Adipose Tissue Dysfunction Occurs Independently of Obesity in Adipocyte-Specific Oncostatin Receptor Knockout Mice

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Objective: This study examined the phenotypic effects of adipocyte-specific oncostatin M receptor (OSMR) loss in chow-fed mice.

Methods: Chow-fed adipocyte-specific OSMR knockout (FKO) mice and littermate OSMRfl/fl controls were studied. Tissue weights, insulin sensitivity, adipokine production, and stromal cell immunophenotypes were assessed in epididymal fat (eWAT); serum adipokine production was also assessed. In vitro, adipocytes were treated with oncostatin M, and adipokine gene expression was assessed.

Results: Body weights, fasting blood glucose levels, and eWAT weights did not differ between genotypes. However, the eWAT of OSMR FKO mice was modestly less responsive to insulin stimulation than that of OSMRfl/fl mice. Notably, significant increases in adipokines, including C-reactive protein, lipocalin 2, intercellular adhesion molecule-1, and insulin-like growth factor binding protein 6, were observed in the eWAT of OSMR FKO mice. In addition, significant increases in fetuin A and intercellular adhesion molecule-1 were detected in OSMR FKO serum. Flow cytometry revealed a significant increase in leukocyte number and modest, but not statistically significant, increases in B cells and T cells in the eWAT of OSMR FKO mice.

Conclusions: The chow-fed OSMR FKO mice exhibited adipose tissue dysfunction and increased proinflammatory adipokine production. These results suggest that intact adipocyte oncostatin M–OSMR signaling is necessary for adipose tissue immune cell homeostasis.

Introduction

A coordination among healthy adipocytes and stromal vascular cells (SVCs; including immune cells, preadipocytes, and endothelial cells) in the local adipose tissue environment is critical to maintain homeostasis of both adipose tissue and systemic metabolism. Obesity is intimately coupled with a chronic, low-grade inflammatory state characterized by leukocyte infiltration of adipose tissue and subsequent modulation of the tissue adipokine profile. Activation of infiltrating leukocytes and promotion of proinflammatory adipokine production can enable adipose tissue dysfunction and lead to temporal effects on metabolic homeostasis (1–4).

Oncostatin M (OSM), an adipokine belonging to the interleukin-6/gp130 family of cytokines, regulates a variety of physiological and pathological processes (5,6). First recognized in 1986 for its antitumorigenic effects (7), it is now evident that OSM can regulate many other biological processes in a cell-type-dependent manner (8). OSM is a unique gp130 cytokine in that it has its own specific receptor subunit (OSM receptor [OSMR] β) that heterodimerizes with gp130 to create a functional OSMR complex, which produces the majority of OSM effects (9). Adipocytes in particular exhibit extensive responses to OSM (10,11), and OSMR is highly expressed in adipose tissue (12). Importantly, though adipocytes express OSMR, they do not produce OSM itself. Rather, OSM is produced in various adipose tissue SVCs, including macrophages, T cells, and B cells (10,12). Increased adipose tissue expression of OSM and OSMR is a correlate of obesity in both mouse and human (12). We have previously shown that adipocyte-specific deletion of OSMR in mice (referred to here as OSMR FKO mice) is associated with augmented adipose tissue inflammation and systemic insulin resistance and obesity (10). Notably, adipose tissue OSM expression itself is elevated in obese OSMR FKO mice (10). While we have demonstrated OSM’s role in perpetuating inflammation and systemic insulin resistance in the obese condition (10), it remains unclear what potential

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Funding agencies: CME is supported by K01 DK106307 and JMS by R01 DK052968 from the National Institutes of Health (NIH). This work used core facilities that are supported in part by COBRE (NIH P30 GM118430) and NORC (NIH P30 DK072478) center grants from the NIH.

Disclosure: The authors declared no conflict of interest. Additional Supporting Information may be found in the online version of this article.

