



## Racial differences in in vivo adipose lipid kinetics in humans

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**Abstract** The storage of lipids in the form of triglycerides (TGs) and the de novo synthesis (lipogenesis) of fatty acids from nonlipid precursors [de novo lipogenesis (DNL)] are important functions of adipose tissue (AT) that influence whole-body metabolism. Yet, few studies have reported in vivo estimates of adipose lipid kinetics in humans. Fifty-two women with obesity (27 African-American and 25 Caucasian; 29.7 ± 5.5 years; BMI 32.2 ± 2.8 kg/m<sup>2</sup>; 44.3 ± 4.0% body fat) were enrolled in the study. In vivo synthesis (or replacement) of TGs ( $f_{TG}$ ) as well as the synthesis of the fatty acid, palmitate [a measure of adipose DNL ( $f_{DNL}$ )], were assessed using an 8 week incorporation of deuterium into lipids (glycerol and palmitate moieties of TGs) in subcutaneous abdominal (scABD) and subcutaneous femoral (scFEM) AT. We report, for the first time, significant race differences in both TG synthesis and absolute DNL, with Caucasians having higher  $f_{TG}$  and  $f_{DNL}$  as compared with African-Americans. The DNL contribution to newly synthesized TG (corrected  $f_{DNL}$ ) was not different between races. Interestingly, our findings also show that the scFEM adipose depot had higher TG replacement rates relative to the scABD. Finally, the replacement rate of TG ( $f_{TG}$ ) was negatively correlated with changes in body weight over the 8 week labeling period. Our results provide the first evidence that in vivo TG replacement (synthesis and breakdown) rates differ by ethnicity. In addition, TG turnover varies by depot location in humans, implying an increased capacity for TG storage and higher lipolytic activity in the scFEM AT.—White, U. A., M. D. Fitch, R. A. Beyl, M. K. Hellerstein, and E. Ravussin. Racial differences in in vivo adipose lipid kinetics in humans. *J. Lipid Res.* 2018. 59: 1738–1744.

**Supplementary key words** adipose tissue • in vivo triglyceride synthesis • in vivo de novo lipogenesis • adipose kinetics • race differences • clinical studies

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Adipose tissue (AT) is a vital organ that is a significant regulator of whole-body energy metabolism and energy balance (1, 2). An important function of AT is the storage of lipids in the form of triglycerides (TGs) to maintain lipid and glucose homeostasis. Evidence suggests that a decreased capacity to synthesize and store TGs in subcutaneous AT may result in increased lipid circulation and deposition in ectopic tissues, such as skeletal muscle, liver, heart, and pancreas, contributing to the development of the metabolic syndrome and T2D (3–5). The majority of published studies utilized in vitro and indirect methods to estimate the synthesis of TGs in humans (6, 7), while only limited investigations have applied an in vivo approach to measure TG kinetics (8, 9). Recent studies reported that in vivo rates of TG synthesis in adipose cells were significantly lower in insulin-resistant as compared with insulin-sensitive subjects (10, 11), providing evidence that TG kinetics in subcutaneous AT plays a significant role in metabolism. To date, no studies have assessed in vivo TG kinetics in human populations of different races.

Another function of AT is the ability to synthesize de novo fatty acids from nonlipid precursors. Although the liver has been implicated as the primary site for de novo lipogenesis (DNL) in humans, recent long-term labeling studies have demonstrated a significant contribution from adipose DNL to adipose lipid stores (8, 12). The role of DNL in human metabolic health and diseases is not established. Studies in subjects with obesity have shown that insulin resistance and hepatic steatosis are positively correlated with liver DNL, but negatively correlated with the expression of DNL genes in AT (13, 14). Other studies have shown suppressed expression of adipose DNL genes in human obesity and T2D (13–15), in agreement with in vivo studies showing reduced DNL in the AT of insulin-resistant

Abbreviations: AIC, Akaike information criterion; AT, adipose tissue; BP, blood pressure; DNL, de novo lipogenesis; EMCL, extramyocellular lipid; <sup>2</sup>H, deuterium; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; OGTT, oral glucose tolerance test; scABD, subcutaneous abdominal; scFEM, subcutaneous femoral; TAT, total abdominal adipose tissue; TG, triglyceride; VAT, visceral adipose tissue.

