Direct control of hepatic glucose production by interleukin-13 in mice

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Introduction

Loss of postprandial glycemic control followed by fasting hyperglycemia is a hallmark of type 2 diabetes (1, 2). The resulting glucotoxicity contributes to metabolic complications, such as pancreatic β cell death and vascular dysfunction (3). Therefore, identification of therapeutic approaches to improve glycemic management will be beneficial for diabetes and related metabolic diseases.

Glucose homeostasis is achieved through tight regulation of its production and utilization. In healthy individuals, these 2 processes are balanced to maintain blood glucose within a narrow physiological range. The liver is the main tissue for glucose production, and the regulation of hepatic gluconeogenic genes, including pyruvate carboxylase (Pcx), phosphoenolpyruvate carboxykinase (Pepck), fructose-1,6-bisphosphatase (Fbp1), and glucose-6-phosphatase (G6p) has been extensively studied. During fasting, gluconeogenic genes are induced in the liver by several transcription factors, including forkhead box protein O1 (FOXO1), PPARγ coactivator-1α (PPARGC1α), and cAMP response element–binding protein (CREB) and its coactivators, CREB-binding protein/ p300 (CBP/p300) and cAMP-regulated transcriptional coactivators (CRTC2) (4, 5). Conversely, in states of abundant glucose, such as after a meal, insulin suppresses hepatic glucose production through AKT kinases that trigger nuclear exclusion of FOXO1 and CRTC2 in a phosphorylation-dependent manner, thereby relieving transcriptional activities of FOXO1 and CREB (4–6). Transcriptional repression of gluconeogenesis is less understood, although STAT3 has been implicated in this process (7, 8). Interestingly, it has been shown that downregulation of hepatic gluconeogenic genes by feeding is maintained in mice lacking Akt1/2 and FoxO1 in the liver (9), indicative of the existence of additional, uncharacterized mechanisms controlling gluconeogenesis (6).

In addition to normal physiological regulation, recent evidence suggests that glucose homeostasis is also modulated by chronic inflammation associated with metabolic stress (10, 11). Genetic models in mice have demonstrated that deletion of key inflammatory mediators improves glucose tolerance in obesity-induced insulin resistance (12). The detrimental effects of proinflammatory pathways, most notably those triggered by Th1 cytokines (e.g., IFN-γ, TNF-α, and IL-1β), on glucose homeostasis are achieved partly through inhibitory serine phosphorylation of IRS1 by JNK. In turn, uncontrolled hyperglycemia could further contribute to chronic inflammation. For example, the activity of NF-kB is increased through O-GlcNAc modification under high-glucose conditions (13). NF-kB is a primary transcription factor downstream of Th1 cytokines, and its activation in the liver promotes systemic insulin resistance (14, 15). Consequently, increased activities of Th2 cytokines (e.g., IL-4 and IL-13) skew the immune response to a Th2 phenotype (16) and restore glucose homeostasis (17–21). Th2 cytokines activate several downstream effectors, including STAT6, PPARγ, and PPARδ, to induce macrophage alternative activation, which dampens inflammation. The Th1/Th2 (or M1/M2) paradigm is thought to play a major role in the progression of white adipose tissue (WAT) inflammation and dysfunction in obesity (22, 23). However, it is unclear whether Th2 cytokines directly interact with metabolic pathways to modulate systemic glucose and lipid homeostasis.

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We previously reported that appreciable amounts of IL-13, but not IL-4, were detected in the liver and WAT (17). Several sources of tissue Th2 cytokines have since been identified, including CD4+ T lymphocytes, eosinophils, type I NKT cells (i.e., αGalCer loaded and CDd reactive), nonimmune cells, and a new class of innate lymphoid-2 cells (ILC2 cells; including nuocytes, Ih2 cells, and natural helper cells) and multipotent progenitor type 2 (MPPtype2) cells (17, 21, 24–29). In the current study, we sought to determine the role of IL-13 in metabolic regulation. Our data revealed a previously unrecognized IL-13/STAT3 pathway in the control of hepatic glucose metabolism.

