Published in final edited form as:

Biochem Biophys Res Commun. 2008 December 12; 377(2): 447–452. doi:10.1016/j.bbrc.2008.09.158.

SOCS-1 Deficiency Does Not Prevent Diet-Induced Insulin Resistance

Brice Emanuelli, Yazmin Macotella, Jérémie Boucher, and C. Ronald Kahn

Hormone and Obesity Research, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts, 02215.

Abstract

Obesity is associated with inflammation and increased expression of suppressor of cytokine signaling (SOCS) proteins, which inhibit cytokine and insulin signaling. Thus, reducing SOCS expression could prevent the development of obesity-induced insulin resistance. Using SOCS-1 knockout mice, we investigated the contribution of SOCS-1 in the development of insulin resistance induced by a high fat diet (HFD). SOCS-1 knockout mice on HFD gained 70% more weight, displayed a 2.3-fold increase in epididymal fat pads mass and increased hepatic lipid content. This was accompanied by increased mRNA expression of leptin and the macrophage marker CD68 in white adipose tissue and of SREBP1c and FAS in liver. HFD also induced hyperglycemia in SOCS-1 deficient mice with impairment of glucose and insulin tolerance tests. Thus, despite the role of SOCS proteins in obesity-related insulin resistance, SOCS-1 deficiency alone is not able to prevent insulin resistance induced by a diet rich in fat.

Keywords

Insulin resistance; diabetes; obesity; cytokines; SOCS

Introduction

Insulin resistance, a central event driving the development of the metabolic syndrome, is associated with various pathological conditions, the most common of which is obesity. Indeed, the prevalence of obesity is increasing worldwide and now affects almost 300,000,000 people. Like humans, mice fed a diet rich in fat become obese and develop insulin resistance [1,2]. Several mechanisms contribute to the development of insulin resistance in obesity, one of the prominent of which is increased inflammation in adipose tissue [3]. Indeed, both increased macrophage accumulation and increased levels of pro-inflammatory cytokines secreted from adipose tissue [4,5] contribute to the activation of a variety of cellular events that impede insulin action in its target tissues.

Suppressor of cytokine signaling (SOCS) proteins are involved in the regulation of the inflammatory response through a negative feedback loop on cytokine action [6]. Indeed, once expressed in response to an elevation of cytokine levels, SOCS proteins can inhibit

© 2008 Elsevier Inc. All rights reserved.

To whom correspondence should be addressed: C. Ronald Kahn, M.D. Joslin Diabetes Center One Joslin Place Boston, MA 02215
Phone: 617-732-2635 Fax: 617-732-2593 c.ronald.kahn@joslin.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cytokine signaling through at least two different mechanisms: SOCS-1 directly binding to Jak kinase to inhibit its tyrosine kinase activity, while SOCS-3 binds to the cytokine receptor to inhibit signaling [6]. In addition to their inhibition of cytokine signaling, SOCS proteins, in particular SOCS-1 and SOCS-3 modulate other signaling pathways, including insulin signaling [7]. Increased expression of SOCS-1 and/or SOCS-3 has been shown to induce insulin resistance in various models of obesity and diabetes via inhibition of the tyrosine kinase activity of the receptor, competition for the binding of the insulin receptor substrates or targeting the insulin receptor substrates to degradation [7-10]. Increased expression of SOCS-1 and/or SOCS-3 has been reported in insulin sensitive tissues in rodents on a high fat diet or in murine genetic models of obesity, as well as in humans with type 2 diabetes [8,11,12]. Therefore, it has been postulated that reducing SOCS expression should help prevent insulin resistance. Indeed, studies performed with SOCS-3 haplo-deficient mice or mice deficient for SOCS-3 in the brain only, have shown that these mice remain insulin sensitive when exposed to HFD-induced obesity [13,14]. Acute inhibition of SOCS-1 and SOCS-3 expression in the liver of obese diabetic db/db mice with antisense oligonucleotides treatment ameliorates some metabolic disorders in these animals [15]. SOCS-1 deficient mice also have been suggested to display increased insulin sensitivity [16], however, the mice do not survive the 3rd week after birth and therefore, it has been impossible to evaluate the role of SOCS-1 in the development of obesity related insulin resistance. Also, since SOCS proteins regulate the inflammatory response, an important link between obesity and insulin resistance, by negatively regulating cytokine signaling, it was important to study in which extent SOCS-1 participates in or protects against the development of this syndrome.

In the present study, we circumvented the problem of early death of the SOCS-1 deficient mice by using mice combining SOCS-1 deficiency with an additional deficiency of RAG2, which rescues the early death of SOCS-1 deficient mice [17]. We were thus able to study the involvement of SOCS-1 in the development of insulin resistance induced by a high fat diet.

