METHODS ARTICLE

Utilizing Confocal Microscopy to Characterize Human and Mouse Adipose Tissue

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Significant advances in our understanding of human obesity, endocrinology, and metabolism have been made possible by murine comparative models, in which anatomically analogous fat depots are utilized; however, current research has questioned how truly analogous these depots are. In this study, we assess the validity of the analogy from the perspective of cellular architecture. Whole tissue mounting, confocal microscopy, and image reconstruction software were used to characterize the three-dimensional structure of the inguinal fat pad in mice, gluteofemoral fat in humans, and subcutaneous adipose tissue of the human abdominal wall. Abdominal and gluteofemoral adipose tissue specimens from 12 human patients and bilateral inguinal fat pads from 12 mice were stained for adipocytes, blood vessels, and a putative marker for adipose-derived multipotent progenitor cells, cluster of differentiation 34 (CD34). Samples were whole-mounted and imaged with laser scanning confocal microscopy. Expectedly, human adipocytes were larger and demonstrated greater size heterogeneity. Mouse fat displayed significantly higher vascular density compared with human fat when normalized to adipocyte count. There was no significant difference in the concentration of CD34-positive (CD34⁺) stromal cells from either species. However, the mean distance between CD34⁺ stromal cells and blood vessels was significantly greater in human fat. Finally, mouse inguinal fat contained larger numbers of brown adipocytes than did human gluteofemoral or human abdominal fat. Overall, the basic architecture of human adipose tissue differs significantly from that of mice. Insofar as human gluteofemoral fat differs from human abdominal adipose tissue, it was closer to mouse inguinal fat, being its comparative developmental analog. These differences likely confer variance in functional properties between the two sources and thus must be considered when designing murine models of human disease.

Keywords: adipocyte, fat, adipose tissue, stromal cell, vascularity, confocal imaging

Impact Statement

Greater understanding of the three dimensional cellular architecture of healthy adipose tissue in humans and mice can also inform research strategies aiming to optimize fat grafting techniques and create durable grafts which resemble endogenous fat in both composition and function.

Introduction

MURINE MODELS ARE COMMONLY USED to simulate limited aspects of human adipose tissue physiology. This practice has accelerated in recent years, as research into the worsening medical burden of obesity and diabetes has intensified.^{1–4} Tremendous advances in our understanding of human obesity, endocrinology, and metabolism have been made possible by comparative studies in mice.^{5–7} In fact,

our understanding of the basic organization of fat depots in the body have been informed by studies incorporating murine models.

As in humans, mouse adipose tissue can be generally designated as subcutaneous or visceral. Within each category, adipocytes are developmentally optimized for energy storage, as in white adipose tissue (WAT), or for thermogenesis, as in brown adipose tissue (BAT). Mouse models have aided in identifying the metabolic and transcriptional differences that

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exist between both types of adipocytes.^{5,8} Moreover, murine models have begun to elucidate the transcriptional pathways involved with “browning” or the acquisition by WAT of characteristics seen in BAT secondary to cold exposure^{8–10} and its implications for the treatment of metabolic syndrome. Transcriptional analyses have revealed differential gene expression among distinct body fat depots in both human and mouse. This genetic variability has contributed to theories on body fat distribution and the propensity toward obesity and metabolic disease in humans.^{1,4} Finally, mouse models have aided in the study of human adipocyte pathophysiology, as evident in the expansive array of transgenic, knockout, and chemically induced mice designed to emulate human type 1 or 2 diabetes mellitus.^{2,3}

Comparative studies typically endeavor to utilize anatomically analogous fat depots. In the case of mice, the major sites of WAT are the inguinal and periovarian/epididymal regions, whereas the major WAT depot in humans is in the subcutaneous adipose tissue of the abdomen.⁶ However, current research has raised questions over how truly analogous these depots are. Fat depots, both within and between species, differ dramatically in their transcriptional, endocrinologic, and metabolic properties.^{1,8,11} Even within a species, current evidence suggests that there exists inherent heterogeneity within a single fat depot.⁵ Whereas prior studies have examined the transcriptional disparities between structures popularly regarded as analogous in murine comparative research, to our knowledge, no study has compared and quantified the objective disparities in cellular architecture that exists between the two species. Thus, as function follows form, an integral step in understanding the fundamental differences between human and mouse adipose tissue—and thus the inherent limitations of murine models—is to compare their basic architecture at the cellular level. This study uses the latest advancements in whole tissue mounting, confocal microscopy, and image reconstruction software to characterize the three-dimensional (3D) structure of the commonly studied inguinal fat pad in mice, the developmentally analogous gluteofemoral fat in humans, and the abundantly available subcutaneous adipose tissue of the abdominal wall.

