Original Article

Decreased Fat Mass in Interleukin-1 Receptor Antagonist–Deficient Mice

Impact on Adipogenesis, Food Intake, and Energy Expenditure

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Interleukin (IL)-1 is a regulator of inflammation but is also implicated in the control of energy homeostasis. Because the soluble IL-1 receptor antagonist (IL-1Ra) is markedly increased in the serum of obese patients and is overexpressed in white adipose tissue in obesity, we studied the metabolic consequences of genetic IL-1Ra ablation in mice. We have shown that IL-1Ra$^{-/-}$ mice have a lean phenotype due to decreased fat mass, related to a defect in adipogenesis and increased energy expenditure. The adipocytes were smaller in these animals, and the expression of genes involved in adipogenesis was reduced. Energy expenditure as measured by indirect calorimetry was elevated, and weight loss in response to a 24-h fast was increased in IL-1Ra$^{-/-}$ animals compared with wild-type mice. Lipid oxidation of IL-1Ra$^{-/-}$ mice was higher during the light period, reflecting their reduction in diurnal food intake. Interestingly, IL-1Ra$^{-/-}$ and IL-1Ra$^{+/-}$ mice presented an attenuation in high-fat diet–induced caloric hyperphagia, indicating a better adaptation to hypercaloric alimentation, which is in line with the role of IL-1Ra as a mediator of leptin resistance. Taken together, we show that IL-1Ra is an important regulator of adipogenesis, food intake, and energy expenditure. *Diabetes* 54:3503–3509, 2005

White adipose tissue (WAT) secretes various cytokines that regulate body weight homeostasis, insulin sensitivity, and inflammation (1). Recently, we have demonstrated that the interleukin (IL)-1 receptor antagonist (IL-1Ra) is strongly elevated in the serum from obese humans (2), that its levels are highly correlated with insulin resistance (2), and that WAT is a major source of this anti-inflammatory cytokine (3). Upon weight loss, the serum IL-1Ra levels decrease (2). In patients with Cushing's syndrome, the IL-1Ra levels are associated with the specific pattern of regional fat distribution (4). IL-1Ra is well known to antagonize the effects of IL-1 by competition for the binding to its receptor (5,6), but its metabolic functions are not well understood despite the fact that the several following connections between IL-1 signaling and energy homeostasis exist.

IL-1 exerts direct effects on adipocytes in culture, such as the inhibition of adipocyte differentiation (7–10) and the stimulation of lipolysis (11). Whereas IL-1Ra$^{-/-}$ mice are lean (12), transgenic mice overexpressing IL-1Ra did not show any alterations in body weight (13,14).

IL-1 and its functional receptor (IL-1 receptor type I) are both expressed in the hypothalamus of rodents and humans (15,16). Although IL-1 is implicated in the pathogenesis of anorexia during lipopolysaccharide administration (17), regulation of feeding by IL-1 under physiological conditions remains more elusive. On the one hand, IL-1 can inhibit food intake through various mechanisms, as illustrated by the leptin resistance in IL-1 receptor type I$^{-/-}$ mice (18–21). On the other hand, chronic central administration of IL-1Ra does not affect food intake in rats (22), and a recent study has reported that IL-1Ra$^{-/-}$ mice had normal food intake and no alterations in the expression of orexigenic and anorexigenic hypothalamic peptides (12).

Although IL-1 has been shown to be antidiabetic in some studies (23,24), this cytokine induces pancreatic β-cell death without affecting pancreatic α-cells (25,26). Moreover, IL-1Ra$^{-/-}$ mice have been reported to have defective insulin secretion, resulting in hypoinsulinemia, and to exhibit increased sensitivity to insulin (12).

Although circulating levels of tumor necrosis factor (TNF)-α and IL-6 are increased in human obesity, they are mostly not above the upper limit of the normal range. In contrast, the mean serum levels of IL-1Ra are at least four times above the upper limit of its reference range (2). Hence, and because of the conflicting findings regarding the metabolic functions of IL-1 and IL-1Ra in rodents, we investigated in detail the implication of IL-1Ra in adipogenesis and in energy homeostasis.

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C/EBP, CAAT enhancer–binding protein; eWAT, epididymal white adipose tissue; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; LPL, lipoprotein lipase; PPAR, peroxisome proliferator–activated receptor; RER, respiratory exchange ratio; TNF, tumor necrosis factor; UCP, uncoupling protein; WAT, white adipose tissue.

