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ARTICLE

Adipocyte-specific deficiency of Janus kinase (JAK) 2 in mice impairs lipolysis and increases body weight, and leads to insulin resistance with ageing

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Abstract
Aims/hypothesis The growing obesity epidemic necessitates a better understanding of adipocyte biology and its role in metabolism. The Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway mediates signalling by numerous cytokines and hormones that regulate adipocyte function, illustrating the physiological importance of adipose JAK–STAT. The aim of this study was to investigate potential roles of adipocyte JAK2, an essential player in the JAK–STAT pathway, in adipocyte biology and metabolism.
Methods We generated adipocyte-specific *Jak2* knockout (A-*Jak2* KO) mice using the Cre-loxP system with *Cre* expression driven by the *Ap2* (also known as *Fabp4*) promoter.
Results Starting at 2–3 months of age, male and female A-*Jak2* KO mice gradually gained more body weight than control littermates primarily due to increased adiposity. This was associated with reduced energy expenditure in A-*Jak2* KO mice. In perigonadal adipose tissue, the expression of numerous genes involved in lipid metabolism was differentially regulated. In addition, adipose tissue from A-*Jak2* KO mice displayed impaired lipolysis in response to isoprenaline, growth hormone and leptin stimulation, suggesting that adipose JAK2 directly modulates the lipolytic program. Impaired lipid homeostasis was also associated with disrupted adipokine secretion. Accordingly, while glucose metabolism was normal at 2 months of age, by 5–6 months of age, A-*Jak2* KO mice had whole-body insulin resistance.
Conclusions/interpretation Our results suggest that adipocyte JAK2 plays a critical role in the regulation of adipocyte biology and whole-body metabolism. Targeting of the JAK–STAT pathway could be a novel therapeutic option for the treatment of obesity and type 2 diabetes.

Keywords Adipocyte · Glucose homeostasis · JAK2 · Janus kinase 2 · Metabolism · Obesity

Abbreviations

A-*Jak2* KO	Adipocyte-specific *Jak2* knockout
AP2	Adipocyte protein 2
CNTF	Ciliary neurotrophic factor
GH	Growth hormone
GHR	GH receptor
GTT	Glucose tolerance test
ITT	Insulin tolerance test
JAK	Janus kinase
LIF	Leukaemia inhibitory factor
OSM	Oncostatin M
RER	Respiratory exchange ratio
STAT	Signal transducer and activator of transcription
$\dot{V}O_2$	Volume of oxygen consumption

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Introduction

Adipose tissue mass is maintained by the balance between lipid synthesis and catabolism. Disruption in this equilibrium has been implicated in the pathophysiology of various metabolic disorders, particularly obesity and type 2 diabetes [1–3]. A better understanding of the regulatory mechanisms governing adipocyte biology and homeostasis is therefore crucial, given the growing worldwide epidemic of obesity [4].

The Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway mediates the signal transduction of numerous cytokines, growth factors and hormones that regulate adipocyte development and function [5]. Several cytokines secreted by adipocytes also use this signalling pathway, illustrating the physiological importance of JAK–STATs in adipocyte biology. We and others have recently shown that hepatocyte-specific deletion of *Jak2*, a ubiquitously expressed member of the JAK kinase family, results in profound lipid accumulation in liver tissue, suggesting a role for JAK2 in lipid metabolism [6, 7]. JAK2 is present in pre-adipocytes and mature adipocytes [8], as well as in adipose tissue [9]. Its mRNA abundance has been shown to be downregulated in human omental adipose tissue under obese conditions [10], suggesting a potential role in the regulation of adipose tissue physiology.

During adipogenesis, JAK2 is activated within 2 h of adipogenic induction and has been shown to act upstream of STAT3 activation [11]. Inhibition of JAK2 with a small-molecule inhibitor or small interfering RNA attenuated the differentiation of 3T3-L1 adipocytes [11]. JAK2 has also been shown to be required for growth hormone (GH)-dependent differentiation of pre-adipocytes via activation of STAT5 [12], a critical regulator of adipocyte development [13–15].

In mature adipocytes, JAK2 can be activated by several cytokines and hormones, most notably leptin, GH, prolactin, IL-6, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and IFN-γ. These cytokines and hormones regulate many aspects of adipose tissue biology. For example, leptin [16], IL-6 [17, 18], IFN-γ [19], GH [20] and prolactin [21] have all been shown to exert lipolytic effects on isolated adipocytes and adipose tissue. Furthermore, leptin, CNTF and LIF can inhibit fatty acid synthesis in white adipose tissue either via a central mechanism [22] or by inhibiting the expression of lipogenic enzymes [23, 24]. In addition to regulating adipose expansion, CNTF enhances the expression of uncoupling protein 1 induced by β3-adrenergic stimulation in brown adipose tissue [25]. OSM, a member of the IL-6 family of cytokines, suppresses adiponectin expression and induces dedifferentiation of adipocytes [26]. Whether all these effects are mediated by JAK2 and to what extent JAK2 activation is required for them to occur has not been established.

At the cellular level, JAK2 functions primarily by transducing signals from cytokines and activating downstream STAT proteins. There is also evidence of STAT-independent functions of JAK2 in adipocytes. It has been shown that fatty acid binding protein 4/adipocyte protein 2 (AP2), a highly-expressed lipid-binding protein in adipocytes, associated with the unphosphorylated form of JAK2 and attenuated its signalling [27]. Nevertheless, the cell-specific functions of adipocyte JAK2 are not well understood.

Recently, adipocyte-specific deletion of *Jak2* driven by the adiponectin promoter has been shown to result in reduced lipolysis and increased body fat [28]. The molecular mechanisms behind this and the metabolic consequences are not clear. In this study, we studied the metabolic effects of adipose JAK2 deficiency. We show that mice with impaired adipose JAK2 signalling driven by the *Ap2* (also known as *Fabp4*) promoter develop profound adiposity when on a regular chow diet, and that this is associated with reduced energy expenditure. Thus, while glucose metabolism in young mice is normal, these mice develop whole-body insulin resistance as they age.