Materials and Methods

Tissue collection

Subcutaneous adipose tissue specimens were obtained from 12 patients undergoing resection of abdominal tissue and gluteofemoral fat. The patients ranged in age from 45 to 60 years, were female, and had no significant medical history or known medical comorbidities. Their body mass index (BMI) range was 25–29. The human adipose tissue specimens from the superficial subcutaneous tissue were cut into 4×4×2 mm pieces for imaging. For mouse subcutaneous adipose tissue, bilateral inguinal fat pads were harvested from twelve 6-month-old female CD-1 mice (Charles River Laboratories, Inc., Wilmington, MA). Each inguinal fat pad was then cut into three 4×4×2 mm long strips for imaging. The human specimens were obtained with the specific approval of Stanford University Institutional Review Board protocol 2188 and biosafety protocol APB 2221. The mouse studies were conducted under the specific approval of Stanford University Administrative Panel on Laboratory Animal Care protocol 9999, assurance number A3213-01.

Tissue staining

Labeling of adipose-derived stromal cells (ADSCs) was accomplished using antibodies against cluster of differentiation 34 (CD34), given this surface antigen's strong association with stem cell-like multipotentiality.^{12,13} This single-marker strategy was validated through an initial experiment, in which specimens from human abdominal, mouse inguinal, and human gluteofemoral adipose tissue depots were stained for three separate conditions: CD34 only; CD105, a commonly accepted progenitor cell marker, only; and for both CD34 and CD105. Human samples were incubated in an anti-CD34 primary stain (ThermoFisher Scientific, 1:100, #MA5-15331) for 24 h at 4°C, followed by a secondary stain conjugated to Alexa Fluor 488 (ThermoFisher Scientific, 1:100, #A-11001), for 1 h at room temperature. In an identical manner, murine specimens were stained in a mouse-specific anti-CD34 primary stain (ThermoFisher Scientific, 1:100, #14-0341-85) followed by a secondary stain conjugated to Alexa Fluor 488 (Abcam, 1:100, #ab150157). This was mirrored in the anti-CD105 primary (Invitrogen, 1:100, #14-1057-82) and secondary stain conjugated to Alexa Fluor 700 (ThermoFisher Scientific, 1:100, #A-21036) used for human samples, as well as the mouse-specific anti-CD105 primary (Invitrogen, 1:100, #14-1051-82) and secondary stain conjugated to Alexa Fluor 647 (ThermoFisher Scientific, 1:100, #A-21247) applied to the mouse inguinal fat pad specimens. Serial staining was accomplished for both species in the case of dual CD34/CD105 staining, thus avoiding the complication posed by a common host species in both primary antibodies. Two, 15-min phosphate-buffered saline (PBS) washes were performed after each staining period. A 1-h room temperature incubation of Hoechst 33342 (ThermoFisher Scientific, 1:10,000, #H3570) in PBS was accomplished for all groups for nuclear staining.

For the main experiment, stromal cells from fresh human tissue samples were incubated in Human CD34 PE-Cy5.5 Conjugate (ThermoFisher Scientific, 1:100, #CD34-581-18) and PBS (ThermoFisher Scientific) for 24 h at 4°C. Similarly, fresh mouse inguinal fat pad strips were incubated in purified anti-mouse CD34 antibody (BioLegend, 1:100, #119302) and PBS for 24 h at 4°C. The mouse samples were then incubated in Alexa Fluor 488 Goat Anti-Rat IgG secondary antibody (BioLegend, 1:100, #405418) and PBS for 1 h at room temperature.

