Loss of Oncostatin M Signaling in Adipocytes Induces Insulin Resistance and Adipose Tissue Inflammation in Vivo*

Oncostatin M (OSM) is a multifunctional gp130 cytokine. Although OSM is produced in adipose tissue, it is not produced by adipocytes. OSM expression is significantly induced in adipose tissue from obese mice and humans. The OSM-specific receptor, OSM receptor β (OSMR), is expressed in adipocytes, but its function remains largely unknown. To better understand the effects of OSM in adipose tissue, we knocked down Osmr expression in adipocytes in vitro using siRNA. In vivo, we generated a mouse line lacking Osmr in adiponectin-expressing cells (OSMRKO mice). The effects of OSM on gene expression were also assessed in vitro and in vivo. OSM exerts proinflammatory effects on cultured adipocytes that are partially rescued by Osmr knockout. Osm expression is significantly increased in adipose tissue T cells of high fat-fed mice. In addition, adipocyte Osmr expression is increased following high fat feeding. OSMRFKO mice exhibit increased insulin resistance and adipose tissue inflammation and have increased lean mass, femoral length, and bone volume. Also, OSMRFKO mice exhibit increased expression of Osm, the T cell markers Cd4 and Cd8, and the macrophage markers F4/80 and Cd11c. Interestingly, the same proinflammatory genes induced by OSM in adipocytes are induced in the adipose tissue of the OSMRFKO mouse, suggesting that increased expression of proinflammatory genes in adipose tissue arises from adipocytes and other cell types. These findings suggest that adipocyte OSMR signaling is involved in the regulation of adipose tissue homeostasis and that, in obesity, OSMR ablation may exacerbate insulin resistance by promoting adipose tissue inflammation.

*This work was supported in part by National Institutes of Health Grant R01DK052968 (to J. M. S.) and National Institutes of Health Nutrition Obesity Research Centers Grant P30DK072476 entitled “Nutritional Programming: Environmental and Molecular Interactions.” The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

1 Supported by National Institutes of Health Centers of Biomedical Research Excellence Grant P20GM103528 entitled “Mentoring Diabetes and Obesity Research in Louisiana.”

2 To whom correspondence should be addressed: Pennington Biomedical Research Center, 6400 Perkins Rd., Baton Rouge, LA 70808. Tel.: 225-763-2648; Fax: 225-578-2597; E-mail: jsteph1@lsu.edu.

Adipose tissue (AT) plays an important role in the maintenance of systemic metabolic homeostasis. Adipokine production is a critical AT function and is highly regulated in several physiological and pathological conditions, including AT expansion, insulin resistance, obesity, and type 2 diabetes. Obesity is closely associated with a chronic, low grade inflammatory state characterized by macrophage infiltration of AT and subsequent proinflammatory adipokine expression (1, 2). Adipokine modulation has been shown to be a key contributor to the insulin resistance often observed in obesity.

Cytokines in the interleukin-6 (IL-6)/gp130 family include IL-6, IL-11, leukemia inhibitory factor, cardiotrophin-1, ciliary neurotrophic factor, and oncostatin M (OSM) (3). The gp130 cytokines regulate several physiological and biological processes (4), and some of these cytokines, namely IL-6 and ciliary neurotrophic factor, have profound effects on metabolism and as such have been previous targets for obesity treatment (5–8). Although originally identified for its ability to inhibit tumorogenesis (9), OSM modulates a host of other biological processes that are cell type-dependent (10). Elevated OSM levels have been observed in a variety of inflammatory diseases in humans, including rheumatoid arthritis and atherosclerosis (11, 12). OSM also has important roles in hepatic insulin resistance and steatosis (13), inflammation (14), and cardiomyocyte remodeling (15) and has several well characterized actions in the liver (16–18). Unlike other gp130 cytokines, OSM has its own specific receptor subunit (OSM receptor β; hereafter referred to as OSMR) that heterodimerizes with gp130 to create the functional OSM receptor complex, and this complex is responsible for the majority of OSM effects (19).

Adipocytes and AT are highly responsive to OSM (20), and Osmr is highly expressed in AT compared with other tissues (21, 22). Significant induction of both Osm and Osmr expres-
sion occurs in AT of obese mice and humans (22). OSM is not produced by adipocytes but is made in cells comprising the stromal vascular fraction (SVF) of human AT (22). We have shown that OSM is expressed in AT macrophages (22) in mice, and others have shown that OSM is produced in liver Kupffer cells where it contributes to steatosis and insulin resistance (13). The majority of studies support a role for OSM in promoting metabolic dysfunction. For example, it is well documented that OSM inhibits adipogenesis (23, 24). In addition, OSM induces plasminogen activator inhibitor-1 (PAI-1) expression in murine adipocytes (22) and in human AT (25). PAI-1 is a known player in metabolic dysfunction (26, 27). Despite a large number of studies showing associations among OSM, inflammation, and metabolic dysfunction, three recent reports from the same group have suggested that OSM is a potential agent for obesity treatment (21, 28, 29). Hence, there is currently a great deal of confounding data on the role of OSM in obesity and metabolic disease states. We have generated a mouse model, the adipocyte-specific Osmr knock-out mouse (OSMR^{FKO}), in an attempt to clarify the function of AT OSM. To our surprise, the loss of adipocyte OSM signaling in vivo was associated with inflammation and insulin resistance. The interpretation of these results is complicated by elevated AT OSM expression in the OSMR^{FKO} mice. Nonetheless, these results suggest that intact adipocyte OSM signaling plays a role in the maintenance of AT homeostasis.