Methods

Animals Adipocyte-specific *Jak2* knockout (A-*Jak2* KO) mice were generated by breeding mice with the *Jak2* gene flanked by loxP sites ($Jak2^{fl/fl}$) [29, 30] (kindly provided by Kay-Uwe Wagner, University of Nebraska Medical Center, Omaha, NE, USA) with mice expressing Cre recombinase under the control of the *Ap2* promoter ($aP2Cre^{+}$; purchased from Jackson Laboratory, Bar Harbor, ME, USA). The resulting $aP2Cre^{+}Jak2^{+/fl}$ mice were intercrossed to generate $aP2Cre^{+}Jak2^{+/+}$, $aP2Cre^{+}Jak2^{+/fl}$ and $aP2Cre^{+}Jak2^{fl/fl}$ (herein referred to as A-*Jak2* KO) mice. Mice were maintained on a mixed 129Sv and C57BL/6 background, and housed in a pathogen-free facility at the Toronto Medical Discovery Tower (Toronto, ON, Canada) with a 12 h light–dark cycle and free access to standard irradiated rodent chow (5% energy from fat; Harlan Teklad, Indianapolis, IN, USA). All animal experimental protocols were approved by the Toronto General Research Institute Animal Care Committee.

In vivo metabolic analyses Blood glucose measurements, the glucose tolerance test (GTT) and insulin tolerance test (ITT), and quantification of glucose-stimulated insulin secretion were done as previously described [6]. For insulin signalling experiments, mice fasted overnight were injected i.p. with human regular insulin (5 U/kg) or PBS. Tissues were removed 10 min later and snap-frozen in liquid nitrogen. Rectal temperature was measured in fed mice between 10:00 and 11:00 hours. To measure energy expenditure, mice were individually housed in a comprehensive laboratory animal monitoring system (Columbus Instruments, Columbus, OH, USA) with free access to food and water. After 24 h acclimatisaton to the apparatus, data for 24 h were collected.

Body composition The body composition of 5-month-old male mice with free access to chow was assessed using nuclear magnetic resonance spectroscopy (Biospec 70/30; Bruker, Ettlingen, Germany). Fat volume was quantified using a combination of semi-automated and manual segmentation tools in MIPAV software (Version 7.0.1; National Institute of Health, Bethesda, MD, USA).

Analyses of serum variables Overnight-fasted mice were anaesthetised and blood was collected by cardiac puncture. Serum insulin levels were measured by a mouse insulin ELISA kit (Crystal Chem, Downers Grove, IL, USA). Serum GH and IGF-1 levels were determined by radioimmunoassay, and serum adiponectin, leptin, TNF-α and IL-6 levels by the Luminex 100 System (Luminex, Austin, TX, USA) at the Mouse Metabolic Phenotyping Centre (Vanderbilt University, Nashville, TN, USA). Serum total cholesterol, triacylglycerol, HDL-cholesterol and NEFA were also assayed at the Mouse Metabolic Phenotyping Centre (Vanderbilt University).

Histological analysis Liver, pancreas and adipose tissue were removed, fixed and processed to paraffin blocks. Tissue sections were stained with haematoxylin and eosin. Adipocyte size was measured using cellSens software (Olympus, Tokyo, Japan). Adipocyte number per fat pad was calculated from the total fat pad volume: average adipocyte volume ratio, using the method developed by Lemonnier [31]. Immunohistochemistry analysis was performed on pancreatic sections using anti-insulin antibody (Dako, Carpinteria, CA, USA) as previously described [32]. Scanned sections were analysed with ImageScope version 11.0.2.716 software (Aperio Technologies, Vista, CA, USA).

Ex vivo lipolysis Perigonadal fat pads were surgically removed from 6-month-old mice, cut into 50 mg pieces and incubated in Krebs–Ringer bicarbonate buffer containing 2% wt/vol. fatty acid-free BSA. After a preincubation period of 1 h at 37°C, isoprenaline (Sigma, St Louis, MO, USA), recombinant murine leptin (PeproTech, Rocky Hill, NJ, USA) or recombinant mouse GH (National Hormone and Peptide Program, Torrance, CA, USA) was added directly to the incubation medium. After 2 h of incubation, glycerol release into the medium was determined using a free glycerol reagent (Sigma).

RNA isolation and quantitative RT-PCR Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse-transcribed with random primers using M-MLV enzyme (Invitrogen), and quantitative real-time PCR was performed using SYBR Green master mix on a 7900HT Fast-Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Each sample was run in triplicate.

Immunoblotting Tissues were mechanically homogenised in ice-cold lysis buffer and centrifuged for 10 min at 14,000*g* and 4°C. The resulting supernatant fraction was separated by SDS-PAGE and immunoblotted with antibodies to phospho-Akt (S473), total Akt, total JAK2 and GAPDH (Cell Signaling Technology, Danvers, MA, USA).

Statistical analysis Data are presented as mean±SEM. Values were analysed by two-tailed independent-sample Student's *t* test or one-way ANOVA, as appropriate, using GraphPad Prism version 5 (GraphPad Software, La Jolla, CA, USA). A value of $p<0.05$ was considered statistically significant.

Results

Disruption of adipocyte JAK2 increases body weight A-*Jak2* KO mice were viable and fertile with no gross abnormalities compared with littermate controls. Quantitative RT-PCR and western blot analyses showed a significant reduction of *Jak2* mRNA and protein abundance in the inguinal, perigonadal and brown adipose tissue (Fig. 1a, b). JAK2 abundance in other tissues, including liver, skeletal muscle, brain and macrophages was not affected (Fig. 1c). Selective deficiency of JAK2 in adipose tissue did not affect circulating levels of GH (which requires JAK2 for signal transduction) and its downstream target IGF-1 (electronic supplementary material [ESM] Fig. 1a, b).

To study the role of adipocyte JAK2 in body weight regulation, we followed A-*Jak2* KO mice and littermate controls on a standard chow diet and monitored their body weight monthly. As shown in Fig. 1d, e, starting from 3 months of age for males and 2 months of age for females, A-*Jak2* KO mice progressively gained more body weight than $aP2Cre^{+}Jak2^{+/+}$ and $aP2Cre^{+}Jak2^{+/fl}$ littermate controls. This was particularly evident in female mice (Fig. 1f). Interestingly, heterozygous $aP2Cre^{+}Jak2^{+/fl}$ mice exhibited a similar growth curve to $aP2Cre^{+}Jak2^{+/+}$ mice, suggesting that gene dosage had no effect on body weight. For subsequent analyses, we combined data from $aP2Cre^{+}Jak2^{+/+}$ and $aP2Cre^{+}Jak2^{+/fl}$ mice, collectively referring to them as controls. By 6 months of age, male and female A-*Jak2* KO mice weighed approximately 40.3% and 65.8% more than their control littermates, respectively (Fig. 1g, h). Body length was slightly greater (Fig. 1i, j), while BMI was significantly higher in A-*Jak2* KO mice (Fig. 1k, l).