A staining master mix was created by diluting Isolectin GS-IB4 from Griffonia simplicifolia, Alexa Fluor 568 (ThermoFisher Scientific, 1:100, I21412) and high-content screening LipidTOX Deep Red Neutral Lipid Stain (ThermoFisher Scientific, 1:100, #H34477) in PBS, to visualize endothelial cells and the lipids within adipocytes, respectively. The human and mouse specimens were separately incubated in the staining master mix at room temperature for 1 h. After a 15-minute PBS rinse, the samples were incubated at room temperature in Hoechst 33342 (ThermoFisher Scientific, 1:10,000, #H3570) stain in PBS for 30 min. The samples were washed twice for 15 min with PBS under agitation. Neither tissue fixation nor permeabilization nor clearing was performed as per the protocol described by Berry *et al.*¹⁴ Microscope slides were prepared by dispensing through a 3-mL syringe, a 4-mm high ribbon of petroleum jelly to the periphery of the slide. Next, Fluoromount-G (Southern Biotech, #0100-01) mounting media was added, into which the washed samples were placed. The

mount was completed with a glass cover, and the edges were sealed with clear nail polish.

The human and mouse adipose tissue samples also were evaluated for the relative composition of BAT and WAT. WAT was labeled using species-specific primary antibodies against the BAT-specific surface antigen ASC-1. A single primary antibody capable of targeting PAT2 in both human and mouse species was used to identify BAT. Human abdominal, mouse inguinal, and human gluteofemoral adipose tissue were stained for three separate conditions: ASC-1 only for BAT, PAT2 only for WAT, and combined ASC-1/PAT2. The groups were incubated in a primary stain of ASC-1 antibody for human (ThermoFisher Scientific, 1:100, #PA5-59771) or for mouse species (ThermoFisher Scientific, 1:100, #MBS540604), PAT2 antibody (Santa Cruz Biotechnology, 1:100, #sc390969), or both, in PBS for 24 h at 4°C. This was followed by a secondary stain of Alexa Fluor 594 (ThermoFisher Scientific, 1:100, #A-11037) for ASC-1, Alexa Fluor 647 (ThermoFisher Scientific, 1:100, #A-21236) for PAT2, or both, for 24 h at 4°C. Two 15-min PBS washes were performed after each staining period. A 1-h room temperature incubation of DAPI (Sigma, 1:10,000, #D9542) in PBS was included for all groups for nuclear staining. Whole mounting of the samples was accomplished as previously described.

Confocal microscopy and 3D imaging analysis

Laser scanning confocal microscopy was performed using a Leica TCS SP8 X confocal microscope (Leica Microsystems, Wetzlar, Germany) with an objective lens (10×HC PL APO, air, N.A. 0.40; 20×HC PL APO IMM CORR CS2, H₂O/Glycerol/oil, N.A. 0.75). Image acquisition was standardized using a 30-μm layer thickness with 4.5-μm steps between layers. Three-dimensional volume rendering of z-stacks was performed on Imaris (Bitplane AG, Zurich, Switzerland), as described by the manufacturer's protocol. Isosurface rendering of cells stained with LipidTOX, Isolectin, and CD34 was accomplished using the Volume Surface tool, then thresholding by volume, ellipsicity, and signal quality. This facilitated discrimination of individual adipocytes for normalization of both Isolectin and CD34 staining to adipocyte numbers. Distance calculations were obtained through the Distance Transformation tool. Quantification of images was then performed on Imaris using the Isosurface Rendering Statistics tool.

Statistical analysis

Biostatistics means and standard error were calculated from numerical data, and bar graphs demonstrated the mean ± standard error. Intergroup variance was evaluated using one-way analysis of variance. A *p*-value of less than 0.05 was considered statistically significant. A Mann-Whitney *U* analysis was used to compare nonparametric data describing mean distance to blood vessels and adipocyte volume. Statistical analysis was performed on Prism (GraphPad Software, Inc., La Jolla, CA). ImageJ (NIH, Bethesda, MD) was used to quantify the relative amounts of BAT and WAT staining in the adipose tissue samples.

