Differential regulation of suppressor of cytokine signaling-3 in the liver and adipose tissue of the sheep fetus in late gestation

Sheridan Gentili,1 Michael J. Waters,2 and I. Caroline McMillen1

1Centre for the Early Origins of Adult Health, Discipline of Physiology, School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, South Australia, Australia; 2Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, Australia

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Gentili, Sheridan, Michael J. Waters, and I. Caroline McMillen. Differential regulation of suppressor of cytokine signaling-3 in the liver and adipose tissue of the sheep fetus in late gestation. Am J Physiol Regul Integr Comp Physiol 290: R1044–R1051, 2006. First published November 10, 2005; doi:10.1152/ajpregu.00573.2005.—It is unknown whether the JAK/STAT/suppressor of cytokine signaling-3 (SOCS-3) intracellular signaling pathway plays a role in tissue growth and metabolism during fetal life. We investigated whether there is a differential profile of SOCS-3 expression in the liver and perirenal adipose tissue during the period of increased fetal growth in late gestation and the impact of fetal growth restriction on SOCS-3 expression in the fetal liver. We also determined whether basal SOCS-3 expression in the fetal liver and perirenal adipose tissue is regulated by endogenous fetal prolactin (PRL). SOCS-3 mRNA abundance was higher in the liver than in the pancreas, spleen, and kidney of the sheep fetus during late gestation. In the liver, SOCS-3 mRNA expression was increased (P < 0.05) between 125 (n = 4) and 145 days (n = 7) gestation and lower (P < 0.05) in growth-restricted compared with normally grown fetal sheep in late gestation. The relative expression of SOCS-3 mRNA in the fetal liver was directly related to the mean plasma PRL concentrations during a 48-h infusion of either a dopaminergic agonist, bromocriptine (n = 7), or saline (n = 5), such that SOCS-3 mRNA expression was lower when plasma PRL concentrations decreased below ~20 ng/ml [y = 0.99 − (2.47/x) + (4.96/x^2); r^2 = 0.91, P < 0.0001, n = 12]. No relationship was shown between the abundance of phospho-STAT5 in the fetal liver and circulating PRL. SOCS-3 expression in perirenal adipose tissue decreased (P < 0.001) between 90–91 (n = 6) and 140–145 days (n = 9) gestation and was not related to endogenous PRL concentrations. Thus SOCS-3 is differentially expressed and regulated in key fetal tissues and may play an important and tissue-specific role in the regulation of cellular proliferation and differentiation before birth.

growth; prolactin; development; pregnancy

ACTIVATION OF THE JAK/STAT intracellular signaling pathway by prolactin (PRL), growth hormone (GH), cytokines, and growth factors regulates tissue growth and metabolism during postnatal life (19). Activation of the JAK/STAT pathway also increases the expression of the suppressors of cytokine signaling proteins, a family of eight structurally similar members (SOCS-1 to -7 and cytokine-inducible SH2 binding protein), which in turn act to inhibit the activation of the JAK/STAT signaling cascade in a classic negative feedback loop (23). It has been demonstrated that regulation of SOCS-3 expression is important for the normal growth and development of the liver, placenta, and immune system. Mice with either a null mutation for SOCS-3 or that overexpress SOCS-3 die during development between embryonic days 9 and 13 (28, 36). SOCS-3−/− lethality is associated with disruption of hepatic hematopoiesis (28), deregulated responses to cytokines such as IL-6 in the liver (8), and placental insufficiency due to deregulated responses to leukemia inhibitory factor (36, 39). It is unknown, however, whether SOCS-3 is expressed and regulated in fetal tissues in species such as the sheep and human, in which there is active neuroendocrine regulation of fetal growth and development during late gestation. The long and short forms of the PRL receptor (PRLR1 and PRLR2, respectively) are expressed in embryonic and fetal tissues in the human (13) and sheep (1), and PRLR1 and PRLR2 expression increases in the fetal sheep liver in the week before delivery (34). In contrast to the liver, the expression of PRLR1 and PRLR2 in the fetal adipose tissue increases between 90 and 125 days gestation and then decreases before delivery (38). Furthermore, experimental restriction of placental growth and development in the sheep results in a decrease in circulating PRL concentrations and a concomitant decrease in fetal body and liver but not in adipose tissue mass (29, 35). Although PRL may play a role in the regulation of tissue growth and metabolism before birth, there have been no studies that have identified whether endogenous PRL acts through the JAK/STAT and SOCS signaling pathway in fetal tissues that express PRLRs.

