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Functional Plasticity of Adipose-Derived Stromal Cells During Development of Obesity

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ABSTRACT

Obesity is a major risk factor for a number of chronic diseases, including diabetes, cardiovascular diseases, and cancer. Expansion of the adipose mass requires adipocyte precursor cells that originate from multipotent adipose-derived stromal cells (ASCs), which in turn also participate in repair activities. ASC function might decline in a disease milieu, but it remains unclear whether ASC function varies during the development of obesity. We tested the hypothesis that microenvironmental inflammatory changes during development of metabolic disorders in obesity affect ASC function. Domestic pigs were fed with an atherogenic ($n = 7$) or normal ($n = 7$) diet for 16 weeks. Abdominal adipose tissue biopsies were collected after 8, 12, and 16 weeks of diet for ASC isolation and immunohistochemistry of in situ ASCs and tumor necrosis factor-α (TNF-α). Longitudinal changes in proliferation, differentiation, and anti-inflammatory functions of ASCs were assessed. At 16 weeks, upregulated TNF-α expression in adipose tissue from obese pigs was accompanied by increased numbers of adipocyte progenitors ($CD24^+/CD34^+$) in adipose tissue and enlarged adipocyte size. In vitro, ASCs from obese pigs showed enhanced adipogenic and osteogenic propensity, which was abolished by anti-TNF-α treatment, whereas lean ASCs treated with TNF-α showed enhanced adipogenesis. Furthermore, obese ASCs showed increased senescence compared with lean ASCs, whereas their immunomodulatory capacity was preserved. Adipose tissue inflammation promotes an increase in resident adipocyte progenitors and upregulated TNF-α enhances ASC adipogenesis. Thus, adipose tissue anti-inflammatory strategies might be a novel target to attenuate obesity and its complications. STEM CELLS TRANSLATIONAL MEDICINE *2016;5:893–900*

SIGNIFICANCE

Adipose-derived stromal cell (ASC) function might decline in a disease milieu, but it remains unclear whether ASC function varies during the development of obesity. This study tested the hypothesis that microenvironmental inflammatory changes during development of metabolic disorders in obesity affect ASC function. It was found that ASCs show increased propensity for differentiation into adipocytes, which is partly mediated by upregulated tumor necrosis factor-α (TNF-α), likely in their adipose tissue microenvironment. Furthermore, TNF-α magnified obese ASC senescence, although it did not regulate their anti-inflammatory properties. Thus, adipose tissue inflammation might be a novel therapeutic target to avert ASC maldifferentiation and senescence.

INTRODUCTION

Obesity is a major risk factor for many chronic diseases, especially cardiovascular diseases. Its prevalence is on a rampant increase, leading to increased morbidity and mortality [1]. By 2030, almost 60% of the world's adult population could be either overweight or obese [2]. Clearly, better understanding of the adipose tissue biology is critical to design targeted novel interventions to minimize the deleterious effects of obesity.

The adipose tissue constitutes almost half the body weight in obese individuals, making it the largest endocrine organ in humans. Even minor metabolic changes in such a large secretory organ have the potential to affect broadly the entire body. Adipose tissue-released adipokines play important roles in the regulation of angiogenesis, blood pressure, glucose homeostasis, lipid metabolism, and vascular hemostasis. Furthermore, in obesity, adipocytes have intrinsic inflammatory properties. They can express receptors for tumor necrosis factor-α (TNF-α), which mediates inflammatory signals and induces secretion of various potent inflammatory cytokines and mediators. In turn, adipocytes not only initiate inflammatory signaling cascades on activation by interleukin (IL)-1β, IL-4, IL-6, and IL-11, as well as interferon-γ (IFN-γ) but also induce the expression of TNF-α,

as well as adiponectin and leptin [3]. In addition, in obese patients, the population of macrophages rises from 5%–10% to up to 60% of all adipose tissue cells [4] and tends to manifest a proinflammatory (M1) phenotype, secreting TNF-α and IL-6 [5]. Therefore, the adipose tissue in obese individuals is characterized by an inflammatory milieu.

Over the past decade, stem cells application in a broad range of diseases has expanded remarkably. Stem cells have been often harvested from the bone marrow, but the adipose tissue is an increasingly preferred source because of the ease of harvesting, abundance of adipose tissue, pluripotency, and proliferative capacity of adipose-derived stromal cells (ASCs). The ASCs can differentiate not only into adipocytes but also into cardiomyocytes, muscle myoblasts, bone-forming osteoblasts, cartilage-forming chondrocytes, endothelial cells, epithelial cells, hematopoietic-supporting cells, hepatocytes, and pancreatic cells [6–8]. Adipose tissue is, therefore, a convenient and useful source of stem-cell harvesting for cellular regenerative therapy.

