Brief communication

SOCS3 deficiency in leptin receptor-expressing cells mitigates the development of pregnancy-induced metabolic changes[a]

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ABSTRACT

Objective: During pregnancy, women normally increase their food intake and body fat mass, and exhibit insulin resistance. However, an increasing number of women are developing metabolic imbalances during pregnancy, including excessive gestational weight gain and gestational diabetes mellitus. Despite the negative health impacts of pregnancy-induced metabolic imbalances, their molecular causes remain unclear. Therefore, the present study investigated the molecular mechanisms responsible for orchestrating the metabolic changes observed during pregnancy.

Methods: Initially, we investigated the hypothalamic expression of key genes that could influence the energy balance and glucose homeostasis during pregnancy. Based on these results, we generated a conditional knockout mouse that lacks the suppressor of cytokine signaling-3 (SOCS3) only in leptin receptor-expressing cells and studied these animals during pregnancy.

Results: Among several genes involved in leptin resistance, only SOCS3 was increased in the hypothalamus of pregnant mice. Remarkably, SOCS3 deletion from leptin receptor-expressing cells prevented pregnancy-induced hyperphagia, body fat accumulation as well as leptin and insulin resistance without affecting the ability of the females to carry their gestation to term. Additionally, we found that SOCS3 conditional deletion protected females against long-term postpartum fat retention and streptozotocin-induced gestational diabetes.

Conclusions: Our study identified the increased hypothalamic expression of SOCS3 as a key mechanism responsible for triggering pregnancy-induced leptin resistance and metabolic adaptations. These findings not only help to explain a common phenomenon of the mammalian physiology, but it may also aid in the development of approaches to prevent and treat gestational metabolic imbalances.

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Keywords Leptin; Suppressor of cytokine signaling; Gestational diabetes; Obesity; Leptin resistance; Hypothalamus

1. INTRODUCTION

The developing embryo and subsequent fetus impose energy demands on pregnant women. Consequently, increased food intake is expected during gestation [1,2]. Additionally, pregnancy leads to benign and transitory insulin resistance [3]. These metabolic adaptations are thought to have evolved to ensure better conditions for offspring development [2]. However, an increasing number of women are developing metabolic imbalances during pregnancy, including excessive gestational weight gain (EGWG) and gestational diabetes mellitus (GDM) [3,4]. These conditions represent a serious health threat to women and their offspring and are becoming a major obstetric complication worldwide. For example, EGWG increases the risk of maternal mortality, GDM, pre-eclampsia, thromboembolism, postpartum hemorrhage and other gestational complications [3]. Furthermore, long-term postpartum weight retention predisposes women to obesity, and GDM increases the risk of diabetes mellitus later in life [4]. Therefore, metabolic imbalances during pregnancy may also aggravate the obesity and diabetes epidemics.

Despite the negative health impacts of pregnancy-induced metabolic imbalances, the precise mechanisms responsible for orchestrating these changes remain largely unknown. Previous studies have

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Abbreviations: ARH, arcuate nucleus of the hypothalamus; DIO, diet-induced obesity; DMH, dorsomedial nucleus of the hypothalamus; EGWG, excessive gestational weight gain; GDM, gestational diabetes mellitus; GTT, glucose tolerance test; IR, insulin receptor; ITT, insulin tolerance test; LepR, leptin receptor; PKC, protein kinase C; GH-V, placental growth hormone; pSTAT3, phosphorylation of the signal transducer and activator of transcription 3; pSTAT3-ir, pSTAT3-immunoreactive; RP, retroperitoneal; SOCS3, suppressor of cytokine signaling-3; STZ, streptozotocin; VMH, ventromedial nucleus of the hypothalamus

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reported lower responsiveness to leptin in pregnant animals [5–7]. Nonetheless, no compelling evidence has been provided about the real contribution of leptin resistance for the onset of pregnancy-induced metabolic changes. Therefore, the objective of the present study was to identify key molecular mechanisms that trigger the metabolic changes observed during pregnancy.

2. MATERIAL AND METHODS

2.1. Generation of conditional knockout mice

All mouse strains were backcrossed at least 4 times to the C57BL/6 background before the initiation of breeding. To induce the *Socs3* gene deletion exclusively in leptin-responsive cells, we bred the LepR-IRES-Cre strain (B6.129-*Lepr*$^{tm2(cre)Rck}$/J, Jackson Laboratories) with mice carrying loxP-flanked *Socs3* alleles (B6.129S4-Socs3^{tm1Ayos}/J, Jackson Laboratories). The SOCS3 KO group was composed of animals homozygous for the loxP-flanked *Socs3* allele and homozygous for the LepR-IRES-Cre allele. The control group was composed of animals homozygous for the LepR-IRES-Cre allele. We used only littermates as controls. The mice were weaned at 4 weeks of age, and the genomic DNA was extracted from tail tips for PCR genotyping (Sigma). The loxP-flanked *Socs3* allele and the wild-type allele were identified by the presence of a 420 bp or 272 bp PCR fragment, respectively. The LepR-IRES-Cre allele and the wild-type allele were identified by the presence of a 213 bp or 800 bp PCR fragment, respectively. After weaning, the mice received a regular low-fat rodent chow diet (2.99 kcal/g; 9.4% calories from fat; Quimtia, Brazil). All animal procedures were approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences at the University of São Paulo, São Paulo, Brazil.