The aims of the present study were therefore to determine whether SOCS-3 is expressed in sheep fetal tissues; in addition, given the different profile of PRLR expression in the fetal liver and adipose tissue in late gestation, we also determined whether there is a differential profile of SOCS-3 expression in these tissues during the period of increased fetal growth in late gestation. We have also investigated the impact of experimental restriction of placental and, hence, fetal growth on SOCS-3 expression in the fetal liver. Finally, we have infused the dopaminergic agonist bromocriptine (Bromo) into the fetal sheep during late gestation to suppress PRL secretion and to determine whether basal SOCS-3 expression in the fetal liver and perirenal adipose tissue is regulated by endogenous fetal PRL. We measured STAT5 protein abundance in the liver and adipose tissue after administration of Bromo to determine whether the actions of endogenous PRL on SOCS-3 expression may be mediated via the classic JAK/STAT signaling cascade during fetal life.

Address for reprint requests and other correspondence: I. C. McMillen, Early Origins of Adult Health Research Group, The Sansom Research Institute, School of Pharmacy and Medical Science, Univ. of South Australia, Adelaide, SA 5005, Australia (e-mail: caroline.mcmillen@unisa.edu.au).

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**MATERIALS AND METHODS**

**Animals.** All procedures were approved by the Animal Ethics Committee of The University of Adelaide. Ewes were housed at The University of Adelaide in individual pens in rooms with a 12:12-h light-dark cycle and provided with food and water ad libitum throughout the duration of the experiments.

**Tissue study.** Fetal organs (liver, adrenal, kidney, perirenal adipose tissue, spleen, pancreas, thymus, heart, lung, thyroid, and skeletal muscle) were collected from four fetuses at 144–145 days gestation and weighed; samples were snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

**Ontogeny study.** A total of 38 pregnant Merino ewes carrying either a singleton or twin fetuses (*n* = 38) between 54 and 145 days gestation were used in the ontogeny studies. Ewes were killed by intravenous overdose of pentobarbital sodium, and fetuses were delivered, weighed, and killed by decapitation. Livers were collected from 20 of 38 fetuses (54 days, *n* = 4; 90–91 days, *n* = 5; 124–125 days, *n* = 4; and 141–145 days, *n* = 7), and perirenal adipose tissue was collected from 21 of 38 fetuses (90–95 days, *n* = 6; 124–145 days, *n* = 6; and 140–145 days, *n* = 9). The fetal liver and the perirenal adipose tissue depots were dissected and weighed, and tissue samples were snap frozen in liquid nitrogen and stored for subsequent analysis.

**Bromo infusion study.** Surgery was performed on 11 pregnant Merino ewes (5 ewes carrying singleton fetuses, 5 ewes carrying twin fetuses, and 1 ewe carrying triplets) between 117 and 124 days gestation under aseptic conditions as previously described (9). Briefly, general anesthesia was induced and maintained with 4% halothane in O<sub>2</sub>, and catheters were inserted into a fetal jugular vein and carotid artery in one fetus in each ewe and in 2 twin fetuses in one ewe (*n* = 12 fetuses). Fetal catheters were exteriorized through an incision made in the ewes’ flank. Animals were allowed to recover from surgery for at least 4 days before blood sampling commenced.

Fetuses were randomly assigned to either a Bromo (*n* = 7) or a saline (Sal; *n* = 5) infusion group. Bromo (0.03 μg/kg/h) or Sal was infused intravenously for a 48-h period between 124 and 138 days gestation. After 48 h of continuous infusion, fetuses infused with Bromo were administered either a Sal (Bromo + Sal; *n* = 4) or a single bolus of ovine PRL (oPRL) (60 μg/kg of predicted fetal body weight; Bromo + oPRL; *n* = 3), and fetuses infused with Sal were administered a Sal bolus (Sal + Sal; *n* = 5). The continuous Bromo or Sal infusion continued after bolus administration. Fetal arterial blood samples (2.5 ml) were collected into heparinized tubes at 24 h and at 0–1 h before the start of the infusion. Blood samples were then collected at +24 and +48 h after the start of the Bromo infusion and at frequent intervals (between +5 to +240 min) after administration of the oPRL or Sal bolus. Samples were centrifuged at 1,500 g for 15 min at 4°C, and plasma was stored at −20°C for subsequent determination of PRL concentrations. In addition, fetal arterial blood (0.5 ml) was collected to assess blood gas and pH status of the fetus (ABL 200 blood-gas analyzer; Radiometer, Copenhagen, Denmark).