Adipogenesis is the process whereby undifferentiated progenitor cells differentiate into fat cells [9]. During the evolution of obesity, expansion of the adipose tissue mass is accomplished through an increase in both adipocyte numbers (hyperplasia) and size (hypertrophy) [10]. Because mature adipocytes are postmitotic, proliferative adipocyte precursor cells contribute to increased adipocyte numbers to respond to metabolic demands. Recent studies suggest that approximately 10% of the body's fat cells are regenerated each year [11]. Furthermore, large adipocytes have an increased lipolytic capacity, which is regulated by proteins in the lipolytic cascade, such as hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) [12].

ASCs from high fat diet-fed rats show increased production of IL-1, IL-6, and TNF-α and increased nuclear factor κ-B (NF-κB) and reduced peroxisome proliferator-activated receptor-γ (PPAR-γ) expression, which may affect ASC function [13]. However, the manner in which ASC function is modulated during the development of obesity, and whether an inflammatory microenvironment in adipose tissue affects adipocyte precursor numbers, remains poorly understood. Thus, we tested the hypothesis that microenvironmental changes during the development of obesity modulate ASC function and that TNF-α is an important mediator of this process.

Materials and Methods

Study Protocols

The Institutional Animal Care and Use Committee approved this study. Fourteen 3-month-old domestic pigs were randomized in two groups (n = 7 each). Obese pigs were fed with a high-fat/high-fructose diet (5B4L fed ad libitum, with protein 16.1%, ether extract fat 43.0%, and carbohydrates 40.8%; Purina TestDiet, Richmond, IN, http://www.testdiet.com) and control (lean) pigs were fed standard chow for a total of 16 weeks. At 8, 12, and 16 weeks, subcutaneous abdominal adipose tissue biopsies were collected under anesthesia and sterile conditions in all pigs. At 16 weeks, the pigs were studied in vivo with a multidetector computed-tomography (MDCT) to assess abdominal fat volume. Pigs were euthanized with pentobarbital-sodium (100 mg/kg i.v.; Sleepaway; Fort Dodge Laboratories, Fort Dodge, IA, https://www.zoetisus.com) 3 days after in vivo studies.

Subcutaneous abdominal adipose tissue biopsies were processed for ASC culture, and tissue studies were performed for assessments of resident ASCs, fat inflammation, and remodeling. Systemic total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured by standard procedures (F. Hoffmann-La Roche AG, Basel, Switzerland, http://www.roche.com), and circulating TNF-α levels using an enzyme-linked immunosorbent assay (ELISA) kit. Systemic glucose and insulin levels were measured at 12 and 16 weeks, and homeostasis model assessment of insulin resistance (HOMA-IR) (fasting plasma glucose $\times$ fasting plasma insulin / 22.5) was used as an index of insulin resistance [14].

Abdominal Fat Volume

MDCT volume scanning (Somatom Sensation-128, Siemens Medical Solution, Forchheim, Germany, http://www.healthcare.siemens.com) was performed at suspended respiration to assess abdominal (subcutaneous and visceral) fat fraction. Fat tissue area and abdominal cross sectional area were both traced in 15 abdominal tomographic slices starting at the middle level of right kidney on MDCT images. The average abdominal fat fraction was expressed relative to the abdominal cross sectional area (percentage), modified from previous methods described [14–17].

ASC Function In Vitro

ASC Culture

Subcutaneous adipose tissue (3–5 g) harvested at each time point in each pig was digested in collagenase-H for 1 hour, filtered, and cultured in Advanced MEM medium supplemented with 5% platelet lysate (PLTMax, Mill Creek Life Sciences, Rochester, MN, http://www.millcreekls.com). The seeding density for the stromal vascular fraction was approximately 4×10^6 cells in a T-75 flask, and the subculture seeding density was 2×10^6 cells. ASCs (passage 3) were subsequently characterized using staining, as we have previously described [18, 19], by cell surface markers positivity to CD90, CD44, and CD105, negativity to CD14, CD34, CD31, and CD45, following criteria set by the International Society for Cellular Therapy [20]. ASCs were quantitatively analyzed with fluorescence-activated cell sorting for CD44, CD90, and CD105 (all $\geq$ 70% positive). The third passages of ASCs were collected and kept in -80°C for later use. ASC functions were further evaluated by their (a) capacity to proliferate and transdifferentiate into adipocytes, chondrocytes, and osteocytes; (b) their anti-inflammatory capacity; and (c) their cell senescence.

ASC Proliferation

ASC proliferation was evaluated by calculating doubling time of cell numbers between the second and third passages. Briefly, approximately 2×10^6 cells were seeded into T-75 flasks and times needed for ASCs to reach 90% confluence were recorded. Confluent ASCs in each flask were counted and doubling time was calculated using an online formula [21].

ASC Transdifferentiation

Passage 3 cells were differentiated into adipocyte, osteocyte, and chondrocyte lineages to evaluate their multipotency, using a commercial kit (catalog no. SC006; R&D Systems, Minneapolis, MN, https://www.rndsystems.com). In parallel experiments, TNF-α (1 μg/ml) or anti-TNF-α antibody (1:200; Abcam) were

added to the culture media throughout the adipogenic differentiation assay. In all cultures, the medium was replaced every 3−4 days, and cells were studied at 21 days.