Results
C57BL/6 Il-13–/– mice show glucose intolerance and insulin resistance on normal chow diet. IL-13 binds to the IL-13 receptor α1 (IL-13Rα1) subunit of the type II receptor complex consisting of an IL-13Rα1/IL-4Rα dimer (30). Both IL-13Rα1 and IL-4Rα were found to be expressed in nonimmune cells, such as hepatocytes (Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI64941DS1), indicative of potential metabolic functions for IL-13. In light of this observation, we used male Il-13–/– mice on the C57BL/6 background as a genetic model to examine the effect of IL-13 depletion on metabolic homeostasis. On normal chow diet (9% fat), C57BL/6 Il-13–/– mice progressively gained more weight over a 9-month period than did WT animals (Figure 1A), although fat mass (determined by dual-energy X-ray absorptiometry; DEXA) was not significantly different. Metabolic cage analyses performed on 4-month-old mice, prior to body weight divergence, revealed that C57BL/6 Il-13–/– mice had reduced oxygen consumption during both day (light cycle; fasting state) and night (dark cycle; feeding state), with no difference in food intake and physical activity compared with control mice (Figure 1B). Interestingly, Il-13 gene deletion led to increased fasting glucose levels (6-hour fast) that were detected at 4 months and peaked at 7 months of age (Figure 1C). Blood chemistries at the latter time point were further analyzed at 10 AM and 10 PM without disturbance of sleep/wake cycles (corresponding
The increase in blood glucose and insulin levels indicated that Il-13 depletion causes dysregulated glucose metabolism. In fact, glucose tolerance test (GTT) and insulin tolerance test (ITT) showed that C57BL/6 Il-13 –/– mice were glucose intolerant and had reduced insulin sensitivity compared with control mice (Figure 2, A and B). Euglycemic-hyperinsulinemic clamp was performed to further characterize the defect in glucose homeostasis.

Figure 2
Il-13–/– mice develop hepatic insulin resistance and systemic metabolic dysfunction on normal chow. (A and B) GTT (A) and ITT (B) of 7- to 8-month-old chow-fed WT and Il-13–/– mice (C57BL/6 background; n = 7–10 per genotype). (C) Euglycemic-hyperinsulinemic clamp demonstrated that Il-13–/– mice were more insulin resistant than WT controls. GIR, glucose infusion rate; HGP, hepatic glucose production (basal and during clamp); GDR, glucose disposal rate. (D) Following the clamp, WT and Il-13–/– mice were given 2-deoxy-d-[1-14C]glucose to determine tissue glucose uptake. (E) Immunoblotting of tissue insulin signaling, assessed by insulin-stimulated AKT phosphorylation. Liver, muscle, and WAT were collected following the clamp. Representative tissue samples from 4 individual mice are shown. (F) Immunoblotting showed insulin-stimulated IRS1 tyrosine phosphorylation in the liver. Quantification of hepatic glycogen content is also shown. (G) Liver histology was performed to assess fat accumulation; shown are sections from 2 representative mice. Scale bar: 100 μm. Quantification of hepatic TG content is also shown. Data are mean ± SEM. *P < 0.05 vs. WT.

to the natural fasting and feeding states, respectively), which demonstrated that glucose concentrations of C57BL/6 Il-13–/– mice were also elevated during the fed state, when lactate production was reduced (10 pm; Figure 1D). Insulin concentrations during the fasted state (10 AM) were increased. In addition, C57BL/6 Il-13–/– mice exhibited higher circulating TG levels, but normal FFA and cholesterol levels (Figure 1E).
C57BL/6 Il-13–/– mice required less exogenous glucose to maintain euglycemia (as demonstrated by a reduced glucose infusion rate), due to their increased hepatic glucose production — both at the basal level and during the clamp — and reduced glucose disposal (Figure 2C). Furthermore, glucose uptake was decreased in both muscle and WAT (Figure 2D). Tissue-specific insulin signaling was assessed by insulin-stimulated AKT phosphorylation. C57BL/6 Il-13–/– muscle and WAT showed decreased levels of phosphorylated AKT (p-AKT) compared with control animals (Figure 2E). The strongest reduction in p-AKT was observed in C57BL/6 Il-13–/– livers, where glycogen content and insulin-induced tyrosine phosphorylation of IRS1 were also lower, while fat accumulation was elevated (Figure 2F and G). These observations suggest that C57BL/6 Il-13–/– mice exhibit systemic metabolic dysregulation with severe hepatic insulin resistance.