Disruption of adipocyte JAK2 leads to increased adiposity To determine the underlying basis for the increased body weight, we measured body composition by nuclear magnetic resonance spectroscopy. Lean body mass was similar in A-*Jak2* KO mice and control littermates (Fig. 2a, b). However, fat mass and body fat content were significantly increased in

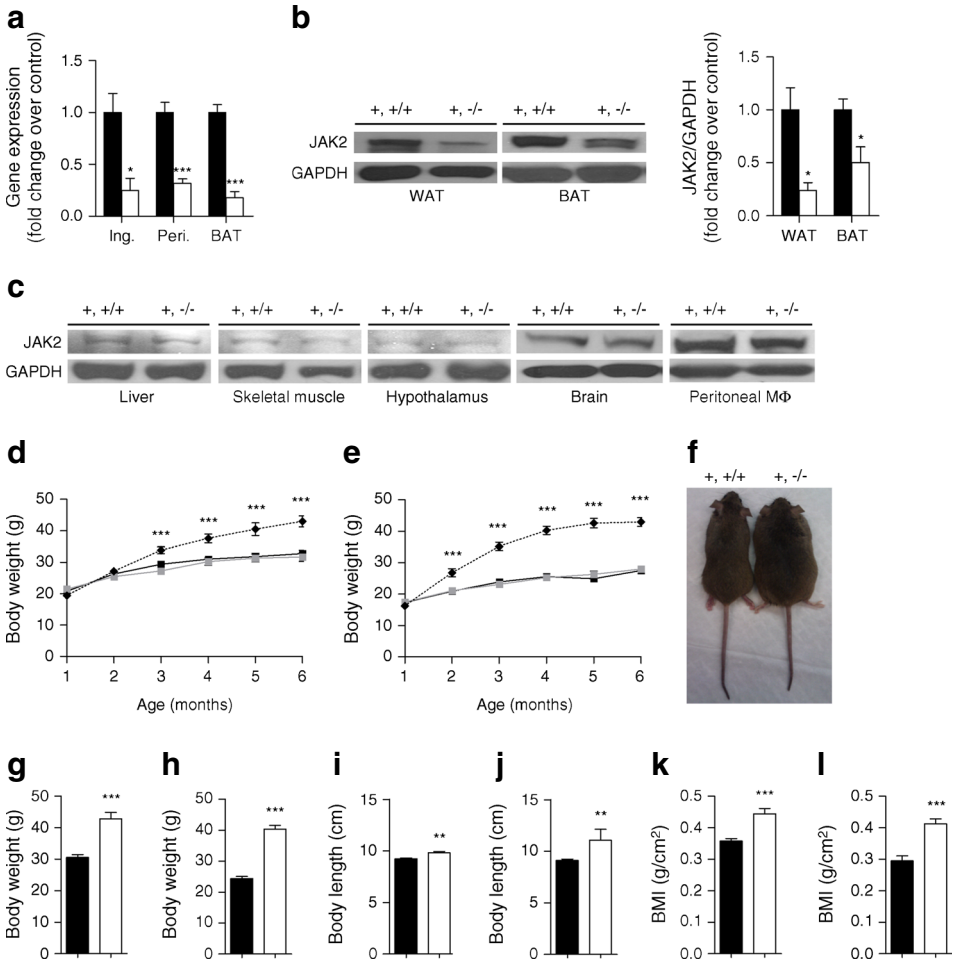

Fig. 1 Increased body weight in A-*Jak2* KO mice. (**a**) mRNA expression of *Jak2* in inguinal (Ing.), perigonadal (Peri.) and interscapular brown adipose tissue (BAT) from *aP2Cre*$^{+}$*Jak2*$^{+/+}$ (black bars) and A-*Jak2* KO (white bars) mice at 5–6 months of age. Values are normalised to 18S mRNA levels and expressed as fold changes relative to the *aP2Cre*$^{+}$*Jak2*$^{+/+}$ group; $n \geq 3$. (**b**) Lysates from perigonadal white adipose tissue (WAT) and BAT from *aP2Cre*$^{+}$*Jak2*$^{+/+}$ and A-*Jak2* KO mice were prepared and processed for immunoblotting for JAK2. Protein band intensity was quantified by ImageJ software and normalised to the *aP2Cre*$^{+}$*Jak2*$^{+/+}$ group; n=4–6. (**c**) Tissue lysates from 5- to 6-month-old mice were prepared and processed for immunoblotting for JAK2. MΦ, macrophages. (**d**) Growth curves of male and (**e**) female *aP2Cre*$^{+}$*Jak2*$^{+/+}$ (black squares), *aP2Cre*$^{+}$*Jak2*$^{+/fl}$ (grey squares) and A-*Jak2* KO (black diamonds) mice and littermate controls; $n \geq 8$. (**f**) A representative photograph of female *aP2Cre*$^{+}$*Jak2*$^{+/+}$ and A-*Jak2* KO littermates at 6 months of age. (**g–l**) Body weight, body length (measured from snout to anus) and BMI at 5–6 months of age in (**g**, **i**, **k**) male ($n \geq 10$) and (**h**, **j**, **l**) female ($n \geq 7$) control (black bars) and A-*Jak2* KO (white bars) mice. All results are mean ± SEM; *$p<0.05$, **$p<0.01$ and ***$p<0.001$

A-*Jak2* KO mice at 5 months of age (Fig. 2a–c). Similarly, when we isolated and weighed fat pads from mice at 5–6 months of age, all fat pads examined from A-*Jak2* KO mice weighed more than those examined from control littermates. The differences reached statistical significance for absolute fat pad weight (Fig. 2d, g) and per cent total body weight (Fig. 2e, h). Notably, greater differences between genotypes were observed in both sexes for the inguinal depot compared with the perigonadal depot. On the other hand, the absolute weight of other organs was comparable between the genotype groups, with the exception of the liver, which weighed more in female A-*Jak2* KO mice (Fig. 2f, i). Together, these results suggest that adipocyte JAK2 deficiency leads to higher body weight due to increased adiposity.

Haematoxylin and eosin staining revealed the presence of enlarged adipocytes in inguinal, perigonadal and interscapular brown adipose tissue in A-*Jak2* KO mice (Fig. 2j). Analysis of adipocyte size, distribution and number suggested a threefold increase in the average size of perigonadal adipocytes, with no change in adipocyte number (Fig. 2k–m). Similar changes were observed for adipocytes from the inguinal depot (data not shown). These results indicate that the adipose tissue expansion observed in A-*Jak2* KO mice is due to adipocyte hypertrophy, not to an increase in cell number.

