

Human IL6 enhances leptin action in mice

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Abstract

Aims/hypothesis Interleukin-6 is an inflammatory cytokine with pleiotropic effects upon nutrient homeostasis. Many reports show that circulating IL6 correlates with obesity and contributes to insulin resistance; however, IL6 can promote energy expenditure that improves glucose homeostasis.

Methods We investigated nutrient homeostasis in C57BL/6J mice with sustained circulating human IL6 (hIL6) secreted predominantly from brain and lung (hIL6^{tg} mice).

Results The hIL6^{tg} mice displayed no features of systemic inflammation and were more insulin-sensitive than wild-type mice. On a high-fat diet, hIL6^{tg} mice were lean, had low leptin concentrations, consumed less food and expended more energy than wild-type mice. Like *ob/ob* mice, the *ob/ob*^{IL6} mice (generated by intercrossing *ob/ob* and hIL6^{tg} mice) were obese and glucose-intolerant. However, low-dose leptin injections increased physical activity and reduced both body weight and food intake in *ob/ob*^{IL6} mice, but was ineffective in *ob/ob* mice. Leptin increased hypothalamic signal transducer and activator of transcription-3 phosphorylation in *ob/ob*^{IL6} mice, whereas *ob/ob* mice barely responded.

Conclusions/interpretation Human IL6 enhanced central leptin action in mice, promoting nutrient homeostasis and preventing diet-induced obesity.

Keywords Diabetes · IL6 · Insulin resistance · Leptin · Leptin sensitivity · Obesity

Abbreviations

AMPK	AMP-activated protein kinase
CNTF	Ciliary neurotrophic factor
DEXA	Dual-energy X-ray absorptiometry
GFAP	Glial fibrillary acidic protein
HFD	High-fat diet
hIL6	Human IL6
hIL6 ^{tg} mice	Mice with sustained circulating human IL6 secreted predominantly from brain and lung
LepRb	Leptin receptor, isoform b
mIL6	Murine IL6
SOCS3	Suppressor of cytokine signalling
STAT3	Signal transducer and activator of transcription-3

Introduction

Understanding how IL6 regulates central and peripheral nutrient homeostasis is complicated by contradictory and multi-systemic effects under various physiological states [1]. IL6 is best known as a pro-inflammatory cytokine that regulates innate immunity and the acute-phase response. However, IL6 also has tissue-specific effects that can differ in humans and rodents, depending on context and timing of stimulation [2]. IL6 promotes chronic inflammation, whereas it displays anti-inflammatory effects during acute inflammatory stimuli [3].

Obesity and its progression to diabetes are associated with chronic inflammation characterised by secretion of the proinflammatory cytokines resistin, TNF α and IL6 from

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adipocytes [4]. Epidemiological data confirm that elevated circulating IL6 correlates with adiposity in humans [5]. IL6 is generally thought to promote systemic insulin resistance, especially during obesity, because it is secreted from fat cells of insulin-resistant humans [5]. However, in type 2 diabetes patients the plasma concentrations of IL6 and TNF α may best reflect the level of adiposity rather than insulin sensitivity during the euglycaemic–hyperinsulinaemic clamp [6]. Yet TNF α might also be a principle cause of dysregulated insulin signalling, as it stimulates production of IL6, IL1 and C-reactive peptide [7]. It is possible that IL6 opposes the action of TNF α upon insulin sensitivity, as physical exercise promotes secretion of IL6 from skeletal muscle, while improving insulin sensitivity and nutrient homeostasis [1, 8]. The question of how IL6 integrates multiple signalling cascades to coordinate nutrient homeostasis in mammals remains unanswered.

Cell-based experiments and in vivo studies in rodents show that IL6 promotes insulin resistance [9]. In vivo, 90 min after IL6 injections plasma glucose and insulin concentrations increase [10]. Infusion of IL6 for 3 h causes hepatic and muscle insulin resistance [11, 12]. In addition, hepatic insulin receptor signalling improves in *ob/ob* mice treated with neutralising antibodies against IL6 [13]. Recently, electrotransfer of murine *IL6* cDNA into skeletal muscle promoted liver inflammation and hyperinsulinaemia in mice [14].

Unlike in rodent studies, infusion of recombinant human IL6 (hIL6) to sustain physiological concentrations in healthy individuals or patients with diabetes increases lipolysis in the absence of adverse effects and enhances glucose infusion rates during a euglycaemic–hyperinsulinaemic clamp [15–17]. Moreover, adipose-derived hIL6 can have autocrine effects that increase leptin secretion and fat oxidation, and reduce expression and activity of lipoprotein lipase in human adipose tissues, a phenomenon that might attenuate progression of obesity and diabetes [18]. Human IL6 also displays anti-inflammatory characteristics by inhibiting TNF α and IL1, and activating IL1 receptor antagonist and IL10 [19–21]. Moreover, in rodents IL6 has central effects similar to those of leptin in promotion of nutrient homeostasis and peripheral insulin sensitivity [1, 22]. Thus, the role of IL6 in the regulation of nutrient homeostasis is contradictory and incompletely resolved, possibly confounded by differences between human and murine cytokine action [1].

Leptin is secreted from adipose tissue in proportion to fat stores, informing the central nervous system of the peripheral energy supply. Dysregulated leptin action (*ob/ob* mice) increases food intake, while reducing energy expenditure. In addition, *ob/ob* mice display severe obesity and insulin resistance that progresses to diabetes [23]. However, ordinary obesity in mice and humans is associated with elevated leptin concentrations, suggesting leptin resistance

in the central nervous system as a principle cause [24, 25]. Interestingly, IL6 might be required for a normal leptin response, as adult *IL6*^{-/-} mice develop hyperphagia and obesity, which is difficult to prevent by peripheral leptin injections [26].

To establish the long-term systemic effect of hIL6 upon nutrient homeostasis in mice, we investigated glucose tolerance, energy expenditure and insulin action in transgenic C57BL/6J mice and *ob/ob* mice that secrete hIL6 constitutively into the circulation. Our results show that hIL6 promotes central leptin action in mice, together with its beneficial effects upon nutrient homeostasis.

Methods

Animals Treatment of mice involved in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of Children's Hospital Boston. *IL6* transgenic mice, which have been previously described [27], were generated by ten backcrosses for pure C57BL/6J background. *Ob/ob* mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). *Ob/ob*^{IL6} mice were generated by mating *ob*^{+/-}/*IL6*^{tg} mice with *ob*^{+/-} mice. Animals were fed either regular chow diet with 9% of energy derived from fat or a high-fat diet (HFD) (Research Diets, New Brunswick, NJ, USA) with 45% of energy derived from fat.

Metabolic analysis Intraperitoneal glucose tolerance test was performed on mice fasted overnight for 16 h. Blood glucose levels were measured on random-fed or overnight-fasted animals in mouse-tail blood using a glucometer (Elite; Bayer, Leverkusen, Germany) and serum samples were collected for insulin measurements. Animals were then injected intraperitoneally with D-glucose (2 g/kg body weight) and blood glucose levels were measured [28]. Blood insulin and leptin levels were determined using rat insulin and mouse leptin ELISA kits (Crystal Chem, Downers Grove, IL, USA). Lean and fat body mass were assessed by dual-energy X-ray absorptiometry (DEXA) (GE Lunar, Madison, WI, USA) [28].

Food intake, physical activity and energy expenditure All measurements were performed over a 72 h period with a comprehensive laboratory animal monitoring system (Oxymax Windows 3.0.3; Columbus Instruments, Columbus, OH, USA). The data presented are average values obtained in these recordings.

Hypothalamic neuropeptide expression Neuropeptide mRNA was analysed using quantitative real-time PCR with customised primers. Actin gene expression was used to

normalise RNA content and the relative gene product amounts were reported as mean±SEM of several animals.

