Adipocyte-specific deletion of IL-6 does not attenuate obesity-induced weight gain or glucose intolerance in mice

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RESEARCH ARTICLE

INTRODUCTION

Interleukin-6 (IL-6) is an inflammatory cytokine that has been implicated in the etiology of obesity and type 2 diabetes (T2D), since patients with metabolic syndrome display elevated levels of this cytokine (2, 21). Furthermore, preclinical studies have demonstrated that IL-6 causes insulin resistance, particularly in the liver, by activating suppressor of cytokine signaling 3 (SOCS3), leading to defective tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) (17, 18).

However, the notion that IL-6 can cause insulin resistance is unclear, since there are many lines of evidence that this cytokine can, in fact, be protective against insulin resistance. First, in humans, infusion of recombinant human IL-6 during a hyperinsulinemic-euglycemic clamp results in a small increase, rather than a decrease, in glucose infusion rate (3). Vigorous physical exercise can increase IL-6 over 100-fold in the circulation from the contracting muscles (19), and, following exercise, insulin sensitivity is markedly increased (23). Furthermore, mice lacking IL-6 are prone to obesity (22), liver inflammation, and insulin resistance when fed a high-fat diet (12), whereas mice that overexpress IL-6 present with the opposite phenotype, in part via enhanced leptin sensitivity (16). More recently, IL-6 was shown in mice to enhance the incretin response (5), which is a drug target for T2D. Notwithstanding this evidence, two important findings suggest that IL-6 release from adipose tissue in obesity can result in liver insulin resistance and inflammation. In a study by Davis et al. (15), the authors observed that a c-Jun NH2-terminal kinase-1 (JNK1)-dependent secretion of IL-6 by adipose tissue caused increased expression of liver SOCS3 and hepatic insulin resistance. Shoelson and colleagues [Cai et al. (1)] blocked IL-6 using an antibody in transgenic mice where IκB kinase-β (IKKβ) was constitutively active in liver, and they observed an improvement in insulin resistance. All of the data taken together, it appears that if IL-6 is released from skeletal muscle during exercise it may have beneficial effects but that IL-6 released from adipose tissue in obesity can lead to liver insulin resistance. This latter hypothesis has, to date, not been comprehensively tested. Accordingly, in the present study, we generated adipocyte-specific IL-6-deficient mice in the context of diet induced and genetic obesity. We demonstrate, contrary to prevailing dogma, that deletion of IL-6 specifically from adipocytes has no effect on obesity-induced metabolic disturbances.
METHODS

Mouse Models of Obesity

AdipoIL-6−/− mice. Adipocyte-specific, IL-6 knockout (AdipoIL-6−/−) mice were generated via breeding of C57BL/6 mice carrying loxP inserts surrounding exon 2 of the IL-6 gene, as previously described (14). Prior to crossing these mice with C57BL/6 mice carrying the Cre recombinase gene under the control of the adiponec- tin promoter (Jackson laboratories B6.FVB-Tg(Adipoq-cre)1Evdhr), we backcrossed these mice to C57BL/6 mice a further 10 generations. Mice were housed at the Alfred Medical Research and Education Precinct Animal Centre (Melbourne, Australia) in a pathogen-free facility under a 12:12-h light-dark cycle. From 8 wk of age, mice were fed either a normal chow diet (NC; 14.3 MJ/kg, 76% of kJ from carbohydrate, 5% from fat, and 19% from protein), or a high-fat diet (HFD) [19 MJ/kg, 36% of kJ from carbohydrate, 43% from fat (42.9% saturated, 35.24% monounsaturated, and 21.86% polyunsaturated fatty acids), and 21% from protein; both Specialty Feeds, Glen Forrest, Australia] for a total of 16 wk. Food and water were provided ad libitum. All experiments were performed in male mice, and on cessation of the study, mice were euthanized after a 5-h fast. Animal experiments were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee in accordance with the Australian code for the care and use of animals for scientific purposes (2013).

Metabolic Phenotyping

Body composition. Fat mass and lean body mass were measured with a 4-in-1 EchoMRI body composition analyzer (Columbus Instruments) and standard laboratory scales.

Oral glucose tolerance test. Glucose tolerance was assessed via gavage of 2 g/kg lean body mass 25% glucose solution and assessment of blood glucose sampled from the tail vein via hand-held glucometer (Accu-check, Roche, Castle Hill, Australia). In all instances, mice were fasted for 5 h. At time points 0 and 15 min, an ~20-μL blood sample was taken for assessment of plasma insulin.

