Increased fat:carbohydrate oxidation ratio in *Il1ra*^{-/-} mice on a high-fat diet is associated with increased sympathetic tone

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Abstract

Aims/hypothesis Proinflammatory cytokines, including IL-1, exert pleiotropic effects on the neuro-immuno-endocrine system. Previously, we showed that mice with knockout of the gene encoding IL-1 receptor antagonist (*Il1ra*^{-/-}, also known as *Il1rn*^{-/-}) have a lean phenotype. The present study was designed to analyse the mechanisms leading to this lean phenotype.

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Methods *Il1ra*^{-/-} mice were fed a high-fat diet following weaning. Energy expenditure, body temperature, heart rate, blood parameters, urinary catecholamines and adipose tissue were analysed.

Results *Il1ra*^{-/-} mice exhibited resistance to obesity induced by a high-fat diet; this resistance was associated with increased energy expenditure and a decreased respiratory quotient, indicating that the ratio of fat:carbohydrate metabolism in *Il1ra*^{-/-} mice is greater than in controls. Activity level in *Il1ra*^{-/-} mice was significantly decreased and body temperature was significantly increased, compared with wild-type (WT) mice. Inguinal white adipose tissues in *Il1ra*^{-/-} mice express increased levels of *Ucp1* and mitochondrial respiratory chain genes compared with WT mice. Histological analysis of adipose tissue in *Il1ra*^{-/-} mice revealed that brown adipose tissue is hyperactive and inguinal white adipose tissue contains smaller cells, which exhibit the distinctive multilocular appearance of brown adipocytes. Urinary epinephrine and norepinephrine excretion in *Il1ra*^{-/-} mice was significantly increased compared with WT mice, suggesting that *Il1ra*^{-/-} mice have increased sympathetic tone. Consistent with this, heart rate in *Il1ra*^{-/-} mice was also significantly increased.

Conclusions/interpretation Our results show that *Il1ra*^{-/-} mice have increased energy expenditure, fat:carbohydrate oxidation ratio, body temperature, heart rate and catecholamine production. All of these observations are consistent with an enhanced sympathetic tone.

Keywords Brown adipose tissue · IL-1 · Proinflammatory cytokines · Sympathetic tone · White adipose tissue

Abbreviations

AR adrenergic receptor
BAT brown adipose tissue

IL1RA	IL-1 receptor antagonist
HFD	high-fat diet
RQ	respiratory quotient
SNS	sympathetic nervous system
UCP-1	uncoupling protein 1
$\dot{V}\text{CO}_2$	carbon dioxide production
$\dot{V}\text{O}_2$	oxygen uptake
WAT	white adipose tissue
WT	wild-type

Introduction

Interleukin-1, a major mediator of inflammation, also performs numerous functions related to host defence by regulating not only the immune system, but also the neural and endocrine systems. Accordingly, IL-1 is produced by a wide variety of cells, including monocytes, macrophages, epithelial cells, endothelial cells and glial cells. Moreover, IL-1 receptors (IL-1Rs) are found in a wide range of immune, neural and endocrine cells, reflecting the pleiotropic activities of this molecule [1]. Leptin, which is released from adipocytes, exerts an inhibitory feedback effect on fat masses by acting on hypothalamic nuclei, which express the cognate signal-transducing leptin receptor ObRb [2]. IL-1 production, which is induced by leptin, is involved in the leptin-induced suppression of feeding [3]. Another member of the IL-1 family, IL-1 receptor antagonist (IL1RA) binds to IL-1 receptors without exerting agonistic activity. This protein is considered to be a negative regulator of IL-1 signalling, participating in the complex regulation of IL-1 activity. Interestingly, serum IL1RA levels are seven times higher in obese human patients than in non-obese study participants [4]. In addition, a large quantity of IL1RA is secreted from adipose tissue, although the biological significance of this phenomenon is not well understood [5]. IL-1 activates sympathetic neural circuits; moreover, i.v. and intracerebroventricular administration of IL-1 activates efferent sympathetic nerve discharge [6–8]. Using *Il1^{-/-}* and *Il1ra* (also known as *Il1rn*)-deficient (*Il1ra^{-/-}*) mice, we demonstrated that IL-1 has a physiological role in feeding behaviour and energy metabolism [9]. *Il1ra^{-/-}* mice, which have a defect in lipid accumulation in adipose tissue, are lean and have reduced serum insulin levels [9, 10]. Recently, Garcia et al. demonstrated that *Il1r1^{-/-}* mice developed maturity-onset obesity, beginning to deviate from the weight of wild-type (WT) mice at 5 to 6 months of age [11].