Results

Increased Proinflammatory Adipokine and OSM Receptor Subunit Expression in OSM-exposed Adipocytes—To understand the function of OSM on adipocytes, we examined the expression of several adipokines in 3T3-L1 adipocytes following OSM exposure for the times indicated in Fig. 1. The expression levels of genes associated with insulin resistance and inflammation (Mcp1, Igfbp3, Pai1, and Spp1) \(A\) and the OSM receptor signaling complex (gp130 and Osmr) \(B\) were examined. Differences in expression levels as compared with vehicle-treated cells were analyzed with a Student’s \(t\) test. \(p\) values are represented with letters \((a, p < 0.0001; b, p < 0.001; c, p < 0.01\) versus vehicle-treated cells), and error bars represent S.D. Values reported represent results from two independent experiments with each treatment condition performed in triplicate.
Oncostatin M (OSM) is a cytokine that is produced by activated immune cells and has been implicated in the regulation of adipose tissue homeostasis. In this study, we investigated the expression of OSM and its receptor in adipose tissue and its role in adipose tissue inflammation and insulin resistance.

Expression of IL-6 receptor α (Il6ra) or leukemia inhibitory factor receptor (Lifr), two other gp130 family receptors, were observed (Fig. 2D). These results suggest that 1) OSM can directly induce expression of proinflammatory adipokines in adipocytes via the OSMR signaling complex and 2) intact OSMR signaling is required in part for transcriptional regulation of both OSMR subunits.

Osm and Osmr Are Differentially Expressed among AT Cell Types—We previously demonstrated in subcutaneous AT from obese humans that the high levels of OSM originated from SVF cells and that OSM was not expressed in human adipocytes (22). In ob/ob mice, we previously observed that Osm expression in AT was readily detectable in macrophages (22). We extended our previous findings by examining the expression of Osm and Osmr in epididymal AT cell types (adipocytes, T cells, and B cells) from C57BL/6J (B6) mice fed low fat diet (LFD) or high fat diet (HFD) for 18 weeks. Compared with LFD-fed mice, HFD-fed mice exhibited a significant increase in T cell Osm expression (Fig. 3A), suggesting that some of the increased AT OSM in obesity is derived from T cells. Increased Osm expression is also observed in adipocytes from HFD-fed mice, which may be due to the presence of lipid-laden macrophages in the adipocyte fraction because adipocytes do not express Osm. Lastly, Osmr expression is increased in adipocytes of HFD-fed mice, although this increase is not significant (Fig. 3B).

Obese OSMR<sup>FKO</sup> Mice Exhibit Increased Fat-free Mass and Are Insulin-resistant—To delete Osmr in adipocytes, we obtained mice from The Jackson Laboratory where exon 2 (first coding exon) of the Osmr gene was flanked by loxP sites (floxed; OSMR<sup>fl/fl</sup>). Floxed mice were crossed with mice expressing Cre recombinase under control of the adiponectin (Adipoq) gene promoter (30). OSMR<sup>FKO</sup> mice exhibited decreased Osmr expression in whole AT, but no changes in gene expression in other tissues, including liver and spleen, were observed (Fig. 4A). Decreased Osmr gene expression was restricted to adipocytes and not SVF cells (Fig. 4B). Decreased OSMR protein expression in the adipocyte fraction from OSMR<sup>FKO</sup> AT is shown in Fig. 4C.

After verification of knock-out specificity, OSMR<sup>FKO</sup> and floxed control mice were fed HFD for 24 weeks. Although body...
weights for OSMRFKO mice were slightly higher starting at week 4 on HFD, the trend did not reach significance until 12 weeks of HFD feeding (Fig. 4D). The increased body weight of OSMRFKO mice was mainly attributed to increased lean mass as no significant differences in fat mass were observed between the groups (Fig. 4E). Interestingly, significant differences in lean mass of OSMRFKO mice were observed starting around 8 weeks of HFD feeding prior to the slight divergence in body weight (Fig. 4E). This difference in lean mass could possibly be attributed to increased bone mass as the mean nasoanal length of the OSMRFKO mice was significantly greater than that of the control mice at the end of the study (10.9 ± 0.075 versus 10.5 ± 0.053 cm, respectively; \( p = 0.0043 \)). After 24 weeks of HFD, OSMRFKO mice had higher serum insulin (Fig. 5A), lower serum total adiponectin levels (Fig. 5B), and blunted responses to an insulin bolus during an insulin tolerance test (Fig. 5C), suggesting impaired insulin-stimulated whole-body glucose handling in these mice. A trend toward impaired glucose disposal capacity was also observed during a glucose tolerance test, although the trend was not significant (Fig. 5D).

**Disrupted AT Homeostasis in Obese OSMRFKO Mice without Any Alterations in Adipocyte Size or Liver Fat**—Histological assessment of epididymal AT in obese OSMRFKO and control mice revealed an apparent increase in the presence of crownlike structures (Fig. 6A), a hallmark of AT inflammation. However, no changes in mean adipocyte area or size were observed.

---

**FIGURE 3.** Expression and regulation of *Osm* and *Osmr* in AT cell types. Relative *Osm* mRNA levels (A) and relative *Osmr* mRNA levels (B) were measured in the floating adipocyte fraction and in immune cells isolated from SVF from mice fed HFD or LFD for 18 weeks. Differences in expression levels were analyzed with a Student’s t test. \( p \) values are represented with asterisks (*, \( p < 0.05 \); ***, \( p < 0.01 \) versus same cell type in LFD mice), and error bars represent S.D. Results are representative of three independent experiments.