Adipogenic differentiation. ASCs ($2.1 \times 10^4/cm^2$) were plated into a 24-well plate with StemXVivo adipogenic medium (R&D Systems) containing hydrocortisone, isobutylmethylxanthine, and indomethacin. Cells were cultured to 100% confluence, and at 21 days adipocytes were fixed for detection of fatty acid binding protein 4.

Osteogenic differentiation. ASCs ($4.2 \times 10^3/cm^2$) were plated into a 24-well plate with StemXVivo Osteogenic medium (R&D Systems) containing dexamethasone, ascorbate-phosphate, and β-glycerolphosphate, cultured to 50%–70% confluence, and at 21 days osteocytes were fixed for detection of osteocalcin.

Chondrogenic differentiation. ASCs (2.5×10^5) were transferred to a 15-ml conical tube, centrifuged, and cultured in a chondrogenic differentiation medium containing insulin, transferrin, selenious acid, linoleic acid, dexamethasone, ascorbate-phosphate, proline, pyruvate, and recombinant TGF-β3. At 21 days the chondrogenic pellet was harvested for detection of aggrecan.

Adipokine Expression in ASCs

ASCs were homogenized using lysis buffer, and protein expression of TNF-α was measured using Western blotting. For adiponectin, ASCs (3×10^5) obtained from lean and obese pigs after 16 weeks of diet were seeded in six-well plates, culture medium was collected after a 24-hour culture, and adiponectin levels were measured using an ELISA kit.

ASC Modulation of Macrophage Phenotype In Vitro

Macrophages were obtained by activating monocytes (U-937, 5×10^5 cells; ATCC, Manassas, VA, http://www.atcc.org) cultured for 7 days in RPMI 1640 supplemented with 20% fetal bovine serum (FBS) in FBS-coated dishes at a density of $1.5 \times 10^5/cm^2$. The culture medium was supplemented with 100 ng/ml of macrophage-colony stimulating factor (R&D Systems), 100 ng/ml of lipopolysaccharide (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com) plus 20 ng/ml IFN-γ (R&D Systems) to induce M1 polarization [22]. M1-polarized cells were then cultured either alone in RPMI 1640 media with 5% FBS or cocultured with ASCs (5×10^5) for 16 hours. Protein expression of inducible nitric oxide synthase (iNOS) and arginase-1 (both 1:200) were evaluated using Western blotting. Culture media were collected, and TNF-α, IL-1β, and IL-12 (which stimulates production of TNF-α) levels measured by Luminex assay (Millipore, Billerica, MA, http://www.emdmillipore.com).

ASC Senescence

ASC senescence was evaluated by the expression of the DNA damage marker H2AX (1:200; Abcam, Cambridge, U.K., http://www.abcam.com) and p16 (1:500, Lifespan BioSciences, Seattle, WA, https://www.lsbio.com) using Western blot, as well as telomerase reverse transcriptase (TERT, the telomerase-specific domains for functional activity of telomerase) using quantitative polymerase chain reaction (qPCR) (primer from Life Technologies, catalog no. Ss03376807_u1). Furthermore, senescence activity of ASCs was measured using a Cellular Senescence Activity Assay kit (Enzo Life Sciences, Farmingdale, NY, http://www.enzolifesciences.com) following the assay protocol. Lysates of ASCs obtained from lean and obese pigs after 16 weeks of diet were incubated with SA-β-Gal substrate for 1 hour at 37°C. Fluorescence was then read with a plate reader at 360 nm (excitation)/465 nm (emission), and results normalized by the number of cells loaded.

Table 1. Systemic measurements in lean and obese pigs

Parameters	Lean (n = 7)	Obese (n = 7)
Body weight (kg)		
Baseline	19.0 ± 0.6	20.0 ± 0.7
12 wk	61.6 ± 4.0	76.9 ± 1.2[a]
16 wk	71.4 ± 4[b]	93.4 ± 0.9[a,b]
Intra-abdominal fat volume (%)	9.1 ± 0.3	13.1 ± 0.5[a]
Adipocyte size (μm^2)	2.9 ± 0.2	3.9 ± 0.2[a]
Mean arterial pressure (mmHg)	101.4 ± 4.0	126.3 ± 4.2[a]
Total cholesterol (mg/dl)	73.3 ± 2.8	523.9 ± 57[a]
HDL (mg/dl)	39.4 ± 4.6	97.8 ± 29.6[a]
LDL (mg/dl)	28.7 ± 2.8	371.1 ± 49[a]
LDL/HDL	0.7 ± 0.1	2.5 ± 0.3[a]
Triglycerides (mg/dl)	7.1 ± 0.7	11.7 ± 2.1[a]
TNF-α (pg/ml)	88.2 ± 8.6	190.1 ± 60.5[a]
Glucose (mg/dl)		
12 wk	106.3 ± 10.0	103.8 ± 7.8
16 wk	137.6 ± 9.4[b]	139.1 ± 17.5[b]
Insulin (mg/dl)		
12 wk	0.11 ± 0.03	0.19 ± 0.03
16 wk	0.13 ± 0.02	0.26 ± 0.06[a]
HOMA-IR		
12 wk	0.4 ± 0.1	1.0 ± 0.1[a]
16 wk	0.7 ± 0.1	1.7 ± 0.5[a,b]

[a] $p < .05$ vs. lean.
[b] $p < .05$ vs. 12 wk.
Abbreviations: HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; TNF-α, tumor necrosis factor-α.