Received: 23 February 2018; Accepted: 5 June 2018; Published online 18 September 2018. doi:10.1002/oby.22254
effects disrupted adipose tissue OSM signaling in the absence of obesity.

With the present study, we examined the function of adipose tissue OSM signaling in the absence of an obesogenic stimulus such as a high-fat diet. Our novel observations demonstrate that, in the absence of obesity, OSMR FKO mice exhibit leukocyte accumulation, increased proinflammatory adipokine production, and a modest reduction of adipose tissue insulin responsiveness. These effects occur in the absence of differences in body weight or adiposity. These results corroborate our previous observations that intact adipocyte OSM signaling is necessary for maintenance of adipose tissue homeostasis and demonstrate that adipose tissue dysfunction precedes systemic metabolic dysfunction in this model.

Methods

Animals and husbandry

Mixed-background OSMR FKO mice and homozygous floxed littermates (OSMR+/+) were obtained from our in-house colony, which was generated using adiponectin–Cre and OSMR+/+ mice as previously described (10). All mice used in these studies were male, were 6 to 9 months of age, and were maintained on LabDiet number 5015 (LabDiet, St. Louis, Missouri) from weaning (3 weeks of age) until study termination. Mice were housed in a temperature-controlled (22°C ± 2°C) and humidity-controlled (45%–55%) room under a 12-hour light–dark cycle and were allowed ad libitum access to food and water. Tissue OSMR and gp130 gene and protein expression, 4-hour blood glucose measurement, and epididymal fat (eWAT) insulin sensitivity studies (acute intra-peritoneal insulin injections) were performed on one cohort of mice. Acute OSM injection studies were conducted in a second cohort of mice, and tissue adipokine studies were conducted in mice from the protein expression cohort and in a third cohort. Serum adipokine studies were also conducted in a third cohort. Flow cytometry studies were performed in an additional cohort of 6-month-old mice. The Pennington Biomedical Research Center Institutional Animal Care and Use Committee approved all studies (protocol #961P).

Acute OSM injection experiments

Fed mice (n = 4 OSMR+/+ and n = 4 OSMR FKO) were given acute injections of 200 ng recombinant murine OSM (catalog number 495-MO-025; R&D Systems, Minneapolis, Minnesota) or vehicle (sterile 0.1% bovine serum albumin [BSA] in phosphate-buffered saline [PBS]) and euthanized 15 minutes later. eWAT pads were excised, snap frozen, and stored at −80°C for immunoblotting analyses as described below.

Cell culture and treatments

Murine 3T3-L1 preadipocytes were grown to 2 days post-confluence in DMEM with 10% fetal bovine serum (FBS) and were differentiated as described previously (10). One day prior to beginning OSM treatment of adipocytes, the medium was replaced with DMEM containing 5% FBS. Cells were pretreated with 50μM extracellular signal-regulated kinase (ERK) inhibitor U0126 for 1 hour, and then 1nM recombinant murine OSM or vehicle (0.1% BSA in PBS) was added as indicated. Six hours later, the medium was removed, and cells were washed with PBS and then harvested for RNA extraction. Experiments were performed in duplicate.

Gene expression analyses

Total RNA was isolated from indicated mouse tissues or 3T3-L1 adipocytes using a RNeasy Mini Kit (Qiagen, Germantown, Maryland), and yield was determined by spectrophotometry (NanoDrop Technologies, Wilmington, Delaware) as previously described (10). Complementary DNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California) with the SYBR Green system (Clontech, Mountain View, California). Relative quantification of mRNA expression was analyzed using an ABI Prism 7900 Sequence Detection System (Applied Biosystems). Sequences for mouse primers (Integrated DNA Technologies, San Diego, California) appear in Table 1.

