Supplemental data

Direct control of hepatic glucose production by interleukin-13

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Supplemental Methods

Lipogenic and fat oxidation assays. For measurement of lipogenesis, hepatocytes were treated \( \pm \) 10 ng/ml rIl-13 overnight followed by incubation with \(^{14}\)C-acetate for 6 h. \(^{14}\)C-lipids were extracted with 2:1 chloroform:methanol and normalized to protein content. Fatty acid oxidation assays were conducted using the \(^{3}\)H palmitate tracer, following overnight rIl-13 treatment. \(^{3}\)H\(_{2}\)O was determined and normalized to the protein concentration.

FACS and F4/80\(^{+}\) cell isolation. Livers and WAT were harvested from mice fasted for 6 h. Liver cells were released by extensive pipetting and filtered through a cell strainer (70 \( \mu \)m), followed by centrifugation at 50g to pellet hepatocytes. Supernatant containing immune cells was washed and collected. WAT was digested for 30 min at 37°C with 2 mg/mL collagenase, filtered through nylon mesh (250 \( \mu \)m) and centrifuged to pellet the stromal vascular fraction. Cells devoid of hepatocytes or adipocytes were subjected to either FACS using antibodies against F4/80 (Life Technologies) and Mgl1 (AbD Serotec) or magnetic beads conjugated with anti-F4/80 antibody (Life Technologies) for RNA isolation to determine M1/M2 gene expression in resident macrophages.
Supplemental table 1

Metabolic parameters of high fat fed BALB/c mice

<table>
<thead>
<tr>
<th>BALB/c HFD</th>
<th>wt</th>
<th>Il-13+/−</th>
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</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>42.78±1.82</td>
<td>43.66±1.31</td>
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<tr>
<td>Liver/body weight (mg/g)</td>
<td>33.44±1.2</td>
<td>34.13±1.27</td>
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<tr>
<td>WAT/body weight (mg/g)</td>
<td>32.7±1.5</td>
<td>33.32±3.12</td>
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<tr>
<td>Glucose (mg/dL)</td>
<td>99.20±9.40</td>
<td>141.20±12.16*</td>
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<tr>
<td>Insulin (ng/mL)</td>
<td>0.47±0.009</td>
<td>0.45±0.004</td>
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<tr>
<td>Triglyceride (mg/dL)</td>
<td>49.53±4.37</td>
<td>67.59±5.43*</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>111.19±5.00</td>
<td>125.64±5.57</td>
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<tr>
<td>Free fatty acid (mMol)</td>
<td>2.05±0.12</td>
<td>1.71±0.14</td>
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<tr>
<td>Lactate (mg/dL)</td>
<td>15.49±1.16</td>
<td>14.42±1.15</td>
</tr>
</tbody>
</table>

Mice were fasted 6 h (n = 8/genotype). *p < 0.05, wt vs. Il-13+/− mice
Supplementary figures and figure legends

Supplemental Figure 1
Body weight and insulin responses in chow-fed and insulin signaling in high fat fed $IL-13^{-/-}$ mice in the BALB/c background. (A) The expression of IL-13 receptors in immune and non-immune cells determined by real-time PCR. IL-13 (and IL-4) binds to type II receptors consisting of IL-4rα/IL-13rα1 dimers. IL-13rα2 is thought to be a decoy receptor. IL-4 also binds to type I receptors consisting of IL-4rα/γc dimers, which are only expressed in immune cells, such as T lymphocytes. (B) Body weight and (C) insulin tolerance test (ITT) in wild-type (wt) or $IL-13^{-/-}$ mice in the BALB/c background on a normal chow diet (9% fat). ITT was conducted in 6 month old animals ($n = 5$/genotype). (D) Immunoblotting of tissue insulin signaling in high fat fed wt and $IL-13^{-/-}$ mice in the BALB/c background assessed by insulin stimulated Akt phosphorylation ($n = 8$/genotype). 5u/kg insulin was i.p. injected and tissues were collected 10 min later. Data are presented as mean ± SEM.
Supplemental Figure 2
Increased hepatic gluconeogenic gene expression in BALB/c $\text{Il-13}^{-/-}$ mice on high fat diet. (A) Metabolic gene expression in the liver. Liver samples from 6 h fasted wt and $\text{Il-13}^{-/-}$ mice in the BALB/c background ($n=6$, high fat diet for 6 months) were collected and gene expression was analyzed by quantitative, real-time PCR. (B) Glucose production and gluconeogenic gene expression are elevated in primary hepatocytes derived from $\text{Il-13}^{-/-}$ mice (BALB/c on high fat diet). rII-13 (10 ng/ml) was given to hepatocytes for two hours followed by a 4 hour glucose production assay in the presence of rII-13. Data are presented as mean ± SEM; *p < 0.05.
Supplemental Figure 3
Assessment of macrophage activation and tissue inflammation in C57BL/6 wt and Il-13–/– mice on normal chow (7 month old males). (A) and (B) Gene expression analyses of inflammatory markers in F4/80+ cells isolated from livers and white adipose tissues (WAT) (n = 4). Cells were isolated using magnetic beads conjugated with anti-F4/80 antibody. Right panel: FACS analyses to examine the percentage of F4/80+ cells in the non-hepatocyte or non-adipocyte fraction and the expression of Mgl1 in F4/80+ cells. (C) Circulating concentrations of cytokines and chemokines determined by ELISA (n = 7). (D) WAT histology (sections from 3 individual mice). Scale bar: 100 µm. Data are presented as mean ± SEM; *p < 0.05.
Supplemental Figure 4

Inflammatory and metabolic gene expression in BALB/c wt and Il-13–/– mice on high fat diet. (A) and (B) Gene expression analyses of inflammatory markers in liver and white adipose tissue (WAT). Tissue samples from 6 h fasted wt and Il-13–/– mice in the BALB/c background (n = 6, high fat diet for 6 months) were collected and gene expression was analyzed by quantitative, real-time PCR. (C) Expression profiling of oxidative metabolism and thermogenic genes in brown adipose tissue (BAT). Data are presented as mean ± SEM; *p < 0.05.
Supplemental Figure 5
Assessment of knockout/knockdown efficiency and the role of Il-13 in fat metabolism in hepatocytes. (A) Hepatic expression of Il-13 and Il-4 at the fed or fasted state determined by quantitative real-time PCR (male C57BL/6 mice, n = 5). (B) The expression of Stat3 and Stat6 in wt, Stat3–/– and Stat6–/– hepatocytes ± rII-13 (10 ng/ml) determined by quantitative real-time PCR. (C) The expression of Stat3, Stat6 and Il-13rα1 in control (sicontrol), Il-13rα1 siRNA (siII-13rα1) and Stat3 siRNA (siStat3) transfected hepatocytes ± rII-13 (10 ng/ml). (D) Il-4 does not suppress glucose production. Glucose production assays were conducted in primary hepatocytes ± rII-13 or rII-4 (10 ng/ml). (E) Lipogenic and fatty acid β oxidation assays in wt and Il-13–/– hepatocytes ± rII-13. Data are presented as mean ± SEM; *p<0.05.