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versus insulin-sensitive subjects (10, 11). Together, these data suggest an important, perhaps beneficial, role for adipose DNL. Yet, studies using direct in vivo measures of adipose DNL in humans remain scarce.

We previously assessed in vivo cell kinetics of subcutaneous AT using an 8 week incorporation of deuterium ( $^2\text{H}$ ), administered as  $^2\text{H}_2\text{O}$ , into the DNA of adipose cells in women with obesity (16, 17). In this same population of women, we have now applied this  $^2\text{H}$ -labeling protocol to measure the in vivo synthesis of new TGs ( $f_{\text{TC}}$ ) via the incorporation of  $^2\text{H}_2\text{O}$  in the glycerol moiety of TGs, as well as the synthesis of the fatty acid, palmitate, providing a measure of adipose DNL ( $f_{\text{DNL}}$ ) in subcutaneous abdominal (scABD) and subcutaneous femoral (scFEM) AT. Our findings demonstrate significant racial differences in TG replacement (synthesis and breakdown) and palmitate synthesis rates, with Caucasians having higher  $f_{\text{TC}}$  and  $f_{\text{DNL}}$  as compared with African-Americans. Our data also show that the scFEM adipose depot had higher TG replacement rates relative to the scABD. Finally,  $f_{\text{TC}}$  was negatively correlated with changes in body weight in women with obesity.

## MATERIALS 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<sup>2</sup>, fasting plasma glucose  $\leq$ 110 mg/dl, blood pressure (BP)  $\leq$ 140/90, absence of major organ disease, normal screening urinary and blood laboratory tests (i.e., complete blood count, renal panel, electrolytes, and liver enzymes), weight stable for  $\geq$ 3 months ( $\pm$  3 kg), and no self-reported significant changes in diet or physical activity in the previous month. Exclusion criteria included a history of diagnosed diabetes, heart or liver disease, chronic use of medications with potential effects on glucose or lipid metabolism, or use of medications or surgical procedures that causes weight gain or loss. Subjects with HIV or hepatitis B or C were excluded, as were pregnant or breastfeeding women. Pennington Biomedical Research Center's Institutional Review Board approved all procedures, and all subjects gave written informed consent.

### $^2\text{H}_2\text{O}$ labeling protocol

After enrollment in the study, aliquots of 99.9%  $^2\text{H}_2\text{O}$  (Sigma-Aldrich) were administered in sterile plastic containers. Participants were instructed to drink three 35 ml doses given three times per day for week 1 (priming period) and two 35 ml doses per day for weeks 2–8. As previously shown, this protocol maintains near-plateau body  $^2\text{H}_2\text{O}$  enrichments (1.5–2.5%) during the 8 weeks (18). Compliance with  $^2\text{H}_2\text{O}$  intake was monitored through weekly return of vials for counting and urine collections to measure  $^2\text{H}$  enrichment.

$^2\text{H}$  enrichments in body water (urine) were measured by isotope ratio MS. Urine samples were cleaned using activated charcoal and filtered. Isotope enrichment was directly analyzed with an H-Device attached to a Delta V Advantage mass spectrometer.  $^2\text{H}_2\text{O}$  enrichments were calculated by comparison to standard curves generated by mixing 100%  $^2\text{H}_2\text{O}$  with natural abundance  $^2\text{H}_2\text{O}$  in known proportions. The integrated (area under the curve) exposure to  $^2\text{H}_2\text{O}$  for each subject was calculated as the area under the  $^2\text{H}$ -water enrichment time course for individual  $^2\text{H}$ -water enrichment measurements using the trapezoid method.

### Study procedures

Anthropometric characteristics {height; metabolic weight; waist-hip ratio; mean BP [1/3 (systolic BP – diastolic BP) + diastolic BP]} were taken at the start of the study. Fasting plasma total TGs, HDL, LDL, and total cholesterol were measured. Following the 8 week  $^2\text{H}$ -labeling period, anthropometric measures were repeated. Despite instructions to maintain body weight and regular meetings with the study staff, small weight changes were observed, and  $\Delta$  body weight was calculated as final weight (week 8) – initial weight (study start).