Immunoblotting

Proteins were separated on 7.5% (OSMR, gp130) or 10% (protein kinase B [Akt], ERK, phosphorylated signal transducer and activator of transcription 3/5 [phosphoSTAT3/5]) polyacrylamide gels containing sodium dodecyl sulfate and transferred to nitrocellulose membranes as previously described (10). After transfer, membranes were blocked and incubated with goat anti-OSMR (catalog number AF662; R&D Systems), rabbit anti-STAT5A (R&D Systems), rabbit anti-phosphoSTAT5A, mouse anti-Akt, and mouse anti-ERK antibodies. Membranes were washed and incubated with horseradish peroxidase-conjugated anti-goat, anti-rabbit, and anti-mouse antibodies. Blots were developed using the ECL Plus Western Blotting detection system (GE Healthcare, Piscataway, New Jersey). Membranes were reprobed for β-actin (Millipore, Billerica, Massachusetts) as a loading control.

TABLE 1. Sequences for qPCR primers used in this study

| Gene  | Forward sequence | Reverse sequence |
|-------|-----------------|-----------------|
| Ccp   | CAG CAG CAT CCA TAG CCA T | TGC TTC CAG AGA CAC GGA GAT CCA G |
| FetA (Ahsg) | CTT CAG GGA TTC AAA CAG GTC T | GAT G TGG GTG AGT GTG GTG |
| Glut1 (Slc2a1) | AGT TGC GCT ATA ACA CTG GTG | GAT G |
| Glut4 (Slc2a4) | TCT TAT TGC AGC GCC TGA G | GAG AAT ACA GCT AGG |
| gp130 | AGG AGA AAT AGA AGC CAT AGT C | TGG AAG GAT CAG GAA CAT TAG G |
| Icam1 | CTG TGC TTT GAG AAG TGT GG | GGC CTC CAT GCA ACA |
| Igfbp6 | TCT ATG TGC CAA ACT GTG ACC | ACC ATC |
| Lcn2 | TGC AAG TGG CCA CCA CGG AC | GGA GAC GC |
| Osmr | CTT TCC CCT GTG AGG CCG AG | GCC GC |
| Ppia | CCA CTG TCG CTT TCC GCC GC | TGC AAA CAG CTC GAA GGA GAC GC |
| Ubb | CCA GTG GGC AGT GAT GG | GCT TAC CAT GCA ACA AAA CCT |

qPCR, quantitative polymerase chain reaction.
Serum and tissue adipokine arrays
Adipokine protein expression levels in serum (n = 3 per genotype) or eWAT (n = 6–7 per genotype) were assessed using mouse Proteome Profiler Adipokine Array Kits (catalog number ARY013; R&D Systems) according to manufacturer instructions. Briefly, eWAT was homogenized in PBS with 10 μg/mL each of aprotinin, leupeptin, and pepstatin. After homogenization, Triton X-100 (Sigma-Aldrich, St. Louis, Missouri) was added to each sample at 1% final concentration. Samples were frozen at −80°C, thawed, and centrifuged at 10,000g for 5 minutes at 4°C to remove cellular debris. Sample protein concentrations were quantified using a bicinchoninic acid assay with BSA as the standard (Sigma-Aldrich). Serum assays were performed using 100 μL of mouse serum as specified in the kit instructions. Membranes were incubated with chemiluminescence reagents and then exposed to autoradiography film for times ranging from 1 to 5 minutes. Film from the 5-minute exposure was used for densitometry analyses, which were performed with Image Studio Lite software (version 3.1; Li-Cor, Lincoln, Nebraska). Densitometry values for each adipokine were normalized to values for the reference spot on each membrane. Resulting values were then compared between genotypes.