Adipose Tissue Histology and Gene Expression

Immunohistochemistry was performed in 5 μm thick either frozen or paraffin-preserved subcutaneous adipose tissue following standard protocols. Inflammation was assessed by TNF-α staining (1:50). To explore adipocyte progenitors in the adipose tissue, adipose tissue sections were stained with the adipocyte progenitor markers CD24 and CD34 [23], as well as with TNF-α. Image analysis utilized a computer-aided image-analysis program (AxioVision Carl Zeiss Micro Imaging, Thornwood, NY, http://www.zeiss.com). In addition, total RNA from 100 mg of adipose tissue was isolated, and HSL (Life Technologies primer, catalog no. ss03383966_u1), ATGL (Life Technologies primer, catalog no. ss03385996_u1), and PPAR-γ (Life Technologies primer, catalog no. ss03394829_m1) expression evaluated using standard qPCR protocol.

Statistical Analysis

Statistical analysis was performed using JMP software package version 8.0 (SAS Institute, Cary, NC, http://www.sas.com). Results were expressed as mean ± SD for normally distributed data. Comparisons within groups were performed using a paired

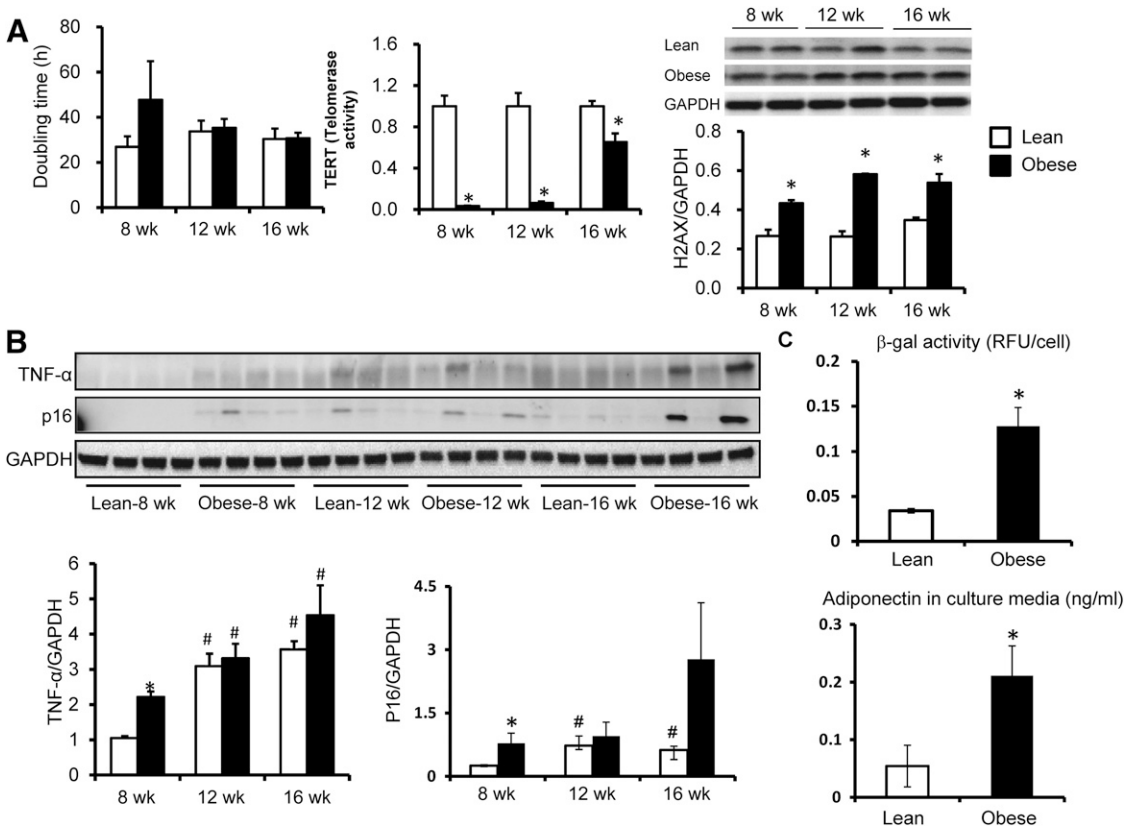

Figure 1. Adipose-derived stromal cell (ASC) proliferation, inflammation, and senescence in lean and obese pigs. **(A):** ASCs from obese and lean pigs showed similar proliferation capacity (doubling time), but obese ASCs had consistently increased H2AX expression and decreased telomerase activity, suggesting increased senescence. **(B):** TNF-α expression was upregulated earlier in obese ASCs compared with lean ASCs but rose at 12 and 16 weeks compared with 8 weeks in both lean and obese ASCs. The expression of p16 in obese ASCs was initially upregulated at 8 weeks but was not noticeably elevated at 12 and 16 weeks compared with lean, due to the upregulation of p16 expression in lean (at 12 weeks) and the large variability in obese (at 16 weeks). **(C):** β-Gal activity and adiponectin release in culture media were increased in obese ASCs compared with lean after 16 weeks of diet. *, $p < .05$, vs. lean at the same time point. #, $p < .05$, vs. 8 weeks at the same group. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RFU, relative fluorescence units; TERT, telomerase reverse transcriptase; TNF-α, tumor necrosis factor-α.