2.2. Breeding strategy and tissue collection

Two-month-old females were bred with sexually experienced males, and we assessed the presence of copulatory plugs on a daily basis. Females that did not mate within 3 weeks were discarded. A breeding strategy was designed to assure that all pups in both groups would have the same genotype (homozygous for LepR-IRES-Cre and heterozygous for LoxP-flanked *Socs3* allele). The day that the copulatory plug was detected was considered to be the first day of pregnancy (P1), and the females were single-housed. All females were euthanized after a 4-h fasting period during the middle of the light phase. Non-pregnant mice (nulliparous and primiparous) were euthanized on the second day of diestrus. To determine adiposity levels, we measured the masses of the uterine, ovarian and retroperitoneal (RP) fat pads. The uterine pad was collected from the midpoint of the cervix and trimmed away along the horn length to the infundibulum region. Ovarian pad was well defined by a circular fat deposit around the ovary. The retroperitoneal pad was removed as a triangular section extending from a vertex in the inguinal region up the midline and across at the lower pole of the kidney, extending laterally as far as fat was visible. The deposits were collected bilaterally and the values presented represent their average.

2.3. Relative gene expression

The tissues were quickly dissected for relative gene expression analysis as previously described in Ref. [8]. Specific primers were designed for each target gene according to the sequences obtained from GenBank or acquired from Applied Biosystems (Supplemental Table 1). The data were reported as fold changes compared with the values obtained from the control group (set at 1.0).

2.4. Leptin sensitivity tests

For all experiments, we used mouse recombinant leptin purchased from Dr. A.F. Parlow (National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases). On P5, mice were anaesthetized (isoflurane) and we performed a surgical procedure, in which osmotic micro-pumps (model 1002; Alzet) were implanted s.c. to deliver 0.5 μg leptin/h. Body weight and food intake were measured daily before (P1–P5) and after (P6–P16) surgery. To determine the region-specific responses to leptin, pregnant control and SOCS3 KO mice received a leptin injection after a 4 h fasting period (2.5 μg leptin/g; s.c.). Three hours later, the mice were perfused with 10% buffered formalin, and their brains were processed to detect leptin-induced phosphorylation of the Signal Transducer and Activator of Transcription 3-immunoreactive (pSTAT3-ir) as previously described in Ref. [7]. We counted the number of pSTAT3-ir cells on one side of a representative rostrocaudal level.

2.5. Western blot

The tissues were homogenized in RIPA buffer (Sigma) containing a cocktail of proteases and phosphatase inhibitors (1:100, Sigma). Protein (40 or 50 μg) was resolved on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Bio-Rad). The membranes were blocked with 5% BSA and incubated overnight at 4 °C with primary antibodies (1:1,000; pSTAT3^{Tyr705}, Cell Signaling; STAT3, Santa Cruz; SOCS3, Cell Signaling; pIR$^{Tyr1162/1163}$, Santa Cruz; pAKT^{Ser473}, Cell Signaling; GAPDH, Santa Cruz). Next, the membranes were incubated for 45 min with secondary antibody (1:10,000; IRDye 800CW, Li-COR). The proteins were detected and analyzed using the Li-COR Odyssey system.

2.6. Hormone levels

ELISA kits were used to determine the serum concentrations of leptin (Crystal Chem), insulin (Crystal Chem) and glucagon (Sigma).

2.7. Glucose homeostasis

A glucose tolerance test (GTT; 2 g glucose/kg; s.c.) and an insulin tolerance test (ITT; 1 IU insulin/kg; s.c.) were performed on P14 and P16, respectively. Insulin sensitivity was evaluated in different tissues by infusing 5 IU insulin/kg s.c. into pregnant mice and euthanizing them 15 min after the injection. GDM was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ; 200 mg/kg; Amresco) dissolved in 50 mM sodium citrate (pH 4.5) on P5 [9]. Glycemia was assessed before (on P1 and P4) and after (on P7 and P13) the STZ injection, followed by a GTT (0.5 g glucose/kg; s.c.) on P14.

2.8. Statistics

The results are expressed as mean ± SEM. The differences between the groups were compared using an unpaired two-tailed Student's t-test. The data obtained from the leptin and insulin sensitivity tests were analyzed by two-way ANOVA and the Bonferroni post hoc test. Glycemic changes after the STZ treatment were assessed using repeated measures ANOVA. Statistical analyses were performed using GraphPad Prism software. We considered *p* values of less than 0.05 to be statistically significant.

3. RESULTS

3.1. Identification of proteins potentially related with leptin resistance during pregnancy

We investigated the hypothalamic expression of several genes potentially related to leptin resistance including components of the

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