**FIGURE 4.** Mice with adipocyte-specific *Osmr* deficiency (OSMRFKO) have increased weight gain and increased fat-free mass. The genotype of OSMRFKO mice was confirmed by qRT-PCR analysis of *Osmr* mRNA in whole tissues (A), qRT-PCR in adipocytes and SVF from fractionated AT (B), and Western blotting in adipocytes from fractionated AT (C). Adipocyte-specific ablation of *Osmr* increases weight gain (D) and fat-free mass accumulation (circles) with no change in fat mass (triangles) in mice on HFD (E). In the panels, gray bars and shapes represent OSMRFKO mice, and black bars and shapes represent OSMRfl/fl mice. Western blots are representative of at least two independent experiments. Differences in gene expression levels, body weights, and NMR values between genotypes at each time point were analyzed using a Student’s t test. \( p \) values are represented with asterisks (*, \( p < 0.05 \); ***, \( p < 0.01 \) versus OSMRfl/fl), and error bars represent S.D. \( n = 8 \) for OSMRFKO mice, and \( n = 10 \) for OSMRfl/fl mice. AU, arbitrary units; iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue.
between OSMR^{FKO} and control mice (Fig. 6, B and C, respectively). In further support of an elevated inflammatory response in OSMR^{FKO} mice, significant increases in the expression of macrophage markers (F4/80 and Cd11c) and T cell markers (Cd4 and Cd8) were observed as well as a trend toward increased expression of several proinflammatory cytokines.

FIGURE 5. OSMR^{FKO} mice exhibit impaired whole-body insulin response. Osmr-deficient mice have higher serum insulin levels (A) and lower serum adiponectin levels (B) after a 4-h fast. There is a significantly decreased whole-body response to an insulin bolus (C) and a trend toward a decreased response to a glucose bolus (D) in the OSMR^{FKO} mice. For statistical analysis of serum values, a Student’s t test was used. For analysis of blood glucose values over time as measured in the insulin tolerance test and glucose tolerance test, repeated measures analysis of variance was used with a Bonferroni post-test. p values are represented with asterisks (*, p < 0.05; **, p < 0.01; ***, p < 0.001 versus OSMR^{fl/fl}), and error bars represent S.D. n = 8 for OSMR^{FKO} mice, and n = 8 for OSMR^{fl/fl} mice.

FIGURE 6. OSMR^{FKO} mice exhibit increased crownlike structure formation but no significant changes in adipocyte size. A, representative H&E sections of epididymal fat from obese control and OSMR^{FKO} mice. Scale bars, 250 μm. Shown are average adipocyte area (B) and size distribution (C) of epididymal adipocytes in control and OSMR^{FKO} mice after 6 months of HFD. Error bars represent S.D. n = 8 for OSMR^{FKO} mice, and n = 10 for OSMR^{fl/fl} mice.
A significant increase in hepatic triglyceride content between genotypes was analyzed using a Student’s t test. *p < 0.05; **, p < 0.01 versus OSMRfl/fl, and error bars represent S.D. n = 8 for OSMRKO mice, and n = 8 for OSMRFKO mice.

Furthermore, quantitative measurements revealed no significant differences in hepatic triglyceride content between genotypes (Fig. 8A). Similar results were found upon histological evaluation of liver sections (Fig. 8B).

A Novel Bone Phenotype in OSMRFKO Mice—A significant increase in femoral length (Fig. 8C) was identified in OSMRFKO mice. Trabecular bone within the distal femur was also increased (Fig. 8D); however, this change was not significantly different (p = 0.058). This change in bone volume density appears to be related to an overall increase (p = 0.050) in trabecular number in the OSMRFKO mice. In addition, trabecular bone structure model index was significantly different where the OSMRFKO mice had significantly more platelike trabeculae (p = 0.021).

Discussion

In this study, we assessed the direct effects of OSM on adipocytes in vitro and characterized the phenotype of a mouse that lacked OSMR in adiponectin-expressing cells (OSMRKO mouse). We used our newly generated mouse model to assess the role of adipocyte OSMR in AT homeostasis and metabolic health. Our results demonstrate that OSM treatment of cultured adipocytes leads to a sustained increase in the expression of several proinflammatory adipokines associated with insulin resistance (Timp1, Mcp1, Pai1, Igfbp3, and Spp1). These observations are consistent with other studies that have shown elevated Mcp1and Pai1 expression with OSM treatment in other cell types, including fibroblasts and hepatic cells (31, 32). In murine adipocytes, we have shown similar effects with acute OSM treatment (22). The roles of MCPI and PAI-1 in obesity, inflammation, and fibrosis are well documented (for reviews, see Refs. 26, 27, and 33).

Our novel finding that OSM treatment induces Timp1, Igfbp3, and Spp1 expression in adipocytes is notable as dysregulated expression of these genes is associated with insulin resistance and metabolic dysregulation in vitro and in vivo (34–39). In obesity, AT Timp1 significantly increases (38, 39), and mice injected with TIMP1 develop insulin resistance and systemic fatty acid overload (38). IGFBP3-treated adipocytes exhibit insulin resistance (36), and mice overexpressing Igfbp3 exhibit decreased glucose uptake in AT and muscle (37). Mice lacking Spp1 (osteopontin) are less likely to develop obesity and insulin resistance and exhibit less AT macrophage infiltration (34, 35). The ability of OSM to promote the expression of these paracrine and endocrine mediators that favor insulin resistance reveals a possible mechanism whereby OSM perpetuates AT inflammation in obesity (see Fig. 9).