Western blotting Mice were fasted overnight (16 h) and then fed for 4 h. Tissues were removed under anaesthetic, homogenised and applied for direct immunoblotting (50 µg) with the indicated antibodies [29]. Antibodies used in this study included: rabbit insulin receptor, IRS1 and IRS2 antibodies (Upstate Biotechnology, Billerica, MA, USA); antibodies against signal transducer and activator of transcription-3 (STAT3), phospho-specific STAT3 (Tyr307), phospho-Akt^{ser473}, Akt and β-actin (Cell Signaling Technology, Danvers, MA, USA); monoclonal antibody to suppressor of cytokine signalling (SOCS3) and phosphotyrosine (Upstate Biotechnology). The intensity of signals was determined using a Kodak molecular imaging system.

Statistical analysis Unless otherwise stated, mean values ± SEM were used to make comparisons between groups. Logistic regression or generalised linear regression (SPSS version 16; SPSS, Chicago, IL, USA) was used where indicated to establish significant difference ($p < 0.05$) when multiple categorical predictors were compared across the experiments. A generalised linear model was used to make comparisons across all samples, using the Bonferroni correction for multiple comparisons.

Results

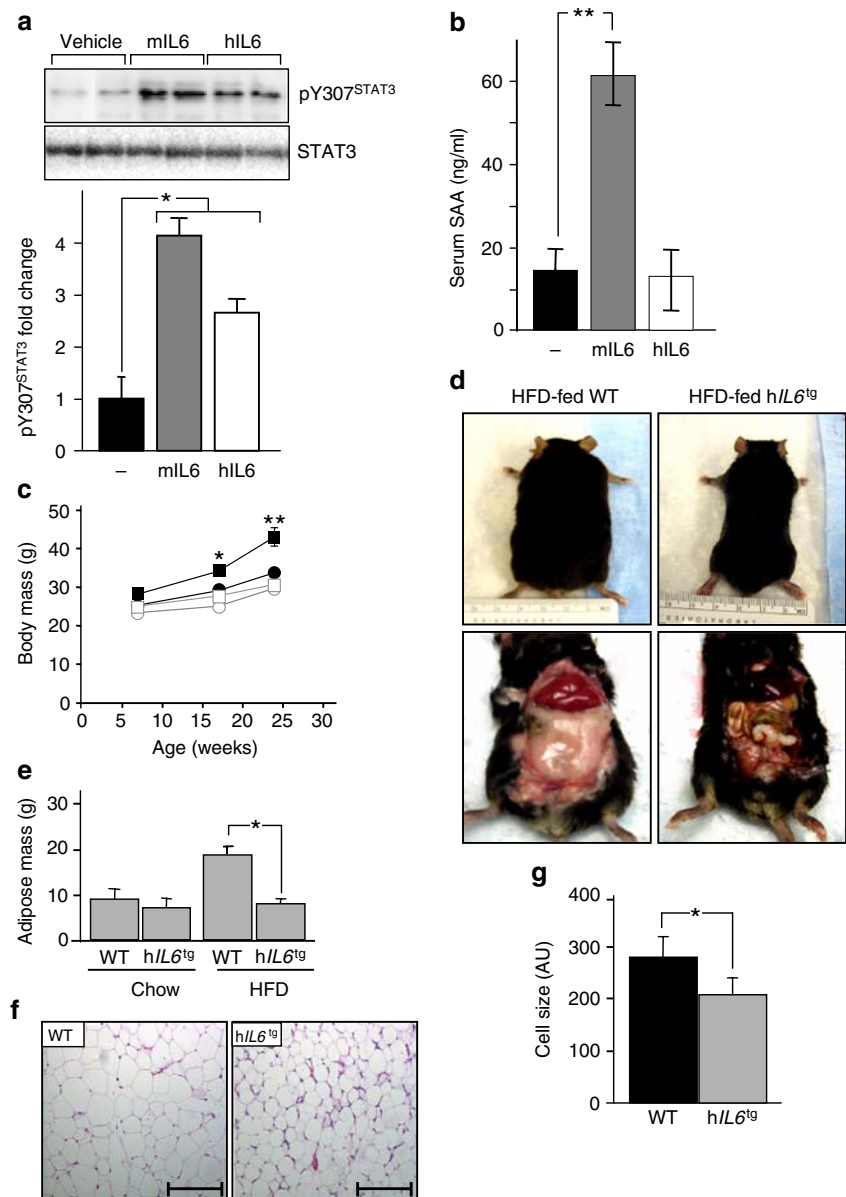
The effect of human IL6 on growth and diet-induced obesity in mice To investigate the effect of hIL6 upon nutrient homeostasis, we created mice with sustained circulating hIL6 secreted predominantly from brain and lung (hIL6^{tg} mice) by backcrossing wild-type C57BL/6J mice 12 times with MUP/hIL6 mice expressing human IL6 cDNA under the control of a mouse urinary protein gene promoter. The hIL6 transgene is expressed predominantly in the brain and the lung (as demonstrated by RT-PCR), (Electronic supplementary material [ESM] Methods, ESM Fig. 1a) [27]. To verify that hIL6 is active in mice, we compared the biological response of wild-type mice to daily injections (50 ng i.p.) of hIL6 or murine IL6 (mIL6) (circulating IL6 143–180 pg/ml, 150 min after injection). Murine IL6 and hIL6 stimulated STAT3 phosphorylation in liver, although the effect of mIL6 was 1.5-fold greater than that of hIL6 (Fig. 1a). Body weight and blood glucose concentrations were unchanged during 3 days of mIL6 or hIL6 injections; however, circulating insulin increased equally during injections with mIL6 or hIL6 (ESM Fig. 1b–d). As previously shown, serum amyloid A protein, a sensitive marker of hepatic acute-phase reaction in mice, increased

significantly after mIL6 injections [14, 30]; despite this, hIL6 had no effect upon serum amyloid A concentrations in mice or cultured mouse hepatocytes (ESM Methods, Fig. 1b, ESM Fig. 1e) [31]. In hIL6^{tg} mice, circulating hIL6 reached 1,150 pg/ml, which was about tenfold higher than the concentration achieved during injection (ESM Methods, ESM Table 1). Nevertheless, hIL6^{tg} mice displayed no evidence of inflammatory or immunological disturbances in tissue histology as demonstrated by immunostaining, white blood cell count or serum chemistry (ESM Methods, ESM Figs 1f, 2b–d, ESM Tables 1 and 2). In addition, IL6 transgene expression in different brain regions did not trigger inflammatory responses within the central nervous system, as demonstrated by RT-PCR (ESM Methods, ESM Table 3). Thus, hIL6 is biologically active in mice, but did not promote an acute inflammatory response.

The hIL6^{tg} mice maintained between 4 and 24 weeks of age on regular chow (9% of energy derived from fat) were slightly smaller than wild-type mice (Fig. 1c). By comparison, HFD (45% energy derived from fat) caused significant weight gain in wild-type mice, whereas HFD-fed hIL6^{tg} mice were slightly smaller than chow-fed wild-type mice (Fig. 1c, d). DEXA confirmed that the lean body mass of 24-week-old wild-type or hIL6^{tg} mice was not influenced by diet. By comparison, HFD increased adipose mass of wild-type mice twofold, but had no effect on hIL6^{tg} mice (Fig. 1e). Haematoxylin and eosin staining confirmed that adipocytes were 30% smaller ($p < 0.05$) in HFD-fed hIL6^{tg} mice than in wild-type mice (Fig. 1f, g). Thus, hIL6^{tg} mice were slightly smaller and had less visceral fat on a chow diet, whereas HFD-induced obesity was prevented.