Isolation of eWAT SVCs for immunophenotyping
Freshly excised eWAT was weighed, pooled (two to three mice per pooled sample; four pooled samples per genotype), and fractionated using previously described methods (10,13), with minor modifications (14) as briefly described here. Tissues were minced in ice-cold digestion buffer (low glucose DMEM without phenol red; 5% heat-inactivated FBS; 1 mg/mL type II collagenase), transferred to 50-mL conical tubes, and placed into a shaking water bath at 37°C. Tissues were digested for 45 minutes at 100 rpm, with tubes shaken vigorously by hand every 10 minutes. In the last 5 minutes of the digestion procedure, EDTA was added at a final concentration of 10 mM. Each slurry was then passed through a prewet 100-μm cell strainer into a 50-mL conical tube, and the strainer was rinsed with 10 mL of PBS. Slurries were centrifuged at 500g for 10 minutes at 4°C. After centrifugation, floating adipocytes and supernatant were removed, and red blood cell (RBC) pellet and incubated for 5 minutes. Lysis buffer was neutralized with PBS, the SFV mixture was passed through a 40-μm cell strainer into a 50-mL tube, the strainer was rinsed twice with 5 mL of PBS, and the tubes were centrifuged at 500g for 10 minutes at 4°C. The resulting SFV pellet was resuspended in 3 mL of PBS and placed on ice for processing for flow cytometry analyses. Viable cells were counted on a hemacytometer using trypan blue exclusion.

Flow cytometry analysis of SVCs
SVCs (5 × 10^5–1 × 10^6 cells per tube) were isolated as described above, suspended in flow cytometry staining buffer (catalog number 00-4222; eBioscience/Thermo Fisher), and incubated in Fc block for 10 minutes on ice. Cells were stained with appropriate antibodies for 45 minutes at 4°C in the dark. All flow cytometry antibodies are listed in Table 2. Stained SVCs were washed twice in PBS, fixed in 1% paraformaldehyde in PBS, and analyzed on FACS Aria or FACS Calibur flow cytometers (BD Biosciences, San Jose, California) using FlowJo software (version 10.0; TreeStar, Ashland, Oregon). Adipose tissue macrophages are identified as CD45<sup>+</sup> and CD64<sup>+</sup> (14,15). Adipose tissue T cells are identified as CD45<sup>+</sup> and CD3<sup>+</sup> (14,15). Adipose tissue B cells are identified as CD45<sup>+</sup>, CD19<sup>+</sup>, and B220<sup>+</sup>. Preadipocytes are identified as CD45<sup>+</sup>, CD31<sup>+</sup>, and Scal<sup>+</sup> (16), and endothelial cells were identified as CD45<sup>+</sup> and CD31<sup>+</sup>. Gating strategies for flow cytometry analyses are based on those previously described for adipose SVCs (3,14,15); autofluorescence (no-antibody) controls, single-stain controls, and fluorescence-minus-one isotype controls were used in each immunophenotyping panel for compensation settings and to determine gating. Data were normalized per gram of eWAT or as a percentage of SVCs, as indicated.

Statistical analyses
GraphPad Prism software was used for all statistical analyses (version 7.0; GraphPad Software, La Jolla, California), and results are expressed as mean ± SEM. Differences between genotypes were analyzed using the Student’s t test, with results considered significant when P < 0.05.

Results
In this study, we examined the phenotypic effects of adipocyte-specific OSMR<sup>−/−</sup> in chow-fed OSMR<sup>FKO</sup> male mice and littermate control OSMR<sup>+/−</sup> mice. Specifically, we examined tissue weight, insulin responsiveness, adipokine production, and stromal cell immunophenotypes in eWAT pads, as well as serum adipokine production.

| Table 2: Flow cytometry antibodies used in this study |
|------------------------------------------------------|
| **Antibody** | **Clone** | **Vendor**          |
| CD45.2      | 104      | eBioscience/Thermo Fisher |
| CD64        | 54-5/7.1 | BD Biosciences        |
| CD31        | 390      | eBioscience/Thermo Fisher |
| CD19        | 1D3      | eBioscience/Thermo Fisher |
| CD20        | RA3-6B2  | eBioscience/Thermo Fisher |
| CD3         | 145-2C11 | eBioscience/Thermo Fisher |
| Sca1        | D7       | eBioscience/Thermo Fisher |
| CD16/CD32   | 93       | eBioscience/Thermo Fisher |
| Mouse IgG2aκ | eBM2a    | eBioscience/Thermo Fisher |
| Mouse IgG1κ | P3.6.2.8.1 | eBioscience/Thermo Fisher |
| Rat IgG2aκ  | eBR2a    | eBioscience/Thermo Fisher |
| Armenian hamster IgG | eBio299Arm | eBioscience/Thermo Fisher |
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Adipose and nonadipose expression of OSMR subunits