Student *t* test and among groups using ANOVA or Wilcoxon when appropriate. A value of $p \leq .05$ was considered statistically significant.

RESULTS

All pigs grew in size during observation, but after 12 and 16 weeks of diet, obese pigs had significantly increased body weight compared with lean pigs, and their abdominal subcutaneous and visceral adipose tissue volumes and adipocyte size were significantly increased (Table 1). Obese pigs also showed elevated blood pressure, systemic total cholesterol, triglycerides, LDL, and HDL (Table 1). Furthermore, circulating TNF-α level was increased in obese pigs (Table 1). Fasting blood glucose levels rose similarly at 16 compared with 12 weeks in both lean and obese pigs, whereas insulin level was elevated only at 16 weeks in obese pigs, as were HOMA-IR levels, suggesting development of insulin resistance in obese pigs (Table 1).

ASC Function In Vitro

As per doubling time, ASCs from obese and lean pigs had similar proliferation capacity (Fig. 1A) throughout the observation period. On the other hand, compared with lean ASCs, obese ASCs had increased H2AX expression and decreased telomerase activity at all time points, and p16 expression started rising at 8 weeks, although at 12 weeks its expression in lean ASCs increased to match obese ASCs. By 16 weeks, p16 expression showed a marked increase in obese ASCs, which did not reach statistical significant difference from lean due to high variability. β-Gal activity in obese ASCs was significantly elevated at 16 weeks, suggesting increased senescence (Figs. 1A, 1B, 1C). TNF-α expression was upregulated in obese compared with lean ASCs 8 weeks after initiation of diet but subsequently increased to similar levels in both groups. The release of adiponectin by obese ASCs in the culture medium was increased compared with lean ASCs at 16 weeks of diet (Figs. 1B, 1C). ASC transdifferentiation toward adipocyte and osteocyte-lineages in obese ASCs was enhanced at 16 weeks compared with lean ASCs, whereas chondrogenesis was transiently enhanced at 8 weeks but fell by 16 weeks of diet (Fig. 2A).

The Effects of TNF-α on ASC Differentiation and Senescence

At 16 weeks, the enhanced adipogenesis observed in obese ASCs was abolished by inhibition of TNF-α, whereas lean ASCs treated with TNF-α showed enhanced adipogenesis. TNF-α also