We previously reported increased OSM expression in AT from obese rodents and humans and demonstrated that AT OSM is produced from SVF cells and not from adipocytes (22). We also observed expression of Osm gene expression in AT macrophages from lean and ob/ob mice; however, the expression levels of Osm in AT macrophages were not significantly altered in obesity (22). Our current observations extend those findings by confirming that Osm expression in AT macro-
phages is not altered by HFD feeding. However, we observed that T cells also express Osm, and its expression is significantly increased in AT by HFD feeding (Fig. 3). Furthermore, we demonstrate that adipocyte Osmr expression is highly up-regulated in HFD feeding. It is widely accepted that T cell recruitment into AT (and subsequent activation) is a hallmark of the chronic, low grade inflammation observed in obesity (40, 41) and that activated T cells can produce OSM (42, 43). For the first time, we demonstrate that AT Thy1.2^+ T cells express Osm and that HFD induces T cell Osm expression in epididymal AT.

OSM produces a proinflammatory response in cultured adipocytes, and AT Osm expression is elevated in obese mice and humans. These facts, coupled with data demonstrating increased adipocyte Osmr expression following HFD feeding, support the hypothesis that adipocyte Osmr knockdown and concomitant reduction of adipocyte OSM signaling in vivo could have metabolically favorable effects in obesity. We tested this hypothesis by creating and phenotyping the OSMR^KO^ mouse. Contrary to our hypothesis, OSMR^KO^ mice are less metabolically healthy following chronic HFD feeding. OSMR^KO^ mice have modest increases in body mass and fat-free mass with no significant changes in fat mass (Fig. 4) and exhibit systemic insulin resistance and AT inflammation. Whole-body Osmr KO mice fed a HFD have higher food intake, body mass, and fat mass than controls with no differences in lean mass (21). These mice also exhibit higher serum leptin and insulin levels than control mice, no significant differences in serum adiponectin levels, and hyperlipidemia when fed HFD for only 8 weeks (21). Our OSMR^KO^ mice also exhibited elevated insulin levels but had lower adiponectin levels after 24 weeks of HFD feeding. It is important to note that after 8 weeks of HFD feeding divergence of the body weights of our control OSMR^KO^ mice had not yet occurred. However, at this stage in the whole-body KO mice, severe metabolic effects in AT, liver, pancreas, and skeletal muscle were already observed, a fact that underscores the non-AT-specific effects in these mice (21). Although the whole-body KO mice exhibited severe hepatic steatosis, we did not observe any notable differences in liver triglyceride content (16, 45) and is expressed in many tissues during both embryonic development and adulthood in mice (46); thus, it is not surprising that the phenotype of whole-body Osmr KO mice is more severe and quite different from OSMR^KO^ mice.

The two major phenotypes of the OSMR^KO^ mice are AT inflammation that is accompanied by increased AT OSM and systemic insulin resistance. Unlike the whole-body KO mice, OSMR^KO^ mice had no significant changes in fat mass or adipocyte size despite increased proinflammatory and profibrotic

FIGURE 8. OSMR^KO^ mice have no changes in liver fat but exhibit a bone phenotype. Mean liver triglyceride (TG) levels (A) and representative H&E sections of livers (B) from obese control and OSMR^KO^ mice are shown. Scale bars, 250 μm. Mean femoral length (C) and mean bone volume/total volume (BV/TV) (D) for obese control and OSMR^KO^ mice fed HFD for 6 months are shown. Differences in triglyceride levels and bone parameters between genotypes were analyzed using a Student’s t test. p values are represented with asterisks (**, p < 0.01 versus OSMR^KO^), and error bars represent S.D. E, midcoronal views of three-dimensional reconstructions of femora from representative control (right) and OSMR^KO^ (left) mice demonstrating increased trabecular bone in the distal metaphysis of the femur of the OSMR^KO^ mice. Scale bar, 1 mm. For liver and bone measurements, n = 9 for OSMR^KO^ mice, and n = 8 for OSMR^KO^ mice.
tissue profiles. These results suggest that loss of adipocyte OSMR signaling negatively alters the AT adipokine profile and influences the development of AT inflammation and insulin resistance without altering the ability of AT to store lipid. Surprisingly, many of the proinflammatory adipokines directly induced by OSM in adipocytes in vitro were also induced in the AT of OSMRFKO mice.

Another notable observation in the OSMRFKO mice was the significant induction of \(Mmp7\) in visceral adipose tissue. This gene has not been well studied in the context of adipose tissue or obesity but serves diverse functions in the innate immune response (47). Although \(Mmp7\) has known roles in tissue fibrosis (48), wound healing, and cell migration (47), its exact function in AT remains to be elucidated. A recent report describes a significant induction of \(Mmp7\) expression in subcutaneous AT from obese, insulin-resistant humans when compared with obese, insulin-sensitive humans (49). Future studies will focus on the relationship of \(Mmp7\) to adipocyte OSM signaling and AT inflammation in obesity.