### Body composition measures (week 8)

Dual-energy X-ray absorptiometry (DXA) was performed using the General Electric Lunar iDXA to determine total body fat. Scans were analyzed with enCORE software version 13.60.033. scABD AT and VAT volumes were defined and quantified with MRI using a 3.0T scanner (GE; Discovery 750w) by obtaining  $\sim$ 581 images from the dome of the liver to the pubic symphysis. Images were analyzed by a single trained analyst. Estimates of VAT and scABD AT volumes were converted to mass using an assumed density of 0.92 kg/l. Visceral AT (VAT):total abdominal AT (TAT) reflects the percentage of abdominal fat that is VAT and was calculated as VAT/(scABD AT + VAT).

Intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) in the soleus and anterior tibialis skeletal muscles, as well as intrahepatic lipid (IHL), were measured with proton MRS ( $^1\text{H}$ -MRS) on a 3.0T whole body imaging and spectroscopy system (GE; Discovery 750w System) using a commercially available  $^1\text{H}$  body coil. IMCL, EMCL, and IHL content was determined with jMRUI (Java-based magnetic resonance user interface) (19) as previously described (20–22). IMCL, EMCL, and IHL peak areas are expressed relative to the peak area of a peanut oil phantom signal of known constant concentration, as previously described (23).

### Oral glucose tolerance test (week 8)

Glucose tolerance was assessed by a 2 h oral glucose tolerance test (OGTT) and insulin sensitivity calculated by the Matsuda Index [10,000/square root of (fasting glucose  $\times$  fasting insulin)  $\times$  (mean OGTT glucose  $\times$  mean OGTT insulin)] (24).

### AT collection (week 8)

AT biopsies were collected with a Bergstrom and a Mercedes needle under sterile conditions and local anesthesia. Samples were taken from the scABD region, between one- and two-thirds of the distance from the iliac spine to the umbilicus, and from the scFEM region, on the anterior aspect of the thigh, one- to two-thirds of the distance from the superior iliac spine to the patella. The tissue was immediately placed in sterile tubes and frozen in liquid N<sub>2</sub> for processing. The procedures were the same and the measurements were consistent for all biopsy collections.

### AT lipid extraction

AT lipids were extracted using the chloroform-methanol (2:1) Folch extraction method, and TGs were isolated via thin-layer chromatography. Adipose TG was incubated with 3 N methanol-HCl. Fatty acid methyl esters were separated from glycerol. The fraction containing the glycerol was further derivatized to the glycerol triacetate derivative for GC-MS analysis (25).

### GC-MS analyses and calculation of fraction of new TGs and DNL

TG-glycerol isotopic enrichments of the glycerol-triacetate derivative were measured by GC-MS (models 6890 and 5973; Hewlett-Packard, Palo Alto, CA) using a DB-225 fused silica column in methane chemical ionization mode monitoring  $m/z$  ratios

of 159, 160, and 161 (representing  $M_0$ ,  $M_1$ , and  $M_2$  isotopomers), as previously described (25). Palmitate methyl ester enrichments were determined by GC-MS using a DB-17 column with helium as carrier gas and electron ionization mode monitoring  $m/z$  270, 271, and 272 for  $M_0$ ,  $M_1$ , and  $M_2$ , as previously described (26). Baseline (unenriched) standards were measured concurrently to correct for abundance sensitivity.

### Calculations for TG-glycerol synthesis from $^2\text{H}_2\text{O}$

The enrichment of the  $M_1$  ion above natural abundance in the TG-glycerol samples was determined by subtracting the  $M_1$  in the standard from the  $M_1$  in the sample. The theoretical maximum  $M_1$  enrichment in TG-glycerol samples was calculated using MIDA equations (27) based on the body  $^2\text{H}_2\text{O}$  exposure integrated over the 8 week period. Newly synthesized TG-glycerol during the  $^2\text{H}_2\text{O}$  administration was calculated using the following equation:

$$f_{\text{TG}} = \frac{\text{EM}_1[\text{TG-glycerol}]}{A^\infty_1[\text{TG-glycerol}]}$$

where  $f_{\text{TG}}$  is the fraction of newly synthesized TG molecules present in the adipose,  $\text{EM}_1$  is the excess mass isotopomer abundance for  $M_1$ -glycerol, and  $A^\infty_1$  is the asymptotic mass isotopomer  $M_1$  abundance of a fully labeled glycerol. The  $A^\infty_1$  was calculated using the body water  $^2\text{H}$  enrichment, assuming that the number of exchangeable C-H bonds in glycerol during the  $^2\text{H}$  labeling is 4 (25). Under conditions of metabolic balance or steady-state,  $f_{\text{TG}}$  represents both synthesis and breakdown (i.e., lipolysis) of TGs and, thus, reflects replacement or turnover of TGs.