A-Jak2 KO mice have normal energy metabolism at 1 month of age, but display reduced energy expenditure as they age To delineate the physiological mechanisms that would account

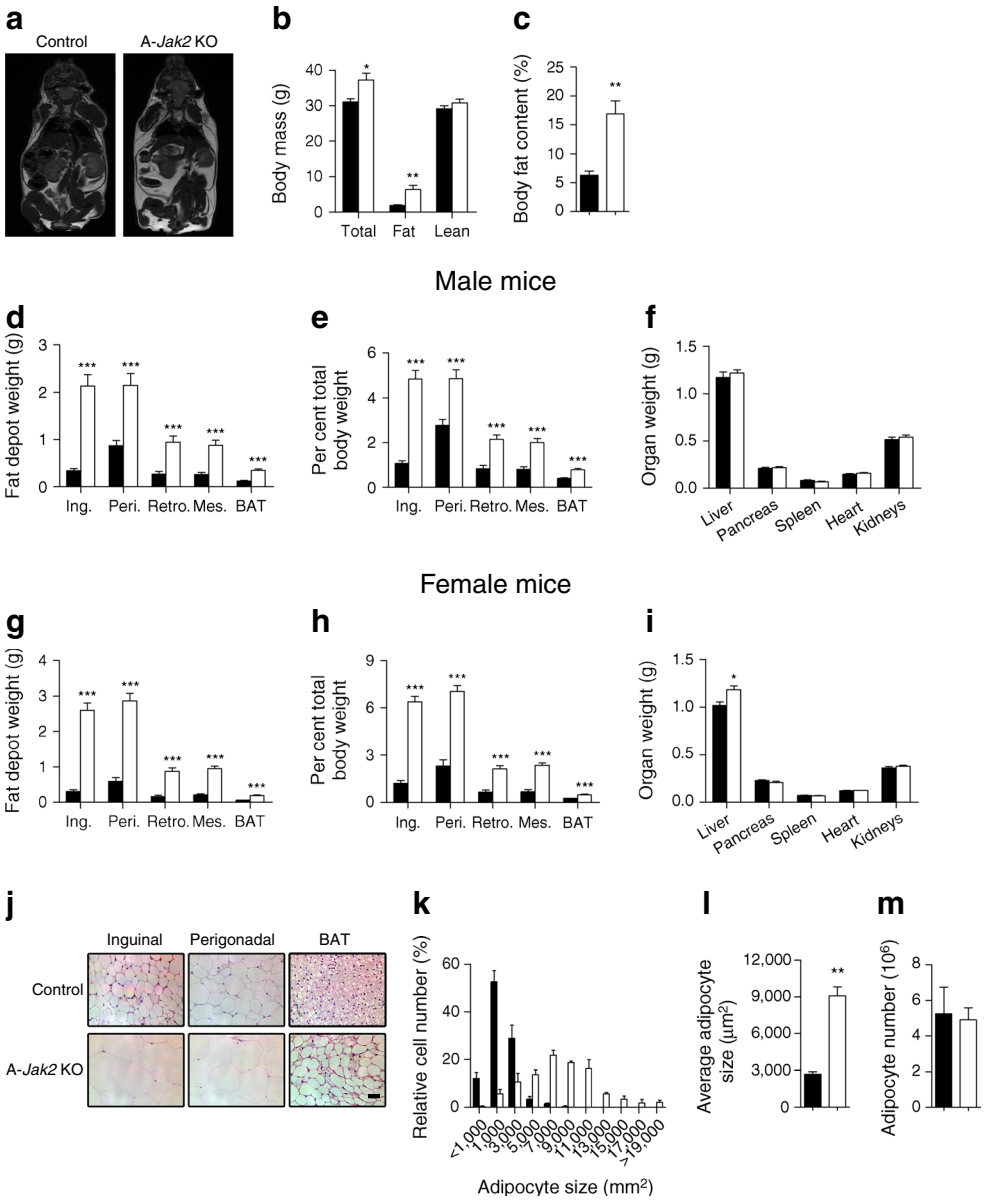

Fig. 2 Increased adiposity in A-*Jak2* KO mice. (**a**) Body composition of male mice at 5 months of age as measured by nuclear magnetic resonance spectroscopy, with (**b**, **c**) quantification as indicated; n=5. Black bars, control; white bars, A-*Jak2* KO. (**d**, **e**) Inguinal (Ing.), perigonadal (Peri.), retroperitoneal (Retro.), mesenteric (Mes.) and interscapular brown adipose tissue (BAT) fat pads were removed from 5- to 6-month-old male (n≥11) and (**g**, **h**) female (n≥10) mice and weighed. Results are expressed as absolute fat pad weight or per cent total body weight. (**f**) Liver, pancreas, spleen, heart and kidneys were removed from 5- to 6-month-old male (n≥10) and (**i**) female (n≥6) mice and weighed. Results are expressed as absolute organ weight. (**j**) Representative micrographs of haematoxylin and eosin staining of inguinal, perigonadal and interscapular BAT sections from 5- to 6-month-old female mice. Scale bar, 40 μm. (**k**) Quantification of adipocyte size distribution, (**l**) average adipocyte size and (**m**) cell number in perigonadal fat pads from tissue sections as above (**j**); n=3. All results are mean ± SEM; *p<0.05, **p<0.01 and ***p<0.001

for the apparent positive energy balance in A-*Jak2* KO mice, we measured their food intake and energy expenditure by indirect calorimetry. At 1 month of age when A-*Jak2* KO mice had a similar body weight to control littermates, there were no significant differences in absolute food or water intake, volume of oxygen consumption ($\dot{V}O_2$), respiratory exchange ratio (RER), physical activity or body temperature between the genotype groups in both sexes (ESM Fig. 2a-l). These data suggest that adipose JAK2 deficiency had no direct effect on energy balance.

By 5–6 months of age, A-*Jak2* KO mice still had normal absolute daily food and water intake, suggesting no change in energy intake (Fig. 3a, b, g, h). A-*Jak2* KO mice were also comparable to control littermates in terms of the energy source utilised, as indicated by a similar RER (Fig. 3c, i). On the other hand, $\dot{V}O_2$ was significantly lower in A-*Jak2* KO mice of both sexes (Fig. 3d, j), consistent with their reduced energy expenditure. This was associated with significantly reduced physical activity in female A-*Jak2* KO mice (Fig. 3k). Male A-*Jak2* KO mice also showed a non-significant decrease in physical activity compared with control littermates (Fig. 3e). In contrast, body temperature was not altered by adipocyte JAK2 deficiency (Fig. 3f, l). Together, these results suggest that the progressive increase in body weight in A-*Jak2* KO mice may be due, at least in part, to decreased energy expenditure.