BALB/c Il-13–/– mice develop hyperglycemia on high-fat diet. We next sought to determine the effect of Il-13 deficiency in the BALB/c genetic background. BALB/c mice exhibit a much stronger Th2 response (accompanied by a weak Th1 response) and are less prone to develop metabolic diseases than are C57BL/6 mice (31, 32). Chow-fed BALB/c Il-13–/– mice had normal body weight and glucose metabolism up to 6 months of age (Supplemental Figure 1, B and C). On high-fat diet (35% fat), BALB/c Il-13–/– mice required less exogenous glucose to maintain euglycemia (as demonstrated by a reduced glucose infusion rate) compared with control animals (Figure 2F). Collectively, our results from Il-13–/– mice in both genetic backgrounds implicated a critical role for IL-13 in maintaining glucose homeostasis.

Il-13 regulates hepatic gluconeogenesis. Insulin is known to suppress glucose output in the liver. The increased glucose production in C57BL/6 Il-13–/– mice (Figure 2) could be due to hepatic insulin resistance. To assess the physiological function of IL-13, pathways involved in glucose synthesis and production were examined in chow-fed C57BL/6 WT and Il-13–/– mice at 2 months of age, when insulin responsiveness (as determined by ITT) and circulating concentrations of fasting glucose, insulin, lactate, and lipids were similar between genotypes (data not shown). Liver samples were collected at 10 AM and 10 PM to examine fasting and feeding metabolic responses. Gluconeogenic gene expression at 10 AM was similar between the 2 C57BL/6 genotypes. As expected, mRNA levels of hepatic gluconeogenic genes (e.g., Pcx, Fbp1, and G6p) were decreased during the fed state (10 PM) compared with the fasted state (10 AM) in WT mice (Figure 4A). However, this downregulation was blunted in C57BL/6 Il-13–/– mice, concomitant with an increase in feeding blood glucose levels (WT, 176.07 ± 2.40 mg/dl glucose, Il-13–/–, 188.66 ± 4.57 mg/dl glucose, *P < 0.05; WT, 1.62 ± 0.37 ng/ml insulin, Il-13–/–, 1.70 ± 0.44 ng/ml insulin). Induction of de novo lipogenic genes (e.g., Sreb1c and acetyl-CoA carboxylase 1 and 2 [Acc1/2]) by feeding was not affected, consistent with the notion that C57BL/6 Il-13–/– mice at this age show a normal hepatic insulin response. Disregulated glucose metabolism at the fed state worsened by 7 months of age, as demonstrated by increased expression of several gluconeogenic genes, including Pcx, Pepck,
Fbp1, G6p, and Ppargc1α, in C57BL/6 Il-13−/− mice (Figure 4B). Subsequently, recombinant IL-13 (rIL-13; 1 μg every other day, total 3 doses) was used to identify primary metabolic targets. rIL-13 treatment normalized gluconeogenic gene expression, but did not affect fatty acid metabolism genes, in C57BL/6 Il-13−/− mice (Figure 4B). rIL-13 also restored glucose and lactate to control levels (Figure 4C), suggesting a direct role for IL-13 in the control of hepatic glucose metabolism. Indeed, primary hepatocytes isolated from C57BL/6 Il-13−/− mice showed increased glucose production and gluconeogenic gene expression, a defect that was rescued by rIL-13 treatment for 6 hours (Figure 4D). Similar results were observed in livers/hepatocytes derived from high-fat diet-fed BALB/c Il-13−/− mice (Supplemental Figure 2, A and B). These data suggest that IL-13 depletion results in loss of postprandial glycemic control, leading to hepatic insulin resistance and fasting hyperglycemia.