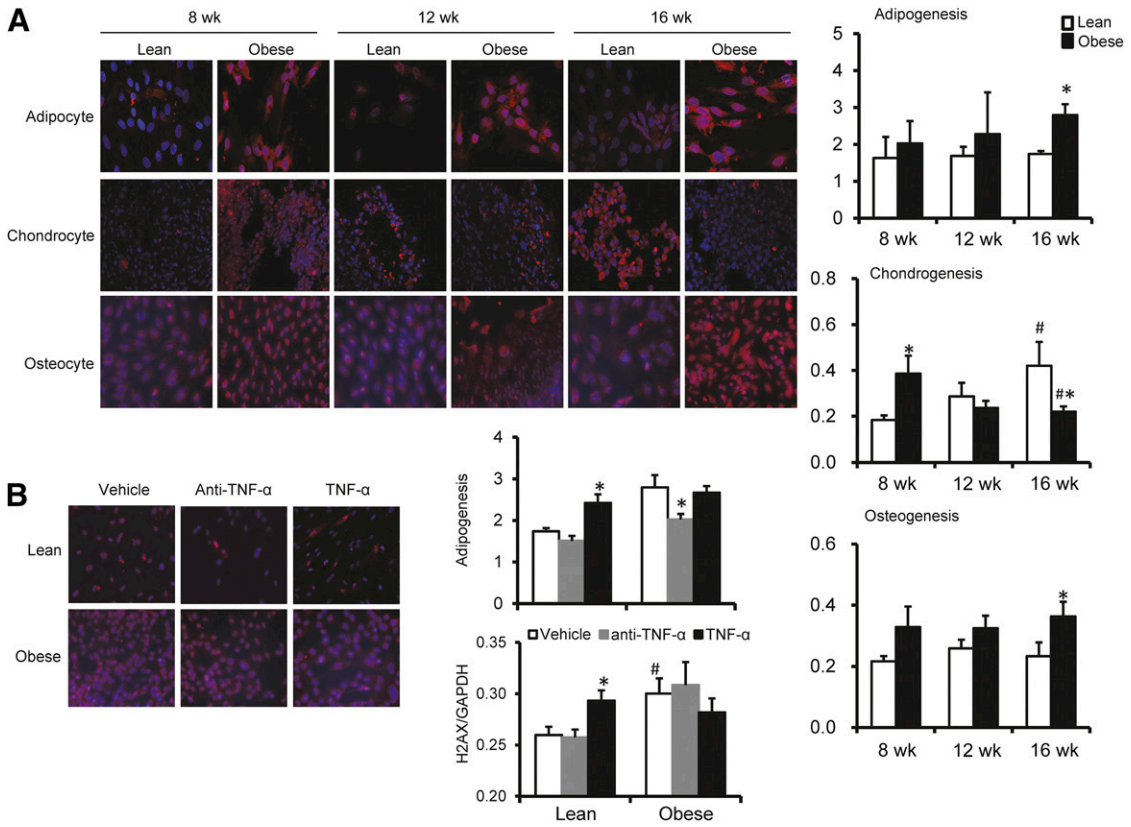

Figure 2. Adipose-derived stromal cell (ASC) trilineage transdifferentiation and the effect of TNF-α. **(A):** ASC transdifferentiation analysis showed that adipocyte (FABP4, red) and osteocyte (osteocalcin, red) lineages in obese ASCs were enhanced at 16 weeks compared with lean ASCs, whereas chondrogenic (aggrecan, red) lineage initially increased but was subsequently blunted. $*$, $p < .05$, vs. lean at the same time point. #, $p < .05$ vs. 8 weeks at the same group. **(B):** Enhanced adipogenesis (FABP4, red) in 16 weeks obese ASCs was abolished by anti-TNF-α treatment, whereas lean ASCs treated with TNF-α showed enhanced adipogenesis. Furthermore, TNF-α stimulates lean ASC senescence (H2AX), whereas anti-TNF-α antibody fails to attenuate obese ASC senescence. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TNF-α, tumor necrosis factor-α.

induced lean ASC senescence, reflected in H2AX expression, but its inhibition failed to rescue senescence in obese ASCs (Fig. 2B).

ASCs Modulate Macrophage Phenotype In Vitro

M1 macrophages activated by M-CSF, LPS, and IFN-γ showed increased iNOS and decreased arginase-1 expression (Fig. 3A). After coincubation with either lean or obese ASCs, both arginase-1 and iNOS expression were restored to control levels, suggesting that obese ASCs retained their capacity for reverting macrophage phenotype. In line with these observations, M1 macrophages were found to secrete inflammatory cytokines (TNF-α, IL-1β, and IL-12) into the culture media, all of which returned to normal levels after coculture with either lean or obese ASCs (Fig. 3A).

Adipose Tissue Microenvironment

Adipose tissue histology showed that TNF-α expression in obese adipose tissue was upregulated at 12 weeks and was extensive by 16 weeks (Fig. 3B). Furthermore, the number of adipocyte progenitor cells ($CD34^+/CD24^+$) in obese compared with lean adipose tissue was elevated at both 8 and 16 weeks, and the M1/M2 macrophage ratio was markedly and consistently elevated. On the contrary, there were no significant differences of HSL, ATGL, and PPAR-γ mRNA expression between lean and obese adipose tissue (Fig. 3C).

Discussion

This study shows that adipose tissue-derived ASCs show increased propensity for differentiation into adipocytes, which is partly mediated by upregulated TNF-α, likely in their adipose tissue microenvironment. Furthermore, TNF-α magnified obese ASC senescence, although it did not regulate their anti-inflammatory properties. Thus, adipose tissue inflammation might be a novel therapeutic target to avert ASC maldifferentiation and senescence.

Obesity is a worldwide health problem, and elucidation of the determinants of adipogenesis during the evolution of obesity is important to design targeted interventional strategies. The adipose tissue not only is considered the primary energy storage organ but also is recognized as an important endocrine tissue and an abundant source of ASCs. ASCs have been applied to address a wide range of disease conditions, thanks to their multipotential differentiation and immunomodulation capacities, although their immunoprivileged features may allow development of allogenic applications. Delivery of autologous ASCs is associated with the least likelihood of cell rejection or extrusion. However, delivery of autologous ASCs derived from patients in need of treatment would require ascertaining that ASC function is intact. Given that ASC differentiation and proliferation are determined by their niche, the obesity-related metabolic milieu may alter their phenotype. A previous study [24] showed that ASCs derived from