A balance between energy intake and expenditure is important for the maintenance of body weight and normal physiology. Energy expenditure represents the sum of the total energy needed for maintaining normal cell and organ

function, metabolism, physical activity and facultative (adaptive) thermogenesis [12]. The sympathetic nervous system (SNS) is thought to be critical for the prevention of obesity associated with a high-fat diet (HFD) [13]. Activation of the SNS induces uncoupling protein 1 (UCP-1) production and activates diet-induced thermogenesis in brown adipose tissue (BAT) [14, 15]. BAT is thought to be involved in the control of body temperature and body weight via cold- and diet-induced thermogenesis, whereas white adipose tissue (WAT) stores and releases energy. WAT is directly innervated by the SNS, as has been established for BAT [16]. Mice lacking BAT due to the production of diphtheria toxin in this tissue are obese and insulin-resistant [17], indicating the importance of BAT in the control of body weight. Administration of β -adrenergic receptor (AR) agonists increases the metabolic rate [18–20], and mice lacking all three β -AR genes become markedly obese when reared on a HFD [21]. In contrast, transgenic overexpression of human $\beta 1$ -AR (also known as *ADRB1*) in WAT resulted in lean mice and an abundance of brown adipocytes in WAT [22]. Brown and white adipocytes are usually located in distinct depots and can be distinguished morphologically [23]; brown adipocytes contain multilocular lipid vacuoles and numerous mitochondria, whereas white adipocytes have a unilocular lipid vacuole and few mitochondria. UCP-1 is specifically produced in BAT; this protein uncouples oxidative phosphorylation from electron transport, resulting in the dissipation of energy as heat [24, 25]. The emergence of these ectopic cells in the WAT of rats [26] and of mice [27] was found to be induced by cold acclimatisation or by the administration of selective $\beta 3$ -AR agonists [27, 28]. Interestingly, the emergence of brown fat cells in white fat depots was associated with a lean phenotype in several transgenic mouse models, including $\beta 1$ -AR transgenic mice [22], *FOXC2* transgenic mice [29] and *Tcf2^{-/-}* (also known as *Ncoa2^{-/-}*) mice [30].

In this study, we further characterised the metabolic phenotype of *Il1ra^{-/-}* mice reared on a HFD. We found that *Il1ra^{-/-}* mice are resistant to obesity associated with decreased respiratory quotient (RQ) (i.e. RQ = carbon dioxide production ($\dot{V}\text{CO}_2$)/oxygen uptake ($\dot{V}\text{O}_2$)), indicating that the ratio of fat:carbohydrate metabolism in *Il1ra^{-/-}* mice is greater than in controls. Histological analysis of BAT suggests that this tissue is hyper-activated in *Il1ra^{-/-}* mice. Gene expression and electron microscopy analyses of inguinal WAT in *Il1ra^{-/-}* mice revealed that this tissue had BAT-like characteristics. The urinary norepinephrine concentration was significantly higher in *Il1ra^{-/-}* mice than in WT mice, indicating that the former had increased sympathetic tone. Moreover, heart rate and body temperature in *Il1ra^{-/-}* mice were significantly increased compared with WT mice, which is also consistent with the idea that *Il1ra^{-/-}* mice

have increased sympathetic tone. Our results suggest that activation of BAT and BAT-like changes of WAT in *Il1ra*^{-/-} mice are responsible for the decreased RQ and resistance to HFD-induced obesity.

Methods

Animals *Il1ra*^{-/-} mice were produced as described previously [31]. These mice were backcrossed to C57BL/6J mice for nine generations. After weaning, 4-week-old mice were housed individually. Age-matched male littermates or adult (9–16 weeks of age) male mice obtained from a breeder (SLC, Shizuoka, Japan) and housed at our facility were used for each experiment. 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–dark cycle (lights on 08:00–20:00 hours) on a normal-chow diet (5.1% of the total energy from fat; total energy 17.5 kJ/g). All experiments were performed according to the institutional ethical guidelines for animal experimentation and safety guidelines for gene manipulation experiments.

Diet study and metabolic measurements At 4 weeks of age, male *Il1ra*^{-/-} and WT mice were placed on and had free access to a high-carbohydrate HFD (50% of total energy from fat; total energy 22.1 kJ/g). Animals were then studied for the next 12 weeks. Total body weight was measured weekly for 12 weeks starting at 4 weeks of age. Blood samples were collected after a 6 h fast at 4, 8 and 12 weeks of age. The mice were killed at 16 weeks of age. Food intake was measured as described previously [32].

Indirect calorimetry Whole-body O₂ consumption and CO₂ production were measured in a respiration chamber that measured 140 × 80 × 90 mm in size and was ventilated with fresh air at a rate of 200 ml/min. The differences in the concentrations of O₂ and CO₂ between inflow and outflow air were measured with a differential O₂ analyser (LC700E; Toray, Tokyo, Japan) and two CO₂ sensors (GMW22D; Vaisala, Helsinki, Finland). 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 first hour were not analysed. The results were then corrected for metabolic body mass ($g^{0.75}$).

Measurement of heart rate and body temperature A telemetric recording system was used to simultaneously record locomotor activity, heart rate and body temperature

from conscious and unrestrained mice as previously described [33, 34]. Briefly, a telemetric transmitter for locomotor activity, electrocardiogram and body temperature was implanted under anaesthesia. The mice were housed in individual cages and placed on a signal-receiving board in a light-proof chamber. Heart rate and body temperature were continuously recorded every 5 min by a Data Quest analysing system (Data Sciences International, St Paul, MN, USA).

