Gender and age-related cell compositional differences in C57BL/6 murine adipose tissue stromal vascular fraction

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ABSTRACT
Adipose tissue is now recognized as a functional organ that contains cellular heterogeneity and diversity within anatomical depots. The stromal vascular fraction (SVF) of adipose contains endothelial progenitors, fibroblasts, lymphocytes, monocyte/macrophages, pericytes, pre-adipocytes, and stromal/stem cells, among others. In recent years, there has been a growing appreciation of the influence of age and gender in the field of stem cell biology. Yet few studies have evaluated the influence of biological age or sex on either SVF cell heterogeneity or immunophenotype. To address this issue, the current study has compared the flow cytometric characteristics between murine SVF of inguinal (iWAT), epididymal (eWAT), and brown (BAT) adipose tissue of male and female, as well as young (6–8 week) and middle-aged (8–12 month) male C57BL/6 mice. Murine gender comparisons revealed male iWAT expressed higher percentages of leukocyte and CD34+ ASC-like sub-populations than female iWAT. Murine age comparisons revealed younger male iWAT, eWAT, and BAT SVF all contained a significantly higher percentage of pre-adipocytes, HSC-like cells, CD25+, and FoxP3+ T-regulatory cells compared to SVF from middle-aged male mice. These findings highlight the potential contribution of biological variables on adipose-derived cell applications and experimental outcomes.

Introduction
While originally viewed solely as a reservoir for energy storage in the form of lipids and as a mechanical cushion, adipose tissue is now recognized as an organ contributing at multiple pathophysiological levels. In addition to metabolizing and storing free fatty acids and triglycerides, adipose tissue secretes adipokines such as adiponectin and leptin [1]. These cytokine and hormonal-like proteins exert local paracrine and systemic endocrine actions influencing bone health, feeding behavior, hematopoiesis, metabolism, and reproduction [1]. Additionally, adipose tissue contributes to the body’s immune status by secreting proteins involved in innate immunity, such multiple complement proteins and pro-inflammatory cytokines. Indeed, adipose tissue can display features of “sterile inflammation” in overweight and obese individuals with type 2 diabetes mellitus (T2DM) [2,3]. The adipose tissue of such patients contains “crown cells” where individual adipocytes surrounded by infiltrating myeloid-derived monocyte/macrophages are undergoing cell death [2,3]. The consequential release of pro-inflammatory cytokines such as interleukin 6 may be the cause of the characteristic T2DM angiogenic and cardiovascular comorbidities. Furthermore, adipose tissue contains hematopoietic stem cells [4,5]. Lethally irradiated mice are rescued as effectively by “stromal vascular fraction” (SVF) cells from adipose tissue as they are by bone marrow cell transplantation [4,5]. Together, these findings reflect the cellular heterogeneity and diversity within adipose tissue depots which contain endothelial progenitors, fibroblasts, lymphocytes, monocyte/macrophages, pericytes, pre-adipocytes, and stromal/stem cells, among others [6]. The SVF cells can be isolated from adipose tissue by collagenase or related enzyme digestion and are characterized, in part, based on the following surface immunophenotype: CD45+CD235a−CD31−CD34+ [6]. The SVF can be further processed by adherence to a plastic surface and cultured in a fetal bovine serum (FBS) or equivalently supplemented medium to expand “adipose-derived stromal/stem cells” (ASC) [6]. The ASC display a CD31−CD45−CD13+CD73+CD90+CD105+ surface immunophenotype and multi-lineage potential
(adipogenic, chondrogenic, osteogenic) [6]. Based on such findings, bioengineers and surgeons consider adipose tissue as an abundant and rich source of stromal/stem cells for tissue engineering and regenerative medical applications [7].

In recent years, there has been a growing appreciation of the influence of age and gender in the field of stem cell biology. Indeed, the National Institutes of Health has now mandated that these biological variables be incorporated into all funded studies through its newly mandated guidelines on “rigor and transparency” [8]. Pioneering studies by Huard and colleagues documented that the characteristics of skeletal muscle stem cells were substantially different if isolated from female vs. male syngeneic mice [9,10]. Likewise, studies of mesenchymal stem cells have determined that their frequency and other functions alter as a function of advancing biological aging [11–14]. While studies have used flow cytometry and related protein-based methodologies to characterize the adipose SVF cells, few studies have extended this approach to evaluate the influence of biological age or sex on either SVF cell frequency or immunophenotype [15,16]. To address this issue, the current study has compared the flow cytometric characteristics between male and female as well as young and older male C57BL/6 mice. The study highlights the potential contribution of biological variables on adipose-derived cell applications and experimental outcomes.