Plasma Analysis

Plasma insulin and IL-6 were determined using mouse ELISA (80-INSMSU-E01; ALPCO, Salem, NH) and (P08505; RayBiotech, Norcross, GA) respectively, per manufacturers’ instructions.

Liver Histology

Hematoxylin and eosin staining was carried out on three liver sections from a minimum of seven mice per group. Three representative images of each section were taken using a Leica DM4000 microscope with ×20 NA 0.5 HCX PL Fluor objective. Particle analysis in ImageJ (National Institutes of Health, Bethesda, MD) was performed to identify and measure likely lipid droplets, with data from nine images for each animal combined.

PCR and Microarray

For confirmation of loxP-Cre recombination, in both AdipoIL-6−/− and AdipoIL-6−/− ob/ob mice, genomic DNA was isolated using the isolate II Genomic DNA kit (Bioline, Alexandria, Australia) per manufacturer’s instructions. PCR was run using the Phire Hot Start II DNA Polymerase (ThermoFisher Scientific) and primer sequences forward CCCACCAAGAAGATAGTCA and reverse ATGCCCA- GCCTTACCTAGGT. For quantitative real-time (qRT)PCR and microarray, RNA was isolated using a Nucleospin RNA extraction kit (Macherey-Nagel, Duren, Germany) according to the manufacturer’s instructions. For microarray analysis, RNA was assessed on a Bioanalyzer using an RNA 6000 Nano chip (Agilent, Santa Clara, CA), setting the threshold for RNA quality at RIN values >7. Samples (n = 3) were subsequently hybridized to a GeneChip Mouse Gene 2.0 ST array and run according to the manufacturer’s instructions (Affymetrix). For qRT-PCR, cDNA synthesis was carried out via Tetro reverse transcriptase (Bioline, Alexandria, Australia) after DNase treatment. Inventoried Taqman primer sequences (Assay on Demand; Applied Biosystems, Thermofisher Scientific) were used for IL-6 (Mm00446190_m1), tumor necrosis factor-α (TNFα; Mm00443258_m1), monocyte chemotactic protein-1 (MCP-1; Mm00441242_m1), and β2-microglobulin (B2M; Mm0437762_m1), with the last used as a housekeeping gene.

RESULTS

Adipocyte-Specific Deletion of IL-6 Does Not Affect HFD-Induced Obesity or Glucose Intolerance

After generating AdipoIL-6−/− mice, we first phenotyped floxed control, Cre control, and wild-type control mice after 16 wk of high-fat feeding. There were no differences in phenotype when we compared all control animals (data not shown). Floxed animals were therefore used in all subsequent experiments as controls. We initially confirmed loxP-Cre recombinase exclusively in white and brown adipose tissue in AdipoIL-6−/− mice (KO) but not littermate floxed controls (FL) (Fig. 1A). To study the role of IL-6 in adipose tissue on HFD-induced obesity and glucose intolerance, we first placed AdipoIL-6−/− mice and floxed control animals on chow or HFD for 16 wk. As expected, the dietary intervention increased body weight (Fig. 1B) and fat mass (Fig. 1C) irrespective of genotype. No differences were observed in these measurements when genotypes were compared (Fig. 1, B and C). After 16 wk on HFD, animals were characterized hyperinsulinemic, a hallmark of insulin resistance, but again we observed no differences when we compared genotypes (Fig. 1D). To determine whether glucose tolerance was influenced by adipocyte-derived IL-6, we next performed an OGTT. As shown previously in our laboratory (20), after only 1 wk of HFD, glucose tolerance was impaired. This effect was observed irrespective of genotype (Supplemental Fig. S1A; all supplemental data are available at https://doi.org/10.25500/edata.bham.00000338). The
HFD-induced glucose intolerance was maintained after 8 wk (Supplemental Fig. S1B) and 16 wk (Fig. 1, E–G). Importantly, however, adipocyte-specific deletion of IL-6 did not affect glucose tolerance at any time point irrespective of diet (Fig. 1 and Supplemental Fig. S1). These data suggest that adipocyte-derived IL-6 plays no role in the HFD-induced insulin resistance and glucose intolerance seen in mice.