Postmortem was performed between 1 and 4 h after administration of the Sal or oPRL bolus. Fetal organs were removed and weighed, and samples of the fetal liver and perirenal adipose tissue were either snap frozen in liquid nitrogen and stored at −80°C for analysis or fixed in 4% paraformaldehyde in 0.2 M phosphate buffer at 4°C for 2 days for histological analysis.

**Placental restriction study.** Fourteen ewes between 140 and 145 days gestation were used in this study. In eight ewes [placental restriction group (PR)], surgery was performed to remove the majority of the endometrial caruncles from the uterus of the nonpregnant ewe before mating occurred as previously described (10). After the carunclectomy surgery, the ewes were monitored for 4–7 days and were mated after ~10 wk. Ewes in the control (*n* = 6) and PR (*n* = 8) groups were transported to the animal holding facility, and tissues were collected between 140 and 145 days gestation, weighed, and snap frozen in liquid nitrogen and stored for subsequent analysis.

**Total RNA extraction from fetal tissues.** Total RNA was extracted from a range of tissues collected from fetuses in the “tissue study” at 144–145 days gestation (liver, adrenal, kidney, perirenal adipose tissue, spleen, pancreas, thymus, heart, lung, thyroid, and skeletal muscle; *n* = 4). Total RNA was also extracted from liver samples collected from fetuses in the “ontogeny study” (54 days, *n* = 4; 90–91 days, *n* = 5; 124–125 days, *n* = 4; and 141–145 days, *n* = 7), from perirenal adipose tissue samples (90–95 days, *n* = 6; 124–125 days, *n* = 6; and 140–145 days, *n* = 9), from both liver and perirenal adipose tissue samples collected from fetuses infused with Bromo + Sal, Bromo + oPRL, or Sal + Sal (*n* = 12), and from the livers of control (*n* = 6) and PR (*n* = 8) fetuses as previously described (44). Briefly, ~100 mg of tissue was homogenized in 1 ml of Tri-reagent (Sigma-Aldrich, St. Louis, MO), and the homogenate was allowed to stand at room temperature for 5 min. 1-Bromo-3-chloro-propane was added, samples were mixed vigorously and allowed to incubate at room temperature for 15 min, and then samples were centrifuged at 4°C at 12,000 g for 15 min. An aliquot of the aqueous layer was recovered, mixed with cold isopropanol and incubated at room temperature for 15 min. RNA was precipitated by centrifugation at 12,000 g for 10 min at 4°C. The pellet was washed in cold 70% ethanol, air dried, and resuspended in molecular grade water. The spectrophotometric absorbance at 260 and 280 nm was used to determine RNA quality and concentration. The quality of the RNA was determined by electrophoresis on a denaturing agarose gel with ethidium bromide staining to visualize 28S and 18S ribosomal RNA using a UV transilluminator.