TABLE 1
Primer sequences used for real-time PCR

	Sense	Antisense
LPL	5′-GCCCAGCAACATTATCCAGT-3′	5′-GGTCAGACTTCCTGCTACGC-3′
Krox20	5′-AGCTGCCTGACAGCCTCT-3′	5′-GGAGATCCAGGGGTCTCTTC-3′
C/EBP-α	5′-GCTGGAGTTGACCAGTGACA-3′	5′-AAACCATCCTCTGGGTCTCC-3′
PPAR-γ_2	5′-CTCCTGTTGACCCAGAGCAT-3′	5′-AATGCGAGTGGTCTTCCATC-3′
28S	5′-TTGAAAATCCGGGGGAGAG-3′	5′-ACATTGTTCCAACATGCCAG-3′

RESEARCH DESIGN AND METHODS

All experimental protocols were approved by the Office Vétérinaire Cantonal (Geneva, Switzerland). Male 4-week-old mice were housed individually from weaning for body weight and food intake measurements, at room temperature (22°C), with a 12-h-light/12-h-dark cycle and free access to water and food. The generation of IL-1Ra$^{-/-}$ mice was previously described (27), and all animals used in this study are backcrossed onto a pure C57BL/6J background for >10 generations. Genotyping was performed by quantitative real-time PCR using a LightCycler (Roche Diagnostics, Rotkreuz, Switzerland) with the DNA Master SYBR Green I kit (Roche Molecular Biochemicals, Rotkreuz, Switzerland) and with genomic DNA extracted from tails tips. The primer set amplified an intronic sequence of IL-1Ra gene (sense, 5′-GAAAATACACACA GACGCTCCCC-3′; antisense, 5′-CACCTGAGGTTGTCATAACCATTG-3′) and a construct sequence (sense, 5′-GCTGCAAGGCGATTAAGTTGG-3′; antisense, 5′-GAGCTGGAAGCATAGCATGATGG-3′).

After weaning at 4 weeks of age, IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type male mice were fed ad libitum a standard diet, in which 7% of the calories were derived from fat, 76% from carbohydrates, and 17% from proteins. At 7 weeks, some of the animals (n = 7–10 in each group) started to receive a high-fat diet in which 40% of the calories were derived from fat, 43% from carbohydrates, and 17% from proteins; the others continued to receive the standard diet. Metabolizable energy of standard diet and high-fat diet are 2.6 and 4.3 kcal/g, respectively. Body weight and food intake were measured weekly from weeks 7 to 17. Mice were killed at 24 weeks in the morning after 3 h of food deprivation. After mice were killed, plasma was frozen. Epididymal white adipose tissue (eWAT) was removed and immediately frozen in liquid nitrogen for RNA preparation or fixed for histological analysis.

For the fasting experiments, body weight was measured in the morning before food removal and after 24 h, before refeeding. For the measurement of food intake during the light-dark cycle, food intake was measured for 5 consecutive days just after light was turned on and just before light was turned off.

Body composition. The mice were anesthetized with intraperitoneal injections of ketamin (100 mg/kg) and xylazinhydrochlorid (rompum, 10 mg/kg) and scanned using a Lunar PIXImus densitometer (Lunar, Madison, WI). Calibration of the instrument was conducted before each run with an aluminum/lucite phantom provided by the manufacturer. Whole-body scans were analyzed using the software provided by the manufacturer. All data used for the analysis of body composition exclude the head and represent the subcranial body composition.

Morphometric analysis of WAT. Five-micrometer sections of eWAT were stained with hematoxylin and eosin stain. The morphometric measurements were performed on 6–12 serial sections taken every 100 μm. Twenty adipocytes were randomly chosen in each section (120–240 cells analyzed in total per mouse), and the area of each adipocyte was determined using the Leica Qwin software (Leica, Glattbrugg, Switzerland) at ×20 magnification.

RNA preparation and mRNA quantification. Total RNA from eWAT was prepared and reverse transcribed as described previously (3). The expression of lipoprotein lipase (LPL), Krox20, CAAT enhancer–binding protein-α (C/EBP-α), and peroxisome proliferator–activated receptor γ_2 (PPAR-γ_2) was determined by quantitative real-time PCR using a LightCycler (Roche Diagnostics) with the DNA Master SYBR Green I or Fast Start DNA Master SYBR Green I (Roche Molecular Biochemicals) kits as appropriate, and the results were normalized for 28S RNA (for primers, see Table 1).

Indirect calorimetry. Oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were simultaneously measured by indirect calorimetry using the Oxymax system (Columbus Instruments, Columbus, OH). The mice were housed in separate chambers for 24 h for acclimatization before starting the measurements with free access to food and water. Measurements were performed every 10 min during 24 h under a constant airflow rate (600 ml/min). An average of three consecutive measurements was calculated for each mouse. For each time point, the measurements for each group were averaged. V_{O_2}, V_{CO_2}, and the respiratory exchange ratio (RER = V_{CO_2}/V_{O_2}) were calculated from V_{O_2} and V_{CO_2}. The average V_{O_2} was calculated during 24 h, during light cycle (0700–1900), and during dark cycle (1900–0700).

Measurement of plasma hormones, cytokines, and metabolites. Circulating leptin was determined using the Lincoplex kit MADPK from Linco (Labodia, Yens, Switzerland) in a 10-μl sample volume, whereas circulating IL-1β, IL-6, and TNF-α were measured with the MCYTO kit in a 25-μl sample volume. Plasma IL-1Ra levels were determined with a commercial ELISA kit (R&D Systems Europe, Abingdon, U.K.).

Statistics. Results are expressed as means ± SE. The unpaired Student's t test and repeated-measures one-way ANOVA were used when appropriate for comparison between groups of mice. These tests were performed with SPSS 13.0 (SPSS, Chicago, IL).