The elevated AT OSM levels observed in the OSMRFKO mice (Fig. 7) support the notion that disrupting the paracrine actions of OSM on adipocytes may contribute to the observed elevation of AT OSM that may influence its paracrine actions on AT SVF cells to promote further proinflammatory adipokine production and thereby further disrupt AT homeostasis (Fig. 9). The concept that intact adipocyte inflammatory signaling pathways are both a requirement and adaptation for AT during obesity is not new as similar requirements for TNF and other inflammatory mediators have recently been demonstrated (50). Mice expressing an adipocyte-specific dominant negative form of TNF that cannot productively engage the TNF receptor are severely glucose-intolerant and have decreased serum adiponectin (50). Similarly, the adipocyte-specific receptor internalization and degradation (RID) complex transgenic mouse has decreased proinflammatory signaling, is glucose-intolerant, and exhibits hepatic steatosis (50). Although further studies are needed, our results support a model where inhibition of adipocyte OSM signaling leads to elevated OSM that likely has paracrine effects on T cells that have high levels of Osmr expression (Fig. 3). However, the elevated expression of Osm and Osmr on T cells in obesity coupled with the increased Cd4 and Cd8 expression we observed in AT of OSMRFKO mice (Fig. 7) greatly enhances the complexity and interpretation of the phenotype of the OSMRFKO mice. Future studies will be needed to elucidate the role of T cell-derived OSM in the context of metabolic disease states.

Taken together, our novel results demonstrate a direct proinflammatory effect of OSM on adipocytes and suggest that abrogation of adipocyte OSM signaling disrupts AT homeostasis to result in inflammation and insulin resistance in the absence of any substantial changes in fat mass. These results reveal a role of OSM in AT and the requirement for intact adipocyte OSM signaling in the maintenance of metabolic health in obesity. Additional research will be required to determine whether the phenotype of OSMRFKO mice is mediated by
Oncostatin M and Adipose Tissue Homeostasis

elevated OSM production in AT and/or altered immune cell OSM signaling in various AT depots.

Experimental Procedures

Cell Culture and Treatments—Murine 3T3-L1 preadipocytes were grown to 2 days postconfluence in DMEM with 10% bovine serum and differentiated as described previously (22). One day prior to beginning the treatment protocol in adipocytes, medium was replaced with DMEM containing 5% FBS. Cells were treated for 24–120 h with 1 nxn OSM (R&D Systems) or vehicle (0.1% BSA in PBS) as indicated. During the treatment period, culture medium was changed every 48 h. At the conclusion of the treatment period, medium was removed, and cells were washed with PBS and then harvested for RNA or protein extraction.

Animals and Diets—Osmr floxed mice were obtained from The Jackson Laboratory (stock number 011081). In these mice, the first coding exon in the Osmr gene (exon 2) is flanked by loxP sites. Adipoq-Cre mice were obtained from a Pennington Biomedical Research Center colony that was originally generated by crossing hemizygous Cre mice (The Jackson Laboratory stock number 010803) to B6 mice. These two mouse strains were crossed to create the adipocyte-specific OSMR knock-out mouse (Osmr flox/flox; Adipoq-Cre; OSMR floxed) and littermate floxed controls (Osmr flox/flox; OSMR floxed). In some experiments, C57BL/6 (B6) mice obtained directly from The Jackson Laboratory were used as indicated. Animals were housed in a temperature (22 ± 2 °C)- and humidity-controlled (45–55%) room under a 12-h light/dark cycle. Mice were allowed ad libitum access to food and water, and body weights were measured weekly. For HFD experiments in OSMR floxed mice, a purified diet containing 45% of calories from fat was used (Research Diets, number D12451). For HFD experiments in B6 mice, a purified diet containing 60% of calories from fat was used (Research Diets, number 12492). All experiments were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

Animal Procedures and Serum Analyses—For studies on OSMR floxed mice and controls, non-fasting body composition was analyzed by NMR (Bruker LF50, Bruker Optics, Germany) at baseline and every other week for the duration of the study (51, 52). Body weights were obtained weekly. Intraportal insulin tolerance tests were performed on mice in the fed state using i.p. injections of 0.04 unit of insulin/mouse (Humulin-R, Eli Lilly). Intraportal glucose tolerance tests were performed after a 4-h fasting period using i.p. injections of 20% dextrose. Tail glucose was measured at baseline (time 0) prior to the administration of insulin or glucose and at the indicated postinjection time points as described previously (51, 52). At study end, blood was collected via cardiac puncture, centrifuged at 3500 rpm for 15 min at 4 °C, and serum was removed and stored at −80 °C until analyzed. ELISA kits were used for the measurement of fasting serum insulin levels (Crystal Chem) and fasting adiponectin levels (Millipore) according to the manufacturers’ instructions.

