Adipose tissue inflammation contributes to short-term high-fat diet-induced hepatic insulin resistance

Michael S. F. Wiedemann,1,2,3 Stephan Wueest,1,2 Flurin Item,1,2 Eugen J. Schoenle,1,2 and Daniel Konrad1,2,3
1Division of Pediatric Endocrinology and Diabetology, University Children’s Hospital, Zurich, Switzerland; 2Children’s Research Center, University Children’s Hospital, Zurich, Switzerland; and 3Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

Submitted 28 March 2013; accepted in final form 30 May 2013

IN OBESITY, ADIPOSE TISSUE EXPANSION is accompanied by local infiltration of different types of inflammatory cells (3). The emerging cross-talk between infiltrating inflammatory cells and local cells, such as adipocytes, results in altered adipokine as well as increased proinflammatory cytokine production and secretion. Consequently, insulin resistance evolves both locally as well as systemically, e.g., in the liver, due to the evolving dysfunctional interorgan crosstalk (6, 8). Consistently impinging on adipose tissue inflammation improves obesity-associated insulin resistance (19, 20, 22, 25). Thus, adipose tissue inflammation may trigger hepatic insulin resistance potentially via the release of cytokines and/or lipids into the circulation. In susceptible mouse strains, obesity and associated insulin resistance may be induced by a fat-enriched diet. It was demonstrated recently that a short period of high-fat diet (HFD), i.e., for 3–4 days, is sufficient to induce hepatic steatosis, hepatic insulin resistance, and adipose tissue inflammation (7, 13). However, it remains unclear whether hepatic insulin resistance (and hepatic steatosis) induced by a short bout of HFD is the result of acute lipid overload or whether it is at least partly mediated by adipose tissue inflammation, as it was demonstrated for long-term HFD feeding.

To assess a potential contribution of adipose tissue inflammation to short-term HFD-induced insulin resistance, we performed experiments in wild-type and adipocyte-specific Fas-knockout mice. We provide evidence that a short challenge of HFD triggers adipose tissue inflammation and hepatic insulin resistance in wild-type mice. Moreover, interfering with adipose tissue inflammation via adipocyte-specific Fas deletion preserved hepatic insulin sensitivity, suggesting that adipose tissue inflammation contributes to HFD-induced hepatic insulin resistance as early as 4 days after initiation of HFD.

MATERIALS AND METHODS

Animals. C57BL/6J mice were obtained from The Jackson Laboratory. Adipocyte-specific Fas-knockout mice (Fas<sup>α adip⁰</sup>) were generated as follows; mice with exon IX of Fas flanked with LoxP sites [a gift of Dr. A. Chervonsky, University of Chicago, and produced as described elsewhere (23)] were crossed with mice expressing Cre recombinase controlled by the Fabp4 promoter [B6.Cg-Tg(Fabp4-cre)1Rev/J; purchased from The Jackson Laboratory]. All mice were genotyped as described (25). Experiments presented in Figs. 1–3 were performed in Fas<sup>α adip⁰</sup> mice. In all experiments, including Fas<sup>α adip⁰</sup> mice, the latter were compared with Fas<sup>α-inf</sup> control littermates. In total, 94 mice were used for this study.

Twelve-week-old male mice were fed ad libitum with standard rodent diet (chow) or HFD (D12331; Research Diets, New Brunswick, NJ) for 4 days. HFD consisted of 58% of calories derived from fat, 25.5% from carbohydrate, and 16.5% from protein. Mice were fasted for 5 h prior to euthanization. All protocols conformed to the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland.

Intraperitoneal glucose tolerance test. Mice were injected intraperitoneally with 2 g/kg body wt glucose after overnight fasting, as described previously (11). Blood glucose concentration was measured with a Glucometer (Accu-Check Aviva; Roche Diagnostics, Rotkreuz, Switzerland) with blood from tail-tip bleedings.