RESULTS

Reduced body weight and increased food intake relative to body weight of IL-1Ra$^{-/-}$ mice. On a standard diet, the body weight of IL-1Ra$^{+/-}$ mice was significantly lower than that of wild-type mice of the same age, whereas the body weight of IL-1Ra$^{-/-}$ animals was reduced by >30% compared with wild type (Fig. 1*A*).

As shown in the Table 2, IL-1Ra was undetectable in the plasma of IL-1Ra–deficient mice, whereas the plasma IL-1Ra levels were 103 ± 30 pg/ml for IL-1Ra$^{+/-}$ and 368 ± 106 pg/ml for wild-type mice. Circulating levels of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were not significantly different among IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice (Table 2), indicating that IL-1Ra gene

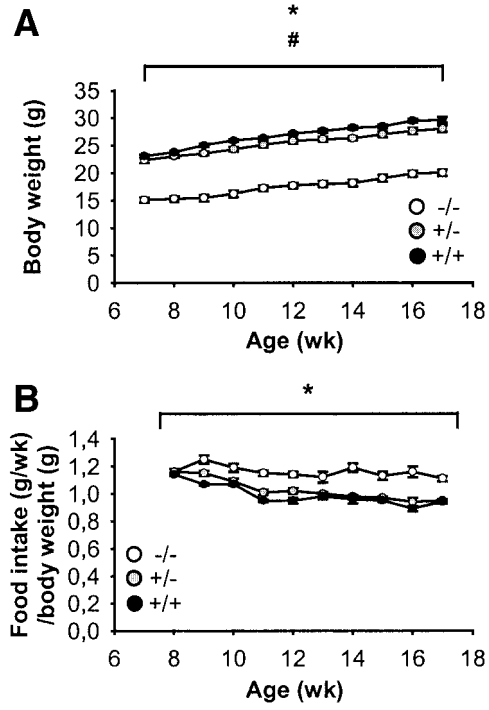

FIG. 1. Reduced body weight and increased food intake relative to body weight of IL-1Ra$^{-/-}$ mice. Body weight gain (*A*) and food intake (normalized per gram body weight) (*B*) of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on standard diet during 10 weeks (n = 7–10 in each group). *P < 0.001 for IL-1Ra$^{-/-}$ vs. wild type; #P < 0.001 for IL-1Ra$^{+/-}$ vs. wild type.

TABLE 2
Circulating cytokines levels in IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type male mice

Genotype	IL-1Ra (pg/ml)	Leptin (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)
IL-1Ra$^{-/-}$	Undetectable	444 ± 106*	63 ± 6	85 ± 23	48 ± 9
IL-1Ra$^{+/-}$	103 ± 30*	2,278 ± 242	108 ± 17	126 ± 48	51 ± 6
Wild type	368 ± 106	2,791 ± 1170	113 ± 26	196 ± 77	69 ± 13

Data are means ± SE of 6–10 animals per group. *$P < 0.05$ compared with wild-type mice.

deletion or haploinsufficiency did not result in a general inflammatory state.

Because IL-1 is known to be an anorexigenic cytokine, it is tempting to speculate that the reduced body weight of IL-1Ra$^{-/-}$ mice was caused by a reduction in food intake. However, at 8 weeks of age, the weekly food intake per gram body weight was similar in IL-1Ra$^{-/-}$ (1.16 ± 0.02 g · week^{-1} · g^{-1} body wt), IL-1Ra$^{+/-}$ (1.17 ± 0.02 g · week^{-1} · g^{-1} body wt), and wild-type (1.15 ± 0.01 g · week^{-1} · g^{-1} body wt) mice. Thereafter, food intake relative to body weight decreased progressively in the wild-type and heterozygous animals, whereas it remained constant in IL-1Ra$^{-/-}$ mice (Fig. 1*B*). Hence, the paradoxical lean phenotype of IL-1Ra$^{-/-}$ mice associated with high food intake relative to their body weight lead us to examine the regulation of adipose depots and of energy expenditure in these animals.

Impaired adipogenesis in IL-1Ra$^{-/-}$ mice. Because previous studies have indicated that adipose fat pads appeared to be smaller in IL-1Ra$^{-/-}$ mice (12), we determined their body fat mass by dual energy X-ray absorptiometry. In 17-week-old mice, the proportion of body fat was significantly lower in IL-1Ra$^{-/-}$ (15.1 ± 0.4%) than in IL-1Ra$^{+/-}$ (19.9 ± 0.8%) or in wild-type (19.9 ± 1.3%) mice (Fig. 2*A*), representing 3.0 ± 0.1, 5.6 ± 0.3, and 5.9 ± 0.4 g fat, respectively. Consistent with these results, we found that circulating leptin concentrations were five- and six-fold reduced in IL-1Ra$^{-/-}$ when compared with IL-1Ra$^{+/-}$ and wild-type mice, respectively (Table 2).