Measurement of blood, serum and urinary parameters Blood glucose levels were measured using the glucose oxidase method (Terumo, Tokyo, Japan), whereas serum triacylglycerol and NEFA levels were examined using colorimetric assays (triacylglycerol-E and NEFA-C tests, respectively; Wako, Osaka, Japan). Serum insulin and leptin levels were both measured with enzyme-linked immunosorbent assays (Seikagaku, Tokyo, Japan) and radioimmunoassays (Eiken, Tokyo, Japan). At 12 weeks of age, *Il1ra*^{-/-} mice and WT mice were placed in metabolism cages that provided free access to tap water and food. Urinary catecholamine excretion was determined by reverse-phase HPLC (SRL, Tokyo, Japan) and corrected by urine creatinine concentration (SRL, Tokyo, Japan). Glucose tolerance and insulin tolerance tests were performed as described in the [Electronic supplementary material \(ESM\)](#).

Electron microscopy Tissues were dissected from the mice under deep anaesthesia and immediately fixed in a solution containing 2.5% (wt/vol.) glutaraldehyde and 2% (wt/vol.) formaldehyde in 0.1 mol/l sodium phosphate buffer (pH 7.4) for 2 h at room temperature. After fixation, tissues were rinsed and post-fixed in 2% (wt/vol.) osmium tetroxide in the same buffer solution on ice. The samples were then washed, dehydrated in a graded series of ethanol and embedded in Epon 812 resin mixture (TAAB, Aldermaston, UK). Semi-thin sections (approximately 0.7 μm thick) were cut on an Ultracut N ultramicrotome (Reichert Depew, NY, USA), stained with 0.2% Toluidine Blue and examined under a microscope (Eclipse E600; Nikon, Tokyo, Japan). Ultra-thin sections were cut, stained with uranyl acetate and lead citrate, and examined with an electron microscope (H-7500; Hitachi, Tokyo, Japan).

Statistical analysis All values are represented as the means ± SEM. Differences between the body weight curves and food intake were evaluated using repeated-measures ANOVA, in which factor 1 was the between-groups factor and factor 2 was the within-subject factor (different ages). Differences between the glucose and insulin level were evaluated using two-way ANOVA followed by Tukey's tests. Comparisons of the two groups were analysed using Student's *t* tests. In all analyses, a two-tailed probability of less than 5% ($p < 0.05$) was considered to be statistically significant.

Results

***Il1ra*^{-/-} mice are protected from diet-induced obesity** To better understand the metabolic phenotype of *Il1ra*^{-/-} mice, we monitored diet-induced changes in these mice. On a HFD, *Il1ra*^{-/-} mice remained lean compared with WT mice (Fig. 1a), whereas HFD-fed WT control mice became obese compared with mice kept on a standard diet (Fig. 1a). The average weights of the epididymal and inguinal fat pads and interscapular brown fat pads of *Il1ra*^{-/-} mice on a HFD were significantly lower than those of WT mice under the same feeding conditions, whereas the relative weights of liver, soleus muscle and spleen were proportional to the decreased body weight (Fig. 1b) of *Il1ra*^{-/-} mice.

Fig. 1 Growth curves, glucose levels and insulin levels in WT and *Il1ra*^{-/-} mice on a HFD. **a** Growth curves in WT mice (littermates; $n=11$, white diamonds, normal chow; $n=10$, black diamonds, HFD) and *Il1ra*^{-/-} mice ($n=9$, white squares, normal chow; $n=8$, black squares, HFD) that had free access to normal chow or HFD. **b** BAT, epididymal WAT (eWAT), inguinal WAT (iWAT), liver, soleus and spleen weight of 12-week-old *Il1ra*^{-/-} mice ($n=6$) under HFD. Data are expressed as a relative value compared with WT mice (littermates; $n=4$). **c** Blood glucose (**c**), serum insulin (**d**) and serum leptin levels (**e**) after 6 h fast in 16-week-old WT (littermates) and *Il1ra*^{-/-} mice (KO) with free access to normal chow or HFD. Data are expressed as means $\pm$ SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. BW, body weight

Altered glucose homeostasis and enhanced insulin sensitivity in *Il1ra*^{-/-} mice We also compared glucose homeostasis in WT and *Il1ra*^{-/-} mice. The fasting blood glucose levels in *Il1ra*^{-/-} mice on a HFD were lower than those of the WT control mice (Fig. 1c), whereas normal chow-fed WT and *Il1ra*^{-/-} mice did not show significant differences in their fasting or their fed blood glucose levels (Fig. 1c and [9]). Consistent with these data, *Il1ra*^{-/-} mice under 20 weeks of age had a considerably enhanced ability to clear glucose from the peripheral circulation during i.p. glucose tolerance tests (ESM Fig. 1a). To determine whether increased insulin sensitivity could account for the improved glucose tolerance, we measured serum insulin levels and performed insulin tolerance tests in vivo. The average serum insulin level decreased by 78% in HFD-fed *Il1ra*^{-/-} mice compared with WT siblings (Fig. 1d). Likewise, *Il1ra*^{-/-} mice under 20 weeks of age on a HFD showed a significantly greater decrease than WT control mice in their blood glucose level during insulin tolerance tests (ESM Fig. 1b). Increased glucose and insulin tolerance were also observed in *Il1ra*^{-/-} mice under 9 weeks of age (data not shown). Thus, insulin sensitivity was enhanced in HFD-fed *Il1ra*^{-/-} mice compared with WT mice.