Materials and methods

Materials

All antibodies were purchased from either eBioscience or Biolegend (San Diego, CA), unless otherwise specified. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Thermo Fisher Scientific (Norcross, GA).

Mice

Animal studies were performed under the veterinary supervision of the Department of Comparative Medicine of the Tulane University School of Medicine under a protocol reviewed and approved by the Institutional Animal Care and Use Committee in accordance with federal, state, and National Institute of Health policies and regulations. The C57BL/6 30cha/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and euthanized by carbon dioxide asphyxiation. The adipose tissue was dissected at necropsy from the following depots: inguinal white adipose tissue (iWAT), epidydimal white adipose tissue (eWAT), and dorsal interscapular brown adipose tissue (BAT).

Adipose tissue harvest and SVF cell preparation

iWAT, eWAT, or BAT was isolated, minced, and digested with collagenase for 60 minutes according to a published protocol from our laboratory[31]. Briefly, the SVF pellets were collected by centrifugation, washed in phosphate buffered saline (PBS), filtered through a 70 µm mesh (Millipore), and the SVF cell concentrations determined by automated Cell Countess (Invitrogen) count. The 1° SVF cells were suspended at a final concentration of 1 × 10^6 nucleated cells per mL in PBS, in preparation for staining.

SVF cell flow cytometric staining

Cell suspensions were incubated with antibodies against the cell surface antigens listed above and in Figure 1 at room temperature (RT) for 30 minutes, protected from light. After two washes with PBS, flow cytometric analysis was performed using a Beckman-Coulter Galios flow cytometer (BD Biosciences, San Jose, CA). For sub-fractionation, relative subpopulations within the SVF cells were determined in freshly isolated SVF.

Age study

SVF cells were isolated from 6–8 week and 8-12-month male C57BL/6 30cha/J mice (Jackson Laboratory, Bar Harbor ME) according to the above methods[32]. Cells were stained for surface or intracellular antigen expression using different combinations of the following antibodies: CD29-APC, CD146-PC7, CD31-FITC, CD34-PE, CD31-APC, CD19-APC, CD117-APC, CD11b-FITC, CD45-PE, CD14-FITC, CD4-PE, CD3-PC7, FoxP3-APC, CD25-PC7, CD8-PE, CD16-PC5, and F4/80-APC (Figure 1 and Table 1). Isotype controls used included

Gender study

SVF cells were isolated from 8-12-month male and female C57BL/6 30cha/J mice (Jackson Laboratory, Bar Harbor ME) according to the same published methods. Cells were stained for cell surface or intracellular antigen expression using the same combinations of indicated antibodies above.
Adipogenic differentiation

Adipogenic differentiation of SVF cells was performed over a 15-day period as previously described [4]. Briefly, SVF cells were plated in 24-well plates in Stromal Medium (Dulbecco’s modified Eagle’s-Ham’s F-12 medium supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin) at $3 \times 10^4$ cells/cm$^2$ for

Table 1. Antibody protocols used for specific sub-populations in the study.

