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Differences in In Vivo Cellular Kinetics in Abdominal and Femoral Subcutaneous Adipose Tissue in Women

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The accumulation of fat in upper-body (abdominal) adipose tissue is associated with obesity-related cardiometabolic diseases, whereas lower-body (gluteal and femoral) fat may be protective. Studies suggest physiological and molecular differences between adipose depots and depot-specific cellular mechanisms of adipose expansion. We assessed in vivo cellular kinetics in subcutaneous adipose tissue from the abdominal (scABD) and femoral (scFEM) depots using an 8-week incorporation of deuterium (^2H) from ^2H_2O into the DNA of adipocytes and preadipocytes in 25 women with overweight or obesity. DNA synthesis rates denote new cell formation of preadipocytes and adipocytes in each depot. Formation of adipocytes was positively correlated to that of preadipocytes in the scABD and scFEM depots and was related to percent body fat in each depot. Notably, preadipocytes and adipocytes had higher formation rates in the scFEM depot relative to the scABD. This method to assess in vivo adipogenesis will be valuable to evaluate adipocyte kinetics in individuals with varying body fat distributions and degrees of metabolic health and in response to a variety of interventions, such as diet, exercise, or pharmacological treatment.

OBESITY STUDIES

Adipose tissue (AT) distribution, versus overall obesity, may be a better determinant of metabolic health risk. Inherent differences exist in fat depots, as abdominal AT (visceral [VAT] and subcutaneous abdominal [scABD]) is associated with obesity complications, whereas lower-body fat (gluteal and femoral [scFEM]) may be protective (reviewed in 1). Adipose expansion involves the enlargement of existing adipocytes (hypertrophy) and the proliferation and differentiation of preadipocytes to adipocytes (hyperplasia). Evidence suggests that limited expandability of subcutaneous AT, i.e., impaired adipogenesis, may contribute to the development of insulin resistance and type 2 diabetes (reviewed in 2) (3,4). Given the intrinsic differences between AT stores, differences in adipogenesis probably contribute to depot heterogeneity with respect to metabolic health.

In vitro studies have shown that preadipocytes from fat depots differ in adipogenic potential, with scABD having higher proliferative and differentiation capacity than scFEM and VAT (5–10). Other evidence suggests that fat expansion post-overfeeding involves depot-specific mechanisms, as the scABD depot expands via hypertrophy and the scFEM via hyperplasia (11). Although in vitro approaches have provided insight into the mechanisms underlying adipogenesis, these measures do not provide a comprehensive in vivo assessment within the natural microenvironment of AT. Hence, little is known about in vivo adipocyte kinetics in human depots. Retrospective analyses by Spalding et al. (12) show constant adipocyte turnover in humans; however, these studies assessed the scABD depot and did not measure preadipocyte kinetics.

To measure the dynamics of AT expansion, we assessed in vivo adipogenesis in scABD and scFEM depots using an 8-week incorporation of deuterium (^2H), administered as ^2H_2O (13), into the DNA of adipocytes and preadipocytes in 25 women with obesity. Measures of DNA synthesis denote new cell formation/proliferation. For the first time, we show that in scABD and scFEM depots, the proliferation

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of preadipocytes correlates with the formation of adipocytes. Importantly, our analyses identify higher in vivo preadipocyte and adipocyte formation in the scFEM depot compared with the scABD. In addition, preadipocyte and adipocyte kinetics highly correlate with body fat content.

RESEARCH DESIGN AND METHODS

Subject Characteristics

Healthy women with overweight or obesity were recruited according to the following inclusion criteria: 18–40 years of age, BMI 27–38 kg/m^2, fasting plasma glucose ≤110 mg/dL, blood pressure ≤140/90 mmHg, absence of major organ disease, normal screening urinary and blood laboratory tests, weight stable for ≥3 months, and no significant changes in diet or physical activity in the previous month. Exclusion criteria consisted of a history of diagnosed diabetes, chronic use of medications with potential metabolic effects (glucocorticoids, adrenergic agents, or thiazolidinediones), or use of medications or procedures that cause weight gain or loss. Subjects with HIV, hepatitis B, or hepatitis C were excluded, as were pregnant or breastfeeding women. The institutional review board of Pennington Biomedical Research Center approved all procedures, and all subjects gave written informed consent.

Anthropometric characteristics (height, weight, and waist-to-hip ratio [WHR]) were taken at the beginning of the study. Following the 8-week labeling period, anthropometric measures were repeated, body composition was measured by dual-energy X-ray absorptiometry using the whole-body scanner GE iDXA, and AT samples were collected.