Th2 cytokines have been shown to modulate high-fat diet-induced tissue inflammation (10) and brown adipose tissue (BAT) thermogenesis (33), both of which contribute to metabolic homeostasis. On normal chow, the expression of proinflammatory (M1) and antiinflammatory (M2) markers in the liver and WAT was similar between WT and Il-13−/− mice on the C57BL/6 background (Figure 5A). FACS and gene expression analyses showed same percentage of F4/80+ cells (e.g., macrophages) with similar M1/M2 expression patterns in the liver and WAT of C57BL/6 Il-13−/− and WT mice (Supplemental Figure 3, A and B). The expression of 2 M2 genes, Mgl1 and Mrc1, was lower by 20% in F4/80+ cells from WAT of C57BL/6 Il-13−/− mice, but the protein levels were not significantly altered (Supplemental Figure 3, A and B). There was also no difference in concentrations of circulating cytokines/chemokines (TNF-α, IL-1β, MCP-1,
and MIP-3α) between genotypes (Supplemental Figure 3C). rIL-13 treatment was able to induce M2 genes (Figure 5A). These results were consistent with the notion that tissue inflammation is more relevant under conditions such as high-fat feeding and obesity (34–36). In fact, immune cell infiltration, typically identified by crown-like structures, was not evident based on WAT histology in these chow-fed C57BL/6 mice (Supplemental Figure 3D). In BAT, the mRNA levels of genes involved in mitochondrial oxidative metabolism and thermogenesis were not affected by IL-13 status (Figure 5B). IL-13 gene deletion in the BALB/c background on high-fat diet led to downregulation of M2 markers, particularly in WAT (Supplemental Figure 4, A and B). Notably, proinflammatory genes were unchanged. There was no difference in BAT metabolic gene expression (Supplemental Figure 4C). Collectively, these results indicate that the defect in glucose metabolism in IL-13−/− mice under these experimental settings is mediated primarily by the function of IL-13 in hepatic gluconeogenic gene expression.

Stat3 mediates the suppressive effect on gluconeogenic gene expression by IL-13. Th2 signaling is known to induce phosphorylation and nuclear translocation of STAT6 to control transcription of M2 genes in immune cells (16). However, STAT3, another member of the STAT family known to suppress gluconeogenic genes (7, 8), has also been shown to interact with IL-13Rα (37, 38). Additionally, feeding is thought to increase p-STAT3 (39). We found that expression of IL-13, but not IL-4, was increased in the liver from fed compared with fasted mice (Supplemental Figure 5A). As reported above, p-STAT3 was readily detectable in WT liver at the fed state (10 pm). In contrast, levels of p-STAT3 were reduced in the liver of C57BL/6 II-13−/− mice, which could be restored by rIL-13 treatment (Figure 6A). The p-STAT6 signal was relatively low in both genotypes, but inducible by rIL-13. To demonstrate a causal relationship between IL-13 and glucose production and identify the downstream mediators, we conducted functional studies in isolated hepatocytes using various genetic models. In primary hepatocytes, rIL-13 treatment led to rapid STAT3 phosphorylation (Figure 6B), which suggests that STAT3 may serve as an effector of IL-13 in regulating gluconeogenesis. In fact, the inhibitory effect of IL-13 on basal glucose production and gluconeogenic gene expression was abolished in Stat3−/− hepatocytes (with the exception of Ppargc1α expression), but was preserved in WT and Stat6−/− cells (Figure 6, C and D). In addition, rIL-13 was unable to suppress the activity of a reporter driven by a 1.7-kb PEPCK promoter element in Stat3−/− hepatocytes (Figure 6E). Reexpression of STAT3 in these cells reduced the promoter activity by 50%, which was further decreased with rIL-13 treatment. ChIP assays further demonstrated that IL-13 increased STAT3 occupancy on Ppck and G6p gene promoters (binding sites located at approximately −1 kb and at −5 kb, respectively; Figure 6F). siRNA knockdown of IL-13 (or Stat3) also abrogated the ability of IL-13 to increase STAT3 phosphorylation, reduce glucose production, and suppress gluconeogenic gene expression (Figure 6, G and H). Knockout or knockdown of Stat3, Stat6, or II-13rt1 was shown to be specific (Supplemental Figure 5, B and C). These findings demonstrated that IL-13 controls glucose homeostasis through the IL-13Rα/STAT3 signaling pathway in the liver.