| Protocols | Sub-population | Protocol | Catalog # | Vendor | Isotype |
|-----------|----------------|----------|-----------|--------|---------|
| 1         | ASC-Like       | CD29-APC | 303008    | Biolegend | IgG1, kappa |
| 2         | EC-Like        | CD34-PE  | 348057    | BD Biosciences | IgG1, kappa |
| 3         | Vascular SMC  | CD31-APC | 17-0311-80| eBioscience | IgG1, kappa |
| 4         | B-cells        | CD45-PE  | 560367    | BD Biosciences | IgG1, kappa |
| 5         | Mast Cells     | CD45-PE  | 560367    | BD Biosciences | IgG1, kappa |
| 6         | Granulocytes   | CD45-PE  | 560367    | BD Biosciences | IgG1, kappa |
| 7         | Monocytes      | CD14-APC | CA385832   | Sysmex | IgG1, kappa |
| 8         | Leukocytes     | CD34-PC7 | 560710    | BD Biosciences | IgG1, kappa |
| 9         | T-cells        | CD4-PE   | 116006    | Biolegend | IgG2a |
| 10        | T-regs         | CD4-PE   | 116006    | Biolegend | IgG2a |
| 11        | NK cells       | CD8-PE   | 100708    | Biolegend | IgG2a |
| 12        | Macrophages    | CD11b-PE | 101208    | Biolegend | IgG2a |
| 13        | Isotype Ctl    | PE       | 555749    | BD Biosciences | Mouse IgG1, kappa |
| 14        | Isotype Ctl    | APC      | AE606921  | Sysmex | Mouse IgG2a |
| 15        | Isotype Ctl    | PC7      | 560373    | Sysmex | Mouse IgG1, kappa |
24 hrs to allow attachment. The plastic-adherent population was referred to as passage 0 (P0) ASC. On day 1 (24 hours after plating), the medium was removed and cells were incubated for three days in adipogenic differentiation medium (Dulbecco’s modified Eagle’s-Ham’s F-12 medium supplemented with 10% fetal bovine serum, 15 mM HEPES (pH 7.4), biotin (33 μM), pantethenate (17 μM, Sigma), human recombinant insulin (100 nM, Boehringer Mannheim), dexamethasone (1 μM), 1-methyl-3-isobutylxanthine (IBMX; 0.25 mM), and rosiglitazone (1 μM). For the remaining 9 days of the adipocyte differentiation maintenance period, the medium was removed every 3 days and replaced with the same medium that did not contain IBMX and rosiglitazone (maintenance medium).

**Colonies Forming Fibroblastic Unit (CFU)-F Assays**

1 × 10⁵ SVF cells were seeded in 100 mm dishes and cultured in a 37°C incubator with humidified 5% CO₂ for 14 days. The media was then removed, and washed 3 times with 1mL PBS. 3.0% crystal violet (Invitrogen) in 100% methanol was added and the plates were incubated for 10 minutes at room temperature. The plates were gently flushed with dH₂O for 15 min., or until the background was clear. The plates with the stained colonies were examined under an inverted microscope and the number of colonies that were 2 mm diameter or larger were counted using a VersDoc Imaging system (Bio-Rad Laboratories, Hercules, CA). The number of colonies per plate divided by the cells plated x 1000 was determined as the “% CFU”.

**Statistics**

All studies were repeated at least in triplicate and values are reported as the mean ± standard deviation (S.D.). For inter-depot comparisons, gender, and age analyses, individual pairs were compared using the Student t test while larger groups were analyzed by Analysis of Variance (2-way ANOVA). Findings were defined as significant with P values ≤ 0.05.

**Results**

**Gating strategies for identification of subpopulations within murine stromal vascular fraction (mSVF)**

Figure 1 and Table 1 provide the gating strategies utilized for analyzing the relative percentages of cell populations within the stromal vascular fraction in the study. Similar to reported values in the literature, initial SVF yields averaged 3–5 × 10⁵ 1⁶ (primary) SVF cells per ml of adipose tissue. CD34 gating was used for identifying endothelial progenitors, CD34⁺ adipose stromal/stem cell (ASC)-like cells, and vascular smooth muscle cells (V-SMC). The endothelial progenitors (EC-like; CD34⁺, CD31⁺, CD146⁺) and CD34⁺-ASC-like (CD34⁺, CD146⁺, CD31⁻) populations were gated using CD34 positivity. CD29⁺ ASC were identified as CD29⁺, CD146⁺, CD31⁻. Vascular smooth muscle cells (V-SMC) were identified as CD34⁺dim, CD31⁺, CD146⁺. Leukocytes and their respective subpopulations that co-express CD45 were identified using CD45 positivity. CD34⁻, CD45⁻ cells represented the pan-leukocytes. These included B cells (CD45⁺, CD3⁻, and CD19⁺), Mast cells (CD45⁺, CD34⁻, and CD117⁺), Granulocytes (CD45⁺, CD11b⁺, and CD3⁻), Monocytes (CD45⁺ and CD14⁺), T cells (CD4⁺, CD3⁺, and CD34⁻), and T-regulatory cells (T-regs; FoxP3⁺, CD25⁻, and CD4⁺). Natural killer (NK) cells were identified using a CD8⁺ gating strategy (CD8⁺, CD16⁺, and CD3⁻). Lastly, Macrophage populations were identified within an un-gated population, expressing the CD11b⁺, F4/80⁺ phenotype.