Materials and Methods

Animals

SOCS-1/RAG2 deficient mice described in [17] were given by Dr J.N. Ihle, Howard Hughes Medical Institute, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee, USA and were obtained from Charles River laboratories (Wilmington, USA). SOCS-1 deletion was confirmed by PCR genotyping. All mice in this study were on a 129Sv-C57BL/6 mixed genetic background. All mice were housed in pathogen-free facilities on a 12-h light-dark cycle and were fed ad libitum either with a standard rodent chow diet (10% fat) or with a high-fat diet containing 55% of calories derived from fat (Harlan Teklad, Madison, WI). Two groups of mice (controls and SOCS-1 KO) were maintained on the standard chow diet, and one group of SOCS-1 KO mice was fed with a high fat diet starting at the age of 9 weeks. The mice were maintained on the different diets for 20 weeks, after which the mice were sacrificed. All protocols for animal use and euthanasia were approved by the Animal Care Use Committee of the Joslin Diabetes Center in accordance with National Institutes of Health guidelines.

Animal Studies

Blood glucose levels were determined from venous tail blood using a One Touch II glucose monitor (Lifescan Inc., Milipitas, California). Insulin and glucose tolerance tests were done on animals that had been fasted for 2 hours or overnight, respectively. For glucose tolerance testing (GTT), blood samples were obtained at 0, 15, 30, 60, and 120 min after intraperitoneal injection of 2 g/kg dextrose. Insulin tolerance tests were performed by injecting 1 U/kg insulin (Novolin, Novo Nordisk, Denmark) intraperitoneally, followed by

blood collection at 0, 15, 30, and 60 min. Histology was done by hematoxylin & eosin staining of liver sections.

RNA extraction and gene expression by real-time PCR

RNA extraction from epididymal adipose tissue and liver was performed using RNeasy kit from Qiagen (Valencia, CA). Gene expression was assessed by quantitative real-time PCR. 1 µg of total RNA was used for cDNA synthesis using a kit from Applied Biosystems (Warrington, UK). Specific primers for leptin, TNF- α (tumor necrosis factor- α), CD68 (cluster of differentiation 68), SREBP1c (sterol regulatory element binding protein), FAS (fatty acid synthase), fructose 1,6 bisphosphatase, PEPCCK (phosphoenolpyruvatecarboxykinase), Glucose-6 phosphatase and TBP (TATA binding protein) were used for the PCR reaction using the ABI Prism 7900HT (Applied Biosystems, Warrington, UK), and analysis was done with the ABI Prism SDS 2.2.2 software.

Results

Weight gain of SOCS-1 deficient mice on a high fat diet

Defining the role of SOCS-1 in insulin resistance has been limited, since SOCS-1 knockout (KO) mice die shortly after birth. To overcome this limitation, we studied mice that were also deficient in expression of RAG2, which prevents the lethality of SOCS-1 deficiency, and thus allows us to study SOCS-1 KO mice after a period of high fat diet induced obesity. Control *socs1*, *rag2* (+/+, -/-) and SOCS-1 KO *socs1*, *rag2* (-/-, -/-) mice were fed with a chow diet and another group of SOCS-1 KO mice was fed with a high fat diet (HFD) for 20 weeks. Both controls and SOCS-1 KO mice gained similar amounts of weight during the experiment, respectively 12.45 ± 0.75 g and 11.30 ± 3.97 g (Fig. 1 A). Epididymal fat pads mass were equivalent in both groups, although there was a tendency for the fat pads mass to be decreased in the KO group (2.63 ± 0.36 g vs 1.83 ± 0.65 g, $p = 0.056$). When placed on a HFD, SOCS-1 KO mice gained 70% more weight than SOCS-1 KO mice on chow diet (Fig. 1 A). High fat diet also induced a 2.3 fold increase of the epididymal fat pads mass (4.24 ± 1.02 g) in SOCS-1 KO mice when compared with normal diet, and this increase is conserved when normalized to total body weight (Fig. 1 B and C). These results indicate that SOCS-1 deficiency does not protect against HFD-induced obesity.

In response to the increased fat mass, there is macrophage infiltration of adipose tissue and change in the expression of various genes in adipose tissue [18]. As expected, the expression of the gene encoding the anti-orexigenic hormone leptin was increased by more than 3-fold in SOCS-1 KO mice on HFD compared with SOCS-1 KO mice on chow diet ($p < 0.005$) and that of the pro-inflammatory cytokine TNF- α was increased by 2-fold, which did not quite reach significance with $p = 0.06$ (Fig. 1 D and E). We also observed a 2-fold increase ($p < 0.05$) in the expression of the macrophage marker CD68 mRNA in the adipose tissue of SOCS-1 KO mice on HFD compared with SOCS-1 KO mice on chow diet (Fig. 1 F), reflecting increased macrophage accumulation in the tissue.