For microcomputed tomography and histological analyses of bone and bone marrow, intact hind limbs from a second cohort of HFD-fed OSMR floxed (n = 9) and littermate controls (n = 8) were collected, fixed in 10% neutral buffered formalin, and stored in 70% ethanol. Femora were disarticulated and dissected free from surrounding soft tissue. AT Isolation and Fractionation—Isolation and fractionation of epididymal or inguinal AT were performed as described previously in detail (53). For OSMR floxed and control mice, floating adipocytes and SVF were processed for RNA or protein extraction. For HFD- and LFD-fed mice, the floating adipocyte fraction and SVF were separated by collagenase digestion, and immune cells from the SVF (Thy1.2+ T cell, CD19+ B cell, F4/80+ macrophage) were isolated sequentially by positive selection using magnetic beads. Briefly, positive isolation of T cells was performed using mouse pan-T (Thy1.2) Dynabeads (Invitrogen) according to the manufacturer’s protocol. For positive isolation of B cells, the cell suspension was incubated with biotinylated CD19 antibody (eBioscience, catalogue number 13-0193), and biotin-binding Dynabeads were used to select the CD19+ cells. Positive isolation of F4/80+ macrophages was performed by incubating cells with an F4/80 antibody (eBioscience, catalogue number 16-4801) and using IgG Dynabeads to isolate the F4/80+ cells. Cells remaining after the sequential isolation of T cells, B cells, and macrophages were also collected. RNA was isolated, and cDNA was prepared. Gene expression was measured using qRT-PCR, and Ppib was used as a control gene.

Adipocyte Area and Size Distribution—For determination of average adipocyte size, hematoxylin- and eosin-stained epididymal AT sections were imaged using a Hamamatsu NanoZoomer digital slide scanner at 20× resolution. The resulting images were subsequently analyzed and quantified using a custom application within VIS software version 5.0.5 (Visiopharm). The number of adipocytes counted per experimental condition ranged from 1,498 to 6,693.

Immunoblotting—Proteins were separated on 5% polyacrylamide gels containing sodium dodecyl sulfate and transferred to nitrocellulose membrane (Bio-Rad). After transfer, the membrane was blocked and incubated with goat anti-OSMR (R&D Systems, catalogue number AF662), rabbit anti-STAT5A (Santa Cruz Biotechnology, catalogue number sc-1081), or mouse anti-phospho-STAT5 (Millipore, catalogue number 05-495) primary antibodies overnight at 4 °C. Results were visualized with the appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) and enhanced chemiluminescence (Pierce).

Gene Expression Analyses—Total RNA was isolated from cells or mouse tissues using an RNeasy Mini kit (Qiagen), and yield was determined by spectrophotometry (NanoDrop Technologies). cDNA synthesized for use with the Extracellular Matrix and Fibrosis RT² Profiler PCR Arrays (Qiagen) was reverse transcribed using the RT² First Strand kit (Qiagen) with the SYBR Green system. All other cDNA was synthesized using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) with the SYBR Green system. Relative quantification of mRNA expression was analyzed using an ABI Prism 7900 Sequence Detection System (Applied Biosystems). Sequences for mouse primers not
including the targeted arrays (Integrated DNA Technologies) appear in Table 1.

**Microcomputed Tomography and Histological Analyses of Bone**—Intact femora were placed in holders for scanning by microcomputed tomography (Scanco Model 40, Scanco Medical AG, Basserdorf, Switzerland). Appropriate fluid (70% ethanol) was used so that bone density as well as bone volume could be determined. The samples were scanned in a coronal plane at 55 kV, 0.3-s integration time, with a 10-μm voxel size in plane and a 10-μm slice thickness. The lengths of the bones were evaluated from the scans ranging from the most proximal portion of the greater trochanter to the most distal aspect of the femoral condyles. The regions of interest for tissue analyses were determined to range from the distal 30% of the femur to the distal physis for trabecular bone and midshaft (50% of the length of the femur) for cortical bone. The proper thresholds for both trabecular and cortical bone were tested, and the same thresholds were used throughout the study. For trabecular bone, bone volume, total volume, bone volume density; and trabecular number, spacing, and thickness among other parameters were evaluated using standard procedures and nomenclature (44).

**Statistical Analyses**—JMP Pro 12.0 and GraphPad Prism 5 software were used for all statistical analyses. Results are expressed as mean ± S.D. Differences between specified groups were analyzed using the Student’s t test with results considered significant when p was <0.05. Differences over time were analyzed using repeated measures analysis of variance with a Tukey’s post hoc test or by Student’s t test at each time point.

**Acknowledgment**—We express gratitude to Dr. Robbie Beyl for guidance regarding statistical analyses.

**References**

1. Greenberg, A. S., and Obin, M. S. (2006) Obesity and the role of adipose tissue in inflammation and metabolism. *Annu. Rev. Physiol.* 83, 4615–4655.

2. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., and Ferrante, A. W., Jr. (2003) Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Investig.* 112, 1796–1808.

3. Fasnacht, N., and Müller, W. (2008) Conditional gp130 deficient mouse mutants. *Semin. Cell Biol.* 19, 379–384.

4. Heinrich, P. C., Behrmann, I., Haan, S., Hermanns, H. M., Müller-Newen, G., and Schaper, F. (2003) Principles of interleukin (IL)-6-type cytokine signaling and its regulation. *Biochem. J.* 374, 1–20.

5. Wallenius, V., Wallenius, K., Ahren, B., Rudling, M., Carlsten, H., Dickson, S. L., Ohlsson, C., and Jansson, J.-O. (2002) Interleukin-6-deficient mice develop mature-onset obesity. *Nat. Med.* 8, 75–79.

6. Feinberg, M. A. (2007) gp130 receptor ligands as potential therapeutic targets for obesity. *J. Clin. Investig.* 117, 841–849.

7. Gloaguen, I., Costa, P., Demartis, A., Lazzaro, D., Di Marco, A., Graziani, R., Paonessa, G., Chen, F., Rosenblum, C. I., Van der Ploeg, L. H., Cortese, R., Ciliberto, G., and Laufer, R. (1997) Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. *Proc. Natl. Acad. Sci. U.S.A.* 94, 6456–6461.