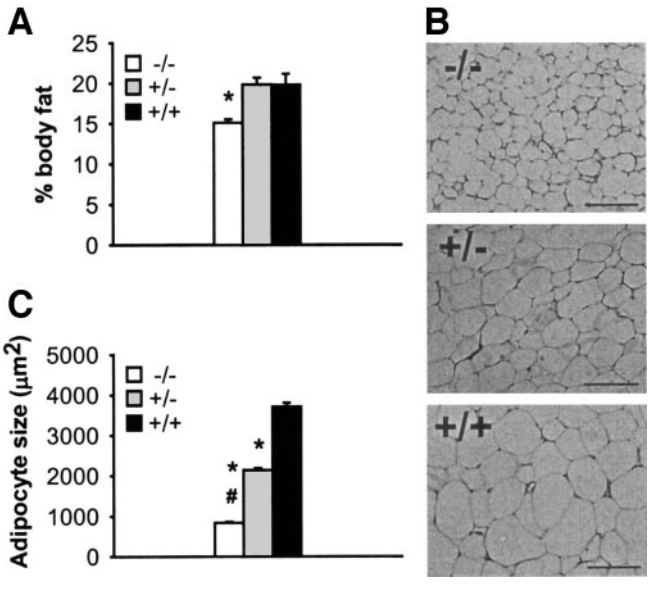

FIG. 2. Reduced adipose storage and adipogenesis in IL-1Ra$^{-/-}$ mice. Percentage body fat (*A*) (n = 8–10 in each group), histological sections of eWAT (*B*) (mark represents 100 μm), and area of adipocytes expressed in micrometers squared (*C*) (n = 4–5 in each group) of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type male mice on standard diet at the age of 17 weeks. *$P < 0.01$ for IL-1Ra$^{-/-}$ vs. wild type (*A*); #$P < 0.0001$ for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$; *$P < 0.0001$ for IL-1Ra$^{-/-}$ and IL-1Ra$^{+/-}$ vs. wild type (*C*).

To determine whether the reduced adipose tissue mass observed in IL-1Ra$^{-/-}$ mice was also associated with qualitative changes, we performed morphometric analysis on histological sections of eWAT (Fig. 2*B*). Quantitative analysis of these sections (Fig. 2*C*) showed that the adipocytes were 77 ± 2 and 26 ± 6% smaller in IL-1Ra$^{-/-}$ and in IL-1Ra$^{+/-}$ than in wild-type mice, respectively, suggesting that normal adipocyte development is modulated by endogenous IL-1Ra.

To understand the mechanism of adipocyte hypotrophy in IL-1Ra$^{-/-}$ mice, we examined the expression levels of LPL, Krox20, C/EBP-α, and PPAR-γ_2, which are enzyme and transcription factors involved in the differentiation process. LPL expression was reduced in eWAT by 64 ± 5% in IL-1Ra$^{-/-}$ and by 44 ± 5% in IL-1Ra$^{+/-}$ when compared with the expression level of wild-type mice (Fig. 3*A*). This observation is compatible with the known inhibitory effect of IL-1 on LPL expression and activity in vitro (9). Interestingly, the intermediate effect of the IL-1Ra haplotype on LPL expression was not observed with the regard to the expression of transcription factors. Krox20 expression was reduced by 71 ± 11% in IL-1Ra$^{-/-}$ mice, whereas it remained unchanged in heterozygous when compared with wild-type mice (Fig. 3*B*). C/EBP-α mRNA was similarly reduced by 52 ± 8 and by 51 ± 6% in IL-1Ra$^{-/-}$ and in IL-1Ra$^{+/-}$ mice (Fig. 3*C*), whereas PPAR-γ_2 mRNA expression was reduced by 61 ± 11 and by 60 ± 4% in IL-1Ra$^{-/-}$ and IL-1Ra$^{+/-}$ mice, respectively (Fig. 3*D*). We also examined in vitro the effect of chronic IL-1 treatment on the differentiation of human primary stromal cells from subcutaneous adipose tissue. The addition of IL-1α during the differentiation process inhibited fat accumulation, and this effect could be completely abolished by the co-treatment with rhIL-1Ra (data not shown).

In summary, these results show that IL-1Ra gene deficiency results in lower body fat and impairs the normal development of adipose tissue by the repression of genes involved in adipogenesis.

Increased energy expenditure of IL-1Ra$^{-/-}$ mice and alterations in circadian substrate use and food intake. A potential explanation for the lean phenotype of IL-1Ra$^{-/-}$ mice despite their relative increased food intake on standard diet might be an increase in their metabolic rate. To test this hypothesis, we measured the V_{O_2} for 24 h by indirect calorimetry of 22-week-old animals on a standard diet. IL-1Ra deficiency led to an increased V_{O_2} throughout the 24-h period (Fig. 4*A*). The average V_{O_2} was increased in IL-1Ra$^{-/-}$ mice during the total 24-h period and during the light or dark cycle (Fig. 4*B*). To exclude that the increased V_{O_2} of IL-1Ra$^{-/-}$ mice is merely reflecting their reduced body weight, we also expressed the results per kg$^{0.75}$, which reflects a normalization for body surface area rather than mass as initially suggested by