We observed significant decreases in OSMR gene and protein expression in two white fat depots from OSMRFKO mice compared with OSMRWT mice (Figure 1A-B). The levels of gp130 gene and protein expression did not differ significantly between the control and knockout mice. There were no significant differences in either OSMR or gp130 gene and protein expression between genotypes in brown adipose tissue (Figure 1A-B) or in nonadipose tissues examined (Figure 1C-D). Notably, expression of OSM itself was significantly increased in the eWAT of OSMRFKO mice (Figure 1E); this increase in OSM expression also was found in obese high-fat-fed OSMRFKO mice (10).

Adipose tissue response to an acute OSM challenge

Acute OSM injection in OSMRWT mice resulted in robust phosphorylation of STAT3 and STAT5 tyrosine residues in eWAT. The reduced STAT phosphorylation in OSMRFKO mice (Figure 2A-B) indicated a substantial decrease in responsiveness to an OSM challenge. The observed effect on STAT5 phosphorylation was significant (P = 0.0084; Figure 2B), while the effect on STAT3 followed the same trend but did not reach significance (P = 0.074; Figure 2B). These data also suggest that adipocyte OSMR is responsible for the majority of OSM signaling in eWAT. Notably, basal levels of STAT3 and STAT5 tyrosine phosphorylation in vehicle-injected mice were lower in the OSMRFKO group (Figure 2A-B). OSM is known to

Figure 1 Expression levels of OSMR and gp130 are not altered in nonadipose tissues. Tissue expression of OSMR and gp130 in various adipose depots at the (A) gene and (B) protein levels in OSMRWT (control [Ctl]) and OSMRFKO (knockout [KO]) chow-fed mice. Nonadipose tissue expression of OSMR and gp130 at the (C) gene and (D) protein levels was also measured. (E) Gene expression of OSM itself was also measured in various adipose depots. Total RNA in various tissues was purified and analyzed by real-time polymerase chain reaction (PCR). Cyclophilin A (Ppia) was used as an endogenous control. Protein (100 μg for adipose tissues, 50 μg for all other tissues) was subjected to Western blot analysis. Data are shown as mean ± SEM. For PCR, n = 8 per genotype (eWAT); n = 3-4 per genotype (inguinal WAT [iWAT], brown adipose tissue [BAT], other tissues). *P < 0.05 versus OSMRWT. AU, arbitrary units.
induce ERK1 and ERK2 phosphorylation, but we did not observe a significant reduction in ERK activation following acute OSM injection in OSMRFKO mice compared with floxed littermate controls.

Body weight and blood glucose

Body weights did not significantly differ between OSMR fl/fl and OSMRFKO mice in any of the experimental cohorts at the time of euthanasia (Figure 3A). Fasting blood glucose levels also were not different between the two genotypes at the time of euthanasia (Figure 3B).

Insulin sensitivity and adipokine levels

As a measure of insulin sensitivity, we examined insulin-stimulated Akt phosphorylation in eWAT. Seven mice from each genotype were given intraperitoneal vehicle or insulin injections and euthanized 10 minutes later. Most of the insulin-injected OSMFKO mice had reduced Akt phosphorylation compared with floxed controls. However, when the results from all the mice were quantitated, the effects did not reach statistical significance (Figure 3C). There were no differences in total Akt levels (Figure 3C-D). Furthermore, we did not observe any significant changes in eWAT Glut1 or Glut4 gene expression levels (Glut1 relative expression: 1.082 ± 0.125 arbitrary units [AU] [OSMR fl/fl] vs. 1.028 ± 0.061 AU [OSMRFKO]; Glut4 relative expression: 1.222 ± 0.237 AU [OSMR fl/fl] vs. 1.238 ± 0.139 AU [OSMRFKO]) in eWAT.

