Biochemical and Biophysical Research Communications 377 (2008) 447–452

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# SOCS-1 deficiency does not prevent diet-induced insulin resistance

Brice Emanuelli, Yazmin Macotela, Jérémie Boucher, C. Ronald Kahn *

*Hormone and Obesity Research, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA*

ARTICLE INFO

*Article history:*
Received 25 September 2008
Available online 16 October 2008

*Keywords:*
Insulin resistance
Diabetes
Obesity
Cytokines
SOCS
Inflammation
High fat diet

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.



## 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 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

* Corresponding author. Fax: +1 617 732 2593.
*E-mail address:* c.ronald.kahn@joslin.harvard.edu (C.Ronald Kahn).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.158

of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN, 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, CA). Insulin and glucose tolerance tests were done on animals that had been fasted for 2 h 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 and 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, PEPCK (phosphoenolpyruvatecarboxykinase), glucose-6-phosphatase, and

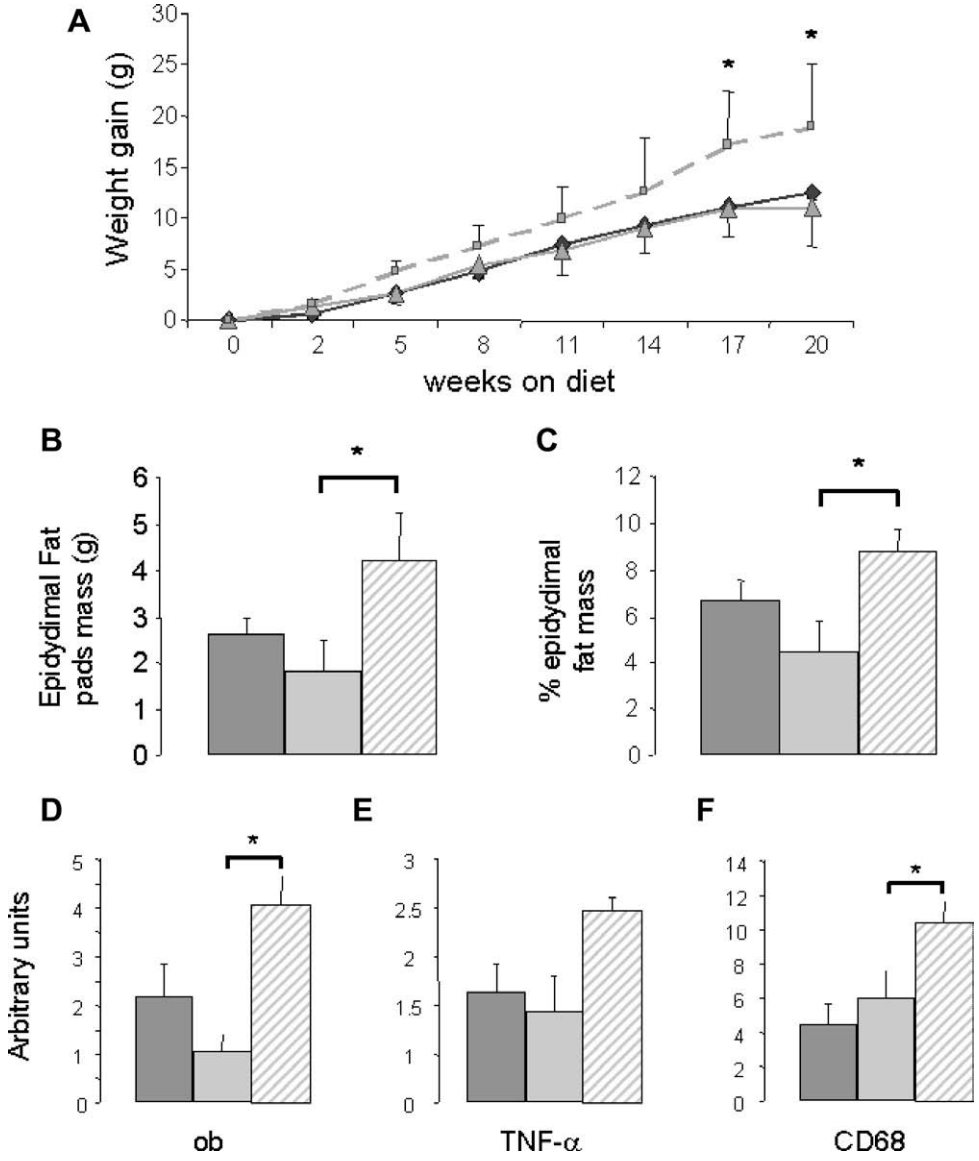


**Fig. 1.** Increased weight gain, fat mass and modulation of gene expression in white adipose tissue of SOCS-1 KO mice on HFD. Nine-week-old SOCS-1 KO mice and littermate controls were fed either with a normal chow diet (ND) or with a high-fat diet (HFD) for 20 weeks. $n = 5$ for the control group on 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 eight 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 ± SEM. The Student $t$ test was used for statistical analysis between two groups. $^{*}p < 0.005$.