**Cellular composition of 8–12 month female mSVF**

Gating strategies described in Figure 1 were applied to detect subpopulations within 8–12-month-old female C57Bl/6 murine inguinal (iWAT), epididymal (eWAT), and brown (BAT) adipose SVF (Table 2). ASC-like cells, pre-adipocytes, and V-SMC, represented the largest subpopulations detected within SVF of all adipose depots of middle-aged, female mice. The three adipose depots displayed no significant difference with respect to the content of their SVF cell sub-populations.

**Cellular composition of 8–12 month male mSVF**

SVF from 8–12 month male C57Bl/6 murine iWAT, eWAT, and BAT adipose depots was analyzed. Like

| Table 2. Cell category percentages within older female mice SVF cells. | iWAT | eWAT | BAT |
|---|---|---|---|
| CD29+ ASC-Like | 18.1 ± 8.5 | 24.6 ± 4.4 | 13.7 ± 4.7 |
| CD34+ ASC-Like | 14.0 ± 4.2 | 8.8 ± 1.8 | 10.0 ± 2.7 |
| Pre-Adipocytes | 10.8 ± 7.5 | 6.5 ± 2.7 | 4.8 ± 1.2 |
| EC-like | 3.1 ± 0.6 | 12.2 ± 9.3 | 2.7 ± 0.9 |
| HSC-like | 7.2 ± 4.0 | 9.9 ± 4.3 | 6.0 ± 1.6 |
| Vascular SMC | 20.8 ± 10.1 | 25.6 ± 3.5 | 21.9 ± 24.7 |
| Leukocytes | 6.9 ± 1.9 | 3.5 ± 3.0 | 7.3 ± 0.2 |
| T-cells | 8.7 ± 2.9 | 3.4 ± 1.4 | 4.8 ± 2.1 |
| B-cells | 2.6 ± 2.2 | 1.9 ± 1.4 | 0.9 ± 0.3 |
| Mast Cells | 1.2 ± 0.5 | 0.6 ± 0.4 | 1.0 ± 1.1 |
| Granulocytes | 6.0 ± 0.9 | 3.8 ± 0.8 | 5.6 ± 0.9 |
| Monocytes | 7.3 ± 2.5 | 8.6 ± 1.4 | 6.8 ± 4.1 |
| Macrophages | 2.0 ± 1.3 | 4.0 ± 1.6 | 2.7 ± 1.9 |
| NK cells | 2.7 ± 1.1 | 4.0 ± 5.0 | 1.5 ± 1.9 |
| CD25- Tregs | 0.8 ± 0.9 | 0.2 ± 0.0 | 0.6 ± 0.1 |
| CD25+ Tregs | 2.5 ± 0.8 | 4.5 ± 1.5 | 0.9 ± 0.2 |
female SVF, male SVF primarily contained ASC-like cells, pre-adipocytes, and V-SMC as the largest percentage subpopulations. Male iWAT SVF expressed higher percentages of Granulocytes and T-cell subpopulations relative to eWAT. Compared to male BAT SVF, male iWAT SVF contained higher percentages of leukocytes and T cells, and lower percentages of HSC-like cells. Conversely, eWAT SVF expressed higher percentages of EC-like cells and V-SMC relative to iWAT SVF. eWAT SVF also contained higher percentages of CD34+ ASC-like cells than BAT SVF.

**Gender-based comparison of male and female mSVF**

Figure 2 provides a comparison of the cellular heterogeneity of subpopulations within 8-12-month female and male iWAT SVF. Again, for both genders, the largest populations detected include ASC, pre-adipocytes, and V-SMC. However, the CD34+ ASC-like and leukocyte populations were significantly different in expression within depots. Male iWAT and eWAT both contained a significantly higher percentage of CD34+ ASC-like sub-populations, compared to their female depots counterparts. In addition, male iWAT also expressed higher percentages of leukocyte sub-populations relative to female iWAT.