Significant increases in C-reactive protein (CRP), intercellular adhesion molecule-1 (ICAM-1), insulin-like growth factor binding protein 6 (IGFBP6), and lipocalin 2 (LCN2) were observed in OSMRFKO eWAT (Figure 4A), while ICAM-1 and fetuin A (FETA) were elevated in OSMFKO serum (Figure 4B). The elevated ICAM-1 in both serum and eWAT in OSMFKO mice suggests that the eWAT may be the source of increasing circulating ICAM-1, although additional studies are required to confirm this hypothesis.

Adipokine expression in 3T3-L1 adipocytes

The lack of adipocyte OSMR signaling was associated with increased OSM expression in eWAT of OSMRFKO mice, both in the current study (Figure 1E) and with high-fat diet feeding (10). As shown in Figure 4A, OSMFKO increased LCN2 levels in eWAT. To determine whether OSM could induce Lcn2 gene expression in a cell-autonomous manner, we studied the effects of OSM in cultured adipocytes. We observed that a 6-hour treatment with OSM strongly induced Lcn2 expression in 3T3-L1 adipocytes. Use of the MAPK/ERK kinase [MEK] inhibitor U0126 demonstrated that this effect was primarily ERK dependent (Figure 5A). The efficacy of ERK inhibition was confirmed by Western blotting that demonstrated a significant reduction in ERK phosphorylation following inhibitor treatment (data not shown). OSM, however, did not induce Igfbp6 expression in adipocytes (Figure 5B). Interestingly, OSM-induced Icam1 expression, and ERK inhibition further increased this effect (Figure 5C). Previous studies have reported Crp and FetA expression and secretion by adipocytes (17,18); however, we were not able to detect either gene in our experiments in 3T3-L1 adipocytes.

Immunophenotyping of SVC populations

Though eWAT weights did not differ (Figure 6A), OSMR fl/fl mice had a significantly higher number of SVCs per gram of eWAT than OSMRFKO mice (Figure 6B). However, immunophenotyping analyses indicated significantly higher CD45+ leukocytes in OSMFKO eWAT compared with OSMR fl/fl eWAT (Figure 6B). While not significant, there were trends toward increased CD19+ B220+ B cells (P = 0.07) and increased CD3+ T cells in OSMFKO eWAT compared with OSMR fl/fl control mice (Figure 6C-D, respectively). No obvious trends were observed in CD64+ adipose tissue macrophages or in endothelial cells between groups (Figure 6E-F). Although not statistically significant, a modest increase in Sca1+ preadipocytes was observed in the OSMFKO mice (Figure 6G).
Discussion

Our previous work indicates that OSMR^FKO^ mice exhibit systemic insulin resistance and adipose tissue inflammation when challenged with a high-fat diet (10). Here, we studied the phenotype of OSMR^FKO^ mice in the absence of a high-fat diet challenge and more closely examined the adipose tissue inflammatory profile in the OSMR^FKO^ mice. There are several significant observations from this study. Notably, adipocyte-specific OSMR deletion does not alter body mass or fasting blood glucose, but it does modestly blunt adipose tissue insulin sensitivity in chow-fed mice. In addition, adipose tissue and serum proinflammatory adipokine expression levels are increased in chow-fed OSMR^FKO^ mice. These results are consistent with the significant increase in the number of adipose tissue leukocytes (Figure 6B) and the trend toward increased T-cell and B-cell numbers, despite no changes in adipose tissue weight. The observed phenotypic effects in chow-fed OSMR^FKO^ mice are not due to alterations in gp130 expression in adipose tissue or in gp130 and/or OSMR expression in nonadipose tissues. Taken together, these results and our previous data (10) demonstrate that the adipose tissue dysfunction in OSMR^FKO^ mice is characteristic of the genotype itself and occurs in the absence of obesity.