^2H_2O Labeling Protocol

After subjects were enrolled in the study, aliquots of 99.9% ^2H_2O (Sigma-Aldrich) were administered in sterile plastic containers. Participants were instructed to drink three 35-mL divided doses per day for week 1 (priming period) and two 35-mL doses per day for weeks 2–8. This protocol maintains near-plateau body ^2H_2O enrichments (1.5–2.5%) during the 8 weeks. Compliance with ^2H_2O intake was monitored through weekly urine collections and the return of vials for counting.

Collection of Blood Monocytes

Blood was collected in Ficoll-Paque solution, and the mononuclear fraction was removed after centrifugation. Monocytes were isolated as CD14$^+$ cells by magnetic beads (EasySep).

Body H_2O Enrichments

^2H enrichments in body water were measured from urine (14). A 15- to 20-μL sample of urine was reacted in an evacuated gas chromatography vial with calcium carbide to produce acetylene. The acetylene gas was transferred into an evacuated gas chromatography vial containing 10% bromine in carbon tetrachloride and incubated at room temperature for 2 h to produce tetrabromoethane. Excess bromine was neutralized with 25 μL of 100% cyclohexene. The tetrabromoethane, containing hydrogen atoms from body H_2O, was analyzed by gas chromatography–mass spectrometry (GC-MS) (14). ^2H_2O enrichments were calculated by comparison with standard curves generated by mixing 100% ^2H_2O with natural abundance ^2H_2O in known proportions.

AT Collection

Adipose biopsies were performed following week 8 of ^2H_2O intake. Two sites were sampled: scABD (two-thirds of the distance from the iliac spine to the umbilicus) and scFEM (anterior aspect of the thigh, one-third of the distance from the superior iliac spine to the patella) regions. After cleansing the skin with povidone-iodine solution, topical anesthesia containing 2% lidocaine/0.5% bupivacaine was administered. A 0.75-cm incision was made in the skin, and AT was aspirated using a Bergstrom and Mercedes needle. The tissue was placed in sterile tubes for processing.

Isolation of Purified Adipocytes and Preadipocytes

scABD and scFEM tissues were treated with a HEPES/type 1 collagenase solution for 1 h at 37°C to isolate adipocytes and the stromal vascular fraction (SVF) (15). The adipocytes were purified by incubation with a cocktail of biotinylated antibodies against markers of endothelial cells (CD31, eBioscience), hematopoietic cells (CD45, BioLegend), and mesenchymal stem cells (CD34, eBioscience) for 15 min at room temperature. Cells attached to these antibodies were bound by magnetic nanoparticles (EasySep), and immunopurified adipocytes were removed and retained. Adipocytes were flash frozen in liquid N_2 and stored at −80°C until DNA extraction.

To isolate preadipocytes, a protocol was used to exploit their property to attach to plastic after a short-term culture of the SVF (16). The SVF was reconstituted in erythrocyte lysis buffer for 5 min at room temperature, centrifuged, reconstituted in 10% FBS in minimal essential medium (αMEM), and cultured for 6 h. The nonadherent cells were washed from the culture plate with PBS. The adherent preadipocytes were detached (0.25% trypsin/ethylenediaminetetraacetic acid [EDTA]) and centrifuged, and the pellet was stored at −80°C until DNA extraction.

DNA Preparation

^2H-labeled DNA was isolated from the adipocytes, preadipocytes, and blood monocytes using DNeasy microDNA extraction kits (QIAGEN). Because mitochondrial DNA (mtDNA) represents a small fraction of genomic DNA and has a slow replication rate, we did not eliminate mtDNA from these analyses. DNA was enzymatically hydrolyzed to free deoxyribonucleosides (17), and hydrolysates were derivatized to pentafluorobenzylhydroxylamine (PFBHA) derivatives for GC-MS analysis.

GC-MS Analyses and Calculation of Fraction of New Cells (f)

The PFBHA derivatives were analyzed for incorporation of ^2H_2O on an Agilent 6890/5973 GCMS equipped with a 30-m DB-225 column using methane negative chemical

ionization and collecting ions in selected ion-monitoring mode at m/z 435, 436, and 437 (representing M_0, M_1, and M_2 mass isotopomers, respectively). Baseline (unenriched) DNA standards were measured concurrently to correct for abundance sensitivity.