**Cellular composition of 6–8 week male mSVF**

SVF from 6–8 week male murine iWAT, eWAT, and BAT was analyzed. Like both the middle-aged male and female SVF, collectively the three largest subpopulations were the pre-adipocytes, the ASC-like, and the leukocyte populations. Young male iWAT expressed significantly higher percentages of V-SMC, leukocytes, T-cells, and B-cells relative to eWAT SVF. Conversely, young iWAT contained lower percentages of EC-like, HSC-like cells, and Macrophages relative to eWAT SVF. Compared to BAT SVF, iWAT SVF contained higher percentages of pre-adipocytes, V-SMC, and leukocytes. This leukocyte population included a significantly higher percentage of the T-cells (more specifically, the CD25FoxP3+ T cells, B-cells, Mast cells, and NK cells). iWAT SVF contained significantly lower percentages of the CD34+ ASC-like cells, EC-like cells, HSC-like cells compared to BAT SVF.

**Age-based comparison of young versus middle-aged male mSVF**

Figure 2 compares the relative percentage subpopulations within younger (6-8 week) versus middle-aged (8-12 month) male murine iWAT SVF. Younger male iWAT SVF contained a significantly higher percentage pre-adipocytes, HSC-like cells, leukocytes, B-cells, Mast cells, NK cells, and CD25FoxP3+ T-regulatory cells than middle-aged iWAT SVF. Comparable SVF composition differences were noted between the younger male eWAT and BAT SVF relative to their middle-aged depot counterparts (Tables 2 and 4).

**Discussion**

**SVF cellular composition as a function of gender**

Inter-depot comparisons revealed that SVF derived from all three depots in middle-aged female mice
expressed similar percentages of cell subpopulations (Table 2). The SVF cells isolated from middle aged male iWAT expressed higher percentages of Granulocytes and T-cell subpopulations relative to eWAT (Table 3). Di Taranto G, et al [17], demonstrated that liposuctioned subcutaneous adipose tissue from human female subjects contained higher stromal markers and indicators of stemness, compared to deep layer tissue. Other supportive data within the literature suggests that males accumulate more visceral fat during development, aging and obesity even after adjusting for BMI [19]. In the present study, murine gender comparisons revealed male iWAT expressed higher percentages of leukocyte sub-populations than female iWAT, while male iWAT and eWAT both expressed a significantly higher percentage of CD34+ ASC-like sub-populations and leukocytes, compared to their age-matched female depot counterparts.

### Table 3. Cell category percentages within older male mice SVF cells.

| Category     | iWAT   | eWAT   | bAT   |
|--------------|--------|--------|-------|
| CD29+ ASC-Like | 23.1 ± 7.0 | 26.6 ± 0.3 | 17.1 ± 5.8 |
| CD34+ ASC-Like | 24.8 ± 10.7 | 18.3 ± 7.1 | 14.9 ± 6.3 |
| Pre-Adipocytes | 12.5 ± 6.1 | 7.2 ± 22 | 5.1 ± 0.9 |
| EC-like       | 4.2 ± 3.3 | 94.5 ± 5.3 | 2.5 ± 0.9 |
| HSC-like      | 4.4 ± 0.2 | 6.5 ± 1.5 | 6.7 ± 0.7 |
| Vascular SMC  | 13.6 ± 0.4 | 23.4 ± 0.5 | 21.9 ± 15.2 |
| Leukocytes    | 19.4 ± 9.7 | 8.4 ± 3.1 | 7.0 ± 0.4 |
| T-cells       | 7.9 ± 2.5 | 4.4 ± 1.1 | 1.8 ± 1.4 |
| B-cells       | 1.2 ± 0.2 | 1.6 ± 1.1 | 0.9 ± 0.2 |
| Mast Cells    | 1.0 ± 0.4 | 0.7 ± 0.3 | 1.0 ± 0.8 |
| Granulocytes  | 6.3 ± 0.8 | 3.6 ± 0.7 | 4.7 ± 1.3 |
| Monocytes     | 9.1 ± 3.5 | 8.6 ± 10 | 8.8 ± 1.4 |
| Macrophages   | 2.0 ± 0.9 | 3.7 ± 13 | 2.2 ± 1.6 |
| NK cells      | 1.4 ± 1.8 | 3.6 ± 3.5 | 1.7 ± 1.0 |
| CD25- Tregs   | 0.1 ± 0.1 | 0.4 ± 0.4 | 0.3 ± 0.3 |
| CD25+ Tregs   | 2.4 ± 0.5 | 2.7 ± 1.1 | 0.6 ± 0.2 |

*value greater compared to iWAT with p value < 0.05.

