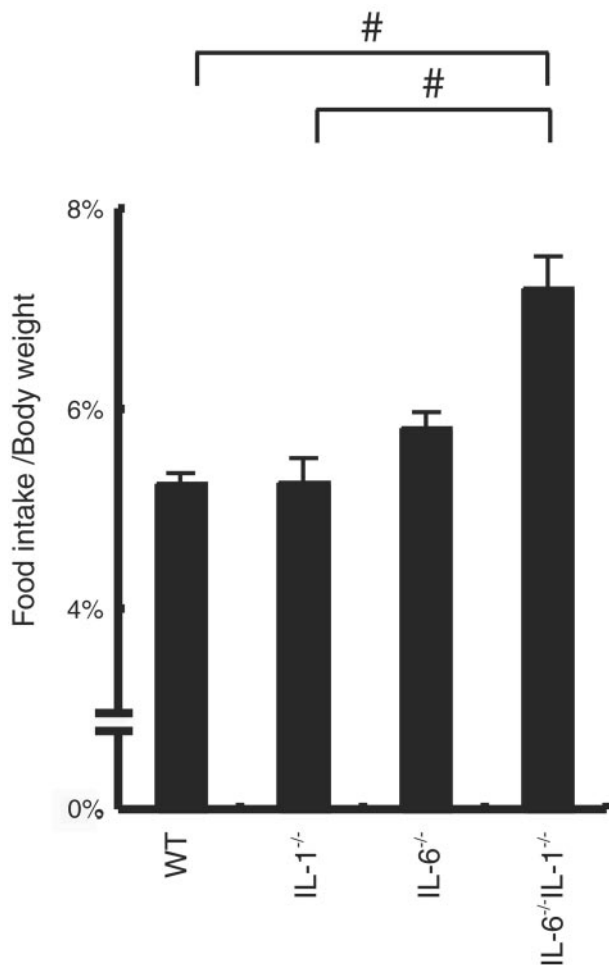


FIG. 4. Food intake after IL-1 α treatment is higher in IL-6^{-/-} IL-1^{-/-} mice. Food intake in wild-type (WT), IL-1^{-/-}, IL-6^{-/-}, IL-6^{-/-} IL-1^{-/-}, and IL-6^{-/-} IL-1^{+/-} mice after IL-1 α injection (1 μ g/kg body wt i.p.) was calculated by subtracting the 24-h value from the value measured before treatment for each animal. The change ratio was calculated by dividing the resulting value by the body weight of the animal. The following numbers of mice were used: WT ($n = 7$), IL-1^{-/-} ($n = 7$), IL-6^{-/-} ($n = 9$), and IL-6^{-/-} IL-1^{-/-} ($n = 6$) mice. Data are expressed as the means \pm SE. Statistical significance was calculated by ANOVA followed by Scheffe's test. # $P < 0.05$.

tial leptin resistance in IL-6^{-/-} IL-1^{-/-} mice. It remains unclear, however, whether leptin resistance is the cause or merely the result of obesity in IL-6^{-/-} IL-1^{-/-} mice as leptin levels correlate with fat mass (27). Another possibility is melanocortin peptides, as it was previously demonstrated that MC3/4R antagonist, SHU9119, reversed IL-1 β -induced reductions in food intake (28). We analyzed the expression levels for proopiomelanocortin and agouti-related protein in IL-1Ra^{-/-} mice (7) and IL-6^{-/-} IL-1^{-/-} mice, but we could not find a significant difference from wild-type mice (data not shown). Another possibility is neuropeptide Y (NPY), as a chronic IL-1 β treatment, reduced expression levels for NPY (29) and NPY blocks and reverses IL-1 β -induced anorexia (30). We also analyzed the expression levels for NPY in IL-1Ra^{-/-} mice (7) and IL-6^{-/-} IL-1^{-/-} mice, but we could not find a significant difference from wild-type mice (data not shown). Further studies are required to clarify the downstream mechanisms responsible for increased food intake in IL-6^{-/-} IL-1^{-/-} mice.

The effects of IL-6 on glucose and lipid homeostasis

were extensively studied. Several articles demonstrated that IL-6 induces insulin resistance in hepatocytes (31), skeletal muscle (32), and adipose tissue (33) and that IL-6 induces lipolysis and fat oxidation (34). The effect of IL-1 on glucose and lipid homeostasis was also extensively studied. IL-1 induces insulin resistance in hepatocytes (35) and mediates glucotoxicity in pancreatic islets (36). We have previously demonstrated that IL-1Ra knockout mice are hypoinsulinemic accompanied with increased insulin sensitivity (7). We found that insulin levels in IL-6^{-/-} IL-1^{-/-} mice were relatively high but not statistically significant compared with control mice. So higher levels of serum insulin in IL-6^{-/-} IL-1^{-/-} mice may be secondary to obesity. To analyze the possible effect of IL-6 deficiency on glucose and insulin homeostasis directly, we performed glucose tolerance test and insulin tolerance test analysis in IL-6^{-/-} IL-1Ra^{-/-} and IL-6^{-/-} IL-1^{-/-} mice but failed to find any significant difference compared with single deficient mice and wild-type mice in young mice (~16 weeks) (data not shown). These results suggest that the effect of IL-6 is primarily dependent on food intake and that IL-6 is not important for insulin release or insulin sensitivity in IL-1Ra^{-/-} mice.

In a classical view, the role of inflammatory cytokines was restricted under pathological conditions. It is well known that IL-1 and IL-6 are induced in the brain in central nervous system infections or injury (37). It should be noted that the roles of inflammatory cytokines, including IL-6 and IL-1 under inflammatory conditions, might be different from those under physiological conditions. In this study, we have demonstrated that IL-1 and IL-6 expressed under physiological conditions reduce food intake to regulate body weight. Recently, it was suggested that obesity is associated with a state of chronic, low-grade inflammation in adipocytes (38,39) and hepatocytes (40,41). Inflammatory cytokines, including IL-1, tumor necrosis factor- α , and IL-6, are induced in adipocytes and hepatocyte, which occurs concomitant with the infiltration of macrophages into adipose and hepatocyte of severe obese mice. Further studies are required for the role of IL-1 signaling under severe obesity.

As human life has always suffered from not only physical stress such as infections or injuries but also mental stresses, which induce inflammatory cytokines in the brain, the role of inflammatory cytokines in obesity and diabetes is potentially of great importance to human obesity. Further analysis of the role of inflammatory cytokines in obesity and diabetes will shed light on new therapeutic strategies for the treatment of metabolic diseases.

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