Serum leptin levels in HFD-fed *Il1ra*^{-/-} mice were significantly lower than those of WT mice, consistent with the decreased fat mass in the former (WT mice 4941 ± 896 pg/ml; *Il1ra*^{-/-} mice 907 ± 161 pg/ml; $p<0.05$) (Fig. 1e). The levels of circulating triacylglycerol were significantly decreased in HFD-fed *Il1ra*^{-/-} mice compared with WT mice (WT mice 8.82 ± 0.55 mmol/l; *Il1ra*^{-/-} mice 6.99 ± 0.55 mmol/l; $p<0.05$), while the levels of circulating NEFA were not significantly different (WT mice 0.52 ± 0.03 mEq/l; *Il1ra*^{-/-} mice 0.55 ± 0.03 mEq/l; $p=0.335$), consistent with increased lipid metabolism in *Il1ra*^{-/-} mice.

Increased energy expenditure and decreased respiratory quotient in *Il1ra*^{-/-} mice The markedly reduced weight of HFD-fed *Il1ra*^{-/-} mice suggests that they may have either reduced food intake and/or increased energy expenditure [12]. Food intake per mouse was significantly decreased in *Il1ra*^{-/-} mice, while food intake normalised to body weight was not significantly different between WT and *Il1ra*^{-/-} mice that were fed a HFD (ESM Fig. 2). Total daily energy expenditure in HFD-fed WT and *Il1ra*^{-/-} mice was assessed by measuring $\dot{V}O_2$ and $\dot{V}CO_2$, and activity was assessed by beam breaks. Daily levels in *Il1ra*^{-/-} mice were significantly lower (Fig. 2a,b). On the other hand, daily energy expenditure (normalised for body weight) was significantly increased by 16% in *Il1ra*^{-/-} mice compared with that of WT mice (Fig. 2c). Interestingly, RQ during the light period was significantly lower than in WT mice, whereas in the dark period RQ was not significantly different (Fig. 2d). Thus, the ratio of fat:carbohydrate metabolism in *Il1ra*^{-/-}

mice is greater than in WT mice, and they expend more energy than WT mice. Furthermore, the body temperature was significantly increased in HFD-fed *Il1ra*^{-/-} mice compared with that of WT mice (Fig. 2e). These results suggest that *Il1ra*^{-/-} mice have increased metabolic rates.

Induction of *Ucp1* mRNA expression in the inguinal WAT of *Il1ra*^{-/-} mice We next assayed the expression of *Ucp1* in WAT, BAT and soleus muscle to survey the possible sites of energy expenditure. The expression level of *Ucp1* mRNA in the inguinal WAT of *Il1ra*^{-/-} mice was significantly

higher than in WT mice (Fig. 3a), whereas levels of *Ucp1* in epididymal WAT and soleus muscles were not significantly different (data not shown) [9]. We also found that the expression of genes encoding rate-limiting enzymes of mitochondrial fatty acid oxidation, such as *Mcad* (also known as *Acadm*) and *Mcpt1*, was increased in inguinal WAT from *Il1ra*^{-/-} mice, suggesting that the increased level of oxidation in the inguinal WAT of *Il1ra*^{-/-} mice probably resulted from an increase in the number of metabolically active mitochondria. The expression level of *Ucp1* mRNA in the BAT was relatively high in *Il1ra*^{-/-} mice compared with that in WT mice, although it was not statistically significant (Fig. 3b). The expression of *Mcad* and *Cox4* (also known as *Cox4i1*) was significantly induced in *Il1ra*^{-/-} BAT (Fig. 3b).

The morphology of brown adipocytes in the inguinal WAT of *Il1ra*^{-/-} mice Histological examinations showed that the lipid vacuoles in the BAT of the *Il1ra*^{-/-} mice were markedly smaller than those of WT mice (Fig. 4a,d), suggesting that the increased activity of BAT also contributes to increased RQ and metabolic rates. Moreover, the mass of the inguinal WAT was smaller in HFD-fed *Il1ra*^{-/-} mice than in HFD-fed WT mice and some of the adipocytes from the *Il1ra*^{-/-} mice exhibited a multilocular phenotype (Fig. 4c,f). Analysis of inguinal WAT using transmission electron microscopy showed a sharp increase in the number of mitochondria in the *Il1ra*^{-/-} mice (Fig. 4g,i) compared with WT mice (Fig. 4h). While the sizes of the epididymal