The enrichment of the M_1 ion above natural abundance in the adipose samples (preadipocytes and adipocytes) was determined by subtracting the M_1 in the standard from the M_1 in the sample. The theoretical maximum M_1 enrichment in adipose cells was calculated using mass isotopomer distribution analysis (MIDA) equations (18) based on the body ^2H_2O exposure integrated over the 8-week period.

$$\text{Fraction of new cells(f)} = \frac{M_1 \text{ enrichment in sample cells}}{\text{Theoretical maximum } M_1 \text{ enrichment}}$$

Blood monocytes were taken at 8 weeks and analyzed to represent a (near) completely turned-over cell. This measurement, used as a reference marker of ^2H_2O exposure, serves to confirm calculations using the theoretical maximum enrichment.

Statistical Analyses

Simple associations were examined using Spearman correlation to assess monotonic nonlinear relationships. A linear mixed-effect model was used to estimate the fraction of new cells (f) and to determine the covariates that may affect "f." Residuals from the model were used to test for normality of "f." The linear model allows for "f" to be adjusted for fixed effects (BMI, percent body fat, and WHR) and random effects (four data measures per subject using a combination of cell types and depots). Differences in "f" values between the preadipocytes and adipocytes from the scABD and scFEM depots were tested using the least square means based on the linear mixed model.

Initial tests for normality indicated that the data (n = 26) was not normally distributed because one subject had a high studentized residual (>3.5). Once this subject was removed, the data were normally distributed. All further analyses used 25 subjects.

RESULTS

The study population included 25 women of African American (n = 14) and Caucasian (n = 11) ancestry, with a mean ± SD age of 31 ± 6 years, BMI of 32.6 ± 2.7 kg/m^2, and 44.3 ± 4.1% body fat. A linear mixed model using overall cellular kinetics (preadipocytes and adipocytes) showed no significant impact of BMI (P = 0.728) or WHR (P = 0.619) on the fraction of new cells, whereas percent body fat had a significant positive effect (P = 0.021). Figure 1 illustrates that the formation of adipocytes was positively correlated to that of preadipocytes in the scABD (Fig. 1*A*) (P = 0.0007) and scFEM (Fig. 1*B*) (P = 0.028) depots.

In Fig. 2, preadipocyte proliferation was significantly higher in the scFEM depot relative to the scABD (Δ = 3.224; P = 0.035). Adipocyte formation was also significantly higher in the scFEM depot relative to the scABD (Δ = 2.877; P = 0.0005).

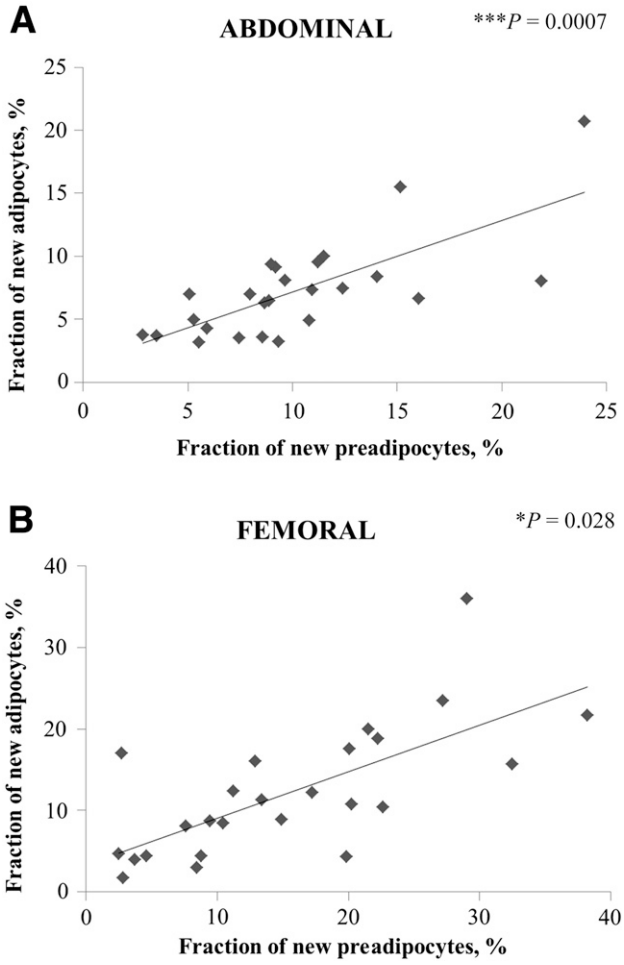

Figure 1—Formation of adipocytes is positively correlated with that of preadipocytes in the scABD (*A*) and scFEM (*B*) depots. Simple associations between the fraction of new adipocytes and preadipocytes were analyzed using Spearman correlation (n = 25). The Spearman correlation between adipocytes and preadipocytes in the scABD depot is 0.6305 (R^2 = 0.3837; P = 0.0007), and it is 0.43932 (R^2 = 0.1835; P = 0.028) in the scFEM depot. *P < 0.05; ***P < 0.01.