Our current results in OSMR^FKO^ mice contrast with those of the global OSMR knockout mice fed a chow diet (19). Global OSMR knockout mice of a similar age to the OSMR^FKO^ mice in our study have increases in body mass, eWAT mass, inguinal WAT mass, liver mass, and food intake compared with OSMR^fl/fl^ mice (19). We did not observe changes in any of these parameters in chow-fed OSMR^FKO^ mice. Furthermore, global OSMR knockout mice also exhibit significant increases in blood glucose and serum insulin and are generally glucose intolerant and insulin resistant (19), while our OSMR^FKO^ mice are not. However, much like the OSMR^FKO^
mice, global OSMR knockout mice exhibit increased adipose tissue inflammation. The vast differences in these phenotypes underscore the importance of OSMR in hematopoiesis, liver development, and other developmental functions and suggest that many of the metabolic effects observed in the global OSMR knockout mice are due to nonadipocyte effects (19–21). Some of the molecular mechanisms by which specific disruption of adipocyte OSM–OSMR signaling axis promotes adipose tissue dysfunction still remain unclear, although our current data provide new information.

As a first step toward examining drivers of the adipose tissue dysfunction in chow-fed OSMR FKO mice, we generated adipokine profiles using antibody arrays. We analyzed 38 adipokines and found that 4 of them were differentially expressed at significant levels: CRP, ICAM-1, IGFBP6, and LCN2 expression levels were significantly increased in eWAT of OSMR FKO mice compared with OSMR fl/fl mice, and we observed significant increases in FETA and ICAM-1 in the serum of OSMR FKO mice compared with OSMR fl/fl mice. Data are presented as mean ± SEM, *P < 0.05 versus OSMR fl/fl.

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Figure 5. Adipokine gene expression in 3T3-L1 adipocytes in response to OSM treatment and/or ERK inhibition. Fully differentiated 3T3-L1 adipocytes were treated with 1 nM of OSM or vehicle (V) for 6 hours in the presence or absence of the ERK inhibitor U0126. Gene expression levels of (A) Lcn2, (B) Igfbp6, (C) Icam1, Crp, and FetA were assessed, but Crp and FetA were not detected. Total RNA was purified from cells and analyzed by real-time polymerase chain reaction (PCR). Ubiquitin b (Ubb) was used as an endogenous control. AU, arbitrary units; tx, treatment; V, vehicle.
IGFBP6, and LCN2. Various cell types produce these proteins, all of which have established effects in inflammation. Immune cells can produce all four of these proteins; however, adipocytes are reported to produce at least two of them (CRP and LCN2). CRP is an acute-phase protein synthesized primarily by the liver in response to cytokines or other inflammatory stimuli. Previous reports have suggested that adipocytes can produce CRP in response to inflammatory stimuli (17,18), although we were unable to detect Crp gene expression in our in vitro 3T3-L1 adipocyte experiments. Adipocytes produce LCN2 in response to proinflammatory cytokine exposure (22–24), and several immune cell types also produce LCN2 (25). However, substantial evidence indicates that circulating LCN2 is liver derived and is produced in response to infection or injury (26,27), so it is not surprising that the changes we observed in LCN2 levels in this study were only at the level of the eW AT itself and not in serum (Figure 4B). The role of LCN2 in adipose tissue is generally thought to be proinflammatory because increasing its expression promotes neutrophil recruitment and induces proinflammatory cytokine production (28,29), although anti-inflammatory effects have been reported in other tissues (30,31). Our in vitro studies clearly indicate that OSM potently induces adipocyte LCN2 expression and that this induction is ERK dependent (Figure 5A). Tumor necrosis factor α and interferon γ are also potent inducers of LCN2 in cultured murine adipocytes (23).