Fig. 2 Activity and metabolic rate of WT and *Il1ra*^{-/-} mice on a HFD. **a** Activity was measured as beam break counts during 24 h in WT (from breeder) ($n=6$, white diamonds) and *Il1ra*^{-/-} mice ($n=6$, black squares). Data are expressed as means. **b** The bars represent the mean activity level over 1 day (LP + DP), light period (LP) or dark period (DP) in WT (from breeder; $n=6$, white bars) and *Il1ra*^{-/-} mice ($n=6$, black bars). **c**, **d** Oxygen consumption relative to body mass (**c**) and RQ (**d**) measured over 1 day, light period or dark period in WT (littermates; $n=6$, white bars) and *Il1ra*^{-/-} mice ($n=5$, black bars). **e** Body temperature was measured during 24 h on HFD in WT (from breeder; $n=6$, white bars) and *Il1ra*^{-/-} mice ($n=6$, black bars). Data are expressed as means±SEM (**b**–**e**). * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Fig. 3 Real-time PCR quantification of *Ucp1*, *Mcad*, *Mcpt1*, *Cox4* and *Cyt-c* (also known as *Cycs*) mRNA expression in BAT and inguinal WAT from WT and *Il1ra*^{-/-} mice on a HFD. Inguinal WAT (**a**) and BAT (**b**) from WT (littermates) or *Il1ra*^{-/-} mice were isolated and the level of mRNA expression as indicated was normalised using *Rps3* values in *Il1ra*^{-/-} mice ($n=6$, black bars) and are shown as a ratio compared with WT mice ($n=6$, white bars). Data are expressed as means±SEM. * $p<0.05$

Fig. 4 Histological analysis of adipose tissue in WT and *Il1ra*^{-/-} mice on HFD. Histology of BAT (a, d), epididymal WAT (b, e) and inguinal WAT (c, f) from WT (littermates) mice (a–c) or *Il1ra*^{-/-} mice (d–f) (magnification $\times 20$) on a HFD. Electron micrographs of adipocytes in inguinal WAT (g) from 12-week-old *Il1ra*^{-/-} mice or WT mice (h). Inset (g) is shown in higher magnification ($\times 7,000$) (i). Note the abundance of large mitochondria that are rich in cristae (well differentiated ‘brown’ mitochondria) and the juxtaposition of mitochondria to small lipid vacuoles. Scale bars, 50 μm (a–f), 2.5 μm (g, h)

WAT were similar in HFD-fed *Il1ra*^{-/-} mice and HFD-fed WT mice, there were some multilocular adipocytes in epididymal WAT in HFD-fed *Il1ra*^{-/-} mice (Fig. 4b,e). The appearance of BAT-like cells was not observed at all in WAT depots in WT mice.

Hyperactivation of sympathetic nervous system in *Il1ra*^{-/-} mice We next analysed catecholamine metabolism as a measure of sympathetic tone in *Il1ra*^{-/-} mice. There was no significant difference between *Il1ra*^{-/-} mice and WT mice in the daily urine volume (data not shown). The urinary epinephrine and norepinephrine concentration normalised to urinary creatinine was significantly higher in *Il1ra*^{-/-} mice than in WT mice (Fig. 5a,b). These results suggest that *Il1ra*^{-/-} mice have increased sympathetic tone. Furthermore, overall, light- and dark-phase values of heart rate in *Il1ra*^{-/-} mice were significantly higher than in WT mice (Fig. 5c). These results are consistent with the notion that *Il1ra*^{-/-} mice have SNS hyperactivity.

Discussion

In this report, we analysed *Il1ra*^{-/-} mice on a HFD and found that they are resistant to HFD-induced obesity, which is consistent with previous reports [9, 10]. The SNS is activated in these mice, resulting in the activation of BAT

and emergence of multilocular BAT-like cells in WAT depots.

Il1ra^{-/-} mice on a HFD had an increased level of energy expenditure normalised to body weight compared with WT mice (Fig. 2). Whereas Somme et al. observed an augmented level of energy expenditure in *Il1ra*^{-/-} mice on a normal diet [10], we only detected a significant increase in energy expenditure on a HFD; the discrepancy is probably due to differences in the diet or housing conditions. As locomotor activity was decreased in *Il1ra*^{-/-} mice under HFD (Fig. 2b, c), basal energy expenditure should increase. We found that HFD-fed *Il1ra*^{-/-} mice had lower RQ than WT mice, indicating that the *Il1ra*^{-/-} mice had an increased fat:carbohydrate oxidation ratio. The result is consistent with the observation that BAT-like cells appeared in WAT depots, because β -oxidation of fatty acids is preferentially used in BAT to produce energy. Our results are also consistent with the recent findings by Garcia et al., which demonstrated that *Il1r1*^{-/-} mice developed maturity-onset obesity with an increased RQ, indicating that *Il1r1*^{-/-} mice had a decreased fat:carbohydrate oxidation ratio [11]. Taken together, these results strongly suggest that IL-1 primarily affects fat:carbohydrate oxidation ratio.