We also examined how cellular kinetics was influenced by overall percent body fat. Preadipocyte (P = 0.019) and adipocyte (P = 0.026) formation rates in the scABD depot were positively associated with percent body fat (Fig. 3*A* and *B*). Similarly, preadipocyte (P = 0.092) and adipocyte (P = 0.056) formation rates in scFEM trended to correlate (Fig. 3*C* and *D*).

DISCUSSION

Given the opposing associations between upper-body versus lower-body adipose accumulation with metabolic health consequences, there is a strong rationale to examine depot differences in cellular mechanisms, notably adipogenesis. The method used for this study has been applied (19) and optimized (20) to assess adipose kinetics and

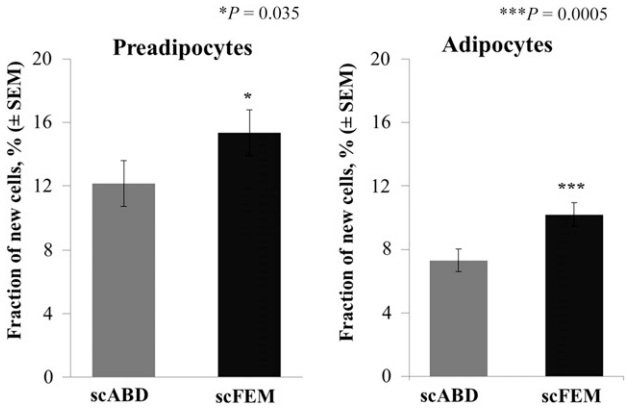

Figure 2—Preadipocytes and adipocytes have higher formation rates in the scFEM depot relative to the scABD. The least square means comparing the fraction of new preadipocytes and adipocytes were derived from the linear mixed model (n = 25). The difference in fraction of new preadipocytes between scFEM and scABD depots is 3.224 (P = 0.0354), and the difference in fraction of new adipocytes between scFEM and scABD depots is 2.877 (P = 0.0005). *P < 0.05; ***P < 0.01.

offers advantages over in vitro techniques, as it provides an integrative view of in vivo adipogenesis within the natural milieu of AT. Recently, an in vivo method assessed fat cell turnover in humans via the incorporation of ^{14}C, derived from the decay of atmospheric radiation after nuclear bomb testing, into adipocyte DNA (12). Relative to our data, this retrospective analysis reported low estimates of adipocyte turnover rates (~10% new cells per year). This difference could be attributed to the inability of the AT to accumulate label over a period of years or decades due to the proliferation and subsequent death of some adipocytes. Our analysis uses a very different experimental protocol with ^{2}H labeling over the course of 8 weeks that has been validated to determine physiological estimates of cell proliferation and adipocyte turnover and has comparable values to other published estimates (reviewed in 17) (13,19,20). In addition, although Spalding et al. (12) assessed scABD adipocyte kinetics, our ^{2}H-labeling study measures both preadipocyte and adipocyte kinetics in metabolically different depots. We present the first evidence that adipose kinetics varies by depot location in humans. Our report reveals higher formation of preadipocytes and adipocytes in the scFEM depot compared with the scABD. Although in vitro assays in primary cultures demonstrated that scFEM preadipocytes exhibit a lower differentiation capacity than scABD (5,9), our data suggest that in vivo scFEM AT has a higher capacity for adipogenesis. Our findings strengthen other experimental observations that implicate the thigh as a more efficient depot to expand and accommodate lipids