**Quantification of SOCS-3 and β-actin mRNA expression by RT-PCR.** Ovine SOCS-3 and β-actin were amplified using RT-PCR. cDNA was generated by reverse transcription of 5 μg total RNA with random sequence hexanucleotides (GeneWorks, Adelaide, Australia) using SuperScript III (Invitrogen, Victoria, Australia) as recommended by the manufacturer. A fragment of ovine SOCS-3 cDNA was amplified though 31 cycles of 60 s at 96°C, 60 s at 58°C, and 90 s at 72°C (Hybaid PCR Express, Teddington, UK) from 3 μl of 1:1 diluted reverse transcription product using native Taq DNA polymerase (Invitrogen) as recommended by the manufacturer with 5′-GCT-GTTGTTGAAACGGATGC-3′ and 5′-GGCTTCTAGCGTGA GCCTG-3′ (GeneWorks) as primers. This produced a 164-bp fragment of ovine SOCS-3 double stranded (ds) cDNA (nucleotides 202–346 of the 850 nucleotide cDNA of human SOCS-3, GenBank accession no. NM_003955). Sequencing with the ABI PRISM dye terminator method (Perkin-Elmer, Wellesley, MA) after QIA-quick purification (Qiagen, Clifton Hill, Australia) confirmed its identity with human SOCS-3 (GenBank accession no. NM_003955). Sequencing with the ABI PRISM dye terminator method (Perkin-Elmer, Wellesley, MA) after QIA-quick purification (Qiagen, Clifton Hill, Australia) confirmed its identity with human SOCS-3 (GenBank accession no. NM_003955) and bovine (GenBank accession no. NM_174466) SOCS-3 sequence. As an internal control, a 349-bp fragment of ovine β-actin cDNA was generated with ovine-specific primers (44) using PCR conditions described above. The measurement of SOCS-3 and β-actin using RT-PCR was optimized and was used as a control in ovine tissues. Briefly, the linearity of the RT-PCR assay was determined by varying the concentrations of Tag DNA polymerase, MgCl<sub>2</sub>, dNTP, SOCS-3, and β-actin-specific forward and reverse primers, and template concentration. Products of RT-PCR (15 μl) were electrophoresed through a 2% (wt/vol) agarose gel in TBE buffer. PCR products were visualized using ethidium bromide and gel scanned using the Molecular FX Scanner (Bio-Rad, Hercules, CA). The band intensity of the RT-PCR products was determined using Quantity One software (Bio-Rad). Molecular sizes of PCR products were estimated by comparing their electrophoretic migration with those of fragments of pUC19 ds DNA digested with HpaII (GeneWorks). The mobility of the SOCS-3 fragment was intermediate between that of 147- and 190-bp markers and was calculated to have an approximate weight, based on molecular marker migration, of 165 bp.
Quantification of STAT5 by Western blot analysis. Protein was extracted from the fetal liver and perirenal adipose tissue samples collected from the Bromo infusion study (n = 12). Briefly, ~50 mg of tissue was homogenized in 500 μl of homogenizing buffer (50 mM Tris·HCl, 150 mM NaCl, 1 mM NaV, 10 mM NaF, 0.6% Triton X-100) containing one “Complete.” mini protease inhibitor cocktail tablet (Roche Diagnostics, Penzberg, Germany). Homogenate was centrifuged at 1,800 rpm for 30 min at 4°C. The supernatant was collected, and protein concentration was determined by Bradford assay (Bio-Rad) using IgG as the standard. Protein (40 μg) from liver and perirenal adipose tissue samples from the three treatment groups were separated by SDS-PAGE, transferred to a PVDF membrane, and immunoprobed for STAT5 (BD Biosciences, San Jose, CA) and phospho-STAT5 (Cell Signaling Technology, Beverly, MA) as previously described (6). Blots were blocked for 1 h at room temperature with 5% nonfat milk-TBST (5% wt/vol nonfat milk powder, Tris buffered saline, and 0.1% Tween 20) for STAT5 and phospho-STAT5 primary antibodies. Primary antibodies were diluted according to manufacturers’ specifications in 2% nonfat milk-TBST for STAT5 and 5% BSA (5% wt/vol BSA powder)-TBST for phospho-STAT5. Blots were incubated with the primary antibody overnight at 4°C, washed 3 × 5 min at room temperature in TBST and incubated with the appropriate peroxidase-conjugated secondary antibody diluted to manufacturers’ specifications in 5% nonfat milk-TBST (STAT5, donkey anti-mouse IgG, Rockland Immunochemicals, Gilbertsville, PA; phospho-STAT5, goat anti-rabbit IgG, Bio-Rad) for 1 h at room temperature. Blots were washed 3 × 5 min in TBST. Immunoreactive proteins were detected by chemiluminescence (100 mM Tris·HCl, 0.009% vol/vol H2O2, 250 mM luminol, and 90 mM cymacur acid) using x-ray film (Agfa, Mortsel, Belgium). Western blots were quantified by scanning densitometry (Bio-Rad).