Interestingly, some multilocular BAT-like cells appeared in the inguinal WAT depots of *Il1ra*^{-/-} mice, whereas no BAT-like cells were observed in WT mice. We have never found any transformations of WAT into BAT in the *Il1ra*^{-/-}

Fig. 5 Urinary excretion of catecholamines and heart rate in WT and *Il1ra*^{-/-} mice on a HFD. Urine epinephrine (**a**) and norepinephrine (**b**) levels expressed as nmol/μmol creatinine in WT (from breeder; *n*=6, white bars) and *Il1ra*^{-/-} mice (*n*=6, black bars). Urine samples (24 h) were collected and assayed for catecholamines. **c** Heart rate was measured during 24 h on HFD in WT (from breeder; *n*=6, white bars) and *Il1ra*^{-/-} mice (*n*=6, black bars). DP, dark period; LP, light period; LP+DP, 1 day. Data are expressed as means±SEM. **p*<0.05. bpm, beats per min

mice under normal diet conditions [9]. Histologically, the BAT-like cells resembled brown adipocytes, and inguinal WAT depots from *Il1ra*^{-/-} mice had increased levels of *Ucp-1* (Figs 3 and 4). There were large differences in the numbers of BAT-like cells that appeared in WAT depots between each animal and between each depot in the same animal. BAT-like cells also appeared in some epididymal WAT depots in *Il1ra*^{-/-} mice. However, we never found BAT-like cells in WT mice. Although it is possible that BAT-like cells in the WAT depots contribute to the increased RQ and increased energy expenditure, we should interpret these findings with caution, as the basal metabolism of WAT is very low.

Urinary norepinephrine and epinephrine excretion were significantly increased in *Il1ra*^{-/-} mice compared with WT mice (Fig. 5), suggesting that sympathetic tone was increased in the *Il1ra*^{-/-} mice. Consistent with the idea that *Il1ra*^{-/-} mice have increased sympathetic tone, *Il1ra*^{-/-} mice had increased heart rate and body temperature (Figs 2e and 5c). The key question is: How might *Il1ra* deficiency result in increased sympathetic tone? It was previously demonstrated that IL-1 acts as an activator of sympathetic neural circuits, and that i.v. and intracerebroventricular administration of IL-1 activates efferent sympathetic nerve discharge [6–8]. Since the balance between IL-1 and IL1RA determines the net IL-1 signalling, *Il1ra* deficiency in the brain may contribute to the increased activity of SNS. Consistent with this notion, we have

previously demonstrated that levels of *Il1β* and *Cox-2* (also known as *Ptgs2*) were increased in *Il1ra*^{-/-} mice compared with WT mice [35], suggesting that excess IL-1 signalling is enhanced in the brain. Furthermore, levels of *Il6*, *Tnfα* (also known as *Tnfa*) and *Il1β* in WAT were decreased (though not significantly) in *Il1ra*^{-/-} mice, indicating that inflammatory changes in response to HFD are relatively low in *Il1ra*^{-/-} mice, as opposed to the possible role of IL-1 signalling in HFD-induced inflammatory change in WAT [35, 36]. Thus, it seems unlikely that excess IL-1 signalling in the periphery is responsible for the lean phenotype.

IL-1 has been recognised as a so-called endogenous pyrogen and is the signal responsible for fever. Considering the fact that *Il1ra*^{-/-} mice on a HFD had increased body temperature, it is possible that the primary effect of *Il1ra* deficiency is to increase body temperature. However, we have previously demonstrated that *Il1ra*^{-/-}*Il-6*^{-/-} mice on a normal diet show a lean phenotype comparable to *Il1ra*^{-/-} mice [32]. Since IL-6 is essential for the IL-1-induced febrile response [37, 38], it is not possible that the lean phenotype could be explained solely by the action of IL-1 as a pyrogen. The activation of SNS and conversion of WAT into BAT may be involved in the elevated body temperature in *Il1ra*^{-/-} mice.

We showed that serum leptin levels in *Il1ra*^{-/-} mice on a HFD were significantly lower compared with WT mice, although food intake was similar between WT and mutant mice. These observations suggest that leptin sensitivity, which plays an important role in protecting against diet-induced obesity by suppressing appetite [39], is increased in *Il1ra*^{-/-} mice. In this regard, it is interesting that leptin signalling in the CNS regulates energy expenditure via activation of the SNS, leading to BAT-like differentiation of WAT [40].

Because animals subjected to chronic β3 adrenergic stimulation show an increased number of multilocular BAT-like cells and decreased WAT stores [18], it is likely that the increased sympathetic tone in *Il1ra*^{-/-} mice is responsible for the increased metabolic activity in BAT and the appearance of BAT-like cells in inguinal WAT of the *Il1ra*^{-/-} mice (Figs 4 and 5). BAT is present throughout the lives of rodents, but in primates it disappears soon after birth; there are no BAT depots in adult humans. Nevertheless, variable quantities of brown adipocytes have been detected in typical WAT depots in humans [41]. Hence, brown adipocytes in WAT depots may play an important role in preventing obesity via a thermogenic mechanism, both in humans and in mice. While we found an increased *Ucp1* expression in inguinal WAT, further studies will be required in order to determine whether the increase in *Ucp1* represents an increase in brown adipocytes within WAT or a WAT-to-BAT conversion or clonal expansion of bona fide BAT depots.