As mentioned above, many immune cells are capable of producing IL-13. To identify potential IL-13–producing cells within the liver, we first used II-13GFP reporter mice, which contained a GFP knockin within the II-13 locus (25). T cells, type 1 NKT cells, B cells, and macrophages were readily detected in the liver by FACS, whereas the presence of eosinophils and LinICOS+Sca-1+CD25+IL-7Rα+ Stat3 mediates the suppressive effect on gluconeogenic gene expression by IL-13.
IL-13 suppresses gluconeogenic gene expression through STAT3. (A) Immunoblotting showing reduced p-STAT3 levels in Il-13–/– liver. Liver samples were collected at 10 pm (2 representative mice per group; cohort as in Figure 4B). (B) Immunoblotting showed that IL-13 induced STAT3/STAT6 phosphorylation in primary hepatocytes from WT, liver-specific Stat3–/–, and Stat6–/– mice. (C) IL-13 suppressed glucose production in hepatocytes in a STAT3-dependent manner. (D) IL-13 decreased expression of gluconeogenic genes through STAT3. Samples were collected 6 hours after rIL-13 treatment for gene expression analyses by quantitative real-time PCR. (E) Suppression of PEPCK promoter activity by IL-13 was mediated by STAT3. Stat3–/– hepatocytes were transfected with a luciferase reporter driven by PEPCK promoter, STAT3 expression vector, and/or rIL-13. RLU, relative luciferase unit. (F) ChIP in WT and Stat3–/– hepatocytes showed STAT3 occupancy on PEPCK and G6p promoters induced by IL-13. ND, not detected. (G) IL-13Rα1 was required for IL-13–dependent inhibition of glucose production. Hepatocytes were isolated from WT mice and transfected with siRNAs targeting either Il-13rα1 (sill-13rα1) or Stat3 (siSTAT3), siControl, control siRNA. Immunoblotting of p-STAT3 and total STAT3 in hepatocytes transfected with control or IL-13Rα1 siRNA is also shown. Samples were run on the same gel but were noncontiguous (white lines). (H) IL-13Rα1 mediated IL-13–dependent inhibition on gluconeogenic gene expression, as assessed by quantitative real-time PCR. Glucose production and gene expression are presented as fold change relative to control. Data are mean ± SEM. *P < 0.05 vs. vehicle, or as indicated by brackets.
The metabolic benefit of Th2 cytokines is believed to resolve, at least in part, the proinflammatory response elicited by Th1 signaling that has been shown to inhibit insulin sensitivity. Using experimental conditions that minimize contributions from metabolic stress–induced inflammation, the current study identified a key function for IL-13 in hepatic glucose production. Depletion of IL-13 by gene targeting resulted in hyperglycemia accompanied by elevated expression of genes required for glucose synthesis and production in the liver, while rIL-13 treatment reversed the dysregulated glucose metabolism. In the C57BL/6 background, the hyperglycemic phenotype of chow fed Il-13−/− mice worsened with age, leading to hepatic and systemic insulin resistance. BALB/c Il-13−/− mice also showed elevated glucose levels with high-fat diet feeding. IL-13 suppressed transcription of gluconeogenic genes through a noncanonical downstream transcription factor, STAT3, and the effect of IL-13 on inhibition of glucose production was lost in Stat3−/− hepatocytes. These results suggest that IL-13 exhibits metabolic functions beyond its role in modulating inflammation.