Hyperinsulinenic euglycemic clamp studies. Hyperinsulinenic euglycemic clamp studies with an insulin infusion rate of 18 mU/kg·min<sup>−1</sup> were performed as described (17). Clamps were performed in freely moving mice. Glucose infusion rate was calculated once glucose infusion reached a more or less constant rate with blood glucose levels at 5 mmol/l (80–90 min after the start of insulin infusion). Thereafter, blood glucose was kept constant at 5 mmol/l for 15–20 min, and glucose infusion rate was calculated. The glucose disposal rate was calculated by dividing the rate of [3-<sup>U</sup>H]glucose infusion by the plasma [3-<sup>U</sup>H]glucose-specific activity (4, 9). Endogenous glucose production during the clamp was calculated by subtracting the glucose infusion rate from the glucose disposal rate (4, 9). Insulin-stimulated glucose disposal rate was calculated by subtracting basal endogenous glucose disposal from total glucose disposal.
glucose production (equal to basal glucose disposal rate) from glucose disposal rate during the clamp (18).

Determination of plasma insulin, free fatty acid, and triglyceride levels. Blood was sampled in mice fasted for 5 h. Plasma free fatty acid and triglyceride levels were determined as described elsewhere (25). Plasma insulin levels were measured using an ELISA kit as described previously (11).

Western blotting. For assessing insulin-stimulated Akt phosphorylation in liver, insulin (1 U/kg) was injected intraperitoneally in mice fasted for 5 h. Liver samples were harvested 15 min after insulin injection, snap-frozen in liquid nitrogen and stored at −80°C until homogenization. Liver samples or isolated adipocytes for Fas determination were homogenized as described previously (24). Protein concentration was determined using BCA assay (Pierce, Rockford, IL), and equivalent amounts of protein (50–75 μg) were resolved by LDS-PAGE (4–12% gel, NuPAGE; Invitrogen). Proteins were electrotransferred onto nitrocellulose membranes (0.2 μm; Bio-Rad, Rechna, Switzerland), and anti-phospho-Akt (Ser473; Cell Signaling Technology, Danvers, MA). Membranes were exposed in an Image Reader and analyzed with Image Analyzer (FujiFilm, Dielsdorf, Switzerland). Antibodies were used: anti-Fas (Upstate, Lake Placid, NY), anti-actin (Millipore, Zug, Switzerland), and anti-phospho-Akt (Ser473; Cell Signaling Technology, Danvers, MA). Membranes were exposed in an Image Reader and analyzed with Image Analyzer (FujiFilm, Dielsdorf, Switzerland). Arbitrary values obtained with Image Analyzer were normalized to an average of 1 in the control group.

RNA extraction and quantitative RT-PCR. Total RNA was extracted from epididymal or mesenteric adipose tissue using the RNeasy Lipid Tissue Mini Kit (Qiagen, Basel, Switzerland), and concentration was determined spectrophotometrically (Nanodrop 1000; Nanodrop Technologies, Boston, MA). The integrity of each mesenteric fat RNA sample was examined by Agilent Lab-on-a-chip technology using the RNA 6000 Nano LabChip kit and a bioanalyzer 2100 (both Agilent Technologies, Basel, Switzerland). An RNA integrity number >8.0 was considered as acceptable for further processing; 0.75–1 μg of RNA was reverse transcribed with Superscript III Reverse Transcriptase (Invitrogen, Basel, Switzerland) using a random hexamer primer (Invitrogen). Taqman (Applied Biosystems, Rotkreuz, Switzerland) was used for real-time PCR amplification. The following PCR primers (Applied Biosystems) were used: TNFα Mm00443258, IL-6 Mm00446190, cd11b Mm0043455, cd11c Mm00498698, F4/80 Mm00802529, MCP-1 Mm00441242, IL-10 Mm00439614, CD36 Mm00432403, CD8 Mm00438116, IFNγ Mm01168134, MIP-1α Mm99990057, and Ela3b Mm00840378. Relative gene expression was obtained after normalization to 18sRNA (Applied Biosystems), using the formula 2−ΔΔCt (16).