Adipose JAK2 deficiency leads to impaired lipolysis To elucidate molecular changes in adipose tissue that could account for the observed phenotype in A-*Jak2* KO mice, we analysed the expression of genes involved in lipid and glucose

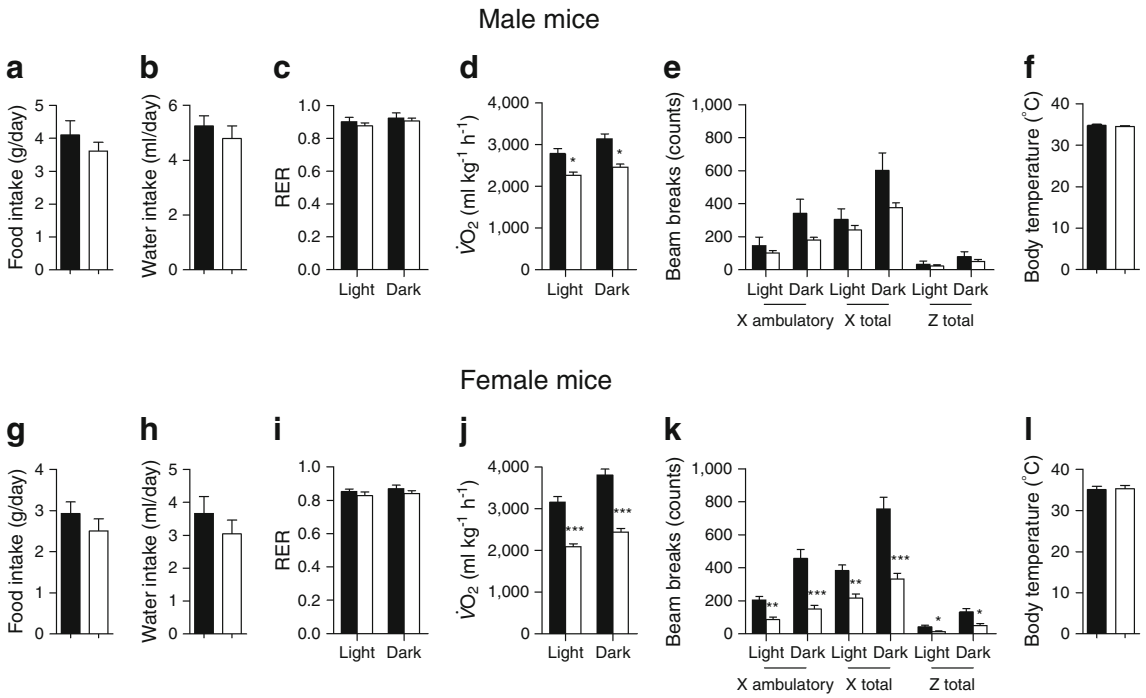

Fig. 3 Reduced energy expenditure in A-*Jak2* KO mice at 5–6 months of age. (**a**–**e**) Male (n=6) and (**g**–**k**) female (n≥6) control (black bars) and A-*Jak2* KO (white bars) mice aged 5–6 months were housed individually in metabolic chambers with free access to food and water, and energy balance data were collected for 24 h. (**a**, **g**) Daily food and (**b**, **h**) water intake were determined by weighing the chow and measuring the volume of drinking water, respectively, before and after the measurement period. (**c**, **i**) RER, calculated as the ratio of volume of carbon dioxide production ($\dot{V}CO_2$):$\dot{V}O_2$. (**d**, **j**) $\dot{V}O_2$ and (**e**, **k**) physical activity expressed as average number of infra-red beam breaks during one measurement interval. (**f**) Rectal temperature of male and (**l**) female control and A-*Jak2* KO mice; n≥3. All results are mean±SEM; *p<0.05, **p<0.01 and ***p<0.001

metabolism in perigonadal adipose tissue. At 1 month of age, gene expression was not altered by JAK2 deficiency (ESM Fig. 3a, b). The genes studied included known STAT target genes, e.g. *Fabp4*, fatty acid synthase (*Fas*, also known as *Fasn*), acyl-CoA oxidase 1 (*Acox1*) and pyruvate dehydrogenase kinase (*Pdk4*). By 5–6 months of age, genes implicated in lipid accumulation became differentially regulated. An overall upregulation of genes implicated in adipogenesis was observed (Fig. 4a). On the other hand, enzymes regulating fatty acid synthesis showed decreased expression. mRNA levels of hormone sensitive lipase (*Lipe*) were significantly increased, whereas expression of adipose triacylglycerol lipase (*Pnpla2*) was reduced in A-*Jak2* KO mice (Fig. 4a). In addition, the mRNA abundance of genes involved in β-oxidation was elevated, suggesting a disruption in adipose lipid homeostasis. In contrast, the expression of genes regulating glucose metabolism was not altered by JAK2 deficiency (Fig. 4b).

Next, given the well-known lipolytic effects of JAK2-activating cytokines and hormones, we measured ex vivo lipolysis in adipose explants from 5- to 6-month-old mice. As shown in Fig. 4c, baseline glycerol release was not affected by adipose JAK2 deficiency. On the other hand, while isoprenaline induced a robust lipolytic response in controls, this effect was significantly attenuated in A-*Jak2* KO mice. Furthermore, glycerol release in response to leptin and GH was completely abolished in A-*Jak2* KO adipose tissue, indicating impaired lipolysis.

Consistent with disrupted lipid homeostasis in adipose tissue, A-*Jak2* KO mice showed higher NEFA levels in the circulation at 5–6 months of age (Fig. 4d), whereas levels of triacylglycerol (Fig. 4e), total cholesterol (Fig. 4f) and cholesterol lipoproteins (ESM Fig. 3c) were not changed. Finally, despite the presence of massive adiposity and increased circulating NEFA concentration, liver tissue from A-*Jak2* KO mice did not accumulate a significant amount of lipid, as shown by histological staining (ESM Fig. 3d). Together, these results suggest that adipose JAK2 deficiency results in defective lipolysis, leading to a disruption of whole-body lipid homeostasis.