**Adipocyte-Specific Deletion of IL-6 Does Not Affect HFD-Induced Liver Steatosis or Inflammatory Gene Expression**

Two previous studies had suggested that IL-6 release from adipose tissue in obesity could result in liver insulin resistance and inflammation (1, 15). Accordingly, we measured liver steatosis and the mRNA expression of inflammatory genes.
(IL-6), TNFα and MCP-1 in liver. As expected, 16 wk on a HFD increased lipid droplet formation (Fig. 2A) and the mRNA expressions of IL-6, TNFα, and MCP-1 (Fig. 2B). Although, as expected, the HFD did not increase liver IL-6 mRNA in AdipoIL-6<sup>−/−</sup> mice, there were no other differences in these measurements when the AdipoIL-6<sup>−/−</sup> mice were compared with floxed controls (Fig. 2, A and B). These data suggest that adipocyte-derived IL-6 plays no role in the HFD-induced hepatic steatosis and inflammation.

**Adipocyte-Specific Deletion of IL-6 Does Play a Minimal Role in Modulating Global Gene Expression in Adipose Tissue**

To this point, we were surprised that the adipocyte-specific deletion of IL-6 had almost no effect on any measured parameters. To ascertain the impact of such deletion on global gene expression, we performed microarray experiments on adipose tissue from AdipoIL-6<sup>−/−</sup> and floxed control mice on both HFD and chow diet and compared the gene expression profile between diets and genotypes. As demonstrated in Fig. 3, A and B, compared with animals on the chow diet, the HFD had a dramatic effect on the adipose tissue gene expression profile in both the AdipoIL-6<sup>−/−</sup> and floxed control mice, with many genes being significantly affected, including a significant enrichment of genes involved in inflammation ($P < 0.0001$). In contrast, however, when the gene expression profile of AdipoIL-6<sup>−/−</sup> was compared with that of the floxed control mice, only two genes, apolipoprotein A-IV (APOA4) and cytochrome P-450, respectively, were significantly increased in the AdipoIL-6<sup>−/−</sup> adipose tissue compared with the floxed controls on a HFD, with no genes being significantly decreased (Fig. 3C). When fed the chow diet, there were no differences whatsoever in AdipoIL-6<sup>−/−</sup> adipose tissue compared with tissue obtained from the floxed controls (Fig. 3C).

**Adipocyte-Specific Deletion of IL-6 Does Not Affect Obesity or Glucose Intolerance in ob/ob Mice**

The proposed mechanism by which IL-6 is thought to mediate liver insulin resistance and inflammation is by acting as an adipokine. Despite a pronounced increase in adiposity
after prolonged high-fat feeding (Fig. 1C), this did not result in a detectable increase in circulating IL-6 in floxed control animals (Fig. 4A). One could argue, therefore, that the experimental model, that being diet-induced obese was an insufficient metabolic perturbation to test the hypothesis. Accordingly, we next crossed the AdipoIL-6^{-/-} mice with a genetic model of obesity, the leptin-deficient (ob/ob) mouse. This model results in a more severe obese phenotype. We first verified that IL-6 was indeed deleted from the adipocytes but not other tissues in the AdipoIL-6^{-/-} ob/ob mice (Fig. 4B). Critically, in this experimental model circulating IL-6 was about threefold higher in floxed ob/ob controls compared with
AdipoIL-6\textsuperscript{-/-} ob/ob mice (Fig. 4C), making it an appropriate model to test the adipokine hypothesis. As expected, ob/ob mice had higher body weight (Fig. 4D) and fat mass (Fig. 4E) than ob/+ controls (deletion of only one leptin allele). No differences were observed in these measurements when AdipoIL-6\textsuperscript{-/-} ob/ob mice were compared with floxed ob/ob control mice or AdipoIL-6\textsuperscript{-/-} ob/+ with floxed ob/+ control mice (Fig. 4, D and E). Despite the fact that circulating IL-6 was about threefold lower in the AdipoIL-6\textsuperscript{-/-} ob/ob mice, this had no effect on insulin resistance, as measured by circulating basal insulin (Supplemental Fig. S2) or glucose tolerance (Fig. 4, F and G).