It was recently demonstrated that *Gnasxl*^{m+P-} mice are lean as a result of increased lipid mobilisation and oxidation in adipose tissue due to increased sympathetic tone [42]. A similar metabolic profile was also observed in mouse models with increased β -adrenergic/G protein α subunit/cAMP signalling in adipocytes, such as adipose-specific overproduction of the forkhead transcription factor FOXO2 [29], and in mice in which the *C/ebp α* (also known as *Cebpa*) gene was replaced with the *C/ebp β* (also known as *Cebpb*) gene [43]. Taken together, these results indicate that β -adrenergic/G protein α subunit/cAMP signalling in adipocytes is an important regulator of whole-body homeostasis. Recently, several inflammatory cytokine-deficient mice, including *Il6*^{-/-} [44], *Il1r1*^{-/-} [11], *Csf2*^{-/-} [45], *Il18*^{-/-} [46, 47] and *Il18r1*^{-/-} [47] mice, were shown to develop maturity-onset obesity, suggesting the involvement of age-related changes in the SNS in the phenotypes of these cytokine-deficient mice. It is possible that inflammatory cytokines are protective against age-related declines in β -adrenergic responsiveness.

Recently, it was demonstrated that recombinant IL1RA (Anakinra) is beneficial in animal models [48] of diabetes and diabetic patients [49], suggesting that excess IL-1 signalling is responsible for the beta cell damage in diabetes. Consistent with this, IL-1 produced in beta cells contributes to glucotoxicity of beta cells [50]. In light of these observations, we histologically analysed islets of HFD-fed mice to examine possible effects of HFD on islets. However, HFD feeding for 16 weeks under our experimental conditions did not affect beta cell mass either in WT or in *Il1ra*^{-/-} mice. Islets in *Il1ra*^{-/-} mice were histologically normal and had normal hormonal content (O. Hashimoto, D. Chida, Y. Iwakura, unpublished data). Thus, the effect of *Il1ra* deficiency under HFD conditions is observed mostly in the SNS, and not in the pancreatic islets, in *Il1ra*^{-/-} mice.

Taken together, these observations suggest that chronically increased sympathetic tone in *Il1ra*^{-/-} mice is responsible for the activation of BAT and the emergence of multilocular BAT-like cells in WAT. Together with low levels of insulin secretion, these changes may contribute to the resistance to HFD-induced obesity observed in these animals. Our findings may suggest novel approaches for developing therapeutics for pathogenic obesity.

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References

1. Tocci MJ, Schmidt JA (1997) Interleukin-1: structure and function. In: Remick DG, Friedland JS (eds) Cytokines in health and disease, second edition. Marcel Dekker, New York, pp 1–27
2. Friedman JM, Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* 395:763–770
3. Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ (1999) Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci U S A* 96:7047–7052
4. Meier CA, Bobbioni E, Gabay C, Assimakopoulos-Jeanet F, Golay A, Dayer JM (2002) IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* 87:1184–1188
5. Juge-Aubry CE, Somme E, Giusti V et al (2003) Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 52:1104–1110
6. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES (2000) The sympathetic nerve—an integrative interface between two super-systems: the brain and the immune system. *Pharmacol Rev* 52:595–638
7. Terao A, Oikawa M, Saito M (1994) Tissue-specific increase in norepinephrine turnover by central interleukin-1, but not by interleukin-6, in rats. *Am J Physiol Regul Integr Comp Physiol* 266:R400–R404
8. Akiyoshi M, Shimizu Y, Saito M (1990) Interleukin-1 increases norepinephrine turnover in the spleen and lung in rats. *Biochem Biophys Res Commun* 173:1266–1270
9. Matsuki T, Horai R, Sudo K, Iwakura Y (2003) IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. *J Exp Med* 198:877–888
10. Somme E, Henrichot E, Pernin A et al (2005) Decreased fat mass in interleukin-1 receptor antagonist-deficient mice: impact on adipogenesis, food intake, and energy expenditure. *Diabetes* 54:3503–3509
11. Garcia MC, Wernstedt I, Berndtsson A et al (2006) Mature-onset obesity in interleukin-1 receptor I knockout mice. *Diabetes* 55:1205–1213
12. Spiegelman BM, Flier JS (2001) Obesity and the regulation of energy balance. *Cell* 104:531–543
13. Friedman JM (2000) Obesity in the new millennium. *Nature* 404:632–634
14. Rothwell NJ, Stock MJ (1979) A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281:31–35
15. Lowell BB, Bachman ES (2003) β -Adrenergic receptors, diet-induced thermogenesis, and obesity. *J Biol Chem* 278:29385–29388
16. Bartness TJ, Song CK (2007) Thematic review series: adipocyte biology. Sympathetic and sensory innervation of white adipose tissue. *J Lipid Res* 48:1655–1672
17. Lowell BB, S-Suselik V, Hamann A et al (1993) Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366:740–742
18. Himms-Hagen J, Cui J, Danforth E Jr. et al (1994) Effect of CL-316,243, a thermogenic β 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol* 266:R1371–R1382
19. Inokuma K, Okamatsu-Ogura Y, Omachi A et al (2006) Indispensable role of mitochondrial UCP1 for antiobesity effect of β 3-adrenergic stimulation. *Am J Physiol Endocrinol Metab* 290:E1014–E1021
20. Strosberg AD (1997) Structure and function of the β 3-adrenergic receptor. *Annu Rev Pharmacol Toxicol* 37:421–450
21. Bachman ES, Dhillon H, Zhang CY et al (2002) β AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297:843–845