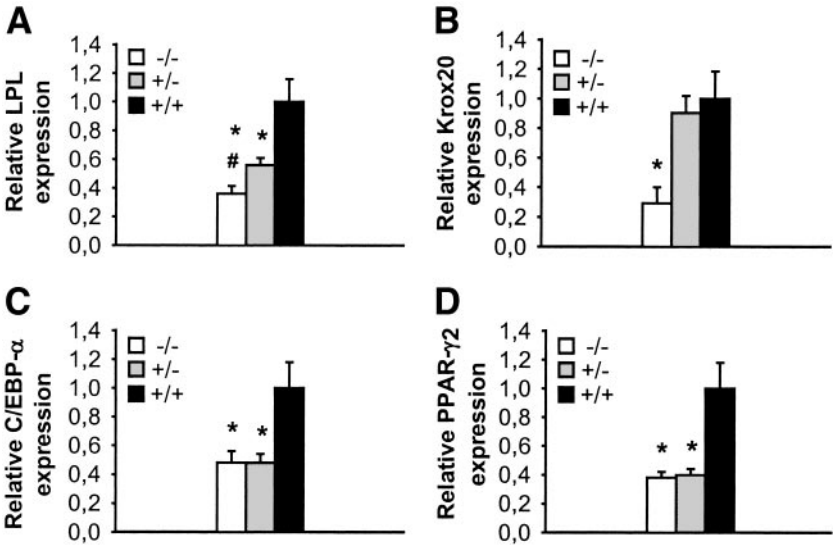

FIG. 3. Reduced adipocyte differentiation in IL-1Ra$^{-/-}$ mice. LPL (*A*), Krox20 (*B*), C/EBP-α (*C*), and PPAR-γ$_2$ (*D*) mRNA in eWAT of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on standard diet. n = 6–9 in each group; $^{\#}P < 0.02$ for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$; $^{*}P < 0.02$ for IL-1Ra$^{-/-}$ or IL-1Ra$^{+/-}$ vs. wild type (*A*); $^{*}P < 0.02$ for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$ or wild type (*B*); $^{*}P < 0.05$ for IL-1Ra$^{-/-}$ or IL-1Ra$^{+/-}$ vs. wild type (*C*); $^{*}P < 0.02$ for IL-1Ra$^{-/-}$ or IL-1Ra$^{+/-}$ vs. wild type (*D*).

Kleiber (28) and discussed by others (29,30). The average V_{O_2} remained significantly higher after this normalization in IL-1Ra$^{-/-}$ mice when compared with heterozygous or wild-type mice (data not shown). Mechanisms underlying this increased energy expenditure of IL-1Ra$^{-/-}$ mice remain to be elucidated because both uncoupling protein (UCP)-1 expression in brown adipose tissue and UCP-3 expression in skeletal muscle are normal in IL-1Ra–deficient animals (data not shown). However, the increased energy expenditure of IL-1Ra$^{-/-}$ mice is also reflected by the faster use of energy stores during fasting. In response to a 24 h-food deprivation, IL-1Ra$^{-/-}$ mice lost 14.6 ± 0.4% of their body weight, whereas wild-type animals lost only 12.3 ± 0.4% (Fig. 4*C*).

This altered adaptation to fasting of IL-1Ra$^{-/-}$ mice could also reflect changes in substrate use. Therefore, the RER was determined during 24 h by indirect calorimetry. Although no difference in the 24-h average RER between IL-1Ra$^{-/-}$ (0.90 ± 0.01) and wild-type mice (0.91 ± 0.01) was present (data not shown), we observed a different circadian pattern in the RER for IL-1Ra$^{-/-}$ when compared with IL-1Ra$^{+/-}$ or wild-type mice (Fig. 4*D*). In fact, the RER of IL-1Ra$^{-/-}$ mice decreased from the start of the light cycle, whereas it remained elevated for IL-1Ra$^{+/-}$ and wild-type mice during this immediate postprandial period. The average RER during the dark cycle was significantly increased in IL-1Ra$^{-/-}$ (0.99 ± 0.01) compared with IL-1Ra$^{+/-}$ (0.96 ± 0.01) or wild-type mice (0.94 ± 0.01) ($P <$ 0.00001 for comparisons among IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice). Inversely, the average RER during the light cycle was significantly reduced in IL-1Ra$^{-/-}$ (0.84 ± 0.03) compared with IL-1Ra$^{+/-}$ (0.91 ± 0.02) or wild-type mice (0.88 ± 0.03) ($P < 0.02$ for comparisons among IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice).

To determine whether the decreased RER observed in IL-1Ra$^{-/-}$ mice during the light cycle was caused by a different circadian feeding behavior, we calculated the ratio of food intake during the light cycle relative to the dark cycle (data not shown). During the light cycle, the wild-type mice ate 29 ± 2.5% of the quantity that they eat during the dark cycle, whereas this proportion was 17 ± 4.4% in IL-1Ra$^{-/-}$ mice ($P < 0.05$), indicating that these animals eat proportionally less during the light time than wild-type mice.