A-Jak2 KO mice have disrupted adipokine secretion To investigate the effects of JAK2 deficiency on adipose function, we also examined expression patterns of adipokines. At 1 month of age, mRNA levels of leptin, adiponectin and *Tnf-a* (also known as *Tnf*) were similar, whereas expression of *Il6* was downregulated in perigonadal adipose tissue from A-*Jak2* KO mice (ESM Fig. 4). As A-*Jak2* KO mice accumulated more adipose mass with age, their perigonadal adipose tissue upregulated the mRNA transcription of leptin and *Tnf-a* (Fig. 5a). Consistent with this, levels of these adipokines were

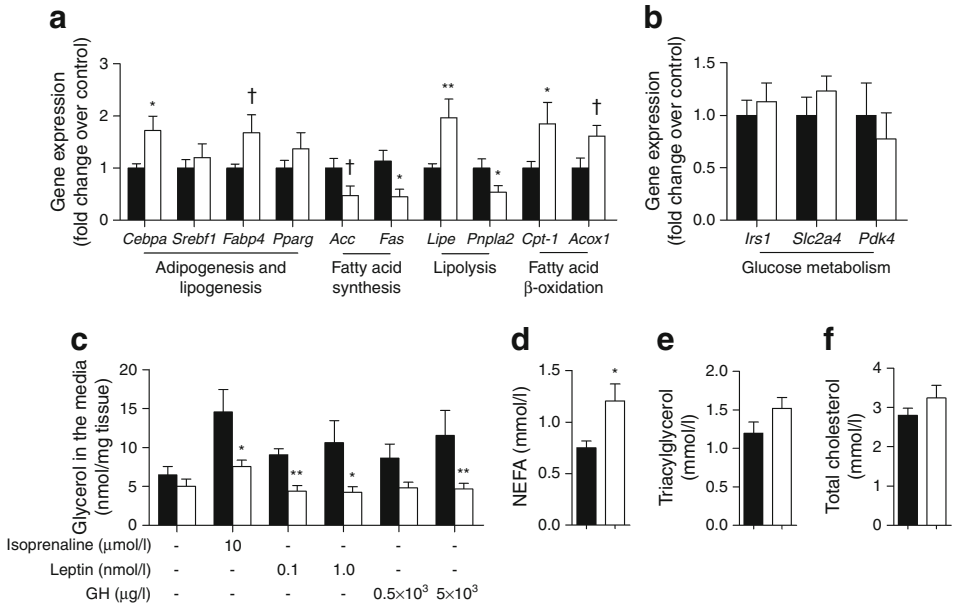

Fig. 4 Impaired lipolysis and disrupted lipid metabolism in A-*Jak2* KO mice. (**a**) mRNA expression of genes involved in lipid metabolism in perigonadal adipose tissue from mice at 5–6 months of age; $n \geq 6$. Values are normalised to 18S mRNA levels and expressed as fold changes relative to the control group. *Cebpa* encodes CCAAT/enhancer binding protein, alpha; *Srebf1*, sterol regulatory element-binding protein 1c; *Fabp4*, fatty acid binding protein 4; *Pparg*, peroxisome proliferator-activated receptor gamma; *Acc*, acetyl-CoA carboxylase; *Fas*, fatty acid synthase; *Lipe*, hormone sensitive lipase; *Pnpla2*, adipose triacylglycerol lipase; *Cpt-1* (also known as *Cpt1a*), carnitine palmitoyltransferase 1; *Acox1*, acyl-CoA oxidase 1, palmitoyl. Black bars, control; white bars, A-*Jak2* KO. (**b**) mRNA expression of genes implicated in glucose metabolism in tissue as above (**a**). *Irs1* encodes insulin receptor substrate 1; *Slc2a4*, solute carrier family 2 (facilitated glucose transporter), member 4; *Pdk4*, pyruvate dehydrogenase kinase isozyme 4. (**c**) Glycerol release from perigonadal fat explants of 6-month-old mice stimulated with isoprenaline, recombinant leptin or GH. Results are from triplicate samples repeated three times. (**d**) Serum levels of NEFA, (**e**) triacylglycerol and (**f**) total cholesterol from overnight-fasted mice at 5–6 months of age; $n \geq 4$. All results are mean±SEM; $^{*}p<0.05$, $^{**}p<0.01$ and $^{\dagger}p=0.06$

also elevated in the circulation (Fig. 5b–e). In addition, while gene expression of adiponectin was not changed (Fig. 5a), circulating adiponectin levels were significantly reduced in A-*Jak2* KO mice (Fig. 5c).

A-Jak2 KO mice show impaired insulin sensitivity as they age To determine the metabolic consequences of adipocyte JAK2 disruption, we assessed glucose metabolism in A-*Jak2* KO mice. At 2 months of age, A-*Jak2* KO mice displayed no changes in random or fasting blood glucose levels (ESM Fig. 5a, b, e, f). Responses of blood glucose to exogenous glucose and insulin administration were also similar in A-*Jak2* KO compared with control mice (ESM Fig. 5c, d, g, h). This normal glucose metabolism was present despite the significant increase in adiposity in female A-*Jak2* KO mice.

By 5–6 months of age, male and female A-*Jak2* KO mice maintained normal levels of random and fasting blood glucose (Fig. 6a, b, f, g). An i.p. GTT suggested the presence of glucose intolerance, especially in female A-*Jak2* KO mice (Fig. 6c, h). However, when we performed an OGTT using a fixed dose of glucose (50 mg) to eliminate the difference in dosing between genotype groups, glucose tolerance in A-*Jak2*

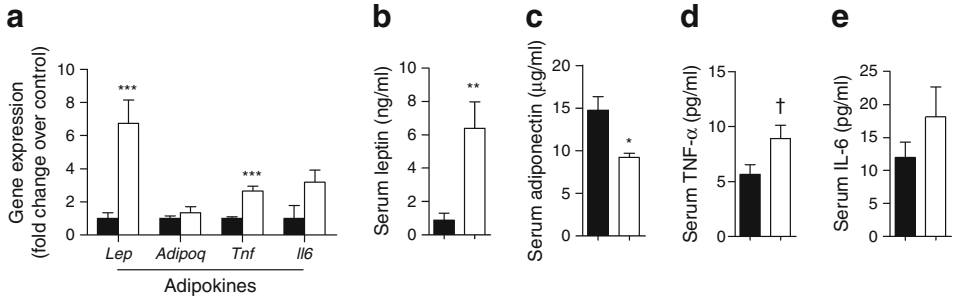

Fig. 5 Disrupted adipokine profile in A-*Jak2* KO mice. (**a**) mRNA expression of genes encoding adipokines, determined in perigonadal adipose tissue from mice at 5–6 months of age; $n \geq 8$. Values are normalised to 18S mRNA levels and expressed as fold changes relative to the control group. *Lep* encodes leptin; *Adipoq*, adiponectin. Black bars, control; white bars, A-*Jak2* KO. (**b–e**) Serum levels of adipokines as indicated from overnight-fasted mice at 5–6 months of age; $n \geq 4$. All results are mean±SEM; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$; $^{\dagger}p=0.07$