### Calculations for DNL

The fraction of palmitate synthesized from the DNL pathway ( $f_{\text{DNL}}$ ) was calculated from body water enrichment and TG-palmitate  $^2\text{H}$  enrichment using precursor-product relationships, as previously described (8). Corrected  $f_{\text{DNL}}$ , which represents the fraction of newly deposited adipose TG-palmitate that derived from the DNL pathway, was calculated by dividing  $f_{\text{DNL}}$  by  $f_{\text{TG}}$ , as previously described (8). This parameter corrects for the large fraction of TG that was preexisting in AT before the 8 week labeling period, for which the palmitates could not have derived from the DNL pathway measured during the study.

### Statistical analysis

A linear mixed effect model was used to estimate the fraction of new TGs ( $f_{\text{TG}}$ ) and DNL ( $f_{\text{DNL}}$ ) over 8 weeks and to determine the covariates associated with " $f_{\text{TG}}$ " and " $f_{\text{DNL}}$ ". Residuals from the model were used to test for normality of " $F$ ". Descriptive statistics for all of the observed covariates have been previously presented (17). Except for plasma TG levels, there were no race differences in any of the observed covariates at baseline (Table 1). Model selection used Akaike information criterion (AIC) to determine which set of covariates best described the data, and every combination of covariates was investigated. AIC combines information about the fit of the data with the number of covariates used. A lower AIC score indicates a better fitting model. Based on the results from these models, simple associations between  $f_{\text{TG}}$  and  $f_{\text{DNL}}$  with the selected covariates were examined using Pearson's correlation.

## RESULTS

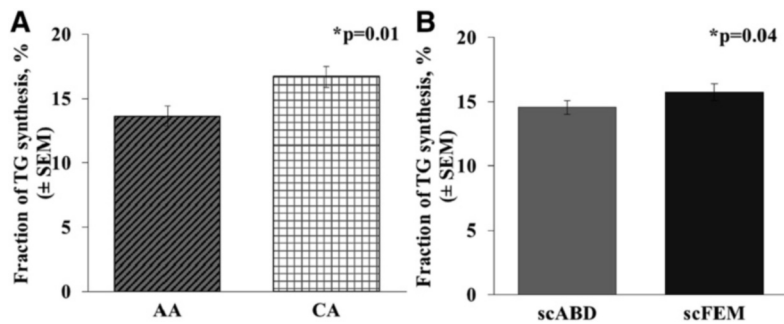
The study population included 52 women, 27 African-Americans and 25 Caucasians. Anthropometric and clinical characteristics of all women in the study have been previously reported (17). The model that best described the data for  $f_{\text{TG}}$  (lowest AIC) included the following: age, percent body fat,  $\Delta$  body weight, VAT:TAT, fraction of new adipocytes, race, and depot location. From this model,  $f_{\text{TG}}$  was significantly associated with depot location ( $P = 0.03$ ), race ( $P = 0.01$ ), and  $\Delta$  body weight ( $P = 0.002$ ). The model that best described the data for  $f_{\text{DNL}}$  (lowest AIC) included age, percent body fat,  $\Delta$  body weight, VAT:TAT, fraction of new adipocytes, race, and depot location. From this model,  $f_{\text{DNL}}$  was significantly associated with race ( $P = 0.003$ ), percent body fat ( $P = 0.02$ ), age ( $P = 0.003$ ), and  $\Delta$  body weight ( $P < 0.0001$ ). Further analyses and presentation of data focus on simple linear correlations, depicted by depot, between  $f_{\text{TG}}$  and  $f_{\text{DNL}}$  and the covariates to which they are significantly associated.