In response to endotoxin, the body exhibits transient systemic insulin resistance, in an attempt to spare glucose for utilization by immune cells (40–42). It is therefore conceivable that in addition to resolving inflammation, one of the main functions of IL-13 is to restore glucose homeostasis that is disrupted by proinflammatory actions. Along this line, nutrient intake may trigger low-grade inflammation through mechanisms including O-GlcNAcylation–mediated activation of NF-κB, as described previously (13). In healthy individuals, insulin is necessary and sufficient to quickly restore glucose levels after feeding. IL-13 is likely required when insulin signaling is compromised or overwhelmed, such as in aged individuals or in cases of overnutrition. Although the glucose-lowering effect of IL-13 may help keep metabolic inflammation under control, this activity is independent of its function in macrophage polarization. Increased circulating glucose levels were observed in C57BL/6 Il-13−/− mice on normal chow, which did not elicit strong tissue inflammation. In the BALB/c background, a strain resistant to metabolic diseases (32), hyperglycemia in Il-13−/− mice was brought about by high-fat diet feeding without altering insulin sensitivity. In the BALB/c background, a strain resistant to metabolic diseases (32), hyperglycemia in Il-13−/− mice was brought about by high-fat diet feeding without altering insulin sensitivity. In the BALB/c background, a strain resistant to metabolic diseases (32), hyperglycemia in Il-13−/− mice was brought about by high-fat diet feeding without altering insulin sensitivity. In the BALB/c background, a strain resistant to metabolic diseases (32), hyperglycemia in Il-13−/− mice was brought about by high-fat diet feeding without altering insulin sensitivity.

Discussion

The metabolic benefit of Th2 cytokines is believed to resolve, at least in part, the proinflammatory response elicited by Th1 signaling that has been shown to inhibit insulin sensitivity. Using experimental conditions that minimize contributions from metabolic stress–induced inflammation, the current study identified a key function for IL-13 in hepatic glucose production. Depletion of IL-13 by gene targeting resulted in hyperglycemia accompanied by elevated expression of genes required for glucose synthesis and production in the liver, while rIL-13 treatment reversed the dysregulated glucose metabolism. In the C57BL/6 background, the hyperglycemic phenotype of chow fed Il-13−/− mice worsened with age, leading to hepatic and systemic insulin resistance. BALB/c Il-13−/− mice also showed elevated glucose levels with high-fat diet feeding. IL-13 suppressed transcription of gluconeogenic genes through a noncanonical downstream transcription factor, STAT3, and the effect of IL-13 on inhibition of glucose production was lost in Stat3−/− hepatocytes. These results suggest that IL-13 exhibits metabolic functions beyond its role in modulating inflammation.

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STAT6 is known as the main effector of Th2 cytokines in immune cells (16, 43). In fact, IL-4/STAT6 signaling is essential for Th2 cell differentiation (44–46). STAT6 has also been shown to mediate certain physiological functions of IL-4, most notably adaptive thermogenesis in BAT (33). In contrast, IL-13 was not required for Th2 differentiation nor thermogenic gene expression (Figure 5B and Supplemental Figure 4C). Interestingly, IL-4 did not appear to control hepatic glucose homeostasis (Supplemental Figure 5D). It is unclear how the differential activities of Th2 cytokines are regulated, as both IL-4 and IL-13 can signal through the type II receptor IL-13Rα1/IL-4Rα heterodimer (30). Structure-based studies suggest that the relative expression of IL-4Rα and IL-13Rα1 may determine the outputs of IL-4 versus IL-13 signaling (30). Although early studies established an interaction between STAT3 and the IL-13Rα1 subunit (37, 38), Th2 cytokines have been shown to weakly activate STAT3 in immune cell lines (30, 38). The ability of IL-13 to induce STAT3 phosphorylation in primary hepatocytes appeared to be rapid and robust (Figure 6), consistent with relatively high expression of IL-13Rα1 in hepatocytes compared with adipocytes and T cells (Supplemental Figure 1A). Levels of p-STAT3 in the liver have been shown to be increased by feeding, although the upstream signaling is not clear (39). Together, these observations suggest that the IL-13/STAT3 axis directs Th2 signaling toward metabolic responses, such as feeding, whereas IL-4 and STAT6 may play roles in adaptive responses. This notion is supported by recent work that identified widely dispersed IL-13–producing cells as non-T, non-B, lineage-negative ILC2 cells that did not express IL-4 (24–26, 29). These cells provide early sources of IL-13 during helminth infection, whereas T cell–derived IL-13–producing cells characterized as non-T, non-B, lineage-negative ILC2 may provide new therapeutic opportunities to control hyperglycemia associated with insulin resistance.