STAT5 immunohistochemistry. STAT5 localization was determined in liver and adipose tissue sections collected from Sal + Sal/Bromo + Sal (n = 7) and Bromo + oPRL (n = 3) groups. After fixation in 4% paraformaldehyde, sections were washed in 0.01 M PBS for 48 h and then stored in 70% ethanol (24 h) before being processed and embedded in paraffin wax. Paraffin sections (5 μm) were dewaxed and rehydrated. Sections were pretreated with 3% H2O2 and boiled in 10 mM citric acid buffer for antigen retrieval. Sections were blocked and using a commercially available blocking solution containing 10% goat nonimmune serum (Zymed Laboratories, San Francisco, CA). Sections were incubated overnight at 4°C with a mouse monoclonal anti-STAT5 (1:100 dilution; BD Biosciences) diluted in a commercially available antibody diluent (Zymed Laboratories). Sections were washed in PBS and incubated with biotinylated secondary antibody (Zymed Laboratories) followed by streptavidin-peroxidase (Zymed Laboratories) for 40 and 10 min, respectively, at room temperature. Immunoreactivity was detected using metal-enhanced DAB substrate kit (Pierce, Rockford, IL) for 10 min. Sections were counterstained with Mayer’s hematoxylin (Merck, Darmstadt, Germany), dehydrated, and mounted.

PRL radioimmunoassay. Plasma PRL concentrations were measured by radioimmunoassay using rabbit anti-ovine PRL (antisemum batch number AP253870691R), generously donated by the National Hormone and Pituitary Program (National Institute of Diabetes and Digestive and Kidney Diseases, Baltimore, MD), and an assay that was previously described and validated for use in fetal sheep plasma (18). The sensitivity of the assay was 1 ng/tube, and the intra- and interassay coefficients of variation were 10% and 20%, respectively.

Statistical analyses. All data are expressed as means ± SE. All SOCS-3:β-actin mRNA, STAT5, and phospho-STAT5 data were generated from duplicate PCR and Western blot analyses, respectively, for each animal. In the tissue study, the intensity of the RT-PCR SOCS-3 signal was used as a measure of SOCS-3 expression because there were tissue-specific differences in the expression of β-actin across the 11 different fetal tissues used. In the ontogeny, Bromo, and PR studies, there were no differences in tissue β-actin expression between treatment groups, and therefore the SOCS-3:β-actin mRNA ratio was used as the measure of SOCS-3 expression. There was no effect of fetal gender on hepatic or adipose tissue SOCS-3 expression, and data for male and female fetuses were therefore pooled. The level of SOCS-3 expression in different fetal tissues or in the liver and adipose tissue at different gestational age ranges were compared by one-way ANOVA with Duncan’s post hoc to determine significant differences between mean values. Mean plasma PRL concentrations were determined during the following time periods: 24 h before the start of the Bromo or Sal infusion, 48 h during the Bromo or Sal infusion, and after the administration of the oPRL or Sal bolus on the day of postmortem. Differences in mean plasma PRL concentrations between Sal- and Bromo-infused fetuses were compared by two-way ANOVA.

The relationships between the relative expression of SOCS-3, relative abundance of STAT5 and phospho-STAT5 in the liver or perirenal adipose tissue, and mean fetal plasma PRL concentration before and during the infusion or on the day of postmortem were determined with the use of linear or polynomial regression analysis. The relationships between either STAT5 or phospho-STAT5 and the expression of SOCS-3 in the liver or perirenal adipose tissue for each of the three treatment groups were determined by linear regression analysis. We compared the difference between SOCS-3 expression in the liver of control and placentaly restricted fetuses using Student’s t-test analysis. P < 0.05 was taken as the level of significance in all analyses.

RESULTS

Differential expression of SOCS-3 mRNA in fetal tissues during late gestation. SOCS-3 mRNA was expressed in a range of fetal tissues at 144–145 days gestation (n = 4; Fig. 1A). The abundance of SOCS-3 mRNA was higher in the fetal liver than in the pancreas, spleen, and kidney (Fig. 1B).

Ontogeny of SOCS-3 mRNA expression in the fetal liver and perirenal adipose tissue. In the liver, there was a significant increase (P < 0.005) in the relative expression of SOCS-3:β-actin mRNA ratio between 124–125 and 141–145 days gestation (Fig. 2A). Furthermore, between 124 and 145 days gestation, there was an inverse relationship between the relative weight of the fetal liver (γ) and SOCS-3 mRNA expression (x) (γ = 40 − 18.8x; r² = 0.40, P < 0.05, n = 11). In fetal perirenal adipose tissue, there was a decrease in the relative expression of SOCS-3:β-actin mRNA between 90–95 and 124–125 days and a further decrease between 124–125 and 140–145 days gestation (P < 0.0001; Fig. 2B). There was no relationship, however, between relative adipose tissue weight and SOCS-3:β-actin mRNA between 124 and 145 days gestation.