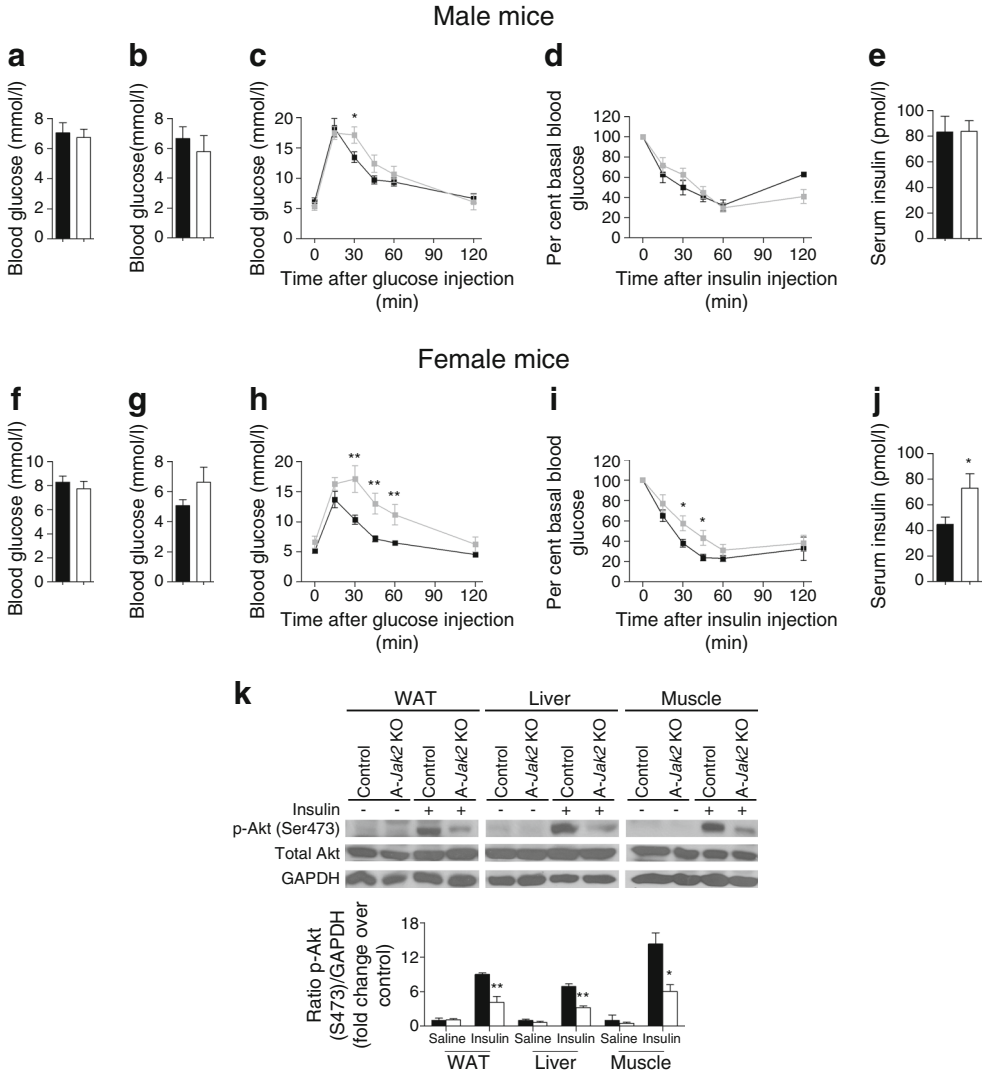

Fig. 6 Impaired insulin sensitivity in A-*Jak2* KO mice at 5–6 months of age. (**a**, **f**) Random blood glucose, (**b**, **g**) fasting blood glucose, (**c**, **h**) i.p. GTT (1 g/kg), (**d**, **i**) i.p. ITT (1.0 U/kg) and (**e**, **j**) fasting serum insulin levels at 5–6 months of age in male ($n \geq 5$) and female ($n \geq 5$) mice. Black, control; white or grey, A-*Jak2* KO. (**k**) Perigonadal white adipose tissue (WAT), liver and skeletal muscle lysates were prepared from 6-month-old female mice that had been injected with insulin (5 U/kg) or PBS, and resolved by SDS-PAGE. Lysates were immunoblotted for phospho-Akt (S473), total Akt and GAPDH. Protein band intensity was quantified by ImageJ software and normalised to the PBS-injected control group; $n=3$. All results are mean±SEM; $^{*}p<0.05$ and $^{**}p<0.01$

KO mice was comparable to that in control littermates (ESM Fig. 6a, b), suggesting that A-*Jak2* KO mice may be able to maintain glucose homeostasis at this age. Nevertheless, while male A-*Jak2* KO mice showed no significant change in insulin sensitivity (Fig. 6d, e), female A-*Jak2* KO mice showed impaired insulin sensitivity during an ITT (Fig. 6i) and higher fasting serum insulin levels (Fig. 6j). Next, to assess organ-specific insulin sensitivity, female A-*Jak2* KO mice and control littermates were challenged with insulin, and tissues removed and processed for analysis of insulin signalling by western blotting. As shown in Fig. 6k, insulin-stimulated Akt phosphorylation was significantly attenuated in perigonadal adipose tissue, liver and skeletal muscle of A-*Jak2* KO mice, suggesting the presence of whole-body insulin resistance. This was associated with increased beta cell area, probably as a compensatory response to increased insulin demand (ESM Fig. 6c, d). However, glucose-stimulated insulin secretion was normal in A-*Jak2* KO mice (ESM Fig. 6e). In summary, selective JAK2 deficiency in adipocytes results in whole-body insulin resistance in association with disrupted lipid homeostasis and adipokine secretion.

Discussion

In this study, we investigated the role of JAK2, a key mediator of cytokine signalling, in adipose tissue biology and whole-body metabolism. Using mice lacking *Jak2* in adipocytes, we show that JAK2 plays an essential role in maintaining adipose mass, such that its deficiency resulted in extensive adipose tissue expansion even on a regular chow diet. This was associated with reduced energy expenditure and impaired insulin sensitivity with age. Notably, the phenotypic differences between A-*Jak2* KO mice and control littermates were more profound in females. The mechanisms underlying this sex-related difference are not clear, but may reflect sexual dimorphic actions of JAK2-activating

cytokines or compensatory pathways activated in response to JAK2 deficiency in male mice.