### Table 4. Cell category percentages within younger male mice SVF cells.

| Category     | iWAT   | eWAT   | bAT   |
|--------------|--------|--------|-------|
| CD29+ ASC-Like | 27.1 ± 7.0 | 20.2 ± 0.2 | 18.3 ± 0.41 |
| CD34+ ASC-Like | 17.1 ± 1.2 | 22.4 ± 2.1 | 35.2 ± 3.2 |
| Pre-Adipocytes | 64.2 ± 5.0 | 56.3 ± 0.3 | 31.4 ± 1.7 |
| EC-like       | 4.0 ± 0.0 | 5.6 ± 0.2 | 6.4 ± 0.1 |
| HSC-like      | 9.8 ± 0.4 | 14.4 ± 0.6 | 33.5 ± 4.6 |
| Vascular SMC  | 9.9 ± 0.3 | 2.5 ± 0.0 | 3.0 ± 0.0 |
| Leukocytes    | 63.5 ± 3.5 | 17.5 ± 3.8 | 8.9 ± 0.9 |
| T-cells       | 3.4 ± 0.2 | 2.3 ± 0.0 | 1.0 ± 0.1 |
| B-cells       | 20.3 ± 0.6 | 3.8 ± 0.3 | 1.5 ± 0.6 |
| Mast Cells    | 21.3 ± 1.5 | 16.5 ± 0.7 | 2.5 ± 0.0 |
| Granulocytes  | 11.9 ± 2.6 | 12.9 ± 0.9 | 2.2 ± 0.2 |
| Monocytes     | 4.9 ± 1.2 | 2.4 ± 0.2 | 7.6 ± 0.4 |
| Macrophages   | 3.1 ± 0.1 | 8.2 ± 0.3 | 3.6 ± 1.2 |
| NK cells      | 11.5 ± 2.1 | 5.2 ± 0.0 | 2.4 ± 0.6 |
| CD25- Tregs   | 6.2 ± 0.7 | 4.5 ± 0.7 | 1.4 ± 0.1 |
| CD25+ Tregs   | 1.4 ± 0.5 | 3.6 ± 1.7 | 3.4 ± 0.3 |

*value greater compared to eWAT with p value < 0.05.

These findings were comparable to those of Contreas, et al [20], who investigated the adipogenic progenitor cell (APC) distribution in SVF of perivascula tissue derived from thoracic aorta and mesenteric resistance arteries of 10-week-old Sprague-Dawley rats, as a function of gender. Sex-related differences were observed in the expression of CD34, where females had fewer CD34+ cells in perivascular adipose. APC proliferation and adipogenic capacity in vitro were not affected by sex. However, APC from aortic adipose had a lower proliferation capacity compared to mesenteric adipose [20]. Collectively, these data suggest the distribution of adipose progenitors differs by gender and is affected by anatomical location. This may affect the adipose tissue functional properties and, therefore the regenerative and immunoregulatory behavior of the SVF within clinical applications.

### SVF cellular composition as a function of age

The young and middle-aged murine cohorts in the present study were equivalent to 20–30 years and 38–47 years in human age, respectively. Recent studies suggest that tissue regenerative properties of autologous adipose stem cells are compromised as a function of age [18–21]. Liu, et. al, demonstrated a decrease in frequency of colony forming units (CFU-F) and SVF cell yield in elderly patients compared to middle-aged. However, Kokai, et al [18] investigated the effects of cell yield and autologous SVF subpopulation composition as a function of age, over a 7-12-year timespan. SVF cells showed neither significant differences in cell yield and composition parameters, nor changes in proliferation rates and differentiation potential [18]. Our murine age comparisons suggest younger male iWAT, eWAT, and BAT SVF all contained a significantly higher percentage of pre-adipocytes, HSC-like cells, and CD25+, FoxP3+ T-regulatory cells compared to SVF from middle-aged mice. These data warrant further investigation into the usage of murine adipose tissue models for analyses of SVF cell composition and functionality in clinical translation.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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biotechnology company focusing research and clinical translation of adipose-derived stromal/stem cells. JMG, XW, and TF are co-owners and co-founders of Obatala Sciences, Inc., a biotechnology company focusing on the use of 3-dimensional adipose depots for drug discovery and research. TF was employed as a post-doctoral fellow at Tulane University at the time of this research and is currently employed at LaCell LLC.