Results

Confocal microscopy of mouse inguinal fat pad, subcutaneous human abdominal adipose tissue, and human gluteo-

femoral adipose tissue yielded detailed, 3D images illustrating the spatial relationship between adipocytes, blood vessels, and CD34-positive (CD34⁺) stromal cells (Fig. 1). Expectedly, the volume of human adipocytes was significantly greater than mouse adipocytes. The majority of mouse inguinal adipocytes were found to have volumes less than $40 \times 10^3 \mu\text{m}^3$, with the smallest adipocyte volume of $10 \times 10^3 \mu\text{m}^3$ and largest adipocyte volume of $120 \times 10^3 \mu\text{m}^3$ (mean = $25 \times 10^3 \mu\text{m}^3$, SEM = 2×10^3). In comparison, human abdominal volumes were more equally distributed over a range of $30 \times 10^3 \mu\text{m}^3$ to $500 \times 10^3 \mu\text{m}^3$ (Fig. 2). Specifically, human abdominal adipose tissue contained, on average, larger adipocytes (mean = $150 \times 10^3 \mu\text{m}^3$, SEM = $5 \times 10^3 \mu\text{m}^3$) compared with mouse inguinal adipocytes (*****p* < 0.0001). Human gluteofemoral fat, which being developmentally analogous to mouse inguinal fat, had smaller adipocytes compared with human abdominal adipose tissue (mean = $126 \times 10^3 \mu\text{m}^3$, SEM = $7 \times 10^3 \mu\text{m}^3$, ***p* < 0.01), but had significantly larger adipocytes versus mouse inguinal fat (*****p* < 0.0001).

Differences in vasculature and adipocyte architecture also were readily apparent, with greater vessel density noted in mouse compared to human fat (Fig. 3A–F). Normalization of Isolectin staining to LipidTOX staining demonstrated a significantly greater ratio of vasculature to LipidTOX stained adipocytes in mouse inguinal fat samples (1.54, SD 0.97) compared with human abdominal adipose tissue (0.38, SD 0.24, ***p* < 0.01) (Fig. 3G). Interestingly, although the vessel density of human gluteofemoral adipose tissue (0.57, SD 0.25) was significantly less than that of mouse inguinal fat, it was nonetheless greater than that of human abdominal fat. More detailed analysis of vasculature and adipocyte distribution in three dimensions revealed that, in comparison to the distance between adipocytes and vessels in human abdominal adipose tissue (mean = 96.49 μm, SEM = 4.74 μm), the mean distances are significantly lower in both mouse inguinal (mean = 59.57 μm, SEM = 7.18 μm, *****p* < 0.0001) as well as in human gluteofemoral (mean = 28.59 μm, SEM = 1.28 μm, *****p* < 0.0001) fat samples (Fig. 3H).

Staining performed for a single ADSC marker, CD34, correlated tightly with that of a second progenitor marker, CD105 (Fig. 4) in all experimental samples from both species, thus validating our single-marker strategy. To that end, differences also were appreciated in CD34⁺ stromal cell distribution between mouse and human adipose tissue (Fig. 5A–C). Staining for CD34 normalized to LipidTOX stained adipocyte number was similar between mouse and human samples (Fig. 5D). However, normalization of CD34 staining to Isolectin staining demonstrated a significantly smaller ratio of CD34⁺ stromal cells to vasculature in mouse inguinal fat samples (0.10, SD 0.06) compared with human abdominal adipose tissue (0.77, SD 0.27, **p* < 0.05) (Fig. 5E). Similarly, the CD34⁺ to vasculature ratio observed in human gluteofemoral fat (0.61, SD 0.26) was significantly higher than that of mouse inguinal fat, but was lower than that of human abdominal adipose tissue.

Three-dimensional images of adipose tissue also were evaluated for distance between vasculature and CD34⁺ stromal cells (Fig. 6A–C). The mean distance between CD34 stained cells and Isolectin-stained vasculature was significantly greater in less vascularized human abdominal adipose tissue (74.63, SD 25.47) compared with mouse inguinal fat tissue (8.98, SD 3.89, ***p* < 0.01) (Fig. 6D). The

FIG. 1. Three-dimensional (3D) reconstructions of z-stack images and isosurface renderings of mouse and human fat. From left to right: Representative confocal images depicted in individual channels and then merged, followed by Imaris 3D isosurface rendering of (A) mouse inguinal, (B) human abdominal, and (C) human gluteofemoral adipose tissue.