Total liver lipid determination. Liver tissue (10–30 mg) was homogenized in PBS, and lipids were extracted in a chloroform:methanol (2:1) mixture. Total liver lipids were determined by a sulphophosphovanillin reaction, as described previously (10). Histology. Liver tissues were fixed in 4% buffered formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin.
Data analysis. Statistical analyses were performed using Student’s t-test. P values < 0.05 were considered significant. All error bars represent SE.

RESULTS

Four days of HFD induces hepatic insulin resistance in C57BL6/J mice. To determine a potential impact of short-term HFD on glucose metabolism, intraperitoneal glucose tolerance test was performed in 12-wk-old mice receiving either a 58 cal% HFD for 4 days or a standard chow diet. As depicted in Fig. 1A, glucose tolerance was deteriorated significantly in HFD-fed littermates [area under the curve (AUC) in HFD-fed mice 1,761 ± 78 mmol·l⁻¹·min vs. AUC in chow-fed mice 1,433 ± 50 mmol·l⁻¹·min; P < 0.01]. Of note is that body weight was similar between both groups (26.4 ± 1.0 g in HFD-fed mice vs. 25.6 ± 1.2 g in chow-fed animals; P =

Fig. 2. Four days of HFD induces liver steatosis. A: representative hematoxylin and eosin-stained liver sections from chow-fed (left) and HFD-fed (right) mice (magnification ×20). B: total liver lipids were determined and expressed relative to liver weight. Results are the means of 4 (chow-fed) or 8 (HFD-fed) animals. C: mRNA expression of respective genes was analyzed in liver of chow-fed (black bars) and HFD-fed (open bars) mice; n = 6–9. All error bars represent SE. *P < 0.05 (Student’s t-test).

Fig. 3. Four days of HFD increases adipose tissue TNFα expression. mRNA expression of respective genes was analyzed in epididymal white adipose tissue (WAT; A) or mesenteric WAT (B) of chow-fed (black bars) and HFD-fed (open bars) mice; n = 3–11. All error bars represent SE. #P = 0.05 (Student’s t-test).
0.55). To assess insulin sensitivity, hyperinsulinemic euglycemic clamp studies were performed. Glucose infusion rate was decreased significantly in HFD-fed mice, reflecting reduced total body insulin sensitivity (Fig. 1B). Whereas insulin-stimulated glucose disposal rate was not significantly diminished (Fig. 1C), insulin-mediated inhibition of endogenous (mainly reflecting hepatic) glucose production was reduced in HFD-fed mice (Fig. 1D), suggesting that a short bout of HFD impacts mainly on hepatic insulin sensitivity. Similarly, insulin-stimulated Akt phosphorylation was reduced significantly in HFD compared with chow-fed mice (Fig. 1E). Of note is that total Akt protein levels were not different between the groups (data not shown).

Four days of HFD induces liver steatosis. Hepatic steatosis is strongly associated with insulin resistance; however, it is presently unclear whether insulin resistance causes hepatic steatosis or whether the increase in triglycerides or lipid metabolites causes the development of hepatic insulin resistance. As depicted in Fig. 2, A and B, 4 days of HFD was sufficient to increase hepatic lipid accumulation, as assessed by histological examinations and biochemical determination of total liver lipid content. In contrast, short-term high-fat feeding had no major impact on liver inflammation, as determined by mRNA expression of inflammatory as well as macrophage markers (Fig. 2C). Of note is that liver TNFα expression was reduced upon HFD, which is in accordance with previously published data reporting a trend toward decreased TNFα liver transcription after short bouts of high-fat feeding (13). Reduced CD8 mRNA expression may suggest lower T cell infiltration in livers of HFD-fed mice.

Four days of HFD increases adipose tissue TNFα expression. Previously, 3–4 days of HFD was reported to induce adipose tissue inflammation (7, 12, 13). Therefore, we determined mRNA expression of proinflammatory cytokines and macrophage markers in epididymal as well as mesenteric adipose tissue. As shown in Fig. 3, A and B, TNFα expression was increased significantly in mesenteric fat pads and trendwise in epididymal fat pads of HFD-fed compared with chow-fed mice. Of note is that harvesting of mesenteric adipose tissue is not that simple and is often contaminated by pancreatic tissue (2). To make sure that a pure fraction of mesenteric adipose tissue was analyzed, expression of Ela3b was determined. All samples included expressed Ela3b at a very low level confirming pure fraction of mesenteric adipose tissue. Therefore, as reported previously (13), a short bout of HFD was sufficient to induce TNFα expression in adipose tissue.