8. Ettinger, M. P., Littlejohn, T. W., Schwartz, S. L., Weiss, S. R., McIlwain, H. H., Heymsfield, S. B., Bray, G. A., Roberts, W. G., Heyman, E. R., Stambler, N., Heshka, S., Vicary, C., and Guler, H. P. (2003) Recombinant variant of ciliary neurotrophic factor for weight loss in obese adults: a randomized, dose-ranging study. *JAMA* 289, 1826–1832.

9. Zarling, J. M., Shoyab, M., Marquardt, H., Hanson, M. B., Lioubin, M. N., and Todaro, G. J. (1986) Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 83, 9739–9743.

10. Bambr, R., Reif, A. R., Haugen, S. H., and Clegg, C. H. (1998) Oncostatin M stimulates excessive extracellular matrix accumulation in a transgenic mouse model of connective tissue disease. *J. Mol. Med.* 76, 61–69.

11. Hui, W., Bell, M., and Carroll, G. (1997) Detection of oncostatin M in synovial fluid from patients with rheumatoid arthritis. *Ann. Rheum. Dis.* 56, 184–187.

12. Albasan-Puig, A., Murray, J., Preusch, M., Coan, D., Namekata, M., Patel, Y., Dong, Z. M., Rosenfeld, M. E., and Wijelath, E. S. (2011) Oncostatin M is expressed in atherosclerotic lesions: a role for Oncostatin M in the pathogenesis of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 31, 292–298.

13. Henkel, J., Gärtnert, D., Dorn, C., Hellerbrand, C., Schanze, N., Elz, S. R., and Todaro, G. J. (1986) Oncostatin M: a growth regulator produced by differentiated histiocytic lymphoma cells. *Proc. Natl. Acad. Sci. U.S.A.* 83, 19170–19175.

14. Wallace, P. M., MacMaster, J. F., Rouleau, K. A., Brown, T. J., Loy, J. K., Donaldson, K. L., and Wahl, A. F. (1999) Regulation of inflammatory responses by oncostatin M, *J. Immunol.* 162, 5547–5555.

15. Kubin, T., Pöling, J., Kostin, S., Gajawada, P., Hein, S., and Todaro, G. J. (1986) Oncostatin M is expressed in atherosclerotic lesions: a role for Oncostatin M in the pathogenesis of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 16, 292–298.

16. Henkel, J., Gärtnert, D., Dorn, C., Hellerbrand, C., Schanze, N., Elz, S. R., and Püschel, G. P. (2011) Oncostatin M produced in Kupffer cells in response to PGE2: possible contributor to hepatic insulin resistance and steatosis. *Lab. Invest.* 91, 1107–1117.

17. Wallace, P. M., MacMaster, J. F., Rouleau, K. A., Brown, T. J., Loy, J. K., Donaldson, K. L., and Wahl, A. F. (1999) Regulation of inflammatory responses by oncostatin M, *J. Immunol.* 162, 5547–5555.

18. Kubin, T., Pöling, J., Kostin, S., Gajawada, P., Hein, S., Rees, W., Wietelmann, A., Tanaka, M., Löchner, H., Schimanski, S., Zbior, M., Warren, H., and Braun, T. (2011) Oncostatin M is a major mediator of cardiomyocyte dedifferentiation and remodeling. *Cell Stem Cell* 9, 420–432.

19. Kamiya, A., Kinoshita, T., Ito, Y., Matsu, T., Morikawa, Y., Senba, E., Nakashima, K., Taga, T., Yoshida, K., Kishimoto, T., and Miyajima, A. (1999) Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. *EMBO J.* 18, 2127–2136.

20. Okaya, A., Kitanaka, J., Kitahara, S., Takase, M., Kim, Y., Terada, K., Sugiyama, T., Takemura, M., Fujimoto, J., Terada, N., Miyajima, A., and...
Oncostatin M and Adipose Tissue Homeostasis

Tsujimura, T. (2005) Oncostatin M inhibits proliferation of rat oval cells, OC15–5, inducing differentiation into hepatocytes. Ann. J. Pathol. 166, 709–719

18. Nakamura, K., Nonaka, H., Saito, H., Tanaka, M., and Miyajima, A. (2004) Hepatocyte proliferation and tissue remodeling is impaired after liver injury in oncostatin M receptor knockout mice. Hepatology 39, 635–644

19. Mosley, B., De Imus, C., Friend, D., Boiani, N., Thoma, B., Park, L. S., and Cosman, D. (1996) Dual oncostatin M (OSM) receptors: cloning and charac-
terization of an alternative signaling subunit conferring OSM-specific receptor activation. J. Biol. Chem. 271, 32635–32643

20. White, U. A., Stewart, W. C., and Stephens, J. M. (2011) Gp130 cytokines inhibit adipogenesis through the RAS/ERK and STAT5 signaling path-
ways. J. Biol. Chem. 286, 37913–37920

21. Komori, T., Tanaka, M., Senba, E., Miyajima, A., and Morikawa, Y. (2014) Oncostatin M is produced in adipose tissue and is regulated in conditions of obesity and type 2 diabetes. J. Clin. Endocrinol. Metab. 99, E217–E225

22. Miyaoka, Y., Tanaka, M., Naiki, T., and Miyajima, A. (2006) Oncostatin M inhibits adipogenesis through the RAS/ERK and STAT5 signaling path-
ways. J. Biol. Chem. 281, 25205–25212