The effect of placental restriction on hepatic SOCS-3 expression. Restriction of placental growth resulted in a lower fetal weight (control, 4.88 ± 0.20 kg; PR, 3.28 ± 0.29; P < 0.005) and absolute liver weight (control, 121.26 ± 12.92 g; PR, 65.61 ± 6.66 g; P < 0.01) but not relative liver weight (control, 24.65 ± 1.87 g/kg; PR, 20.10 ± 1.21). There was also a significant decrease in the relative expression of SOCS-3 in the liver of PR fetuses at 140–145 days gestation compared with control fetuses (Fig. 3).

Effect of Bromo and exogenous oPRL on fetal plasma PRL concentrations. There was no difference between experimental treatment groups or between singleton and twin fetuses in mean fetal arterial PO2 (Sal + Sal, 21.4 ± 2.3 Torr; Bromo + Sal, 20.5 ± 1.4 Torr; Bromo + oPRL, 22.3 ± 0.8 Torr) or arterial blood pH (Sal + Sal, 7.397 ± 0.011; Bromo + Sal, 7.397 ± 0.011).
7.434 ± 0.011; Bromo + oPRL, 7.395 ± 0.004). There was no difference in mean plasma PRL concentrations before the start of either the Sal or Bromo infusion (Sal + Sal, 49.6 ± 7.6 ng/ml; Bromo + Sal, 46.2 ± 10.5 ng/ml; Bromo + oPRL, 40.7 ± 11.4 ng/ml). During the 48-h infusion period, plasma PRL concentrations decreased in the Bromo-infused fetuses but not in the Sal-infused fetuses (Fig. 4A). On the day of postmortem, plasma PRL concentrations were higher ($P < 0.005$) after the administration of the PRL bolus (see Fig. 7A).

Relationship between SOCS-3 expression in the fetal liver or perirenal adipose tissue and circulating PRL concentrations. There was a significant relationship between the relative expression of SOCS-3 mRNA in the fetal liver and mean plasma PRL concentrations during the 48-h Bromo or Sal infusion period such that SOCS-3 mRNA expression was lower when plasma PRL concentrations decreased below $\sim 20$ ng/ml [$y = 0.99 - (2.47/x) + (4.96/x^2)$; $r^2 = 0.91$, $P < 0.0001$, $n = 12$; Fig. 5]. Therefore, although the relative expression of SOCS-3 mRNA tended to be lower in the Bromo-compared with the Sal-infused fetuses, SOCS-3 mRNA expression was only significantly lower in the fetal liver in the Bromo + oPRL group compared with the Sal + Sal group (Fig. 4B). There was no relationship between the relative expression of SOCS-3 mRNA
in the liver and fetal plasma PRL concentrations measured either before the start of the infusion or on the day of postmortem (data not shown). There was also no relationship between the relative weight of the fetal liver and mean SOCS-3 expression when data from all three treatment groups were combined.

In the perirenal adipose tissue, there was no difference between the mean SOCS-3-β-actin mRNA ratio in the three treatment groups (Sal + Sal, 0.67 ± 0.02; Bromo + Sal, 0.67 ± 0.02; Bromo + oPRL, 0.75 ± 0.09). Furthermore, there was no relationship between relative expression of SOCS-3 mRNA in adipose tissue and mean plasma PRL concentrations measured either before or during the infusion period or on the day of postmortem.

**STAT5 abundance in the fetal liver and perirenal adipose tissue.** Immunoreactive STAT5 was localized within the cytoplasm and nucleus of the fetal hepatocytes (Fig. 6) and was not detected when liver sections were incubated in the absence of primary antibody. Immunoreactive STAT5 was not detected in the cytoplasm or nucleus of adipocytes in fetal perirenal adipose tissue (data not shown). The total abundance of STAT5 protein in the fetal liver was greater in the Bromo-oPRL group compared with the Sal-Sal but not the Bromo-Sal-infused animals (Fig. 7B). There was no effect of Bromo infusion or oPRL administration on the relative abundance of phospho-STAT5 (Sal + Sal, 776.5 ± 33.6; Bromo + Sal, 843.0 ± 83.2; Bromo + oPRL, 811.0 ± 17.3 arbitrary units) in the fetal liver or on STAT5 (Sal + Sal, 568.2 ± 84.8; Bromo + Sal, 732.4 ± 113.4; Bromo + oPRL, 842.9 ± 53.9 arbitrary units) or phospho-STAT5 (Sal + Sal, 881.7 ± 48.9 arbitrary units) in the perirenal adipose tissue. Furthermore, there was no relationship between either STAT5 or phospho-STAT5 and plasma PRL concentrations (before the start of the infusion, during the infusion, or on the day of postmortem) or SOCS-3 expression in the fetal liver or perirenal adipose tissue.