Fig. 4. Adipocyte-specific Fas-knockout mice are protected from HFD-induced adipose tissue inflammation. A: total cell lysates were prepared from isolated epididymal adipocytes harvested from wild-type C57Bl6/J mice fed either a chow diet or HFD for 4 days. Lysates were resolved by LDS-PAGE and immunoblotted with anti-Fas antibody; n = 3–5. B and C: mRNA expression of respective genes was determined in epididymal (B) or mesenteric WAT (C) of HFD-fed Fasff (open bars) and FasΔadipo (gray bars) mice; n = 5–6. All error bars represent SE. *P < 0.05; **P < 0.01 (Student’s t-test).
Insulin 60 adipo HFD adipo HFD Fas 90 Fas 30 Fas

The present study aimed to determine whether adipose tissue inflammation contributes to short-term HFD-induced hepatic insulin resistance. We next assessed glucose metabolism in HFD-fed adipocyte-specific Fas-knockout and control mice. As depicted (Fig. 5A), glucose tolerance was improved significantly in HFD-fed Fas-knockout mice compared with control mice (AUC in Fas-knockout mice 1,564 ± 66 mmol·l⁻¹·min vs. AUC in control mice 1,717 ± 42 mmol·l⁻¹·min; P < 0.05). In hyperinsulinemic euglycemic clamp studies, glucose infusion rate was not different between Fas-knockout and control mice (Fig. 5B). In contrast to insulin-stimulated glucose disposal rate, which was not different between the two groups

Table 1. Phenotypic characteristics of HFD-fed Fas⁰⁷ and FasΔadipo mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fas⁰⁷</th>
<th>FasΔadipo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>27.6 ± 0.5</td>
<td>28.5 ± 0.4</td>
</tr>
<tr>
<td>Epididymal fat mass, mg</td>
<td>288.6 ± 33.6</td>
<td>304.4 ± 21.3</td>
</tr>
<tr>
<td>Mesenteric fat mass, mg</td>
<td>248.4 ± 21.3</td>
<td>290.4 ± 23.2</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>8.8 ± 0.2</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>0.85 ± 0.3</td>
<td>1.06 ± 0.22</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.58 ± 0.10</td>
<td>0.65 ± 0.11</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>78.1 ± 10.4</td>
<td>104.3 ± 18.5</td>
</tr>
</tbody>
</table>

Results are means ± SE of 6–25 mice fasted for 5 h. FFA, free fatty acids; TG, triglycerides. Differences between both groups were statistically not significant.

HFD-fed adipocyte-specific Fas-knockout mice express decreased levels of proinflammatory cytokines in white adipose tissue. In the present study, we aimed to determine whether adipose tissue inflammation contributes to HFD-induced hepatic insulin resistance as early as 4 days after initiation of high-fat feeding. Previously, we found that adipocyte-specific Fas-knockout mice (FasΔadipo) were protected from long-term HFD-induced insulin resistance (25). Importantly, adipose tissue inflammation was reduced in FasΔadipo mice and was associated with improved hepatic insulin sensitivity. Therefore, if adipose tissue inflammation contributes to short-term HFD-induced hepatic insulin resistance, FasΔadipo mice may be a good model to study the presence of such cross-talk. As depicted in Fig. 4A, 4 days of HFD increased Fas protein content significantly in isolated adipocytes of wild-type mice. Moreover, expression of proinflammatory cytokines and macrophage markers was reduced significantly in both epididymal as well as mesenteric fat pads of FasΔadipo mice challenged by a short bout of HFD compared with control littermates (Fig. 4, B and C), suggesting that Fas depletion diminished adipose tissue inflammation. Further phenotypic characteristics of HFD-fed Fas⁰⁷ and FasΔadipo mice are described in Table 1.