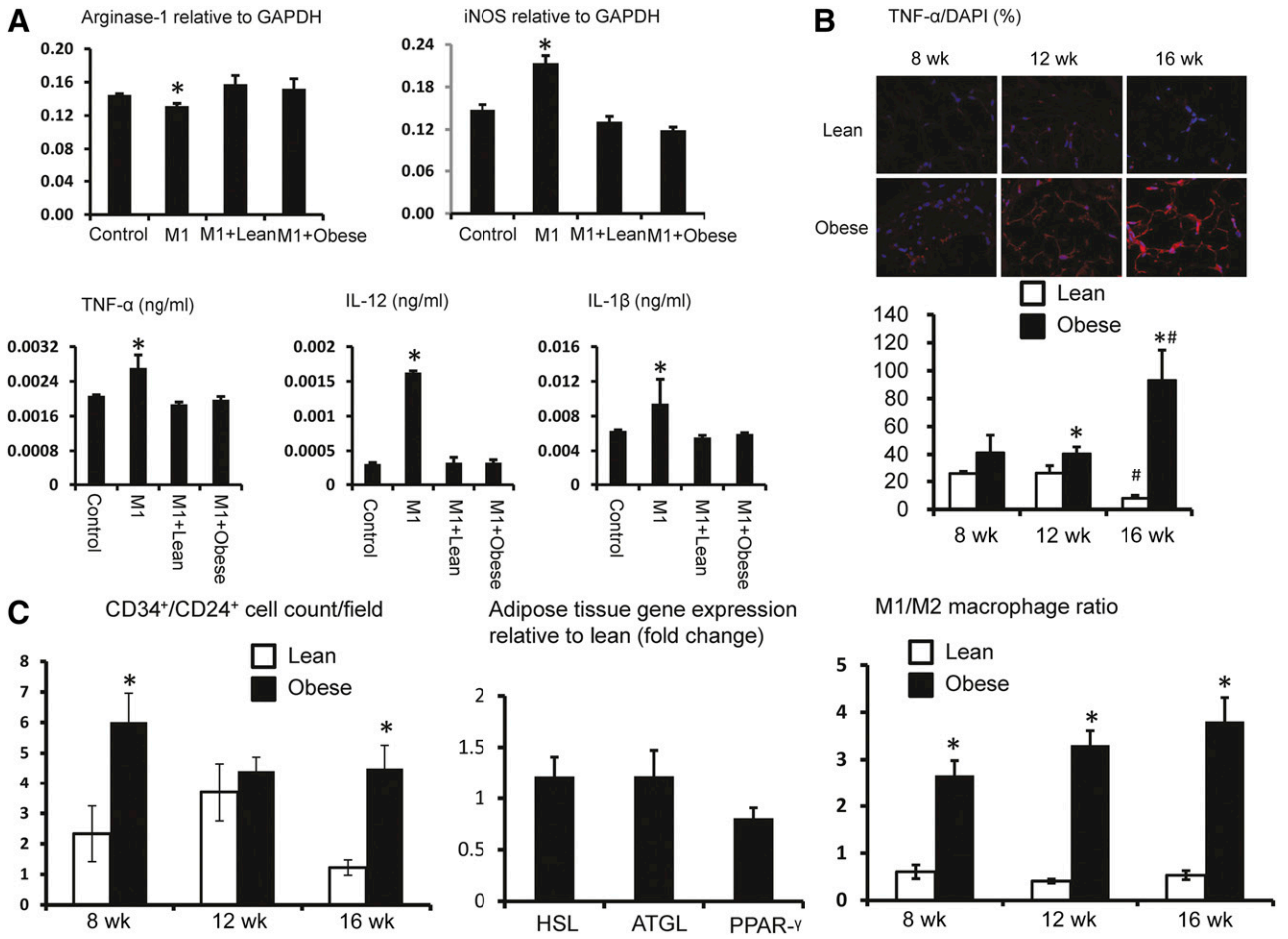

Figure 3. Anti-inflammatory and immunomodulatory capacity of adipose-derived stromal cells (ASCs) obtained from lean and obese pigs. **(A):** Activation of macrophages toward M1 (proinflammatory) phenotype increased expression of iNOS and decreased arginase-1. After coincubation with either lean or obese ASCs, both arginase-1 and iNOS expression were restored. M1 macrophages-release of inflammatory cytokines (TNF-α, IL-1β, IL-12) into the culture media also returned to normal levels after coculture with lean or obese ASCs. $*$, $p < .05$, vs. control. **(B):** TNF-α expression in obese adipose tissue was elevated at 12 weeks and rose markedly by 16 weeks. **(C):** The numbers of $CD34^+/CD24^+$ adipocyte progenitors in obese adipose tissue were significantly increased at 8 and 16 weeks. There were no significant differences of HSL, ATGL, and PPAR-γ mRNA expression between lean and obese adipose tissue, indicating unaltered lipolytic capacity. The ratio of M1/M2 macrophages was significantly increased from 8 weeks to 16 weeks in obese fat, suggesting a proinflammatory phenotype shifting. $*$, $p < .05$ vs. lean at the same time point. #, $p < .05$ vs. 8 weeks at the same group. Abbreviations: ATGL, adipose triglyceride lipase; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSL, hormone-sensitive lipase; IL, interleukin; iNOS, inducible nitric oxide synthase; PPAR-γ, proliferator-activated receptor-γ; TNF-α, tumor necrosis factor-α.