Taken together, these results show that IL-1Ra deficiency induces an increase in the metabolic rate throughout the day. Moreover, IL-1Ra$^{-/-}$ mice lose more weight

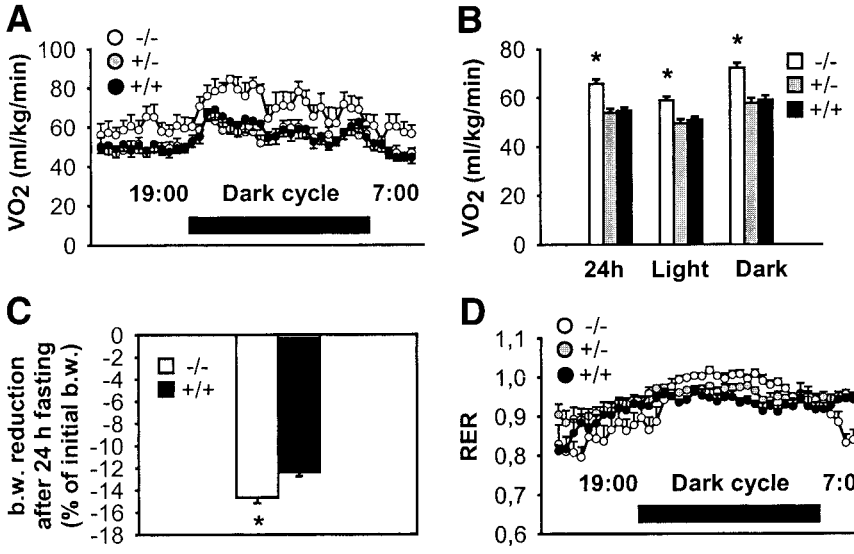

FIG. 4. Increased energy expenditure and altered circadian rhythm of substrate utilization and food intake in IL-1Ra$^{-/-}$ mice. *A* and *B*: V_{O_2} of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on standard diet. *B*: n = 6–7 in each group; $^{*}P < 0.05$ for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$ or wild type. *C*: Weight reduction of IL-1Ra$^{-/-}$ and wild-type mice after 24 h of fasting. *D*: n = 4 in each group; $^{*}P < 0.01$ for IL-1Ra$^{-/-}$ vs. wild type. The RER of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on standard diet was measured during 24 h by indirect calorimetry (n = 6–7 in each group).

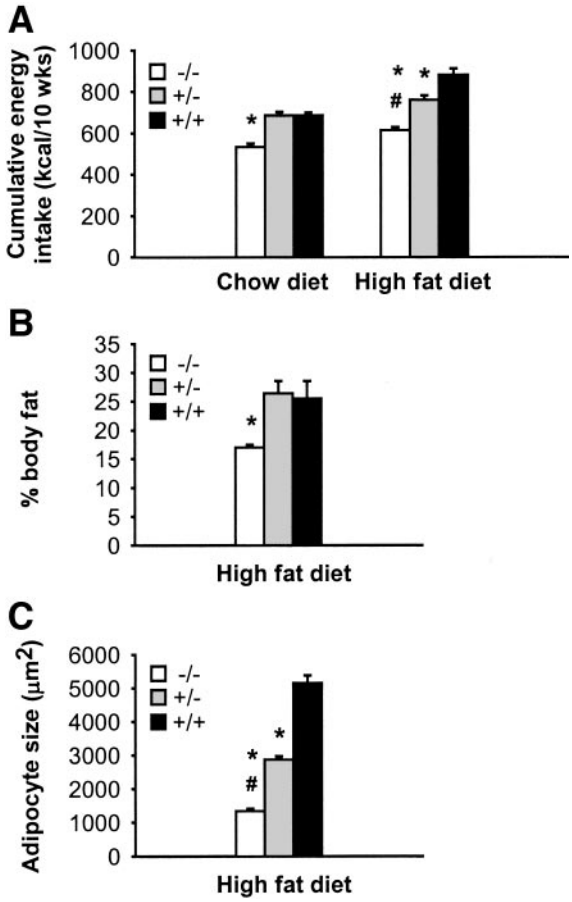

FIG. 5. Resistance to diet-induced caloric hyperphagia and fat gain in IL-1Ra$^{-/-}$ mice. *A*: Cumulative energy intake of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on standard chow diet and high-fat diet for 10 weeks. n = 7–10 in each group; *P < 0.0001 for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$ or wild type (standard diet); #P < 0.0001 for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$; *P < 0.0001 for IL-1Ra$^{-/-}$ or IL-1Ra$^{+/-}$ vs. wild type (high-fat diet). Percentage body fat (*B*; n = 7–10 in each group) and area of adipocytes expressed in micrometers squared (*C*; n = 4–5 in each group) of IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice on high-fat diet at the age of 17 weeks. *P < 0.01 for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$ or wild type (*B*), #P < 0.0001 for IL-1Ra$^{-/-}$ vs. IL-1Ra$^{+/-}$, *P < 0.0001 for IL-1Ra$^{-/-}$ or IL-1Ra$^{+/-}$ vs. wild type (*C*).

than wild-type mice in response to fasting, which is likely the result of increased energy expenditure.

Furthermore, IL-1Ra$^{-/-}$ mice oxidate more lipid during the light cycle and more carbohydrate during the dark cycle than wild-type mice, which is probably the consequence of their proportionally lower food intake during light time.