Adipocyte-specific Fas-knockout mice are protected from short-term HFD-induced hepatic insulin resistance. We next assessed glucose metabolism in HFD-fed adipocyte-specific Fas-knockout and control mice. As depicted (Fig. 5A), glucose tolerance was improved significantly in HFD-fed Fas-knockout mice compared with control mice (AUC in Fas-knockout mice 1,564 ± 66 mmol·l⁻¹·min vs. AUC in control mice 1,717 ± 42 mmol·l⁻¹·min; P < 0.05). In hyperinsulinemic euglycemic clamp studies, glucose infusion rate was not different between Fas-knockout and control mice (Fig. 5B). In contrast to insulin-stimulated glucose disposal rate, which was not different between the two groups
(Fig. 5C), insulin-mediated inhibition of hepatic glucose production was almost completely preserved in Fas<sup>Δ adip</sup> mice, whereas it was clearly blunted in Fas-expressing littermates (Fig. 5D). Similarly, insulin-stimulated Akt phosphorylation was sustained in livers of Fas<sup>Δ adip</sup> mice compared with control mice (Fig. 5E). Of note, total Akt protein levels were not different between the groups (data not shown). Thus, adipocyte-specific Fas-knockout mice are protected from HFD-induced hepatic insulin resistance.

**Adipocyte-specific Fas depletion has no impact on liver lipid accumulation.** In contrast to improved hepatic insulin sensitivity, total liver lipid content was not different between HFD-fed adipocyte-specific Fas-knockout and control mice (Fig. 6, A and B). Similarly, expression of several inflammation markers was similar between both groups (Fig. 6C). Thus, Fas depletion in adipocytes protects mice from developing short-term HFD-induced adipose tissue inflammation and hepatic insulin resistance but not hepatic steatosis.

**DISCUSSION**

The present study suggests that adipose tissue inflammation contributes to short-term HFD-induced hepatic insulin resistance. Such a notion is based on the following findings: 1) 4 days of HFD induces adipose tissue TNFα expression; 2) glucose intolerance as well as hepatic insulin resistance develops 4 days after initiation of HFD; and 3) Fas depletion specifically in adipocytes reduces both HFD-induced adipose tissue inflammation and hepatic insulin resistance.

Several lines point toward a role of adipose tissue inflammation in the development of hepatic insulin resistance in obesity. It is thought that increased production and release of proinflammatory cytokines from visceral, i.e., omental and mesenteric adipose tissue into the portal vein, may contribute to the development of hepatic insulin resistance (6). A role for such dysfunctional fat-liver cross-talk in the pathogenesis of obesity-associated hepatic insulin resistance is supported by the fact that impinging on adipose tissue inflammation protects against HFD-induced insulin resistance and steatosis (15, 20, 25). Our findings presented here suggest that adipose tissue inflammation occurs early on in the course of HFD-induced metabolic alterations and contributes to hepatic insulin resistance. Such results seem to be in contrast to previous findings claiming that short-term HFD-induced insulin resistance is independent of inflammation (13). Although this study demonstrates induction of adipose tissue inflammation as manifested by an increase in macrophage infiltration of adipose tissue as well as by increased expression of proinflammatory cytokines (mainly TNFα), it fails to show a beneficial effect of macrophage and lymphocyte depletion as well as of hematopoietic cell-specific Jun NH2-terminal kinase (JNK)-deficiency on short-term HFD-induced insulin resistance in mice (13). In contrast, we find herein a favorable effect of adipocyte-specific Fas deficiency on both adipose tissue inflammation and hepatic insulin sensitivity. Of note, differences in TNFα expression were stronger in mesenteric compared with epididymal adipose tissue, supporting the “portal theory.” The latter proposes that the liver is exposed directly to increas-

![Fig. 6](http://ajpendo.physiology.org/)