ICAM-1 is a cell surface glycoprotein found on endothelial cells and leukocytes that is induced by proinflammatory cytokine production and interacts with β-integrins to facilitate leukocyte migration (32). Several studies have reported that adipocytes can express ICAM-1 (33–35). Our in vitro experiments indicate that, while Icam1 gene expression is very low in basal conditions, OSM treatment substantially induces Icam1 mRNA levels in adipocytes (Figure 5). The significant elevations of ICAM-1 in both serum and eWAT in OSMRFKO mice compared with floxed controls (Figure 4) was consistent with the elevated OSM levels we observed in OSMRFKO mice. Our flow cytometry data also demonstrate increased leukocyte numbers in OSMRFKO adipose tissue. Given its known functions in leukocyte migration and recruitment, we hypothesize that ICAM-1 is either induced in adipocytes by immune-cell-derived factors, produced by immune cells alone, or both and may act to effectively trap leukocytes in the eWAT of OSMRFKO mice. Further studies are required to evaluate this hypothesis.

Increased levels of FETA, a negative acute-phase protein primarily secreted from liver (36), are associated with metabolic syndrome and insulin resistance in humans and animals (37–39). In adipose tissue, FETA serves as an endogenous toll-like receptor 4 ligand, where it binds fatty acids for presentation to toll-like receptor 4 and subsequently triggers proinflammatory cytokine production via the nuclear factor-κB pathway (38). FETA can also promote macrophage migration into adipose tissue (37) and directly interfere with insulin receptor action to mediate insulin resistance (39). Data from these previous studies have coincided with results obtained in OSMRFKO mice in which we...
observed significant increases in serum FETA (Figure 4B) and a modest blunting of eWAT insulin signaling compared with OSMRfl/fl mice.

A family of six IGFBPs controls the activity of insulinlike growth factors (IGFs); the IGFBPs all serve to inhibit IGF actions. Of these IGFBPs, IGFBP6 has garnered interest for its high specificity for IGF2 and for its IGF-independent roles, such as the promotion of apoptosis and inhibition of angiogenesis (40,41). Literature addressing the possible role of IGFBP6 in adipose tissue has been scant; however, recent evidence has suggested that, in some inflammatory conditions, IGFBP6 may be a possible chemotactic factor (40). In this study, we observed significant increases in IGFBP6 levels in OSMRFKO eWAT compared with OSMRfl/fl eWAT. It is possible that, if IGFBP6 acts with chemotactic ability in adipose tissue, the increased IGFBP6 in OSMRFKO eWAT could be partially responsible for the significantly higher number of leukocytes measured in the tissue.

Collectively, our prominent adipocyte array results suggest a local proinflammatory environment in the adipose tissue of OSMRFKO mice that occurs in the absence of obesity. In further support of these observations, flow cytometry analyses revealed elevations of leukocytes, B cells, and T cells in OSMRFKO eWAT (Figure 6). While it remains unclear as to which cells in the adipose tissue are responsible for the increased adipokine production, it is clear that the loss of adipocyte OSM-OSMR signaling disrupts adipose tissue homeostasis in the absence of obesity. Likely, more than one cell type is contributing to this effect because there is known cross talk among cell types in adipose tissue. Future studies will more closely examine each cell type in the adipose tissue of OSMRFKO mice to better determine their roles in contributing to disrupted adipose tissue homeostasis. Despite the evident adipose tissue dysfunction in chow-fed OSMRFKO mice, a systemic metabolic challenge, such as an obesogenic diet, is required to produce whole-body insulin resistance while perpetuating adipose tissue inflammation in these animals (10). Taken together, these results and our previous results strongly suggest the need for intact adipocyte OSM-OSMR signaling in maintaining adipose tissue homeostasis.

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