As shown in Fig. 1A, newly synthesized adipose TG ( $f_{\text{TG}}$ ) was significantly higher in Caucasians versus African-Americans ( $\Delta = 3.05\%$ ;  $P = 0.01$ ). Figure 1B demonstrates that

TABLE 1. Race differences in the descriptive statistics for covariates

Variable	African-Americans (n = 27)	Caucasians (n = 25)	P
Age (years)	30.85 ± 3.91	28.64 ± 6.59	0.14
BMI (kg/m <sup>2</sup> )	32.50 ± 2.63	31.53 ± 2.79	0.20
Waist-hip ratio	0.87 ± 0.07	0.83 ± 0.06	0.10
Body weight (kg)	87.06 ± 8.21	85.94 ± 8.85	0.63
$\Delta$ Body weight (kg) (week 8-week 0)	-1.03 ± 1.81	-0.52 ± 2.21	0.37
Percent body fat	43.70 ± 3.77	44.52 ± 3.86	0.44
TAT (kg)	8.92 ± 8.34	9.00 ± 8.24	0.87
scABD AT (kg)	7.81 ± 1.36	7.86 ± 1.51	0.91
VAT (kg)	0.94 ± 0.38	1.14 ± 0.44	0.09
VAT:TAT (%)	10.66 ± 3.58	12.64 ± 3.56	0.05
Leg fat (%)	45.39 ± 4.68	45.96 ± 4.47	0.66
IHL (%)	3.78 ± 6.07	2.30 ± 1.84	0.24
IMCL (%)	0.85 ± 0.49	0.78 ± 0.32	0.56
EMCL (%)	3.59 ± 2.36	3.31 ± 1.95	0.63
Matsuda index	3.69 ± 2.13	4.62 ± 1.77	0.11
Mean BP (mmHg)	84.27 ± 8.08	80.61 ± 6.26	0.08
Total cholesterol (mg/dl)	175.85 ± 41.46	185.16 ± 38.26	0.40
LDL (mg/dl)	105.2 ± 35.62	109.1 ± 26.49	0.65
HDL (mg/dl)	58.07 ± 15.27	57.18 ± 11.99	0.81
HDL:total cholesterol	0.36 ± 0.07	0.34 ± 0.06	0.47
TRIG (mg/dl)	65.51 ± 23.59	102.24 ± 68.22	0.01

Data are expressed as mean ± SD.

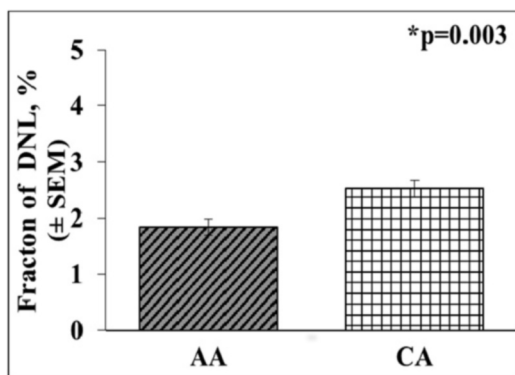


**Fig. 1.** TG synthesis (i.e., replacement) was significantly higher in Caucasians versus African-Americans (A) and in the scFEM depot relative to the scABD depot (B). The least square means comparing the fraction of new TGs ( $f_{TG}$ ) were derived from the linear mixed model [27 African-Americans (AA) and 25 Caucasians (CA)]. A: The difference in TG replacement between African-Americans and Caucasians is 3.05% ( $P = 0.01$ ). B: The difference in  $f_{TG}$  between scFEM and scABD depots is 1.18% ( $n = 52$ ;  $P = 0.04$ ).

TG replacement was significantly higher in the scFEM depot compared with the scABD ( $\Delta = 1.17\%$ ;  $P = 0.04$ ).

**Figure 2** demonstrates that newly synthesized palmitate ( $f_{DNL}$ ) was significantly higher in Caucasians versus African-Americans ( $\Delta = 0.68\%$ ;  $P = 0.003$ ). Corrected  $f_{DNL}$ , or the contribution of DNL to new TGs, was not different between the races (data not shown). TG synthesis was significantly correlated with DNL in the scABD ( $r = 0.63$ ;  $P < 0.0001$ ) and scFEM ( $r = 0.66$ ;  $P < 0.0001$ ) adipose depots (data not shown).

We then investigated how in vivo TG synthesis and DNL (palmitate synthesis) rates were associated with anthropometric and clinical characteristics using simple correlation analyses. **Figure 3** shows that  $f_{TG}$  was negatively associated with  $\Delta$  body weight in both scABD ( $P = 0.01$ ) and scFEM (trend;  $P = 0.11$ ) adipose depots. Age, percent body fat, and VAT:TAT were also investigated, but none of these variables correlated with  $f_{TG}$  in either depot. The  $f_{DNL}$  was also negatively associated with 8 week  $\Delta$  body weight in the scABD ( $P = 0.0004$ ) and scFEM ( $P = 0.0006$ ) depots (data not shown). DNL rates in the scABD depot ( $P = 0.03$ ), but not the scFEM depot ( $P = 0.91$ ), were negatively correlated with percent body fat (data not shown). Age and VAT:TAT were also investigated, but not significantly associated with  $f_{DNL}$  in either depot.