Resistance to diet-induced caloric hyperphagia and fat gain in IL-1Ra$^{-/-}$ mice. The lean phenotype observed in IL-1Ra$^{-/-}$ mice despite a relatively high food intake on standard diet lead us to examine the regulation of food intake, adipose mass, and adipocyte differentiation in IL-1Ra$^{-/-}$, IL-1Ra$^{+/-}$, and wild-type mice exposed to a hypercaloric high-fat diet.

During the 10 weeks of high-fat feeding, IL-1Ra$^{-/-}$ mice ingested a total of 615 ± 15 kcal (vs. 535 ± 15 on standard diet), IL-1Ra$^{+/-}$ mice ingested 761 ± 20 kcal (vs. 687 ± 17), and wild-type mice ingested 916 ± 12 kcal (vs. 687 ± 12) (Fig. 5*A*), representing an excess of caloric intake of 33 ± 2% for the wild-type mice and of only 11 ± 3% for IL-1Ra$^{+/-}$ and 15 ± 3% for IL-1Ra$^{-/-}$ mice. The proportion of body fat remained significantly lower in IL-1Ra$^{-/-}$ when compared with IL-1Ra$^{+/-}$ or wild-type mice (Fig. 5*B*).

Quantitative analysis of sections from eWAT of mice on the high-fat diet showed that the adipocytes were 74 ± 1% smaller in IL-1Ra$^{-/-}$ and 44 ± 2% smaller in IL-1Ra$^{+/-}$ than in wild-type mice (Fig. 5*C*).

Taken together, the results show that IL-1Ra$^{-/-}$ mice are resistant to diet-induced caloric hyperphagia and fat gain. The feeding of a high-fat diet cannot correct the defect in adipocyte differentiation in these animals.

DISCUSSION

We have examined the impact of the IL-1Ra gene deletion and haploinsufficiency on adipose tissue development, feeding behavior, and energy expenditure. The lean phenotype of our IL-1Ra$^{-/-}$ mice under physiological conditions was more marked than those previously reported with other genetic constructions on the same C57BL/6 genetic background (12,13). Similar to Matsuki et al. (12), we detected a reduction in insulin levels in IL-1Ra–deficient mice (31), but our findings clearly indicate that the causes of this lean phenotype are much more complex than just the result of an hypoinsulinemic state as proposed by Matsuki et al. (12). We show that IL-1Ra–deficient mice have increased food intake relative to their body weight on standard diet and increased energy expenditure, as well as a defect in adipogenesis. However, these changes are not the result of a general pro-inflammatory state as demonstrated by normal or even lower circulating levels of IL-1β, IL-6, and TNF-α.

First, we studied the effect of IL-1Ra deficiency on energy storage. The reduction in fat mass in our IL-1Ra$^{-/-}$ mice was associated with a marked change in WAT morphology, which is in contrast to Matsuki et al. (12), who reported no alteration in WAT histology and who did not measure adipocyte size. Interestingly, we demonstrated that IL-1Ra haploinsufficiency also led to impaired adipocyte differentiation, although these animals had a virtually unaltered fat mass. Hence, an increase in adipocyte recruitment might compensate for the adipocyte hypotrophy in heterozygous mice, because a moderate excess of IL-1 signaling has been reported to enhance the proliferation of preadipocytes in vitro (7).

The process of adipocyte differentiation follows a highly synchronized program involving different transcription factors, all of which (including Krox20 [32]) are reduced in WAT of IL-1Ra$^{-/-}$ mice. These observations suggest that endogenous IL-1Ra is involved in adipocyte differentiation, and the locally increased ratio of IL-1Ra to IL-1β in WAT of obese patients (3) might contribute to obesity by limiting the auto/paracrine antilipogenic action of endogenous IL-1 (33). Finally, the observation that adipocyte size of IL-1Ra–deficient mice remains markedly reduced on a high-fat diet confirms that the defect in adipogenesis is not a simple consequence of an altered energy balance.

In fact, we have observed that IL-1Ra$^{-/-}$ mice have an increase in food intake with respect to their body weight when fed a normal diet, and this is possibly the consequence of decreased blood leptin levels, reflecting reduced WAT stores. This observation is at odds with Matsuki et al. (12), who reported no alteration in food intake when normalized for body weight. A potential factor explaining this difference might be the fact that Matsuki et al. calculated the food intake-to-body weight ratio on only one occasion at an unspecified age. However, the observation that IL-1Ra$^{+/-}$ mice placed on a high-fat diet have a reduced excess of caloric intake despite circulating leptin

levels similar to those of wild-type mice requires another explanation. A possible hypothesis is that IL-1 is a mediator of leptin action and the excess of IL-1Ra contributes to the acquired resistance to leptin. Luheshi et al. (21) have shown that mice lacking IL-1 receptors are also resistant to leptin, raising the possibility that IL-1Ra might function as a mediator of the obesity-associated acquired resistance to the central effects of leptin (2). The role of IL-1Ra as a regulator of food intake was confirmed by the effects of a high-fat diet on energy intake in our IL-1Ra$^{-/-}$ mice. When the cumulative caloric intake was increased by 33% in wild-type mice, this increase was limited to 15% in IL-1Ra$^{-/-}$ mice, corroborating the concept that IL-1Ra enhances caloric intake (2).