Methods

Animals and metabolic studies. IL-13–/– mice in the BALB/c background were generated as described previously (54). These animals were backcrossed 5 generations to the C57BL/6 strain (96.875%). Background- and age-matched littermates (males) were used as WT controls. Metabolic studies in chow-fed C57BL/6 WT and IL-13–/– mice were conducted in 4 cohorts (n = 6–10 per genotype, 2–9 months of age). For high-fat diet–fed BALB/c IL-13–/– mice (n = 8 per genotype; 2 cohorts), 10- to 12-week-old mice were placed on a high-fat diet (Bio-Serv) for 6 months. WT and BALB/c IL-13–/– mice (n = 5 per genotype, 4–6 months of age; 2 cohorts) fed normal chow were also studied. ITT and GTT were performed as described previously (55) using 1 U/kg body weight insulin and 1.5 g/kg glucose, respectively, by i.p. injection after a 6-hour fast. For PTT, mice were fasted for 16 hours followed by i.p. injection of sodium pyruvate (2 g/kg). Blood glucose concentrations were measured at the indicated time points using a glucometer (OneTouch Ultra; LifeScan). Fasting blood chemistries, including TGs, FFAs, cholesterol, and lactate, were measured following a 6-hour fast using commercial kits (Wako, Thermo-Fisher). In certain experiments, blood chemistries, serum cytokines/chemokines, and tissue samples were determined at 10 AM and 10 PM, corresponding to the natural fasting and feeding states, respectively. These mice were fed ad libitum and housed in a quiet room without disturbance of their sleep/wake behavior to reduce environmental stress. Serum cytokines were measured using Luminex multiplex kits (Millipore). Quantification of liver and muscle TGs and glycogen was based on published protocols (56). Metabolic cage studies and DEXA were performed as described previously (55). For in vivo rescue studies, mice were given either 1 μg rIL-13 (Peprotech) or an equal volume of PBS every other day for 1 week (total 3 doses; n = 5 per treatment). Stat6–/– mice (C57BL/6 background) were purchased from the Jackson Laboratory. Liver-specific Stat3–/– mice were generated as described previously (7) and provided by J. Mizgerd (Boston University, Boston, Massachusetts, USA). Mice were housed in the Harvard School of Public Health barrier facility on a 12-hour light, 12-hour dark cycle.

Euglycemic-hyperinsulinemic clamp. Mice were implanted with catheters followed by a 5-day recovery (57). After an initial 5-mCi bolus, [3-3H]glucose was infused (0.05 μCi/min for 2 hours) to measure basal glucose turnover. A 2-hour euglycemic-hyperinsulinemic clamp was conducted with a prime and continuous insulin infusion (2.5 mU/kg/min), coupled with a variable infusion of 40% glucose to maintain blood glucose at 6 mM. Every 5 minutes, blood glucose was measured via tail bleed during the first hour to stabilize blood glucose levels and every 10 minutes thereafter until the end of the 2-hour clamp to maintain constant blood glucose levels. The rate of whole-body glucose turnover was estimated using a continuous infusion of [3-3H]glucose (0.1 μCi/min). Tissue-specific glucose uptake was estimated by a bolus administration of 2-deoxy-d-[1-14C]glucose (10 μCi) 45 minutes prior to the end of clamp experiments.

Primary hepatocytes and glucose production assays. Primary hepatocytes were isolated and cultured as described previously (56) using Liberase (Roche) from WT, IL-13–/–, Stat6–/–, and liver-specific Stat3–/– mice. To measure glu-
cose production, hepatocytes were treated with rIL-13 (10 ng/ml) or vehicle in DMEM low-glucose media for 2 hours, washed, and incubated for 4 hours in glucose production media (DMEM without glucose or phenol red, supplemented with 1 mM pyruvate, 10 mM lactate, and 10 ng/ml rIL-13). Glucose concentrations in the media were measured enzymatically and normalized to total protein content. For siRNA experiments, hepatocytes seeded in 12-well plates were transfected with 40 pmol siRNA pools (Qiagen) using Lipofectamine 2000 (Invitrogen) for 48 hours, followed by glucose production assays.