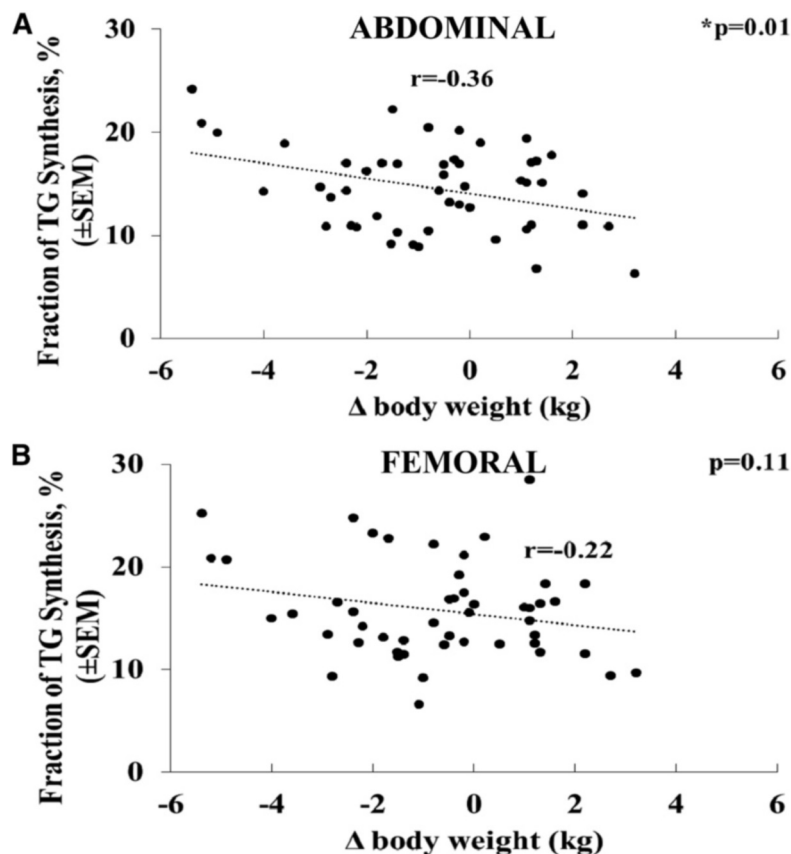
**Fig. 2.** Palmitate synthesis ( $f_{DNL}$ ) was significantly higher in Caucasians versus African-Americans. The least square means comparing the fraction of new palmitates ( $f_{DNL}$ ) were derived from the linear mixed model [27 African-Americans (AA) and 25 Caucasians (CA)]. The difference in palmitate synthesis ( $f_{DNL}$ ) between African-Americans and Caucasians is 0.68% ( $P = 0.003$ ).

## DISCUSSION

The objective of this study was to examine the in vivo synthesis of TGs ( $f_{TG}$ ) and DNL ( $f_{DNL}$ ), which both represent markers of subcutaneous AT lipid dynamics, in a population of women with obesity. Our novel findings demonstrate significant racial differences in both TG replacement and DNL, with Caucasians having higher  $f_{TG}$  and  $f_{DNL}$  as compared with African-Americans. Corrected  $f_{DNL}$  was not different between racial groups. Importantly, our data also show that the scFEM adipose depot had higher TG replacement rates relative to the scABD adipose depot. Finally,  $f_{TG}$  and  $f_{DNL}$  were negatively correlated with changes in body weight over the 8 week study period, suggesting higher lipid kinetics in women that lost more weight. Our mean estimates of both  $f_{TG}$  ( $14.55 \pm 0.54\%$  in the scABD depot;  $15.73 \pm 0.65\%$  in the scFEM depot) and  $f_{DNL}$  ( $2.26 \pm 0.11\%$  in the scABD depot;  $2.09 \pm 0.11\%$  in the scFEM depot) are comparable to previously reported measures of TG synthesis and absolute DNL rates (8, 11). Our findings also estimate the contribution from DNL to newly deposited TG (corrected  $f_{DNL}$ ) to be  $\sim 15\%$  in the scABD depot and  $\sim 13\%$  in the scFEM depot, similar to prior estimates of  $\sim 20\%$  (8).