In addition to the impaired adipogenesis and the increase in food intake on normal diet relative to their body weight, we also found that IL-1Ra–deficient mice exhibit a pronounced elevation in $V\text{O}_2$. This increase in energy expenditure, despite normal UCP expression in muscle and brown adipose tissue, is in contrast to Matsuki et al. (12), who did not detect any alteration in the $V\text{O}_2$. Possible explanations for this divergence might be the shorter acclimatization before the measurements, the shorter length of measurements, unspecified differences in the environment and diet, or differences in the activity levels (e.g., exercise). The elevated metabolic rate of IL-1Ra$^{-/-}$ mice that we observed in the fed state likely explains the increased weight loss in response to fasting. The analysis of the circadian profile of the $V\text{O}_2$ and RER revealed an increase of the latter during the dark cycle in IL-1Ra$^{-/-}$ mice, indicating that IL-1Ra$^{-/-}$ animals oxidate more carbohydrates during the period of nocturnal alimentation.

However, the RER of IL-1Ra$^{-/-}$ mice decreased much more rapidly after the start of the light time compared with wild-type mice. This reduction in daytime RER in IL-1Ra$^{-/-}$ mice seems to reflect a difference in circadian food intake, as confirmed by their reduced food intake during the light cycle. This, in turn, can possibly be explained by a reduction of their physical activity and/or an increase in their sleep, which would be in keeping with the known role of IL-1 signaling in sleep induction and the regulation of activity (34,35).

Although any animal model with the genetic ablation of a gene is susceptible to exhibit phenotypic features related to the compensatory mechanisms, rather than the deficiency of the gene under study, several arguments support the concept that the IL-1Ra deficiency and the resulting constitutive IL-1 oversignalling explain the metabolic phenotype that we describe.

1) The basal production of IL-1Ra in wild-type mice and humans is likely to be sufficient to block IL-1 activity at a systemic and local level. The serum IL-1Ra concentrations in the wild-type mice are at a 2.6-fold molar excess with regard to IL-1. Given the equal affinity of IL-1 and IL-1Ra for the IL-1 receptor (36,37), such an excess at least partially blocks IL-1 action. Within the WAT from obese subjects, we have shown that IL-1Ra is produced at a 1.4-fold molar excess (compared with 0.3-fold in lean subjects), which is again compatible with a constitutive inhibitory activity of basal IL-1Ra (3).

2) Irikura et al. (38) have characterized the inflammatory phenotype of IL-1Ra$^{-/-}$ mice and have crossed them with IL-1 receptor knock-out animals. These experiments have revealed that the low body weight of IL-1Ra–deficient mice can be rescued either by deleting the IL-1 receptor or by reintroducing a transgene coding for IL-1Ra.

3) Finally, a mouse harboring an IL-1–secreting tumor has recently been reported, and these animals have decreased food intake and body weight, which is mainly due to a reduction in fat mass. In contrast, these alterations are not observed in control animals bearing a nonsecreting tumor (39).

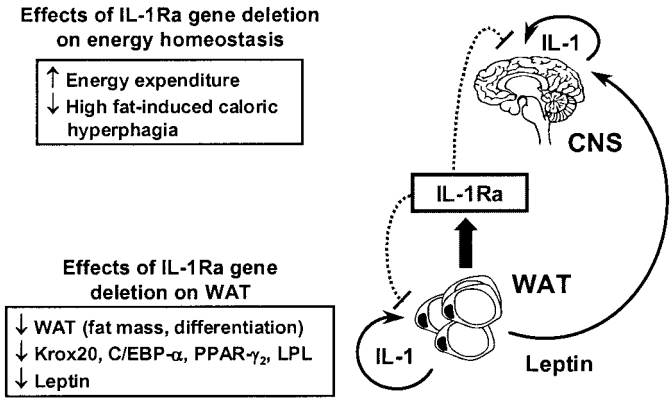

FIG. 6. Metabolic actions of IL-1 and IL-1Ra. See DISCUSSION for details.

Hence, we provide evidence in vivo that endogenous IL-1Ra is a pro-adipogenic factor and that it limits energy expenditure by opposing the IL-1–induced increase in $V\text{O}_2$ (Fig. 6). In addition, IL-1Ra deficiency or haploinsufficiency protects against diet-induced caloric hyperphagia, suggesting that IL-1Ra might act as a mediator of obesity-induced leptin resistance (2). However, IL-1Ra provides not only a mechanistic link to type 2 diabetes through its obesity-promoting actions, but we have recently shown that it reduces the sensitivity to insulin in skeletal muscle independently of obesity (31). In contrast, one might speculate that IL-1Ra excess is protective for islets with regard to IL-1–mediated β-cell apoptosis (40,41). However, we were unable to see any alterations in the morphology or insulin content of the pancreas from IL-1Ra$^{-/-}$ mice (31). Hence, the overall effects of IL-1Ra promote weight gain and insulin resistance, thus favoring the development of type 2 diabetes.

In conclusion, we show that IL-1Ra is involved in the regulation of adipogenesis, energy expenditure, and energy intake, suggesting that markedly increased serum levels in obese humans might contribute to obesity-associated anomalies, including the acquired resistance to leptin.

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