Glucose tolerance and insulin sensitivity in hIL6^{tg} mice Although acute treatment of C57BL/6J mice with IL6 causes insulin resistance and hyperinsulinaemia, fasting insulin was unexpectedly low in chow- and HFD-fed hIL6^{tg} mice (Fig. 2a). The HOMA2 (<http://software.informer.com/getfree-homa2-calculator-download/>) of insulin resistance confirmed that HFD-fed wild-type mice developed insulin resistance, whereas HFD-fed hIL6^{tg} mice remained insulin-sensitive (Fig. 2b). Moreover, glucose tolerance in HFD-fed hIL6^{tg} mice was indistinguishable from that in chow-fed wild-type and hIL6^{tg} mice, whereas the HFD caused glucose intolerance in wild-type mice (Fig. 2c, d). Consistent with the expected compensatory response to insulin resistance, beta cell mass and islet density increased significantly in HFD-fed wild-type mice; however, beta cell mass in hIL6^{tg} mice on chow or HFD was indistinguishable from that in chow-fed wild-type and hIL6^{tg} mice (Fig. 2e, f). Thus, hIL6 prevented diet-induced insulin resistance and compensatory beta cell growth in hIL6^{tg} mice.

Fig. 1 The effect of human IL6 on growth and diet-induced obesity in mice. **a** Western blot and densitometric analysis of pSTAT3 and total STAT3 in liver of C57BL/6 mice after three daily injections of mIL6 (50 ng), hIL6 (50 ng) or vehicle. Intensity of pSTAT3 was normalised to STAT3 ($n=10$). **b** Serum amyloid A (SAA) levels measured from random fed mice 150 min after injection of mIL6, hIL6 or vehicle ($n=10$). **c** Average body weights of male $hIL6^{tg}$ mice on chow diet (white circles; $n=15$) and on HFD (white squares; $n=10$), or of wild-type mice on chow diet (black circles; $n=15$) and on HFD (black squares; $n=10$). **d** Representative images of 24-week-old mice as labelled. **e** Body composition of 24-week-old wild-type and $hIL6^{tg}$ mice on chow and HFD measured by DEXA ($n=10-12$). **f** Representative haematoxylin and eosin staining of epididymal adipose tissue of wild-type and $hIL6^{tg}$ mice aged 24 weeks. Scale bar, 500 μm . **g** Morphometric analysis of epididymal adipose tissue ($n=5$ animals per genotype). Values (where applicable) are mean \pm SEM; * $p<0.05$ and ** $p<0.01$ for wild-type vs $hIL6^{tg}$ mice



Hepatic metabolism and signalling in $hIL6^{tg}$ mice IL6 has contradictory effects upon hepatic glucose metabolism due at least in part to the opposing action of hepatic STAT3 signalling. Under ordinary conditions, STAT3 mediates the suppressive effect of IL6 on hepatic glucose production [32]. However, during obesity IL6 inhibits peripheral insulin signalling by stimulating STAT3 to SOCS3 signalling [33]. In the present study, postprandial STAT3 phosphorylation increased in chow-fed wild-type and $hIL6^{tg}$ mice; however, STAT3 phosphorylation was barely detected in HFD-fed wild-type mice (Fig. 3a, b). Basal and postprandial STAT3 phosphorylation increased significantly in HFD-fed $hIL6^{tg}$ mice, consistent with the improved glucose tolerance under these conditions (Fig. 3a).

As expected, liver SOCS3 levels were related to STAT3 phosphorylation. Compared with chow-fed wild-type mice, SOCS3 increased in chow-fed $hIL6^{tg}$ mice, decreased in HFD-fed wild-type mice and increased tenfold in HFD-fed $hIL6^{tg}$ mice (Fig. 3c, d). The postprandial IRS1 concentration decreased significantly in the liver of chow-fed and HFD-fed $hIL6^{tg}$ mice, which correlated with increased SOCS3 concentration as previously described (Fig. 3c, e) [33]. By comparison, hIL6 had no effect on liver IRS2 concentrations in chow-fed mice, whereas it prevented loss of IRS2 in HFD-fed $hIL6^{tg}$ mice (Fig. 3d, f). Thus changes in IRS1 and IRS2 concentrations were poor predictors of the effect of hIL6 upon systemic glucose homeostasis and peripheral insulin sensitivity.

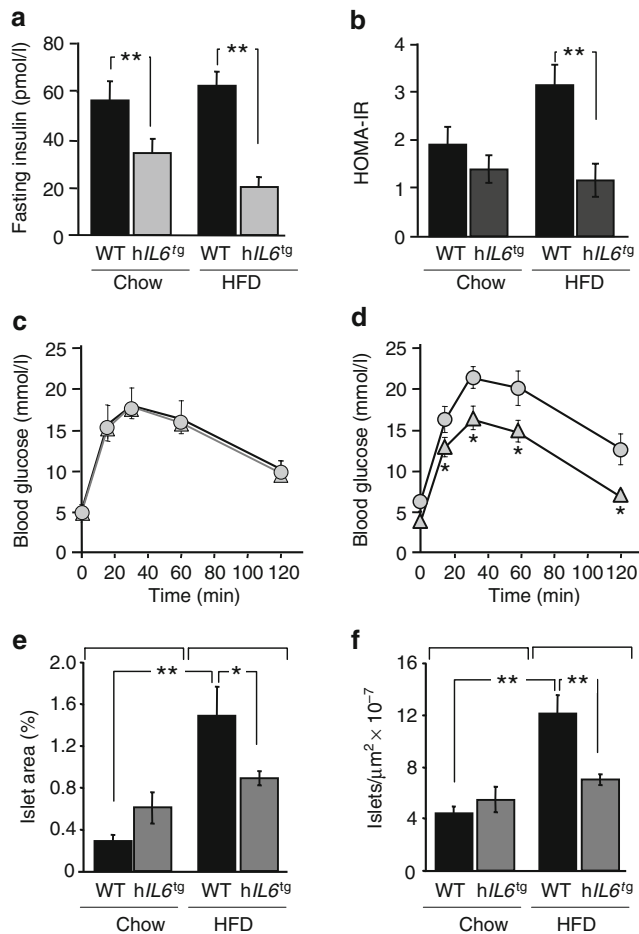


Fig. 2 Glucose tolerance and insulin sensitivity in *hIL6^{tg}* mice. **a** Fasting serum insulin levels ($n=10$) at 24 weeks. **b** HOMA index of insulin resistance (IR). **c** Glucose tolerance test of 24-week-old male mice fed a chow ($n=15$) diet or **d** HFD ($n=10$). Circles, wild-type; triangles, *hIL6^{tg}*. **e** Percent of pancreas area that consisted of islets ($n=4$ animals per group). **f** The number of islets per μm^2 ($n=4$ animals per group). Data are expressed as average \pm SEM. * $p<0.05$ and ** $p<0.01$ for wild-type vs *hIL6^{tg}* mice

The insulin receptor mediates tyrosine phosphorylation of IRS1 and IRS2, which directly activates the phosphatidylinositol-3-kinase to Akt cascade in all cells. Insulin stimulated equally insulin receptor phosphorylation and Akt^{Ser473} phosphorylation in liver of chow-fed wild-type and *hIL6^{tg}* mice; however, insulin-stimulated insulin receptor phosphorylation in HFD-fed *hIL6^{tg}* mice was significantly stronger than in HFD-fed wild-type mice (Fig. 3g, h). Consistent with these results, insulin-stimulated Akt^{Ser473} phosphorylation in HFD-fed *hIL6^{tg}* mice was also increased compared with HFD-fed wild-type mice (Fig. 3g, i). This pattern of insulin receptor and Akt phosphorylation might explain in part the positive effect of *hIL6* upon insulin sensitivity in *hIL6^{tg}* mice.