**Fig. 6.** Adipocyte-specific Fas depletion has no impact on liver lipid accumulation. A: representative hematoxylin and eosin-stained liver sections from HFD-fed Fas<sup>Δ adip</sup> (left) and Fas<sup>Δ adip</sup> (right) mice (magnification ×20). B: total liver lipids were determined and expressed relative to liver weight. Results are the means of 8 (Fas<sup>Δ adip</sup>) or 5 HFD-fed animals (Fas<sup>Δ adip</sup>). C: mRNA expression of respective genes was determined in liver of HFD-fed Fas<sup>Δ adip</sup> (open bars) and Fas<sup>Δ adip</sup> mice (gray bars); n = 9. All error bars represent SE. *P < 0.05 (Student’s t-test).
ing amounts of free fatty acids and/or proinflammatory factors released from visceral fat into the portal vein, promoting the development of hepatic insulin resistance (6). Our findings presented here may suggest that short-term HFD-increased TNFα expression in adipose tissue induces hepatic insulin resistance. In support of such a notion, TNFα was shown recently to induce insulin resistance in hepatocytes in culture (5). Moreover, it was reported that inhibition of TNFα by the monoclonal antibody infliximab improves HFD-induced hepatic insulin resistance in rats (1).

There is a strong association between hepatic steatosis and insulin resistance, although it is still debated whether insulin resistance is the cause of hepatic steatosis or whether the increase in triglycerides (or of lipid metabolites such as ceramides, diacylglycerol, and acyl-CoAs) causes the development of hepatic and/or systemic insulin resistance (14). In this regard, our observation that Fas\textsuperscript{Adipo} mice were protected from hepatic insulin resistance but not hepatic steatosis is intriguing and indicates that the latter may be the result of an acute lipid overload rather than a dysfunctional adipose tissue-liver cross-talk at an early stage of HFD. Moreover, it may suggest that short-term HFD-induced hepatic steatosis develops independently of concomitant hepatic insulin resistance. Supporting this notion, it was reported previously that 3 days of HFD induced hepatic insulin resistance and steatosis as well as hepatic inflammation and Kupffer cell activation. Interestingly, depletion of the latter improved hepatic insulin sensitivity, whereas it did not affect hepatic steatosis (12). Conversely, Samuel et al. (21) reported in rats that treatment with 2,4-dinitrophenol normalized short-term HFD-induced hepatic steatosis, whereas it improved only partly the ability of insulin to suppress endogenous glucose production. Of note is that Fas\textsuperscript{Adipo} mice depicted both improved hepatic insulin sensitivity as well as reduced total liver lipid content after 6 wk of HFD (25), suggesting that, later in the course of HFD-induced obesity, adipose tissue inflammation and its resulting dysfunctional adipose tissue-liver cross-talk may impact on both hepatic insulin sensitivity as well as hepatic steatosis.

In conclusion, our results identify adipose tissue inflammation and consecutive dysfunctional adipose tissue-liver cross-talk as an early event in the development of HFD-induced deterioration of hepatic insulin sensitivity.

ACKNOWLEDGMENTS

We thank Giatgen Spinas for continuous support and Dr. A. Chervonsky, University of Chicago, for providing Fas\textsuperscript{−/−} mice. We are grateful to Roche Diagnostics, Rotkreuz, Switzerland, for providing test strips for the measurement of blood glucose.

GRANTS

This work was supported by research grants from the Swiss National Science Foundation (no. 310030–141238 to D. Konrad), the Wolferrman Nägeli Stiftung (to D. Konrad), and the Forschungskredit of the University of Zurich (to F. Item).

DISCLOSURES

No conflicts of interest, financial or otherwise, exist for any of the authors.

AUTHOR CONTRIBUTIONS

M.S.W., S.W., and F.I. performed the experiments; M.S.W., S.W., F.I., and D.K. analyzed the data; M.S.W., S.W., F.I., and D.K. interpreted the results of the experiments; M.S.W., S.W., F.I., and D.K. prepared the figures; M.S.W., S.W., E.J.S., and D.K. edited and revised the manuscript; M.S.W., S.W., F.I., E.J.S., and D.K. approved the final version of the manuscript; S.W. and D.K. contributed to the conception and design of the research; D.K. drafted the manuscript.

REFERENCES