CD34⁺ stromal cell to vasculature distance in human gluteofemoral fat (45.09, SD 17.8) also was found to be significantly greater than that of mouse inguinal adipose tissue; however—as was observed in the prior metrics—insofar as human gluteofemoral fat differs from human abdominal adipose tissue, it was closer to mouse inguinal fat, being its comparative developmental analog.

Finally, remarkable variability was observed in the relative composition of BAT among the adipose tissue depots investigated. Specifically, there was a significantly larger mean area of PAT2 labeled pixels in mouse inguinal fat (36271, SD 15781) compared with human abdominal fat (4023, SD 3577, $*p < 0.05$) and human gluteofemoral fat (11321, SD 5276, $*p < 0.05$) (Fig. 7A, B). However, the mean area of ASC-1-labeled pixels was not significantly different between the fat samples (Fig. 8A, B).

Discussion

Obesity has been described as an emerging international epidemic affecting 35% of adults and 17% of children in the United States,¹⁵ and ~600 million people worldwide.¹⁶ The burden of metabolic disease extends beyond individual

FIG. 2. Frequency distribution of adipocyte volume in mouse and human samples. Human adipocytes, both abdominal and gluteofemoral, were significantly larger than mouse adipocytes ($****p < 0.001$), and also demonstrated a broader distribution of volumes ($****p < 0.001$). Additionally, human gluteofemoral fat had smaller adipocytes compared with human abdominal adipose tissue ($**p < 0.01$).

FIG. 4. Cluster of differentiation 34 (CD34) costains with CD105. Maximum intensity projection images of mouse inguinal fat pad, human abdominal fat, and human gluteofemoral adipose tissue stained with CD34, Hoescht, and the ASC marker CD105. Merged images demonstrate a high degree of overlap between CD34 and CD105 antibody binding. ASC, adipose-derived stromal cells.

health outcomes such as quality of life, disability, and mortality, and impacts entire economies through disproportionate and massive utilization of healthcare services.¹⁷ Given this reality, it is not surprising that considerable resources are dedicated to understanding the pathophysiology of human metabolic disease. In fact, obesity research accounts for approximately \$997 million in annual grant funding reported by the National Institutes of Health in 2017.

Much of this research relies heavily on murine comparative models to emulate the physiology of human obesity. To wit, human abdominal fat and mouse inguinal fat pad tissue are among the more commonly used samples in these analyses. However, there is a paucity of research evaluating the relevant similarities and differences between human and murine fat, as well as the overall translatability of the findings made.

Human and murine adipose tissue share many structural features. Both species organize fat into anatomically specific depots, which generally can be categorized as either subcutaneous or visceral. Moreover, correlates to human omental and mesenteric fat are observed in mice. To this point, mouse mesenteric fat has been advanced as the archetypal analogous fat structure, as its location, biology, and access to the portal vein mirror that of humans.⁶ As in humans, female rodents possess greater fat mass compared with males.¹⁸

In addition to structural similarities, human and mouse fat share notable hormonal factors. Returning again to sex-specific attributes, females of both species compared with males have higher plasma levels of leptin, a protein involved in energy balance and hunger signaling.¹⁹ Furthermore, unlike in males, leptin is preferentially excreted from peripheral stores in females versus visceral in males. Commonalities in hormonal regulation that influence the accumulation and distribution of fat also have been identified. Accumulation of visceral adipose tissue in both species has been linked to

higher levels of the protein GCC 11-*beta* HSD-1.²⁰ Likewise, Collagen VI *alpha* 3—a protein secreted by adipocytes during times of stress—has been implicated as a mediator of adipocyte expansion in both murine genetic knockout models, in addition to studies involving human populations genetically predisposed to insulin resistance.²¹

The aforementioned structural, metabolic, and endocrinologic characteristics of adipose tissue shared by humans and rodents argue for the validity of the murine model of human disease. However, marked differences exist between the fat tissue of both species, and these contrasting attributes must be given equivalent consideration. For instance, some fat depots have no analog such as the well-studied perigonadal fat pads in mice, which are not found in human beings.²²