23. Neufang-Infantes, D., White, U. A., Elks, C. M., Morrison, R. F., Gimble, J. M., Considine, R. V., Ferrante, A. W., Ravussin, E., and Stephens, J. M. (2014) Oncostatin M is produced in adipose tissue and is regulated in conditions of obesity and type 2 diabetes. J. Clin. Endocrinol. Metab. 99, 21861–21875

24. Eguchi, J., Wang, X., Yu, S., Kershaw, E. E., Chiu, P. C., Dushay, J., Estall, J. L., Klein, U., Maratos-Flier, E., and Rosen, E. D. (2011) Transcriptional control of adipose lipid handling by IRF4. Cell Metab. 13, 249–259

25. Schnittker, D., Kwofie, K., Ashkar, A., Trigatti, B., and Richards, C. D. (2013) Oncostatin M and TLR-4 ligand synergize to induce MCP-1, IL-6, and VEGF in human aortic adventitial fibroblasts and smooth muscle cells. Mediators Inflamm. 2013, 1537053

26. Vollmer, S., Kappler, V., Kaczor, J., Fliegel, D., Roveling, C., Kato, N., Kietzmann, T., Behrmann, I., and Haan, C. (2009) Hypoxia-inducible factor 1α is up-regulated by oncostatin M and participates in oncostatin M signaling. Hepatology 50, 253–260

27. Panee, J. (2012) Monocyte chemotactic protein 1 (MCP-1) in obesity and diabetes. Cytokine 60, 1–12

28. Kahlen, F., Findeisen, H. M., and Brummer, D. (2014) Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes. Mol. Metab. 3, 384–393

29. Nomiyama, T., Perez-Tilve, D., Ogawa, D., Gizard, F., Zhao, Y., Heywood, E. B., Jones, K. L., Kawamori, R., Cassis, L. A., Tschöp, M. H., and Brummer, D. (2007) Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. J. Clin. Invest. 117, 2877–2888

30. Chan, S. S., Twigg, S. M., Firth, S. M., and Baxter, R. C. (2005) Insulin-like growth factor binding protein-3 leads to insulin resistance in adipocytes. J. Clin. Endocrinol. Metab. 90, 6588–6595

31. Silva, J. V., Gui, Y., and Murphy, L. I. (2002) Impaired glucose homeostasis in insulin-like growth factor-binding protein-3 transgenic mice. Am. J. Physiol. Endocrinol. Metab. 283, E937–E945

32. Meissburger, B., Stachorski, L., Röder, E., Rudofsky, G., and Wolfrum, C. (2011) Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) controls adipogenesis in obesity in mice and in humans. Diabetologia 54, 1468–1479

33. Chevay, C., Mari, B., Menthoul, M.-N., Bonnafous, S., Anglard, P., Van Obberghen, E., and Tartare-Deckert, S. (2003) Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J. Biol. Chem. 278, 11888–11896

34. Sell, H., Habich, C., and Eckel, J. (2012) Adaptive immunity in obesity and insulin resistance. Nat. Rev. Endocrinol. 8, 709–716

35. Wua, H., Ghosh, S., Perrard, X. D., Feng, L., Garcia, G. E., Perrard, I. L., Sweeney, J. F., Peterson, L. E., Chan, L., Smith, C. W., and Ballantyne, C. M. (2007) T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation 115, 1029–1038

36. Brown, T. J., Liobin, M. N., and Marquardt, H. (1987) Purification and characterization of cystotatic lymphokines produced by activated human T lymphocytes. Synergistic antiproliferative activity of transforming growth factor beta 1, interferon-γ, and oncostatin M for human melanoma cells. J. Immunol. 139, 2977–2983

37. Malik, N., Kallestad, J. C., Gunderson, N. L., Austin, S. D., Neubauer, M. G., Ochs, V., Marquardt, H., Zarling, J. M., Shoyab, M., and Wei, C. M. (1989) Molecular cloning, sequence analysis, and functional expression of a novel growth regulator, oncostatin M. Mol. Cell. Biol. 9, 2847–2853

38. Boussinse, M. L., Boyd, S. K., Christiansen, B. A., Guldberg, R. E., Jepsen, K. J., and Müller, R. (2010) Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J. Bone Miner. Res. 25, 1468–1486

39. Tanaka, M., Hirabayashi, Y., Sekiguchi, T., Inoue, T., Katsuki, M., and Miyajima, A. (2003) Targeted disruption of oncostatin M receptor results in altered hematopoiesis. Blood 102, 3154–3162

40. Tamura, S., Morikawa, Y., Tanaka, M., Miyajima, A., and Senba, E. (2002) Developmental expression pattern of oncostatin M receptor β in mice. Mech. Dev. 115, 127–131

41. Parks, W. C., Wilson, C. L., and López-Boado, Y. S. (2004) Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat. Rev. Immunol. 4, 617–629

42. Giannandrea, M., and Parks, W. C. (2014) Diverse functions of matrix metalloproteinases during fibrosis. Dis. Model. Mech. 7, 193–203

43. Lawler, H. M., Underkoffler, C. M., Kern, P. A., Erickson, C., Bredbeck, B., and Rasouli, N. (2016) Adipose tissue hypoxia, inflammation and fibrosis in obese insulin sensitive and obese insulin resistant subjects. J. Clin. En-

44. understating the underlying mechanisms of adipose tissue homeostasis. Am. J. Clin. Investig. 117, 195–209