Energy balance in *hIL6^{tg}* mice Excess food intake relative to energy expenditure promotes obesity, a process

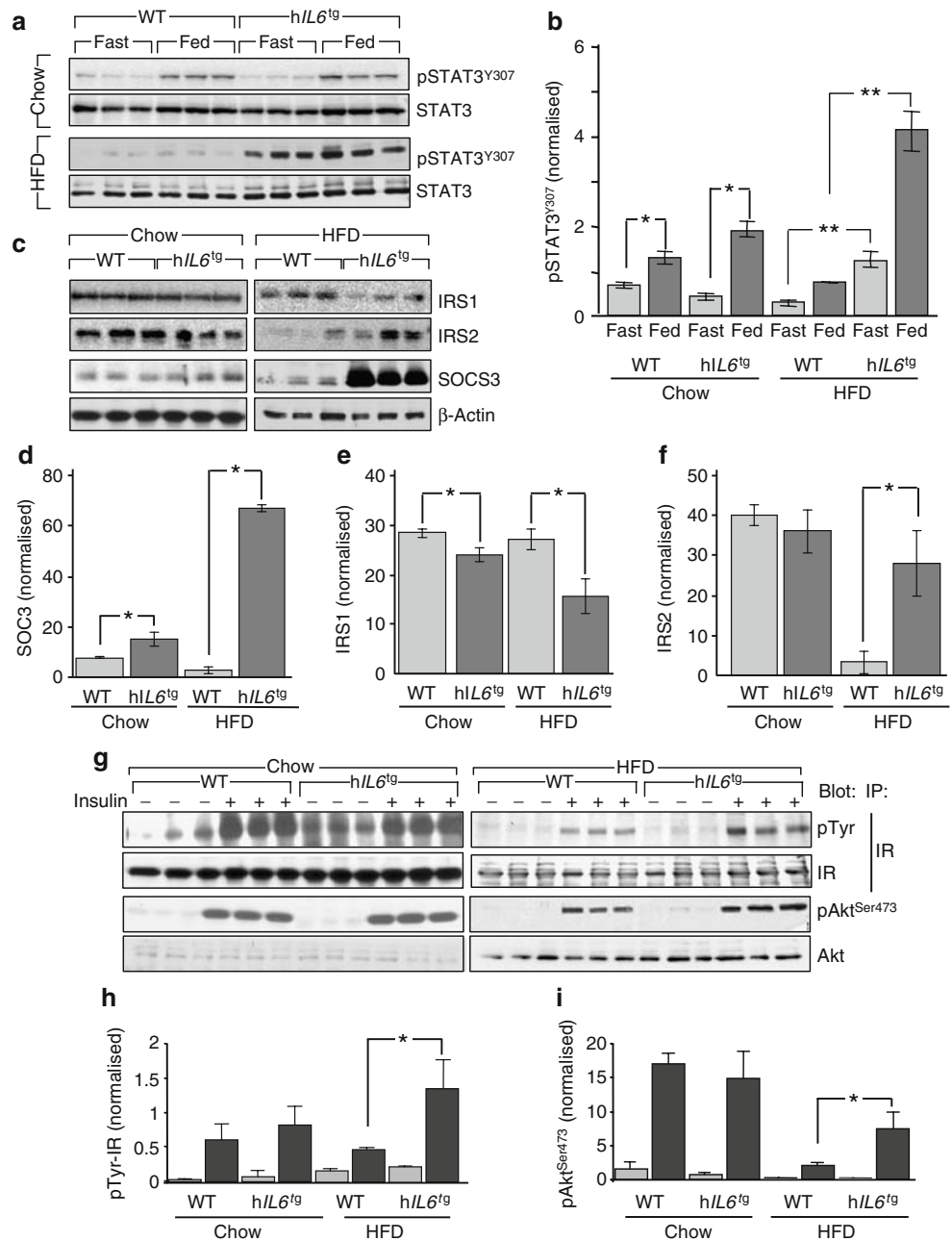
in which leptin plays an important regulatory role [34]. At 6 months of age, wild-type and *hIL6^{tg}* mice were monitored for 72 h in the comprehensive lab animal monitoring system. Food intake by chow-fed wild-type and *hIL6^{tg}* mice was indistinguishable, whereas circulating leptin decreased significantly in the latter (Fig. 4a, b). These results suggest that chow-fed *hIL6^{tg}* mice were more sensitive to leptin than wild-type mice. Compared with chow-fed wild-type mice, leptin increased significantly in HFD-fed wild-type mice, while food intake in both groups was indistinguishable, suggesting that wild-type mice developed leptin resistance on HFD (Fig. 4a, b). By comparison, leptin decreased sevenfold in HFD-fed *hIL6^{tg}* mice, while food intake decreased. Since adipose mass was equal in *hIL6^{tg}* mice on chow or HFD (Fig. 1e), these results suggest that *hIL6* promotes leptin sensitivity in *hIL6^{tg}* mice.

Regardless of diet, the *hIL6^{tg}* mice consumed more oxygen and expelled more CO₂ than wild-type mice during the light and dark phases (Fig. 4c, d). They also displayed 20% more voluntary movement than wild-type controls, when controlling for age, time of day and diet (Fig. 4e). Thus, diet-induced obesity was probably avoided in *hIL6^{tg}* mice by a combination of reduced food intake and increased activity and energy expenditure, which was associated with increased leptin sensitivity.

Central regulation of feeding behaviour in *hIL6^{tg}* mice Intracerebral IL6 injections can prevent obesity in *Il6^{-/-}* mice, suggesting that IL6 might affect central regulation of nutrient homeostasis [26]. We investigated the STAT3 to SOCS3 signalling cascade in the hypothalamus of wild-type and *hIL6^{tg}* mice, where this pathway plays an important role in regulating expression of *Pomc* and *Agrp* [35]. Compared with wild-type mice and regardless of diet, STAT3 phosphorylation was significantly increased in fasted *hIL6^{tg}* mice; however, feeding increased STAT3 phosphorylation only in HFD-fed wild-type mice (Fig. 5a, b). Consistent with these results, chow- and HFD-fed *hIL6^{tg}* mice displayed a significant tenfold increase in hypothalamic SOCS3 protein compared with wild-type mice (Fig. 5c, d).

We used semi-quantitative RT-PCR to determine whether *hIL6* altered expression of *Agrp* and *Pomc*. As expected, feeding suppressed expression of the former and increased expression of the latter in chow-fed wild-type and *hIL6^{tg}* mice (Fig. 5e, f). By comparison, expression of *Agrp* and *Pomc* was reduced and insensitive to feeding in HFD-fed wild-type mice, whereas expression in HFD-fed *hIL6^{tg}* mice responded normally to feeding (Fig. 5e, f). Thus, *hIL6* had multiple effects in the hypothalamus of mice on HFD; these effects were consistent with increased energy utilisation, reduced food intake and changes in peripheral glucose homeostasis.