In addition, structures previously regarded as analogous do not meet this criterion when subject to gross anatomic scrutiny. To this end, Chusyd *et al.* elaborate on the nature of human versus rodent subcutaneous fat. Adipose tissue from the human abdomen (i.e., abdominoplasty fat), buttocks, and thigh—the latter two collectively termed the gluteofemoral region—constitute the major subcutaneous depots of the human body. In mice, the predominant subcutaneous depots are located anteriorly in the interscapular region of the neck, and posteriorly in the inguinal fat of the gluteofemoral region. Thus, the inguinal fat pad tissue of mice is more closely related structurally to the gluteofemoral fat of the human buttock or thigh.

This fact is relevant in the context of the current study, in which we have conducted a structural analysis of human abdominoplasty fat compared with mouse inguinal fat pad tissue, given the ubiquity of both types of samples in the current literature. The differences we observed can, in part, be attributed to the fact that these structures are not truly analogous, as they derive from unique depots. The notion that separate fat depots are functionally unique is supported

FIG. 5. The ratio of CD34-positive (CD34⁺) stromal cells to blood vessels is significantly reduced in mouse compared to human fat. Maximum intensity projection images demonstrating CD34-stained stromal cells (*green*) and Isolectin-stained blood vessels (*red*) from representative (A) mouse inguinal fat pad, (B) human abdominal fat, and (C) human gluteofemoral fat. (D) Quantification of ASC as normalized to LipidTOX-stained adipocytes is not significantly different between mouse and human samples. (E) The ratio of ASC to blood vessels is significantly greater in human samples compared to mouse (* $p < 0.05$).

FIG. 6. Mean CD34⁺ stromal cells to vessel distance is significantly greater in human compared to mouse fat. Maximum intensity projection images demonstrating Isolectin-stained blood vessels color-coded based on distance from the nearest blood vessel in representative (A) mouse inguinal fat pad, (B) human abdominal fat, and (C) human gluteofemoral fat. (D) The mean distance between ASC and vessels is significantly lower in mouse compared to human abdominal (** $p < 0.01$) or human gluteofemoral fat (* $p < 0.05$).

by transcriptional analysis. Multiple studies have demonstrated differential gene expression between the separate fat depots of a single species, in particular, specific members of the *HOX* superfamily of developmental genes.^{23,24} In addition, microarray studies have identified increased expression

of leptin and plasminogen activator inhibitor in mouse inguinal fat tissue, compared to upregulated angiotensinogen, *PPAR*-gamma, and adiponectin in murine epididymal fat.²³

In our present study, localization of CD34 expression was evaluated given its potential as a cell surface marker for

FIG. 7. Relative abundance of brown adipocytes in human and mouse white fat. **(A)** Mouse inguinal fat pad, human abdominal fat, and human gluteofemoral adipose tissue samples were stained with the brown adipocyte tissue-specific marker PAT2 and Hoescht. Representative confocal images show more PAT2 cells in mouse inguinal adipose tissue versus human gluteofemoral fat or human abdominal fat. **(B)** Quantification of the mean area of PAT2-labeled pixels demonstrated significantly higher staining in mouse inguinal fat compared to both human fat depots ($*p < 0.05$). There was no difference in the number of PAT2-stained pixels between human gluteofemoral and human abdominal fat.

adipose-derived multipotent progenitor cells. Multiple studies have highlighted the importance of CD34⁺ cells in adipose tissue health, adipogenesis, and angiogenesis. Suga *et al.* observed the correlation between CD34⁺ progenitors and innate cell immaturity or “stemness,” whereas a relative paucity of the population conferred commitment to a developmental fate. This and other studies characterized the role of CD34⁺ cells in adipocyte replicative capacity and vasculogenesis.^{25,26} The translational value of the CD34⁺ cell population was highlighted by Matsumoto *et al.*, in a first-ever study establishing the benefits in soft tissue augmentation, of enriching fat grafts with adipose resident multipotent progenitor cells.²⁷ Ideally, multiple progenitor cell markers would be used to identify ADSCs. Unfortunately, limitations in spectral bandwidth

prevented the addition of a fifth, distinct fluorophore channel to do so. We validated our single-marker protocol by staining separately for CD105, a second surface antigen with a well-documented connection to progenitor cell multipotentiality. The results revealed a tight correlation between CD34 and CD105 staining in all fat depots considered.