subcutaneous adipose tissue of obese donors have impaired cell proliferation, chondrogenic ability, and immunophenotype, but their differentiation toward osteogenic and adipogenic lineages remains intact. The biological microenvironment in vivo, such as obesity-related inflammation, may play a key role in ASC impairment. Our study extends previous studies by showing that obesity, at least at its early stage, has a relatively minor effect on several aspects of adipose-derived ASC functions. Compared with lean ASCs, obese ASCs had intact proliferation and immunomodulatory capacity and enhanced adipogenesis and osteogenesis. We also observed a significant increase in adipocyte progenitor numbers in fat tissue obtained from obese animals, which may contribute to expansion of adipose tissue volume during the development of obesity. It is possible that at a later stage of obesity, impaired ASC proliferation may affect adipogenesis as well. Nonetheless, fat inflammation may increase ASC senescence substantially. Thus, autologous ASCs isolated from obese individuals may be useful for cell based therapy but may show a propensity to differentiate toward adipocytes and develop a senescent phenotype, which is toxic for neighboring cells. Therefore, in obese individuals, interventions that attenuate adipose tissue inflammation may improve adipose ASC function. Furthermore, systemic anti-inflammation strategies may potentially blunt ASC transdifferentiation into adipocytes in situ, and thereby attenuate fat deposition. A retrospective study using anti-inflammatory (aspirin and/or statin) therapy on body weight in individuals with type 2 diabetes mellitus indeed showed that attenuation of inflammation was associated with weight loss [25].

The adipose tissue in obesity also showed notable tendency toward overproduction of proinflammatory adipokines, accompanied by reduced production of anti-inflammatory adipokines. Multiple mechanisms may be involved in adipose tissue inflammation. A high-fat diet causes imbalance of energy intake and expenditure, leading to adipocyte hypertrophy. Hypertrophied adipocytes are known to release large amounts of monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2

(CCL2), a central mediator of macrophage infiltration, and TNF-α, [26] which increase the adipose tissue inflammatory response. In obese human individuals, the number of infiltrating macrophages is positively correlated to body mass index and C-reactive protein levels with the size of adipocytes [27]. In fact, as shown in mice [5], we have found that our obese pig model manifests transformation of adipose tissue macrophages from an anti-inflammatory "alternatively activated" (M2) phenotype, seen in lean fat, to preponderance of inflammatory, "classically activated" (M1) macrophages, which also secrete TNF-α and cause tissue inflammation. ASCs also expressed TNF-α, although it was upregulated at 12 and 16 weeks in both lean and obese pigs, implying that TNF-α may be required for normal ASC function during growth. However, in lean animals, ASC TNF-α does not necessarily contribute to tissue inflammation, because the observed adipose tissue TNF-α level gradually fell over time. The increased adiponectin release from obese ASCs may be related to their enhanced potential for adipogenesis, because adiponectin secretion steadily increases as adipogenesis proceeds in ASCs [28].

Large adipocytes show an increased lipolytic capacity, probably due to enrichment of distal regulatory proteins in the lipolytic cascade, such as HSL and ATGL [12]. However, we observed no significant differences in the expression of HSL and ATGL between lean and obese adipose tissue. On the contrary, obese pigs had increased plasma triglycerides levels, which may promote their accumulation in organs such as liver and adipose tissue, and magnify fat cell hypertrophy in obesity. TNF-α treatment of rodent adipocytes results in downregulation of HSL expression [29] and ATGL mRNA expression [30], implicating TNF-α in lipolytic function in advanced obesity. TNF-α may not only induce adipocyte hypertrophy but also contribute to the expansion of adipose tissue mass via hyperplasia. Indeed, TNF-α enhances adipose stem cell proliferation in vitro [31]. Our study also showed that increased TNF-α expression in the adipose tissue of obese animals was associated with increased numbers of adipocyte progenitors, suggesting a role of TNF-α in development of obesity.

Our study may be limited by the use of relatively young pigs, with early obesity but no diabetes or cardiovascular diseases. We also cannot rule out the effect of mild hypertension on ASC function, and evaluated ASC function only in subcutaneous fat tissue, the most accessible source for ASCs in clinical studies. Some assays were performed only at the 16-week endpoint because of limited sample availability. Nevertheless, our results demonstrate that in early swine obesity, adipose-derived ASCs preserve most of their normal functions, such as proliferation and immunomodulatory capacity, yet show enhanced adipogenesis, osteogenesis, and senescence, which may be mediated by increased tissue inflammation.

Conclusion

Our study suggests that ASCs derived from adipose tissue of obese subjects may be useful for autologous delivery but may warrant pretreatment with anti-inflammatory strategies to blunt their transdifferentiation into adipocytes and their early aging.

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Author Contributions

X.-Y.Z.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; S.M.: collection and assembly of data, data analysis and interpretation; A.E. and L.J.H.: data analysis and interpretation; J.R.W.: administrative support; D.S.: collection of data; A.L.: conception and design; L.O.L.: conception and design, financial support, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

L.O.L. is a consultant for Stealth Biopharmaceuticals, has honoraria from Novartis, and has research funding from AstraZeneca. The other authors indicated no potential conflicts of interest.

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