Fig. 3 Hepatic metabolism and signalling in *hIL6^{tg}* mice. **a** Western blot analysis and **b** quantification of pSTAT3 normalised to STAT3 in liver of wild-type and *hIL6^{tg}* mice aged 24 weeks and maintained on chow and HFD. **c** Western blot analysis of IRS1, IRS2 and SOCS3 normalised to β -actin in liver of mice as above (**a**), with bar graph quantification for SOCS3 (**d**), IRS1 (**e**) and IRS2 (**f**). **g** Insulin receptor (IR) level and tyrosine and Akt^{ser473} phosphorylation in mice as above (**a**), but aged 17 weeks and on chow and HFD stimulated with insulin by i.v. injection. **h, i** Quantification of blot (**g**). Light grey, no insulin; dark grey, insulin. Results, where applicable, were normalised to loading controls and are expressed as mean \pm SEM, $n=6$. * $p<0.05$ and ** $p<0.01$ for wild-type mice vs *hIL6^{tg}* mice



The effect of human *IL6* in *ob/ob* mice *Ob/ob* mice are hyperphagic, obese and develop severe insulin resistance that progresses to diabetes; however, peripheral injections of leptin can restore nutrient homeostasis [36]. We created *ob/ob^{IL6}* mice to determine whether *hIL6* could restore nutrient homeostasis without leptin. As expected, *ob/ob* mice on a chow diet were heavier than wild-type mice between 4 and 12 weeks of age (Fig. 6a). Until 7 weeks, both *hIL6^{tg}* and *ob/ob^{IL6}* mice had reduced body mass compared with wild-type mice (Fig. 6a). However, at 8 weeks the *ob/ob^{IL6}* mice were slightly heavier than wild-type and *hIL6^{tg}* mice, gaining weight until the *ob/ob^{IL6}* mice were only 3.3 ± 0.3 g ($p<$

0.0001) lighter than *ob/ob* mice at 12 weeks of age (Fig. 6a). DEXA analysis showed that *ob/ob^{IL6}* mice accumulated less adipose mass than *ob/ob* mice at 8 weeks of age, whereas the adipose mass was indistinguishable by 12 weeks (Fig. 6b). Compared with wild-type and *hIL6^{tg}* mice, food intake during a 48 h period was significantly greater and indistinguishable in *ob/ob* and *ob/ob^{IL6}* mice (Fig. 6c). By 48 weeks, the *ob/ob* mice were heavier than *ob/ob^{IL6}* mice (*ob/ob* 78 g, *ob/ob^{IL6}* 65 g; $p<0.05$), whereas wild-type and *hIL6^{tg}* mice had identical weights (wild-type 34.7 g, *hIL6^{tg}* 32.8 g; $p=0.7$) (Fig. 6a). Thus, *hIL6* largely failed to restore nutrient homeostasis in *ob/ob^{IL6}* mice.

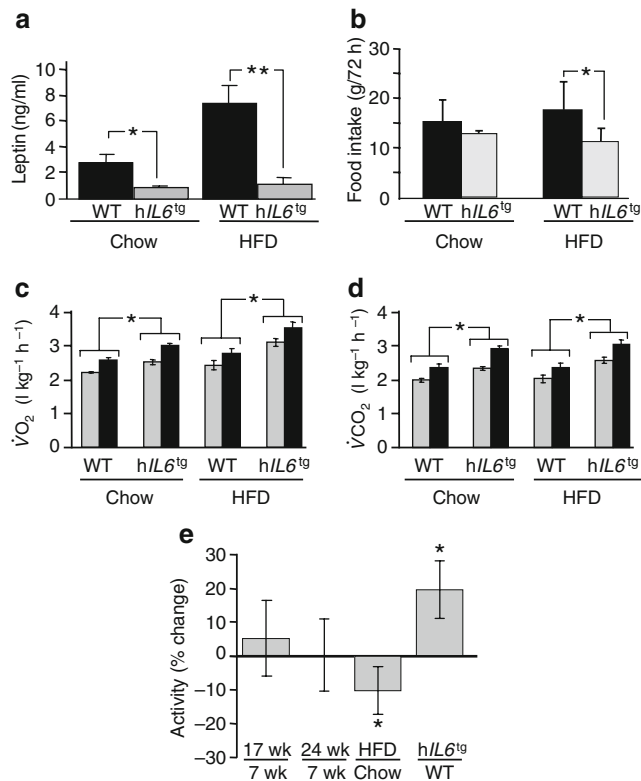


Fig. 4 Energy balance in *hIL6^{tg}* mice. **a** Serum leptin concentrations ($n=10$ – 12) in 24-week-old wild-type and *hIL6^{tg}* mice maintained on chow diet or HFD. **b** Male mice at 24 weeks of age were monitored for 72 h in the comprehensive lab animal monitoring system ($n=10$ mice per group) to assess food intake, **c** oxygen consumption ($\dot{V}O_2$) and **d** carbon dioxide production ($\dot{V}CO_2$). Significance ($*p<0.05$) was determined by generalised linear model. **e** Locomotor activity of mice aged 7, 17 and 24 weeks during the light and dark cycles was analysed by logistic regression (SPSS) of the beam breaks classified above or below the median of 96 breaks in a 5-min test interval. Values for change relative to the control are mean \pm SEM. All other values (**a**–**d**) are means \pm SEM. $*p<0.05$ and $**p<0.01$ for wild-type vs *hIL6^{tg}* mice. Wk, weeks

Despite the fact that the adipose mass was equal in *ob/ob* and *ob/ob^{IL6}* mice at 12 weeks, the fed blood glucose and fasted insulin concentrations were reduced 50% in the latter (Fig. 6d, e). Despite this, *ob/ob* and *ob/ob^{IL6}* mice were diabetic, as their fasting blood glucose and glucose tolerance were dysregulated compared with wild-type and *hIL6^{tg}* mice (Fig. 6f). By 48 weeks of age, fed blood glucose was indistinguishable in wild-type, *hIL6^{tg}* and *ob/ob^{IL6}* mice, and significantly lower than in *ob/ob* mice (Fig. 6d). Thus, *hIL6* promotes glucose homeostasis in old obese *ob/ob^{IL6}* mice.

To directly establish the effect of *hIL6* upon leptin action, we injected leptin intraperitoneally into 12-week-old chow-fed mice. A typical daily dose of leptin (1 mg/kg body weight) decreased food intake and body weight of *ob/ob* and *ob/ob^{IL6}* mice (data not shown). To better distinguish the effect of leptin in these two groups, we

injected a low dose of leptin (0.1 mg/kg) daily for 16 days. Compared with *ob/ob* mice, low-dose leptin significantly decreased body weight and increased locomotor activity of obese *ob/ob^{IL6}* mice; however, low-dose leptin had no effect in chow-fed wild-type or *hIL6^{tg}* mice, and was too low to significantly reduce food consumption by *ob/ob* mice ($\Delta\text{food}=-0.5\pm 0.3$ g, $p>0.05$) (Fig. 6g–i). Low-dose leptin did, however, significantly reduce food consumption by *ob/ob^{IL6}* mice ($\Delta\text{food}=-1.0\pm 0.2$ g/day, $p<0.001$) (Fig. 6i). To establish whether *hIL6* increases central leptin signalling, we compared STAT3 phosphorylation in the hypothalamus of 1-year-old wild-type, *hIL6^{tg}*, *ob/ob* and *ob/ob^{IL6}* mice at 2 h after a single low-dose leptin injection. Compared with wild-type mice, basal and leptin-stimulated STAT3 phosphorylation was significantly increased in *hIL6^{tg}* mice, but significantly decreased in *ob/ob* mice (Fig. 6j). Remarkably, basal and leptin-stimulated STAT3 phosphorylation in *ob/ob^{IL6}* mice was indistinguishable