To disrupt JAK2 specifically in adipocytes, we used *Ap2-Cre* transgenic mice, a widely used model to study the adipocyte-specific function of genes of interest [33, 34]. However, the *Ap2* promoter/enhancer has been shown to drive transgene expression during embryonic development [35]. Therefore, we cannot rule out a contribution of JAK2 deficiency in other tissues to the observed phenotype of A-*Jak2* KO mice. Nevertheless, our results are in agreement with a recent report characterising mice with adipose-specific *Jak2* deletion driven by the adiponectin promoter [28]. Similarly to our mice, male knockout mice in this model had normal body weight, but significantly increased body fat at 8–10 weeks of age.

The marked adiposity observed with adipocyte JAK2 deficiency is consistent with previous studies in which various components of the JAK2–STAT pathway were disrupted in adipocytes. In particular, disruption of the GH receptor (GHR) [36] and STAT3 [37] using the *Ap2* promoter, and knockdown of the leptin receptor using antisense RNA [38] all resulted in increased body weight and adiposity. Of all the models, A-*Jak2* KO mice had the most striking increase in body weight. This is probably due to the combined disruption of signalling from multiple cytokines as a consequence of JAK2 deficiency. Interestingly, the perigonadal fat depot had the smallest increase in mass, especially in male A-*Jak2* KO mice. This is in line with observations in adipocyte-specific *Ghr* knockout mice [36] and may be due to different responses of various fat depots to the action of GH [39].

Previous studies in vitro have suggested a critical role of JAK2 in adipogenesis [11, 12]. Although direct in vivo evidence is lacking for JAK2, studies in different mouse models support the importance of its downstream protein, STAT5, in adipocyte differentiation [40, 41]. Therefore, JAK2 deficiency may have an impact on adipocyte development in our model. However, A-*Jak2* KO mice probably did not have impaired adipogenesis because they maintained a similar number of adipocytes compared with control littermates. This lack of an effect on adipogenesis may be due to the late deletion of *Jak2* induced by the *Ap2* promoter, as AP2 is commonly regarded as a marker of terminal adipocyte differentiation [42].

With no change in adipogenesis and no direct effect on energy balance, adipocyte-specific JAK2 deficiency might alter body composition by modulating lipid turnover in the adipose tissue. Indeed, a number of JAK2-activating cytokines and hormones including leptin [16], IL-6 [17, 18], IFN-γ [19], GH [20] and prolactin [21] have well-documented lipolytic effects. In agreement with this, adiponectin promoter-driven *Jak2* deletion resulted in impaired lipolysis in white adipose tissue [28]. In this work, we further showed that adipocyte JAK2 is required for leptin- and GH-stimulated lipolysis. Our results are consistent with previous work showing defective leptin- and GH-stimulated lipolysis in adipocytes from mice lacking STAT3, and STAT5A and STAT5B, respectively [37, 43]. However, in contrast to these models, adipose JAK2 deficiency also significantly attenuated catecholamine-induced lipolysis, albeit to a lesser degree, indicating that adipose JAK2 directly modulates the lipolytic program.

We postulate that whole-body metabolic changes later in life in A-*Jak2* KO mice are likely to be secondary to increased adiposity and the ensuing insulin resistance, as A-*Jak2* KO mice were metabolically normal at a younger age despite profound obesity. Specifically, with blunted insulin action, insulin-stimulated expression of lipogenic genes including acetyl-CoA carboxylase (*Acc*, also known as *Acaca*) and *Fas* would be diminished [44]. The inhibition of lipolysis via suppression of *Lipe* would also be attenuated [45]. This might explain the higher circulating levels of NEFA in older A-*Jak2* KO mice.

In line with insulin resistance resulting from disrupted adipose tissue homeostasis, we observed higher circulating levels of leptin and TNF-α, and lower levels of adiponectin, which could all contribute to the metabolic disturbances in older A-*Jak2* KO mice. While histological analysis of the liver did not suggest excessive lipid deposition, it is possible that with age and more severe insulin resistance, fatty liver may eventually develop in A-*Jak2* KO mice. Importantly, our knockout animals were phenotypically distinct in terms of whole-body metabolism compared with other models of disrupted JAK–STAT signalling. The disruption of GHR in adipocytes did not affect glucose homeostasis or hepatic lipid content up to 20 weeks of age [36], whereas mice with diminished adipose leptin signalling displayed glucose intolerance by as early as 6–7 weeks of age, and liver steatosis by 19–20 weeks [38]. In contrast, although glucose homeostasis was relatively normal in mice with adipose-specific STAT3 deficiency, fatty liver was present by 20 weeks of age [37]. These differences in whole-body metabolism are present despite the same primary defect in body weight homeostasis, demonstrating the complexity of metabolic regulation and the diverse functions of the JAK–STAT pathway in adipose physiology.

Results from this work and our previous study on hepatocyte-specific *Jak2* deletion suggest that JAK2 acts to keep lipid content in check in metabolic tissues with high lipid turnover. We propose that disruption of this important regulator leads to excessive lipid accumulation locally. This promotes neutral lipid storage and prevents spillover into the circulation and other organs. Consequently, despite massive liver steatosis, hepatocyte-specific *Jak2* knockout mice did not develop inflammatory liver damage and were in fact protected from high-fat diet-induced obesity and glucose intolerance [6]. In the case of adipose JAK2 deficiency, lipid

accumulation in adipose tissue was initially benign with no adverse effects on metabolic variables. However, the progressive deposition of lipid eventually exceeded the storage capacity of existing adipocytes, leading to the release of NEFA into the circulation and the ensuing metabolic consequences as mice grew older.

In summary, A-*Jak2* KO mice develop increased body mass and adiposity due to defective lipolysis. With age, this increased adiposity leads to dyslipidaemia, insulin resistance and dysregulation of adipocyte gene expression. Therefore, JAK2 in adipocytes is required for body weight homeostasis and prevents excessive lipid accumulation in adipose tissue. Targeting the JAK–STAT pathway may provide a new therapeutic option for the treatment of obesity and associated type 2 diabetes.

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Contribution statement SYS designed and performed experiments, interpreted and analysed data, and wrote and edited the manuscript. CTL, JJB, TS, S-YL and SAS performed experiments, analysed data and edited the manuscript. MW designed experiments, interpreted and analysed data, and critically edited the manuscript. All authors have read and approved the final version of the manuscript. MW is responsible for the integrity of the work as a whole.

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