22. Soloveva V, Graves RA, Rasenick MM, Spiegelman BM, Ross SR (1997) Transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue are resistant to obesity. *Mol Endocrinol* 11:27–38
23. Himms-Hagen J (1989) Brown adipose tissue thermogenesis and obesity. *Prog Lipid Res* 28:67–115
24. Nicholls DG, Locke RM (1984) Thermogenic mechanisms in brown fat. *Physiol Rev* 64:1–64
25. Nedergaard J, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B (2001) UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim Biophys Acta* 1504:82–106
26. Cousin B, Cinti S, Morroni M et al (1992) Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J Cell Sci* 103:931–942
27. Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP (1998) Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest* 102:412–420
28. Nagase I, Yoshida T, Kumamoto K et al (1996) Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic beta 3-adrenergic agonist. *J Clin Invest* 97:2898–2904
29. Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, Enerback S (2001) FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106:563–573
30. Picard F, Gehin M, Annicotte J et al (2002) SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell* 111:931–941
31. Horai R, Asano M, Sudo K et al (1998) Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *J Exp Med* 187:1463–1475
32. Chida D, Osaka T, Hashimoto O, Iwakura Y (2006) Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. *Diabetes* 55:971–977
33. Ishii K, Kuwahara M, Tsubone H, Sugano S (1996) Autonomic nervous function in mice and voles (*Microtus arvalis*): investigation by power spectral analysis of heart rate variability. *Lab Anim* 30:359–364
34. Furuzawa M, Kuwahara M, Ishii K, Iwakura Y, Tsubone H (2002) Diurnal variation of heart rate, locomotor activity, and body temperature in interleukin-1 alpha/beta doubly deficient mice. *Exp Anim* 51:49–56
35. Xu H, Barnes GT, Yang Q et al (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830
36. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808
37. Chai Z, Gatti S, Toniatti C, Poli V, Bartfai T (1996) Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J Exp Med* 183:311–316
38. Kagiwada K, Chida D, Sakatani T et al (2004) Interleukin (IL)-6, but not IL-1, induction in the brain downstream of cyclooxygenase-2 is essential for the induction of febrile response against peripheral IL-1alpha. *Endocrinology* 145:5044–5048
39. Scarlett JM, Jobst EE, Enriori PJ et al (2007) Regulation of central melanocortin signaling by interleukin-1 beta. *Endocrinology* 148:4217–4225
40. Plum L, Rother E, Munzberg H et al (2007) Enhanced leptin-stimulated pi3k activation in the CNS promotes white adipose tissue transdifferentiation. *Cell Metabolism* 6:431–445
41. Tiraby C, Langin D (2003) Conversion from white to brown adipocytes: a strategy for the control of fat mass? *Trends Endocrinol Metab* 14:439–441
42. Xie T, Plagge A, Gavrilova O et al (2006) The alternative stimulatory G protein alpha-subunit XLalphas is a critical regulator of energy and glucose metabolism and sympathetic nerve activity in adult mice. *J Biol Chem* 281:18989–18999
43. Chiu CH, Lin WD, Huang SY, Lee YH (2004) Effect of a C/EBP gene replacement on mitochondrial biogenesis in fat cells. *Genes Dev* 18:1970–1975
44. Wallenius V, Wallenius K, Ahren B et al (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 8:75–79
45. Reed JA, Clegg DJ, Blake Smith K et al (2005) GM-CSF action in the CNS decreases food intake and body weight. *J Clin Invest* 115:3035–3044
46. Zorrilla EP, Sanchez-Alavez M, Sugama S et al (2007) Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency. *Proc Natl Acad Sci USA* 104:11097–11102
47. Netea MG, Joosten LA, Lewis E et al (2006) Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat Med* 12:650–656
48. Sauter NS, Schulthess FT, Galasso R, Castellani LW, Maedler K (2008) The anti-inflammatory cytokine IL1Ra protects from high fat diet-induced hyperglycemia. *Endocrinology* 149:2208–2218
49. Larsen CM, Faulenbach M, Vaag A et al (2007) Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 356:1517–1526
50. Maedler K, Sergeev P, Ris F et al (2002) Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* 110:851–860