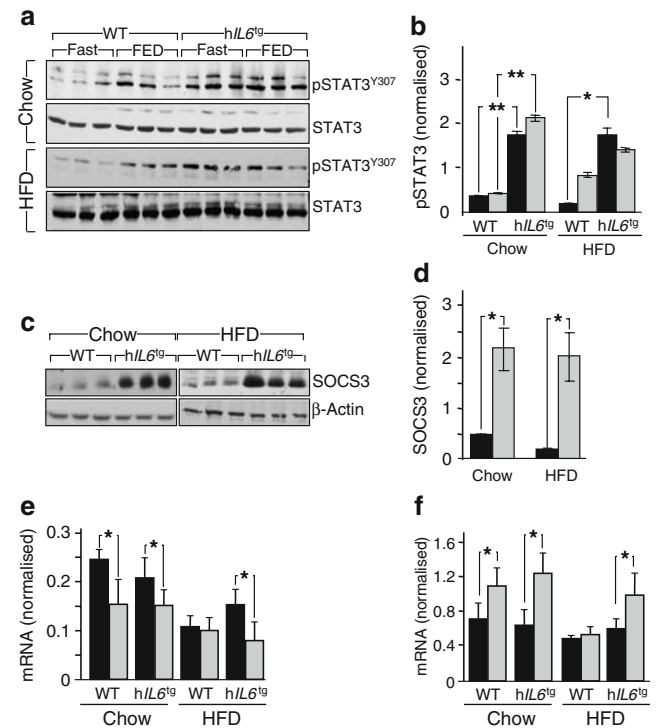


Fig. 5 Central regulation of feeding behaviour in *hIL6^{tg}* mice. **a** Western blot analysis of pSTAT3, normalised to STAT3, in hypothalamus of wild-type and *hIL6^{tg}* mice aged 24 weeks and on chow or HFD (16 h fasting [Fast] and 4 h refeeding [FED]), with **b** quantification as mean \pm SEM ($n=6$). Black bars, fasted; grey bars, fed. **c** Western blot analysis of SOCS3 normalised to β -actin in the hypothalamus of wild-type and *hIL6^{tg}* mice aged 24 weeks and on chow or HFD, with **d** quantification as mean \pm SEM ($n=6$). Black bars, wild-type; grey bars, *hIL6^{tg}*. **e** Levels of *Agrp* mRNA and (**f**) of *Pomc* mRNA from hypothalamus of wild-type and *hIL6^{tg}* mice, as determined by semi-quantitative RT–PCR. Mice were 24 weeks old and maintained on chow or HFD (24 h fasted, black bars; 8 h feeding, grey bars), $n=6$, values means \pm SEM. $*p<0.05$ and $**p<0.01$ for wild-type mice vs *hIL6^{tg}* mice

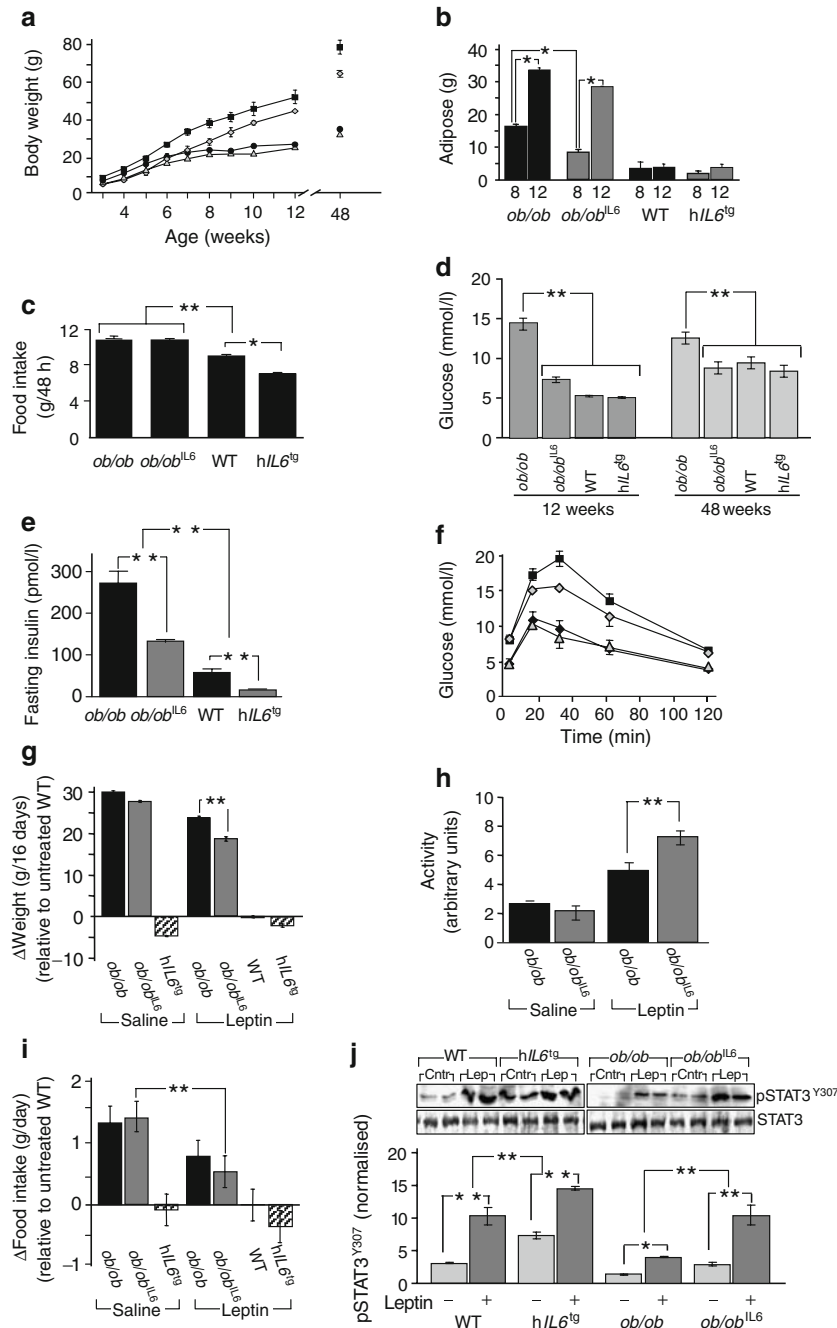


Fig. 6 Effect of human IL6 in *ob/ob* mice. **a** Average body weights of male *ob/ob* (black squares), *ob/ob^{IL6}* (grey rhombs), wild-type (black circles) and *hIL6^{tg}* (grey triangles) mice ($n=6-8$) on chow diet. $p<0.001$ for *ob/ob^{IL6}* vs *ob/ob*; $p=0.7$ for wild-type vs *hIL6^{tg}*. **b** Adipose mass determined by DEXA of mice at 8 and 12 weeks of age ($n=6-8$). **c** Food intake (average \pm SEM, $n=6$) of 12-week-old male mice maintained on a chow diet. **d** Fed blood glucose at 12 and 48 weeks of age; **e** fasting circulating insulin concentration; and **f** glucose tolerance test (key as above [a]). Blood glucose was plotted at the indicated time intervals after intraperitoneal injection of 0.75 g of D-glucose/kg body weight. **g** Body weight relative to chow-fed wild-type mice was determined over 16 days. **h** Spontaneous activity

determined in the comprehensive lab animal monitoring system. **i** Daily food intake (mean \pm SEM) relative to untreated wild-type mice was determined over 16 days. Significant differences in body weight (**g**) and food intake (**i**) were determined by generalised linear model with the presence or absence of leptin injections, *hIL6* transgene or the *ob/ob* genotype were used as categorical predictors, as the initial body weight and day of measurement were fixed at 4.82 and 42.1 g, respectively. **j** Western blot analysis of pSTAT3, normalised to STAT3, in hypothalamus of *ob/ob*, *ob/ob^{IL6}*, wild-type and *hIL6^{tg}* mice, with quantification by bar graph. Values are mean \pm SEM; significant differences were determined by a generalised linear model, using the Bonferroni correction for multiple comparisons. $*p<0.05$; $**p<0.01$

from that in wild-type mice (Fig. 6j). These results support the hypothesis that circulating hIL6 augments central leptin signalling and action, revealing the principle mechanism by which hIL6 promotes nutrient homeostasis in *hIL6^{tg}* mice.

Discussion

Despite evidence of pleotropic and contradictory actions of IL6 upon glucose tolerance in rodent models and human studies, our experiments show clearly that overexpression of *hIL6* in brain and lung of *hIL6^{tg}* mice reduces daily food consumption and promotes energy expenditure. Consistent with the reduced adiposity, circulating insulin decreases and glucose tolerance improves, confirming that hIL6 promotes systemic insulin sensitivity, especially in animals on HFD. Moreover, circulating leptin and daily food consumption decreases, suggesting that hIL6 improves central leptin sensitivity or action.