Metabolic parameters of SOCS-1 deficient mice on a high fat diet

No difference was detected in fasting glucose in the three groups at the beginning of the diet (61.7 ± 9.2 mg/dL for the control group on ND, 70.2 ± 10.4 mg/dL for SOCS-1 KO group on ND and 75.2 ± 10.4 mg/dL for SOCS-1 KO group on HFD). Blood glucose levels remained very similar in both control and SOCS-1 KO groups on ND for the entire 20 weeks of the diet study (Fig. 2 A), with a tendency to be lower in the SOCS-1 KO (99.3 ± 21.5 mg/dL for the control group on ND, 87.8 ± 10.8 mg/dL for SOCS-1 KO group on ND at week 18). Fasted blood glucose levels progressively increased in the group of SOCS-1 KO mice on HFD consistent with increasing insulin resistance, and the mice eventually

became hyperglycemic with a value almost 70% higher than mice on ND by the end of the experiment (146.6 ± 27.1 mg/dL vs 87.8 ± 10.8 mg/dL).

Insulin tolerance test was performed after 18 weeks on the test diet. Both groups on ND had similar insulin sensitivity, whereas SOCS-1 KO mice on HFD developed insulin resistance, as indicated by a lesser fall in glucose levels following the IP insulin injection (Fig. 2 B). Glucose tolerance tests were performed after 7 or 19 weeks on the test diets. After 7 weeks on the diet, SOCS-1 KO mice on ND displayed a slightly better glucose tolerance than control littermates on the same diet, although this did not reach statistical significance (Fig. 2 C). SOCS-1 KO mice on HFD developed glucose intolerance when compared to SOCS-1 KO mice on ND as determined by quantitation of the area under the curve (Fig. 2 C and D). After 19 weeks on the different diets, all three groups displayed similar glucose tolerance curves (Fig. 2 C and D), suggesting that impaired glucose tolerance developed in all groups with aging, and that neither SOCS-1 deletion nor HFD had any additional effect. Altogether, these results indicate that SOCS-1 deletion doesn't protect against HFD-induced insulin resistance.

High fat diet triggers hepatosteatosis and modulates gene expression in SOCS-1 deficient mice on a high fat diet

Hematoxylin and eosin staining of liver sections taken from each group of mice revealed increased hepatic steatosis in livers from SOCS-1 KO mice on HFD as compared to the SOCS-1 KO mice or normal mice on ND (Fig. 3 A). Non-alcoholic hepatic steatosis occurs in response to increased fat accumulation in the liver. Interestingly, although the expression of SREBP1c and FAS, two of the most important genes regulating lipid synthesis in liver, was unchanged in control and SOCS-1 KO groups on ND, the expression levels of SREBP1c and FAS were increased by 280% and 60%, respectively, in the livers of SOCS-1 KO mice on HFD when compared to SOCS-1 KO on ND (Fig. 3 B). No difference was observed in the expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6 phosphatase and fructose-1,6 biphosphatase, key genes involved in the regulation of gluconeogenesis, between all three groups (Fig. 3 C).

Discussion

Cytokine and inflammatory pathways are activated and play important roles in obesity and type 2 diabetes related insulin resistance through a variety of mechanisms [3,19]. One mechanism involves an increase in expression of SOCS proteins, especially SOCS-1 and SOCS-3, which have been suggested to further propagate the insulin resistance by directly inhibiting insulin action [8,11,12,20]. Thus, increasing levels of SOCS-1 and SOCS-3 have been shown to reduce insulin action *in vitro* and *in vivo* in fibroblasts, muscle cells, adipocytes, beta cells and in the liver [7-9,11,21] and reduction of the expression of SOCS-1 and SOCS-3 has been shown to improve insulin sensitivity [15]. Mice with heterozygous deletion of SOCS-3 at the whole body level or homozygous deletion of SOCS-3 in brain have been shown to remain insulin sensitive when exposed to HFD-induced obesity [13,14]. SOCS-1 deficient mice also have been suggested to display increased insulin sensitivity [16], however, the role of SOCS-1 in the development of insulin resistance in obesity has been unclear, since SOCS-1 (-/-) mice die in the first few weeks of life [16].