**Reporter assays and ChIP.** For reporter assays, a 1.7 kb mouse PEPC promoter element was cloned into the pGL3-basic vector. Hepatocytes were transfected with the promoter construct together with CMV-STAT3 or empty vector followed by overnight treatment with rIL-13. The relative luciferase unit was normalized using the dual-luciferase reporter control (Cell Signaling). STAT3 antibody and IgG control were from Cell Signaling. Real-time PCR was performed to determine STAT3 occupancy using primers flanking putative STAT3 binding sites on the promoters of Pepek (approximately –1 kb relative to the transcription initial site) and G6p (approximately –5 kb) (8) as well as off-target sites (–10 kb) for each promoter. The following primers were used: Pepek –1 kb forward, 5′-GGTTGCTCAAGTGCCAC-3′; Pepek –1 kb reverse, 5′-GTAGACCTTCTAGTGC-3′; Pepek –10 kb forward, 5′-CTGGTTGTAAGTGGGAGTC-3′; Pepek –10 kb reverse, 5′-GGAGAGCTCTGTTGTAAGTCGC-3′; G6p –5 kb forward, 5′-GGCTTGTTGGTCTGGCTTACG-3′; G6p –5 kb reverse, 5′-GCTGACCTTAACTCTCTGTAAGC-3′; G6p –10 kb forward, 5′-GAATCCAGCTAGTGGACATTG-3′; G6p –10 kb reverse, 5′-GTGTCTGAAAGTACTGATCTAC-3′.

**Gene expression.** RNA was isolated from tissues (TRIZol) or cells (USB-Affymetrix) and reverse transcribed with random hexamer and oligo-dT primers (1:1 ratio). Relative gene expression was determined by SYBR green-based real-time PCR. Transcripts were normalized to 36B4 expression.

**Immunoblotting.** Cells and tissues were lysed in IP buffer (20 mM Tris HCl, pH 8.0; 100 mM NaCl, 1 mM EDTA; 100 mM NaCl; 1 mM DTT; and protease and phosphatase inhibitors). For IRS1 tyrosine phosphorylation, tissue samples were immunoprecipitated with anti-IRS1 antibody, followed by immunoblotting with anti-p-tyrosine and anti-IRS1 antibodies. Antibodies against the following proteins were used (all from Cell Signaling): p-AKT (pS473), AKT, IRS1, p-STAT3 (pY705), STAT3, p-STAT6 (pY641), STAT6, β-tubulin, and p-tyrosine (Cell Signaling). Anti-iNOS was from Santa Cruz Biotechnology.

**113-Producing cells in the liver.** To determine potential sources of IL-13, liver samples from all libitum-fed WT BALB/c and IL-13Gfp+ mice (female, aged 6–8 weeks, backcrossed 11 generations to BALB/c) (25) were collected at 10 am and 10 pm (n = 3–4 per genotype per time point). Hepatocytes were removed from the preparation through a 44% Percoll gradient. FACS was performed to examine the presence of CD45 and CD8 T cells, CD19 B cells, and CD11b+ macrophages as well as CD4+ and CD4+CD11c+ NK cells (25). Nuocytes (Lin ICOS-Sca-1 CD25 IL-7Rα) and eosinophils (SiglecF−) were not detected. Similar results were obtained from both genotypes, and the data were combined in Figure 7A. IL-13+ cells in each cell population were further determined with GFP signal. A second approach was performed to identify IL-13+ and IL-4+ cells: liver immune cells from fasted or fed mice (8-week-old male C57BL/6; n = 4 per time point) were purified using percoll gradient and cultured in RPMI1640 culture medium (10% FBS, 50 μg/m 2-mercaptoethanol, and 2 mM glutamine) with 50 ng/ml phorbol myristate acetate (Sigma-Aldrich), 10 μg/ml brefeldin A (Invitrogen), and 10 μM monensin (Sigma-Aldrich) for 3 hours before FACS analyses using antibodies against CD45, CD4, CD11c, and IL-13 (28).

**Statistics.** Data are presented as mean ± SEM. Statistical differences between WT and IL-13−/− mice or between 10 am and 10 pm were assessed using 2-tailed Student’s t test. For in vitro assays, the mean and SEM were determined from 3–4 biological replicates for 1 representative experiment. Experiments were repeated at least 3 times. A P value less than 0.05 was considered significant.

**Study approval.** Animal studies were approved by the Harvard Medical Area Standing Committee on Animals.

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