Previous reports have shown that central leptin signalling requires IL6-mediated signals for a normal response. Thus *Il6^{-/-}* mice slowly develop obesity while circulating leptin increases, and obese *Il6^{-/-}* mice do not respond to intracranial leptin injections [26]. By contrast, circulating leptin decreases significantly in *hIL6^{tg}* mice on chow or HFD. Since leptin signalling is required in the hypothalamus to suppress appetite and promote energy expenditure, hIL6 apparently augments leptin action: otherwise the *hIL6^{tg}* mice would consume more food and accumulate adipose mass [23]. In our study, only *ob/ob^{IL6}* mice responded significantly to low-dose leptin injections with greater locomotor activity accompanied by decreased body weight and food consumption. Thus our results support the hypothesis that life-long hIL6 promotes central leptin signalling, which prevents diet-induced obesity in mice.

The signalling subunit gp130 of the IL6 receptor complex is similar structurally to the intracellular tail of the signalling-isoform of the leptin receptor, isoform b (LepRb) [37]. Consistent with the shared regulation of STAT3 phosphorylation by leptin and IL6, *Pomc* and *Agrp* expression in our study was nearly normal in *hIL6^{tg}* mice on a HFD. However, the effect of hIL6 upon *Pomc* and *Agrp* regulation appears to occur through its effects upon leptin signalling, as *ob/ob* and *ob/ob^{IL6}* mice were equally hyperphagic.

STAT3 to SOCS3 signalling is stimulated by leptin in the hypothalamus and throughout the body by numerous factors including IL6, IFN- γ , IL10, CNTF (ciliary neurotrophic factor) and other gp130 signalling cytokines [2]. However, the leptin response increased while SOCS3 production also increased in the hypothalamus of lean *hIL6^{tg}* mice, suggesting that SOCS3 does not inexorably block the leptin signal. Direct comparison of hypothalamic

STAT3 phosphorylation in *ob/ob* and *ob/ob^{IL6}* mice shows that hIL6 weakly promoted STAT3 phosphorylation in the absence of leptin. Thus, hIL6 largely promotes the leptin-stimulated STAT3 to SOCS3 cascade, which maintains the normal relation between leptin and SOCS3.

We posit that IL6 receptor α -neurons are separate from LepRb-neurons, since hIL6 failed to normalise body weight or food intake in *ob/ob^{IL6}* mice. However, IL6 receptor α -neurons might converge upon a common efferent circuit, ordinarily regulated by LepRb neurons, to augment leptin signalling in *ob/ob* mice or wild-type mice on the HFD. A similar relation appears to exist between CNTF receptor neurons and LepRb neurons [2]. LepRb and the CNTF receptor share structural homology and can activate similar signalling pathways in the hypothalamus. In the absence of CNTF receptor, CNTF can activate gp130 through a homodimer of IL6 receptor and leukaemia inhibitory factor receptor (LIFR) [38]. CNTF can ameliorate obesity by circumventing diet-induced leptin resistance [39]. It remains to be investigated whether CNTF mediates any of the central effects of IL6.

Cell-based experiments suggest that the IL6-stimulated STAT3 to SOCS3 cascade causes hepatic insulin resistance by inhibiting insulin receptor signalling and increasing IRS1 degradation [10]. In parallel with increasing SOCS3 concentrations, IRS1 concentrations in the present study decreased in the postprandial liver of *hIL6^{tg}* mice; however, hIL6 prevented the near complete loss of insulin-stimulated insulin receptor autophosphorylation and the downstream phosphorylation of IRS2 and Akt^{ser473} in animals on HFD, a finding consistent with improved systemic glucose tolerance. Recently, the negative effect of SOCS3 on insulin action has been questioned, since liver-specific *Stat3^{-/-}* mice with low SOCS3 concentrations were unable to suppress hepatic glucose production [32]. The strongest effect of mIL6 upon liver metabolism might depend upon hypothalamic STAT3 signalling, which mediates the normalising effect of leptin on hepatic insulin action in rats on a HFD [35]. Intracerebral ventricular insulin infusion has been shown to increase levels of mIL6 in the liver, which can increase hepatic STAT3 and through that suppress expression of gluconeogenic enzymes [32]. Thus hepatic IL6 to STAT3 signalling triggered by brain insulin action could play an important role in nutrient homeostasis. However, in animals on a chow diet hIL6 might not be sufficient, because STAT3 phosphorylation did not increase in fasted *hIL6^{tg}* mice, while increasing equally in wild-type and *hIL6^{tg}* mice. Whereas the HFD inhibited hepatic STAT3 phosphorylation in our study, hIL6 strongly promoted basal and postprandial STAT3 phosphorylation. Thus a postprandial signal, perhaps initiated by insulin and/or leptin in the hypothalamus, appears to be essential for hepatic STAT3 phosphorylation.

The question of whether IL6 has positive or negative effects on metabolism is the subject of continuing controversy [8]. The hypothesis that IL6 induces insulin resistance is challenged by findings that regular physical exercise increases insulin sensitivity while promoting production and release of IL6 from contracting skeletal muscle [40, 41]. IL6 can also increase peripheral insulin sensitivity and glucose tolerance by activating AMP-activated protein kinase (AMPK) in muscle [17, 42]. Here, however, hIL6 had no effect on AMPK phosphorylation or activity in *hIL6^{tg}* mice (data not shown). Further investigation regarding a potential of AMPK to mediate some of the effects of hIL6 is required.

The relation between IL6 and leptin in the central nervous system might play an important role on the effect of exercise upon nutrient homeostasis. Moderate exercise promotes peripheral insulin sensitivity and suppresses weight gain [43]. Human IL6 secretion from skeletal muscle is dramatically increased during and after exercise [44]. Our results are consistent with the hypothesis that the effect of exercise upon nutrient homeostasis and insulin sensitivity might be mediated through central effects of muscle-derived IL6 in promoting central leptin signalling.

Our results are consistent with the hypothesis that decreased fat mass in *hIL6^{tg}* mice, especially those on HFD, arises through increased energy expenditure. Thus oxygen consumption, CO₂ production and physical activity were increased in the *hIL6^{tg}* mice. These data are consistent with previous reports that a single intracranial injection of IL6 increases oxygen consumption and energy expenditure by rats [22, 26].

Chronic cerebral expression of mIL6, using an *IL6* transgene under the control of glial fibrillary acidic protein (*GFAP*) promoter, activates the hypothalamic–pituitary–adrenal axis, which increases corticosterone concentrations in stressed mice [45]. However, in our experiments, plasma corticosterone concentrations were barely increased in unstressed *hIL6^{tg}* mice compared with control mice and increased equally in both mice during stress (data not shown). However, as in our *hIL6^{tg}* mice, the plasma leptin concentration was reduced in *GFAP-IL6* transgenic mice [45]. Since circulating IL6 was not elevated in those *GFAP-IL6* mice, those results support the hypothesis that hIL6 promotes leptin action in the central nervous system.

Previous reports have shown that transgenic *IL6* causes various pathologies of the immune system that can be fatal to mice [46, 47]. Human *IL6* in C57BL/6J mice under the control of human immunoglobulin heavy-chain enhancer develop mesangial proliferative glomerulonephritis with massive IgG1 plasmacytosis [48]. *MTI/IL6* transgenic mice expressing murine IL6 constitutively in the liver developed progressive kidney damage and died between 12 and 20 weeks of age [49]. Hepatic inflammation occurs in

transgenic mouse secreting mIL6 from muscle [14]. Despite the above, our *hIL6^{tg}* mice with circulating hIL6 secreted from brain and lung never displayed hepatic inflammation, acute inflammatory response or systemic inflammation. Perhaps the sites of IL6 secretion are critical for its systemic effect. In any case, *hIL6^{tg}* mice provide a unique system to investigate the role of hIL6 in central and peripheral nutrient homeostasis.