**DISCUSSION**

It is generally accepted that elevated circulating IL-6 due to obesity results in systemic insulin resistance, in part by reducing hepatic insulin action secondary to increased expression of...
SOCS3, liver inflammation, and hepatic steatosis. This hypothesis has, to date, not been experimentally tested. Here, using two models of obesity, we report that adipocyte-specific deletion of IL-6 plays no role in diet-induced or genetic obesity-driven insulin resistance or glucose intolerance.

The current thinking that elevated circulating IL-6 mediates liver insulin resistance is largely based on two previous studies. In the study by Davis et al. (15), the authors deleted JNK1 from adipose tissues and observed a complete amelioration of circulating IL-6 and improvements in liver inflammation, steatosis, SOCS3 protein expression, and liver insulin action. Intriguingly, however, in this previous paper, the HFD-induced IL-6 levels in wild-type animals were ~30 pg/mL. These levels were 30-fold higher than what we report here. The reason for these discrepancies is not readily apparent; however, HFD-induced increases in IL-6 of 30 pg/mL are at odds with most values reported in the literature. In the study by Shoels et al. and colleagues (1), the circulating IL-6 levels in the control animals were consistent with those reported by us but increased approximately twofold in the liver-specific IKK transgenic mice. Neutralizing IL-6 in this model ameliorated insulin resistance. It must be pointed out, however, that in the aforementioned study IKK was overexpressed in the liver and, hence, the IL-6 levels in the liver of the mice were directly increased. Taking our current data together with this previous study, we can only conclude that liver-derived IL-6 as a result of increased IKK results in systemic insulin resistance but that the IL-6 in adipocytes plays no role in obesity-induced systemic insulin action.

In the DIO model of obesity, although high-fat feeding led to pronounced adiposity, this did not translate to any observable increase in circulating IL-6. Therefore, it was possible that the DIO model was not appropriate to test our hypothesis. This necessitated further experiments using a genetic model of obesity. The ob/ob mouse, irrespective of IL-6 presence/absence in adipocytes, weighed ~55 g at the cessation of the study, and in this model the circulating IL-6 in the floxed ob/ob mice were ~30 pg/mL, which was reduced to ~10 pg/mL in the AdipoIL-6-/- ob/ob mice (Fig. 4C). These levels were roughly similar to those in the previous paper discussed above (15). Despite the approximately threefold decrease in circulating IL-6, glucose tolerance (Fig. 4, F and G) and hyperinsulinemia (Supplemental Fig. S2) were unaffected. Of note, when we deleted IL-6 from adipocytes specifically in this model, circulating IL-6 was nonetheless ~10-fold higher than what we reported in our DIO model. It must be noted that the FasCre line exhibits no recombination in other tissues, whereas another common adipose-specific Cre mouse, the adipocyte protein-2 (aP2)-Cre mouse, results in recombination in many other cells and tissues (10). Indeed, when IL-6 is deleted, driven by aP2-Cre promoter, differences were observed in metabolism of female mice (13). The ob/ob mouse is known to display systemic inflammation (6), and it is likely that other cells, such as those of the immune system, contributed to this elevation. This may have been sufficient to blunt any effect of reduced adipocyte IL-6 release. Notwithstanding this, it is clear from our data that adipocyte-specific deletion of IL-6 plays no role in obesity-induced metabolic disturbances.

To our surprise, adipocyte-specific deletion of IL-6 had almost no effect on global gene transcription in the adipose tissue. Although the HFD had major effects on the transcrip-

tome (Fig. 3), no genes were significantly changed when we compared the AdipoIL-6-/- with the floxed control mice on the chow diet, whereas only two genes were increased by adipocyte IL-6 deletion on the HFD. apoA-IV is known to play a role in chylomicrons and very-low-density lipoprotein secretion and catabolism (9). Cytochrome P-450 belongs to a group of hemoproteins that perform varied roles such as the oxidation of structurally unrelated compounds, including steroids and fatty acids (4). Whether the upregulation of these genes in the adipose tissue of high-fat-fed AdipoIL-6-/- mice has functional significance requires further investigation. What is clear, however, is that deleting IL-6 from the adipocytes of mice has almost no effect on global gene transcription.

In summary, we demonstrate that deleting IL-6 specifically from adipocytes in obese mice has no role in mediating glucose metabolism. Hence, our data do not support the prevailing model that in obese states IL-6 is released from adipose tissue to induce liver steatosis, inflammation, and insulin resistance.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

ADIPOCYTE IL-6 AND GLUCOSE TOLERANCE