**DISCUSSION**

We have demonstrated for the first time that SOCS-3 is widely expressed in fetal tissues during late gestation and that SOCS-3 expression is differentially regulated in the liver and perirenal adipose tissue of the normally grown fetus during the last month of pregnancy and is suppressed in the liver of the growth-restricted fetus. Furthermore, the levels of SOCS-3 expression in the liver, but not adipose tissue, were related to circulating PRL concentrations during late gestation in the sheep fetus.

In the adult mouse, SOCS-3 is expressed at relatively high levels in the lung, spleen, and thymus and at relatively low levels in the liver and heart (37). In contrast, we found that SOCS-3 expression was higher in the fetal liver than in the fetal spleen, kidney, and pancreas. These different tissue profiles of SOCS-3 expression may relate to the differences in the hemopoietic roles of the liver and spleen before and after birth.
or to differences in the responsiveness of these tissues to circulating cytokines in the rodent and sheep.

In the present study, there was a ~30% increase in hepatic SOCS-3 expression between 125 and 145 days gestation and an inverse relationship between the relative weight of the fetal liver and hepatic SOCS-3 expression in late gestation. In the adult mouse, SOCS-3 acts to inhibit cytokine stimulation of the JAK/STAT pathway in the liver and is a major inhibitor of hepatocyte proliferation following partial hepatectomy (4). One possibility is that there is an active stimulation of SOCS-3 expression in the fetal liver during late gestation to inhibit hepatocyte proliferation and promote the metabolic differentiation of the hepatocytes, which is required for the transition to extrauterine life. It is well established that the prepartum increase in fetal cortisol concentrations from around 135 days gestation plays a major role in the functional differentiation of fetal organ systems, including the liver, gut, and lungs in preparation for birth. Cortisol acts to decrease hepatic IGF-II expression and to increase expression of both the adult liver-specific GH receptor mRNA transcript and IGF-I, as well as stimulating hepatic glucogenesis during late gestation (11, 24–26). GH acts to stimulate the JAK/STAT/SOCS signaling cascade in adult tissues, and one possibility therefore is that GH acts at the functional adult-type GH receptor in the fetal liver in the week before delivery to upregulate SOCS-3 mRNA expression (22, 43). It has also been demonstrated, however, that there is a cortisol-dependent increase in the expression of PRLR1 and PRLR2 in the fetal liver during late gestation (34). Furthermore, although circulating fetal PRL concentrations may vary between animals depending on the external photoperiod and level of fetal nutrition (2, 17, 35), plasma PRL concentrations tend to increase in fetal sheep during the last week of gestation (15, 30).

Fig. 6. Representative images of liver sections collected from fetuses infused with Sal + Sal (A), Bromo + Sal (B), or Bromo + oPRL (C). Immunoreactive STAT5 is present in the cytoplasm and nucleus of fetal hepatocytes, depicted by white arrow. CV, central vein.

Fig. 7. A: effect of Bromo infusion and oPRL administration on plasma prolactin concentrations on the day of postmortem. B: effect of Bromo infusion and oPRL administration on hepatic STAT5 protein abundance on the day of postmortem in the Sal + Sal (n = 5), Bromo + Sal (n = 4), and Bromo + oPRL (n = 3) groups, where postmortem was performed between 1 and 4 h after the administration of the Sal or oPRL bolus. Different superscripts denote mean values that are significantly different from each other (P < 0.05).
We investigated the role of endogenous PRL in the maintenance of hepatic SOCS-3 expression using the dopamine agonist Bromo to suppress pituitary PRL secretion in the late gestation fetus (16, 27). Bromo is an agonist specific to the D2 subclass of dopamine receptors present on lactotrophic cells (12) and has been shown to have no effect on plasma concentrations of placental lactogen (41). Infusion of Bromo for 48 h suppressed fetal plasma PRL concentrations by ~85%, and there was a significant relationship between hepatic SOCS-3 expression and the mean plasma PRL concentrations achieved during the 48-h infusion period. One feature of this relationship was that SOCS-3 expression was decreased when plasma PRL concentrations fell below ~20 ng/ml. Although there was no relationship between PRL concentrations and the abundance of phospho-STAT5, the total abundance of STAT5 protein (phosphorylated and unphosphorylated) was increased in the livers of animals infused with Bromo, and there was no difference in the total abundance of STAT5 in the livers of Bromo-infused fetuses treated with either PRL or Sal. Thus basal SOCS-3 expression in the fetal liver may be regulated by PRL through an intracellular pathway, which is not dependent on phospho-STAT5 activation. There is evidence that PRL can upregulate the activation of a range of intracellular kinases, including MAPK (3) and PKC (21), and that SOCS-3 expression can be upregulated through the p38 MAPK pathways, independent of STAT activation (5, 7). Furthermore, when PRL concentrations are decreased, there may be an increase in bioavailable STAT5 protein within the fetal hepatocyte, potentially as a consequence of a decrease in STAT5 turnover utilization in the cell.