In summary, hIL6 protects mice from insulin resistance and obesity. Since this effect was not observed in *ob/ob* mice, hIL6 apparently augments central leptin action without substituting for leptin. Due to its immunoreactive nature, IL6 might never be a successful therapeutic treatment strategy; however, prolonged treatment with IL6 homologues with high accessibility to the central nervous system might show therapeutic promise in anti-obesity therapy.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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References

1. Glund S, Krook A (2008) Role of interleukin-6 signalling in glucose and lipid metabolism. *Acta Physiol (Oxf)* 192:37–48
2. Febbraio MA (2007) gp130 receptor ligands as potential therapeutic targets for obesity. *J Clin Invest* 117:841–849
3. Gabay C (2006) Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 8(Suppl 2):S3
4. Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* 444:860–867
5. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 9:414–417
6. Carey AL, Bruce CR, Sacchetti M et al (2004) Interleukin-6 and tumor necrosis factor- α are not increased in patients with type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. *Diabetologia* 47:1029–1037
7. Petersen AM, Pedersen BK (2006) The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J Physiol Pharmacol* 57 (Suppl 10):43–51
8. Pedersen BK, Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev* 88:1379–1406
9. Senn JJ, Klover PJ, Nowak IA, Mooney RA (2002) Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 51:3391–3399
10. Senn JJ, Klover PJ, Nowak IA et al (2003) Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-

- dependent insulin resistance in hepatocytes. *J Biol Chem* 278:13740–13746
11. Kim HJ, Higashimori T, Park SY et al (2004) Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. *Diabetes* 53:1060–1067
 12. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA (2003) Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* 52:2784–2789
 13. Klover PJ, Clementi AH, Mooney RA (2005) Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* 146:3417–3427
 14. Franckhauser S, Elias I, Rotter SV et al (2008) Overexpression of Il6 leads to hyperinsulinaemia, liver inflammation and reduced body weight in mice. *Diabetologia* 51:1306–1316
 15. van Hall G, Steensberg A, Sacchetti M et al (2003) Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab* 88:3005–3010
 16. Petersen EW, Carey AL, Sacchetti M et al (2005) Acute IL-6 treatment increases fatty acid turnover in elderly humans in vivo and in tissue culture in vitro. *Am J Physiol Endocrinol Metab* 288: E155–E162
 17. Carey AL, Steinberg GR, Macaulay SL et al (2006) Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* 55:2688–2697
 18. Trujillo ME, Sullivan S, Harten I, Schneider SH, Greenberg AS, Fried SK (2004) Interleukin-6 regulates human adipose tissue lipid metabolism and leptin production in vitro. *J Clin Endocrinol Metab* 89:5577–5582
 19. Pedersen BK, Bruunsgaard H, Ostrowski K et al (2000) Cytokines in aging and exercise. *Int J Sports Med* 21(Suppl 1):S4–S9
 20. Pedersen BK, Fischer CP (2007) Physiological roles of muscle-derived interleukin-6 in response to exercise. *Curr Opin Clin Nutr Metab Care* 10:265–271
 21. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK (2003) Exercise and IL-6 infusion inhibit endotoxin-induced TNF- α production in humans. *FASEB J* 17:884–886
 22. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO (2002) Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochem Biophys Res Commun* 293:560–565
 23. Myers MG, Cowley MA, Munzberg H (2007) Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 70:537–556
 24. Takahashi N, Patel HR, Qi Y, Dushay J, Ahima RS (2002) Divergent effects of leptin in mice susceptible or resistant to obesity. *Horm Metab Res* 34:691–697
 25. Spiegelman BM, Flier JS (2001) Obesity and the regulation of energy balance. *Cell* 104:531–543
 26. Wallenius V, Wallenius K, Ahren B et al (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 8:75–79
 27. Lieskovska J, Guo D, Derman E (2002) IL-6-overexpression brings about growth impairment potentially through a GH receptor defect. *Growth Horm IGF Res* 12:388–398
 28. Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF (1999) Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* 23:32–40
 29. Dong X, Park S, Lin X, Capps K, Yi X, White MF (2006) Irs1 and Irs2 signaling is essential for hepatic glucose homeostasis and systemic growth. *J Clin Invest* 116:101–114
 30. Han CY, Chiba T, Campbell JS et al (2006) Reciprocal and coordinate regulation of serum amyloid A vs apolipoprotein A-I and paraoxonase-1 by inflammation in murine hepatocytes. *Arterioscler Thromb Vasc Biol* 26:1806–1813
 31. Migita K, Miyashita T, Maeda Y et al (2005) An active metabolite of leflunomide, A77 1726, inhibits the production of serum amyloid A protein in human hepatocytes. *Rheumatology (Oxford)* 44:443–448
 32. Inoue H, Ogawa W, Asakawa A et al (2006) Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* 3:267–275
 33. Lebrun P, Van Obberghen E (2008) SOCS proteins causing trouble in insulin action. *Acta Physiol (Oxf)* 192:29–36
 34. Myers MG Jr (2004) Leptin receptor signaling and the regulation of mammalian physiology. *Recent Prog Horm Res* 59:287–304
 35. Buettner C, Poci A, Muse ED, Etgen AM, Myers MG Jr, Rossetti L (2006) Critical role of STAT3 in leptin's metabolic actions. *Cell Metab* 4:49–60
 36. Seufert J, Kieffer TJ, Habener JF (1999) Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proc Natl Acad Sci U S A* 96:674–679
 37. Ernst M, Jenkins BJ (2004) Acquiring signalling specificity from the cytokine receptor gp130. *Trends Genet* 20:23–32
 38. Schuster B, Kovaleva M, Sun Y et al (2003) Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an alpha-receptor for CTNF. *J Biol Chem* 278:9528–9535
 39. Steinberg GR, Watt MJ, Fam BC et al (2006) Ciliary neurotrophic factor suppresses hypothalamic AMP-kinase signaling in leptin-resistant obese mice. *Endocrinology* 147:3906–3914
 40. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK (2004) Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 53:1643–1648
 41. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund PB (2000) Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* 529(Pt 1):237–242
 42. Ruderman NB, Keller C, Richard AM et al (2006) Interleukin-6 regulation of AMP-activated protein kinase. Potential role in the systemic response to exercise and prevention of the metabolic syndrome. *Diabetes* 55(Suppl 2):S48–S54
 43. Flores MB, Fernandes MF, Ropelle ER et al (2006) Exercise improves insulin and leptin sensitivity in hypothalamus of Wistar rats. *Diabetes* 55:2554–2561
 44. Pedersen BK (2007) IL-6 signalling in exercise and disease. *Biochem Soc Trans* 35:1295–1297
 45. Raber J, O'Shea RD, Bloom FE, Campbell IL (1997) Modulation of hypothalamic–pituitary–adrenal function by transgenic expression of interleukin-6 in the CNS of mice. *J Neurosci* 17:9473–9480
 46. Suematsu S, Matsuda T, Aozasa K et al (1989) IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc Natl Acad Sci U S A* 86:7547–7551
 47. Hirano T, Suematsu S, Matsusaka T, Matsuda T, Kishimoto T (1992) The role of interleukin 6 in plasmacytomagenesis. *Ciba Found Symp* 167:188–196
 48. Katsume A, Miyai T, Suzuki H et al (1997) Interleukin-6 overexpression cannot generate serious disorders in severe combined immunodeficiency mice. *Clin Immunol Immunopathol* 82:117–124
 49. Fattori E, Della RC, Costa P et al (1994) Development of progressive kidney damage and myeloma kidney in interleukin-6 transgenic mice. *Blood* 83:2570–2579