This study demonstrates, for the first time, a significant difference between African-Americans versus Caucasians in in vivo TG replacement in AT depots of women with obesity, with higher rates (TG synthesis and breakdown) in Caucasians. Previous studies have reported race differences in cardiometabolic health, with African-Americans tending to have increased hypertension, abdominal obesity, and insulin resistance compared with Caucasians (28–31). Additional investigations have documented race differences in plasma lipid profiles (32–34). However, to our knowledge, this is the first study to describe differences in in vivo adipocyte lipid kinetics among races.

Many lines of evidence suggest that the impaired capacity for TG storage in subcutaneous AT may impact whole-body metabolism and contribute to the development of obesity-related disorders (9–11). Recent studies in humans reported that the AT of insulin-resistant subjects had impaired in vivo TG synthesis ( $f_{TG}$ ) relative to insulin-sensitive subjects (10, 11). This perspective suggests that higher  $f_{TG}$ , or TG synthesis, should be associated with better metabolic health. It is important to note that, theoretically, the  $f_{TG}$  measurement reflects both synthesis and breakdown of adipose TG stores under steady-state or near-steady state



**Fig. 3.** TG synthesis (i.e., replacement) in the scABD and scFEM depots negatively correlates with  $\Delta$  body weight. Simple associations between the fraction of new TGs and  $\Delta$  body weight were analyzed using Spearman's correlation ( $n = 52$ ). The Spearman's correlation between the fraction of new TGs and  $\Delta$  body weight is  $-0.36$  ( $P = 0.01$ ) in the scABD (A) and  $-0.22$  ( $P = 0.11$ ; trend) in the scFEM (B).

conditions, as were present in our study. Therefore, high  $f_{TG}$  could alternatively be interpreted as high lipolysis rates. Indeed, our data show higher  $f_{TG}$  and higher plasma TG concentrations in Caucasians, consistent with this model (Table 1). Although our study did not portray any significant racial differences in clinical characteristics and variables associated with metabolic health between African-Americans and Caucasians, with the exception of higher plasma TGs in Caucasians, the present analysis represents the first to report that the in vivo synthesis of TG in adipose depots varies by race in healthy women with obesity. Future prospective investigations are necessary to examine how the dynamics of in vivo TG kinetics in AT may be associated with the development of metabolic health disorders in different racial groups.

Given the opposing associations between upper-body (scABD) versus lower-body (scFEM) adipose accumulation with metabolic health biomarkers, there is a strong rationale to examine depot differences in adipocyte kinetics. Interestingly, this study presents the first evidence that in vivo TG synthesis varies by depot location in humans and suggests that scFEM AT has a higher capacity for TG storage. These findings, together with our previous data reporting higher in vivo adipogenesis (i.e., fat cell formation) in the scFEM depot (16), strengthen other experimental observations that implicate the thigh as a more efficient depot to expand and accommodate lipid (35, 36).

The manner by which DNL in white AT is altered in human obesity and insulin resistance is poorly understood.

Clinical interest in adipose DNL has been sparked by recent studies in rodents demonstrating that palmitoleate, which is derived from adipose DNL, is an insulin-sensitizing fatty acid (37, 38), suggesting a plausible role for AT DNL in systemic glucose homeostasis. Subsequent studies in humans revealed that the AT of insulin-resistant individuals had reduced rates of in vivo DNL relative to insulin-sensitive subjects (10, 11). Hence, these recent studies highlight a potentially significant role for adipose DNL in humans.

Previous investigations did not observe race differences in adipose DNL rates (12). Our findings show significantly higher in vivo adipose DNL (palmitate synthesis) in Caucasians versus African-Americans. However, the fraction of newly deposited palmitate in adipose TG that derived from the DNL pathway (corrected  $f_{DNL}$ ) is not different between Caucasians and African-Americans. Corrected  $f_{DNL}$  is a sensitive measure for palmitate turnover that corrects for dietary palmitate and circulating unlabeled palmitates that also get reesterified onto the new glycerols. The lack of significant race differences in corrected  $f_{DNL}$  indicates that the higher  $f_{DNL}$  measures (i.e., storage of DNL-derived palmitate) in Caucasians may be due to higher TG turnover, rather than a greater contribution of DNL to TG-palmitate.