Overall, this investigation demonstrates that mouse fat is more vascularized compared with human fat. Specifically, the mean distance between adipocytes and blood vessels is greater in human compared to mouse fat. Similarly, the mean distance between CD34⁺ stromal cells and blood vessels is significantly greater in human fat. To control for the inherent heterogeneity of the whole mounted fat samples, multiple samples were prepared for each specimen

FIG. 8. Relative paucity of white adipocytes in human and mouse white fat. **(A)** Mouse inguinal fat pad, human abdominal fat, and human gluteofemoral adipose tissue samples were stained with the white adipose tissue-specific marker ASC-1 and Hoescht. Representative confocal images show equal numbers of ASC-1-labeled cells among all samples in both species. **(B)** Quantification of the mean area of ASC-1-labeled pixels demonstrated no difference in the number of ASC-1-stained pixels between human gluteofemoral and human abdominal fat.

obtained from the 12 human and 12 mouse subjects. Moreover, quantification of vasculature and CD34⁺ stromal cells were normalized to each sample's total adipocyte cell count. Interestingly, there was no significant difference in the concentration of CD34⁺ stromal cells in samples from either species. As anticipated, human fat cells are larger compared with mouse cells, possessing a greater degree of size heterogeneity.

The variability observed also could be accounted for by the stark difference in species size. It is known that small animals such as mice function at a relatively higher metabolic rate compared to humans due to their size and ther-

moregulatory requirements.²⁸ For this reason, mice possess a greater percentage of heat-dissipating BAT, which is more vascularized than WAT.²⁹ A potential mechanism for this phenomenon involves brown adipocytes developing within deposits of WAT either upon cold or sympathetic nervous stimulation.³⁰ These brown adipocytes are thought to differentiate from unique precursor cells which originate in WAT, and express genes characteristic of both white and brown adipocytes.^{31–34} In the current study, we explored the heterogeneity of WAT stores with respect to the relative contribution of brown and white adipocytes by staining additional samples for PAT2, a surface marker of brown adipocytes,

and ASC-1 for white adipocytes.³⁵ The expression of PAT2 correlates strongly with the BAT-specific protein UCP1. The protein ASC-1 is a plasma membrane localized sodium-independent neutral amino acid exchange transporter, encoded by the SLC7a10 gene.³⁵ There was significantly more PAT2 staining in the mouse inguinal fat specimens compared with human abdominal fat or human gluteofemoral fat; however, there was no significant difference between the degree of ASC-1 labeling among the samples from both species. These data are compatible with the literature showing that BAT is a key thermogenic tissue in small mammals, including rodents.³¹

Beyond comparative anatomy, the cellular architecture gleaned from this study provides metrics on putative healthy adipose tissue from both human and mouse. This could serve as a baseline for comparison in studies that depend on following structural changes in adipocytes over time. To wit, murine models are popularly used to study human lipoaspirate grafting, such as in reconstructive surgery. Autologous adipose tissue grafting is a ubiquitous technique used by reconstructive surgeons, but despite its universality, the practice of fat grafting is complicated by unpredictable long-term volume retention. Understanding the structural, metabolic, and genetic factors influencing graft retention has been the focus of ongoing translational research in mice. One key lies in understanding the endogenous fat depots that naturally exist in humans; cumulatively, they represent the healthiest, most durable adipocyte populations in the body. One hypothesis is that the most durable fat grafts mature to more closely resemble the architecture of endogenous fat. Three-dimensional confocal microscopy provides the baseline for healthy, endogenous fat that will empower this analysis.

Conclusion

The basic architecture of human adipose tissue differs significantly from that of mice. Thus, more research needs to be conducted into establishing whether these structural differences confer variance in functional properties between adipocytes of both species, specifically when designing murine models of human disease.

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Disclosure Statement

No competing financial interests exist.

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