In the present study, we have been able to determine the role of SOCS-1 in HFD induced obesity by taking advantage of mice with combined SOCS-1/RAG2 deficiency, which can survive to adulthood. We find that deficiency of SOCS-1 does not prevent the development of high fat diet-induced obesity or HFD-induced insulin resistance. Indeed, SOCS-1 KO mice gained weight when fed with a high fat diet in a similar manner than wild type mice on HFD [2]. As previously reported in other models, increased weight gain in SOCS-1 KO

mice is due to increased fat mass, indicating that SOCS-1 deficiency does not alter adipose tissue development. Indeed, it has been reported that adipocyte differentiation may actually be increased in mouse embryonic fibroblasts isolated from SOCS-1 KO animals [16], although we did not observe any increase adipose tissue mass in SOCS-1 deficient mice on normal diet. Rather SOCS-1 KO mice on normal diet tended to have lower adipose tissue mass than controls.

The high fat diet-induced obesity that occurred in SOCS-1 KO animals was accompanied with increased inflammation in the adipose tissue, as reflected by higher levels of expression of the pro-inflammatory cytokine TNF- α and the macrophage marker CD68. In addition, SOCS-1 KO mice on HFD developed hepatic steatosis with increased expression of lipogenic genes. Both of these changes are central to the development of insulin resistance. This ultimately led to impaired glucose tolerance and hyperglycemia. While these results seem to be contradictory with a report showing that SOCS-1 KO mice are hypoglycemic and more insulin sensitive [16], in the latter study, all of these mice died very shortly after birth, making it impossible to study any potential beneficial effect of SOCS-1 deficiency in a context of obesity-induced insulin resistance. Thus, our results indicate that insulin resistance can develop despite the absence of SOCS-1, an inhibitor of its signaling.

In summary, our study clearly demonstrates that insulin resistance induced by high fat feeding can develop independently of the presence of SOCS-1. This is in contrast to SOCS-3 haplo-deficient mice that are protected against high fat diet induced obesity and metabolic complications [13]. Similarly, mice deficient for SOCS-3 specifically in the brain also display resistance to HFD-induced obesity and insulin resistance [14]. This is also in contrast to the beneficial effect we previously described that decreasing SOCS-3 or both SOCS-1 and SOCS-3 expression by antisense oligonucleotides treatment in liver of db/db mice decreased the expression of SREBP-1c and FAS and the resulting hepatosteatosis [15]. Thus, SOCS-1 deletion alone may not be sufficient to ameliorate the metabolic disorders of insulin resistance, but SOCS-1 may act to complement the effects of SOCS-3 in insulin resistance. This may be partly explained by the different manner in which these two proteins have been shown to alter insulin receptor signaling [11]. It remains of interest to determine how each of these inflammatory pathways works in vivo and which are complementary or synergistic in their effects on insulin resistance. This would ultimately allow targeting these pathways for the therapy of this important disorder.

Acknowledgments

This work was supported by National Institutes of Health Grant DK33201. B.E. acknowledges support from the mentor-based American Diabetes Association award. Y.M. was supported by the Pew Latin American Fellows Program in the Biomedical Sciences and by the minority mentor-based American Diabetes Association award.

Reference List

1. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. *Diabetes*. 1988; 37:1163–1167. [PubMed: 3044882]
2. Almind K, Kahn CR. Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. *Diabetes*. 2004; 53:3274–3285. [PubMed: 15561960]
3. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest*. 2005; 115:1111–1119. [PubMed: 15864338]
4. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003; 112:1796–1808. [PubMed: 14679176]

5. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003; 112:1821–1830. [PubMed: 14679177]
6. Alexander WS, Hilton DJ. The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. *Annu Rev.Immunol.* 2004; 22:503–529. [PubMed: 15032587]
7. Emanuelli B, Peraldi P, Filloux C, Sawka-Verhelle D, Hilton D, Van Obberghen E. SOCS-3 is an insulin-induced negative regulator of insulin signaling. *J.Biol.Chem.* 2000; 275:15985–15991. [PubMed: 10821852]
8. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol.Chem.* 2001; 276:47944–47949. [PubMed: 11604392]
9. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem.* 2002; 277:42394–42398. [PubMed: 12228220]
10. Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y, Furlanetto RW. Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. *J Biol.Chem.* 2001; 276:25889–25893. [PubMed: 11342531]
11. Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol.* 2004; 24:5434–5446. [PubMed: 15169905]
12. Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H. Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes.* 2004; 53:2232–2241. [PubMed: 15331532]
13. Howard JK, Cave BJ, Oksanen LJ, Tzamelis I, Bjorbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat.Med.* 2004; 10:734–738. [PubMed: 15220914]
14. Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat.Med.* 2004; 10:739–743. [PubMed: 15208705]
15. Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl.Acad Sci U S.A.* 2004; 101:10422–10427. [PubMed: 15240880]
16. Kawazoe Y, Naka T, Fujimoto M, Kohzaki H, Morita Y, Narazaki M, Okumura K, Saitoh H, Nakagawa R, Uchiyama Y, Akira S, Kishimoto T. Signal transducer and activator of transcription (STAT)-induced STAT inhibitor 1 (SSI-1)/suppressor of cytokine signaling 1 (SOCS1) inhibits insulin signal transduction pathway through modulating insulin receptor substrate 1 (IRS-1) phosphorylation. *J Exp Med.* 2001; 193:263–269. [PubMed: 11208867]
17. Marine JC, Topham DJ, McKay C, Wang D, Parganas E, Stravopodis D, Yoshimura A, Ihle JN. SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell.* 1999; 98:609–616. [PubMed: 10490100]
18. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest.* 2003; 112:1785–1788. [PubMed: 14679172]
19. Lebrun P, Van Obberghen E. SOCS proteins causing trouble in insulin action. *Acta Physiol.(Oxf.).* 2008; 192:29–36. [PubMed: 18171427]
20. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat.Med.* 2005; 11:183–190. [PubMed: 15685173]
21. Emanuelli B, Glondou M, Filloux C, Peraldi P, Van Obberghen E. The potential role of SOCS-3 in the interleukin-1beta-induced desensitization of insulin signaling in pancreatic beta-cells. *Diabetes Suppl.* 2004; 3:S97–S103.

Figure 1. Increased weight gain, fat mass and modulation of gene expression in white adipose tissue of SOCS-1 KO mice on HFD

Nine week-old SOCS-1 KO mice and littermate controls were fed either with a normal chow diet (ND) or with a high fat diet (HFD) for 20 weeks. n=5 for the control group on ND (dark grey), n=5 for the SOCS-1 KO group on ND (light grey), and n=6 for the SOCS-1 KO group on HFD (dashed light grey). (A) Total body weight was measured throughout the all experiment in all groups and weight gain was calculated. (B) Epididymal fat pad mass was assessed at week 20 after sacrifice. (C) Fat pad mass was normalized by dividing by total body weight. Expression of leptin (D), TNF- α (E) and CD68 (F) from epididymal fat pads was analyzed by real-time PCR. Expression levels were normalized to TBP and presented as the means $\pm$ SEM. The Student t test was used for statistical analysis between two groups. * p<0.005

Figure 2. HFD-induced hyperglycemia, glucose intolerance and insulin resistance in SOCS-1 KO mice on HFD

Nine week-old SOCS-1 KO mice and littermate controls were fed either with a normal chow diet (ND) or with a high fat diet (HFD) for 20 weeks. n=5 for the control group on ND (dark grey), n=5 for the SOCS-1 KO group on ND (light grey), and n=6 for the SOCS-1 KO group on HFD (dashed light grey). (A) Fasting blood glucose levels were measured at weeks 2, 5, 12 and 18 after starting the testing diets. (B) Intraperitoneal insulin tolerance tests were performed on week 19 of HFD. After a 2 h fast, insulin was injected at 1 mU/kg of body weight, and blood glucose levels were measured. Results are expressed as percent of basal. (C) Intraperitoneal glucose tolerance test (IPGTT) were performed on week 7 and 18 of HFD. After an overnight fast, glucose was injected at 2 g/kg of body weight, and blood glucose levels were measured. (D) Area under the curve of IPGTT was estimated by linear integration, i.e., by summing the numerical integration values of successive linear segment approximations of the glucose curve for 0–15, 15–30, 30–60 and 60–120 min post-injection. The Student t test was used for statistical analysis between two groups. *p<0.05

Figure 3. Hepatosteatosis and modulation of gene expression in the liver of SOCS-1 KO mice on HFD

(A) Hematoxylin and eosin staining of liver section is shown at a 40x magnification. (B-C) Gene expression analysis was performed on liver extracts from SOCS-1 KO mice and littermate controls fed either with a normal chow diet (ND) or with a high fat diet (HFD) for 20 weeks, n=5 for the control group on ND (dark grey), n=5 for the SOCS-1 KO group on ND (light grey), and n=6 for the SOCS-1 KO group on HFD (dashed light grey). Hepatic expression of various genes was analyzed by real-time PCR as mentioned using SREBP1c, FAS, F1,6 BPase, G6Pase, PEPCK specific primers. Expression levels were normalized to TBP and presented as the means $\pm$ SEM. * $p < 0.01$.