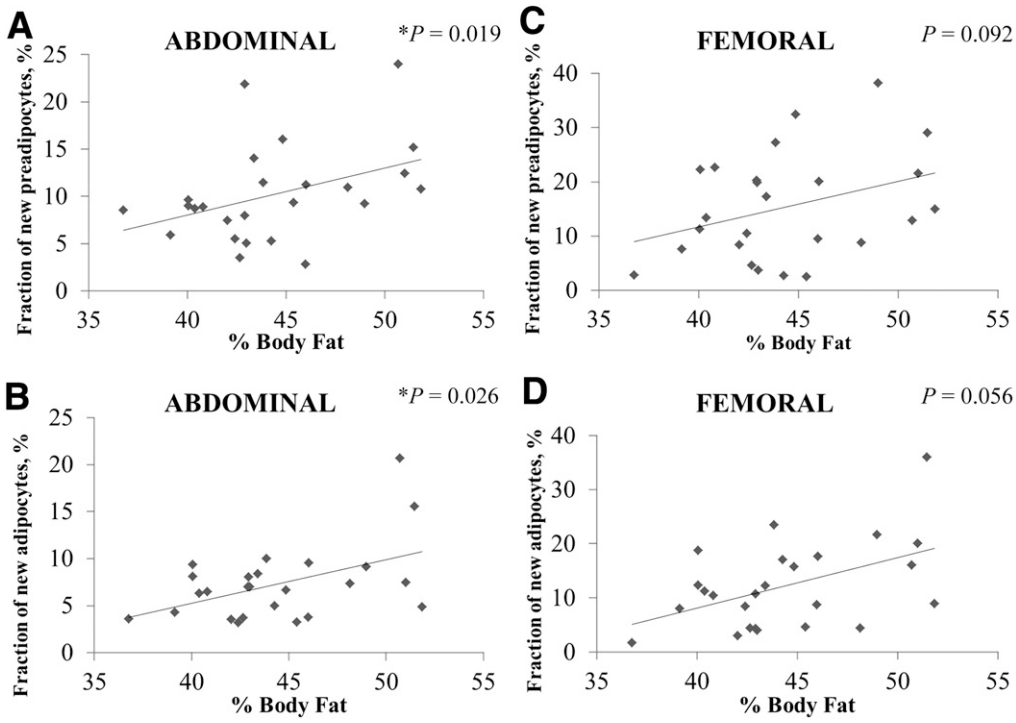

Figure 3—Preadipocyte and adipocyte formation in both scABD (A and B) and scFEM (C and D) depots positively correlates with overall percent body fat. Simple associations between the fraction of new preadipocytes or adipocytes and percent body fat were analyzed using Spearman correlation (n = 25). The Spearman correlation between fraction of new preadipocytes (scABD) and percent body fat is 0.4263 (R^2 = 0.2472; P = 0.019), and it is 0.3291 (R^2 = 0.2346; P = 0.026) between new adipocytes (scABD) and percent body fat. The Spearman correlation between fraction of new preadipocytes (scFEM) and percent body fat is 0.2761 (R^2 = 0.1123; P = 0.092), and it is 0.5358 (R^2 = 0.2116; P = 0.056) between new adipocytes (scFEM) and percent body fat. *P < 0.05.

(reviewed in 21,22), especially in women with a higher percent body fat (Fig. 3).

Interestingly, our data show that preadipocyte formation is higher than that of adipocytes in scABD (P = 0.0006) and scFEM (P = 0.008) depots (Fig. 2). Likewise, the ratios of new preadipocytes-to-adipocytes are correlated between depots (ρ = 0.587; P = 0.002). This may suggest constant preadipocyte recruitment to accommodate energy surplus. Also, because adipocytes arise from preadipocytes, one expects an increase in preadipocytes to precede adipocyte formation.

Our findings suggest that women with a higher percent body fat generate more preadipocytes and adipocytes. Notably, we have not measured cell death, a significant component of adipocyte turnover. In women who are weight-stable (week 0 to week 8 Δ = –0.028; P = 0.99) and assuming a relative steady state in AT during the 8-week labeling period, new cell formation is likely balanced by loss of cells. Therefore, although our study design does not include analysis of cell death, under steady-state conditions, replacement rate measured by ^{2}H label incorporation does legitimately reveal turnover or kinetics of the adipose cell population.

One study limitation is that we have not assessed kinetics in VAT, which is associated with facets of the metabolic syndrome. However, scABD adiposity has also been linked to the pathogenesis of insulin resistance and type 2 diabetes (23,24). Our analyses are strengthened by the inclusion of two functionally divergent depots. Another limitation is that we only assessed overweight and obese women. Hence, our results cannot be extrapolated to cellular kinetics in lean individuals or, because of sex differences in adipose characteristics (reviewed in 25), in men.

In conclusion, our report depicts novel regional differences in adipose kinetics and confirms this in vivo method as an innovative approach to assess individuals with varying adipose distributions and in response to interventions, including diet, exercise, or pharmacological treatment.

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Author Contributions. U.A.W. planned the study design, performed the experiments, analyzed and reviewed the data, and wrote the manuscript. M.D.F. provided guidance for performing the in vivo adipogenesis method, performed the DNA extraction, performed the GC-MS analysis, and provided editorial assistance. R.A.B. analyzed experimental data, described statistical methods, and provided editorial assistance. M.K.H. provided guidance for performing the in vivo adipogenesis method, maintained quality assurance of the GC-MS methods, reviewed the data, contributed to the discussion, and provided editorial assistance. E.R. provided the funding, planned the study design, reviewed the data, contributed to discussions, provided guidance for development of the manuscript, and provided editorial assistance. E.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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