Although there was no increase in SOCS-3 expression or in phospho-STAT5 abundance in the fetal liver after acute administration of PRL, these studies were only carried out in a limited number of fetuses. In the adult rabbit and mouse mammary gland, ovary, adrenal, and liver, prior suppression of endogenous PRL secretion enhances JAK/STAT activation and SOCS-3 expression in response to acute PRL stimulation (20, 33, 40, 42). Clearly, further studies are required to determine whether acute PRL stimulation can activate the JAK/STAT signaling pathway and upregulate SOCS-3 expression in the liver during fetal life.

Interestingly, plasma PRL concentrations are suppressed by almost 50% in the placentally restricted fetus compared with normally grown fetuses (35), and we have found in the present study that SOCS-3 expression was suppressed by ~10% in the liver of the growth-restricted fetuses compared with controls. The magnitude of the decrease in SOCS-3 expression in the placentally restricted group appears consistent with the observation in the fetuses infused with Bromo that, when the relative expression of SOCS-3 in the fetal liver is greater than ~0.65, there is a direct relationship between hepatic SOCS-3 expression and circulating PRL concentrations in the range of ~0–20 ng/ml. This suggests that there may be active regulation of hepatic SOCS-3 expression in response to a decrease in placental substrate supply by either PRL or other cytokines and growth factors.

In contrast to the increase in SOCS-3 expression in the fetal liver during late gestation, there was a significant decrease in SOCS-3 expression in the perirenal adipose tissue during this period. Although this decrease is coincident with the fall in PRLR1 and PRLR2 expression in the perirenal adipose tissue, which also occurs in late gestation (38), there was no effect of Bromo infusion or acute PRL stimulation following Bromo on SOCS-3 expression in this major fetal fat depot. There was also no effect of PRL suppression or PRL stimulation on the abundance of STAT5 or phospho-STAT5 in fetal perirenal adipose tissue. Although this suggests that PRL does not play a role in the regulation of the JAK/STAT/SOCS pathway in the fetal adipocytes during late gestation, one possibility is that PRL plays a greater role in the regulation of SOCS-3 expression and adipogenesis earlier in gestation, when the PRLR is expressed at relatively high levels in the adipocytes. Mice with a null mutation of the PRLR have a reduced abdominal fat mass compared with their wild-type counterparts, implicating PRL in the regulation of adipogenesis (14). PRL has been implicated as playing a major role, via PRLR1 and PRLR2, in the regulation of uncoupling protein-1 abundance in fetal and neonatal adipose tissue depots (31) and in the maintenance of core body temperature and thermoregulation in the neonatal lamb (32). It would appear, from the data reported in the present study, that PRL may upregulate uncoupling protein-1 expression and lipolysis via intracellular signaling pathways other than the JAK/STAT/SOCS pathway.

In summary, we have demonstrated that SOCS-3 is widely expressed in fetal tissues during late gestation and that SOCS-3 expression is differentially regulated in the liver and perirenal adipose tissue of the normally grown fetus during the last month of pregnancy and is suppressed in the liver of the growth-restricted fetus. In contrast to the fetal liver, basal SOCS-3 mRNA expression in fetal adipose tissue is not dependent on endogenous PRL during late gestation. Thus SOCS-3 is differentially expressed in a range of key fetal tissues and may play an important role in the tissue-specific regulation of cellular proliferation and differentiation before birth.

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REFERENCES