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**References**

[1] Trayhurn P, Wood JS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutri. 2004;92:347–55.

[2] Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112:1821–30.

[3] Weisberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112:1796–808.

[4] Cousin B, Andre M, Arnaud E, et al. Reconstitution of lethally irradiated mice by cells isolated from adipose tissue. Biochem Biophys Res Commun. 2003;301:1016–22.

[5] Han J, Koh YJ, Moon HR, et al. Adipose tissue is an extramedullary reservoir for functional hematopoietic stem and progenitor cells. Blood. 2010;115:957–64.

[6] Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15:641–8.

[7] Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circulation Res. 2007;100:1249–60.

[8] Lauer, M. Implementing Rigor and Transparency in NIH & AHRQ Research Grant Applications. National Institutes of Health. 2016. https://grants.nih.gov/reproducibility/index.htm.

[9] Deasy BM, Lu A, Tebbets JC, et al. A role for cell sex in stem cell-mediated skeletal muscle regeneration: female cells have higher muscle regeneration efficiency. J Cell Biol. 2007;177:73–86.

[10] Deasy BM, Schugar RC, Huard J. Sex differences in muscle-derived stem cells and skeletal muscle. Critical Rev Eukaryotic Gene Expression. 2008;18:173–88.

[11] Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9:641–50.

[12] Ock SA, Lee YM, Park JS, et al. Evaluation of phenotypic, functional and molecular characteristics of porcine mesenchymal stromal/stem cells depending on donor age, gender and tissue source. J Veterinary Medical Sci. 2016;78:987–95.

[13] Pandey AC, Semon JA, Kaushal D, et al. MicroRNA profiling reveals age-dependent differential expression of nuclear factor kappaB and mitogen-activated protein kinase in adipose and bone marrow-derived human mesenchymal stem cells. Stem Cell Res Therapy. 2011;2:49.

[14] Scruggs BA, Semon JA, Zhang X, et al. Age of the donor reduces the ability of human adipose-derived stem cells to alleviate symptoms in the experimental autoimmune encephalomyelitis mouse model. Stem Cells Translational Med. 2013;2:797–807.

[15] Kheterpal I, Ku G, Coleman L, et al. Proteome of human subcutaneous adipose tissue stromal vascular fraction cells versus mature adipocytes based on DIGE. Journal of Proteome Research. 2011;10:1519–27.

[16] Zimmerlin L, Donnenberg VS, Pfeifer ME, et al. Stromal vascular progenitors in adult human adipose tissue. Cytometry A. 2010;77:22–30.

[17] Di Taranto G, Cicione C, Visconti G, et al. Qualitative and quantitative differences of adipose-derived stromal cells from superficial and deep subcutaneous lipoaspirates: a matter of fat. Cytotherapy. 2015;17(8):1076–89. Epub 2015 May 1.

[18] Kokai LE, Traktuev DO, Zhang L, et al. Adipose stem cell function maintained with age: an intra-subject study of long-term cryopreserved cells. Aesthet Surg J. 2017;37(4):454–463.

[19] Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93(1):359–404.

[20] Contreras GA, Thelen K, Ayala-Lopez N, et al. The distribution and adipogenic potential of perivascular adipose tissue adipocyte progenitors is dependent on sexual dimorphism and vessel location. Physiol Rep. 2016;4(19):e12993.

[21] Liu M, Lei H, Dong P, et al. Xiao R Adipose-derived mesenchymal stem cells from the elderly exhibit decreased migration and differentiation abilities with senescent properties. Cell Transplant. 2017;26(9):1505–1519. [Epub ahead of print]