Of note, our analyses cannot distinguish between fatty acids (i.e., palmitate) that are synthesized by AT from those that are synthesized by the liver and transported to the adipose depots. It has been previously estimated that hepatic


DNL contribution to adipose DNL is minor, i.e.,  $\sim 0.2\text{--}0.3\%$  fatty acids (palmitate) over an 8 week labeling period (8). This calculation is based on the rate of VLDL-TG secretion by the liver ( $\sim 10\text{--}20$  g/day), the fraction of palmitate in VLDL-TG-fatty acids ( $\sim 20\%$ ), and the fraction of VLDL-TG-palmitate derived from the DNL pathway ( $\sim 10\%$ ), giving a maximum total contribution from hepatic DNL of  $<200\text{--}400$  mg/day. Even if all this hepatic DNL were deposited in AT, 8 weeks of labeling would result in  $\sim 10\text{--}20$  g DNL-palmitate. With  $>7$  kg of AT palmitate (20% of  $>35$  kg adipose TG mass), this represents  $<0.2\text{--}0.3\%$  of adipose-palmitate, which is a small fraction of the  $\sim 2.5\%$  of adipose palmitate measured as  $f_{\text{DNL}}$ . Further studies are needed to elucidate the role of adipose DNL in human metabolism and health.

Though the goal was weight maintenance throughout the duration of the study, some of the participants experienced small changes in body weight (gain and loss). Interestingly, our data demonstrate a negative association between  $f_{\text{TG}}$  and  $\Delta$  body weight, suggesting higher TG synthesis and DNL in women that lost more weight. As TG replacement rates reflect both synthesis and removal (lipolysis), this inverse correlation may implicate increased lipolysis in subjects that lost more weight, which may, in turn, facilitate increased reesterification leading to more newly synthesized TGs. Of note, our data show higher  $f_{\text{TG}}$  as well as higher plasma TG concentrations in Caucasians. We also previously reported a negative correlation between in vivo adipose cell formation and  $\Delta$  body weight, suggesting higher adipogenesis in women that lost more weight (17). Notably, body weight change in these subjects over the 8 week period was small [on average  $-0.77 (\pm 2)$  kg], which equates to only a minimal change in total body fat ( $<1.5\%$  of total body mass). Notably, there was no significant association of in vivo lipid synthesis ( $f_{\text{TG}}$ ) and percent body fat in the scABD ( $r = 0.09$ ;  $P = 0.52$ ) or the scFEM ( $r = 0.19$ ;  $P = 0.18$ ) depot. Additional investigations are necessary to examine how significant dynamic changes in body weight and percent body fat may impact in vivo adipose kinetics.

We did not observe any associations between in vivo adipocyte kinetics and markers of metabolic health. Previous analyses demonstrated that  $f_{\text{TG}}$  was associated with facets of the metabolic syndrome, including insulin resistance and lipid profile measures (10, 39). Notably, our study included only women who were metabolically healthy. Future investigations will encompass more diverse subject populations to better understand how the dynamics of in vivo AT lipid kinetics can influence metabolic health in humans.

We expected subjects with greater fat mass to exhibit slower TG replacement rates. As described in the Results, neither total body fat nor regional fat masses were significantly associated with  $f_{\text{TG}}$ . Additional analyses demonstrated that the inclusion of total fat mass, TAT mass, scABD AT mass, and percent leg fat (or leg fat mass) in the statistical models did not change the overall results. In addition, race differences in the fractional replacement of TG remained significant with minimal changes in the mean in African-Americans (0.03–0.64% difference) and Caucasians (0.01–0.67% difference) relative to the reported

estimates. Hence, our findings do not support the idea of lower TG turnover in subjects with higher fat mass and may suggest that TG turnover per kilogram of AT is relatively constant in this population of women.

A major strength of our study is the use of the  $^2\text{H}$ -labelling protocol to directly measure in vivo TG synthesis and DNL in the AT, which is vastly different from previous methods that employed indirect approaches or utilized circulating blood plasma samples for analysis. Furthermore, the assessment of both scABD and scFEM depots is an additional strength, as depot differences in AT function and metabolism are well-documented. There are also limitations to this study. Although we have a generous sample size, our analysis includes only premenopausal women with obesity, so these results cannot be applied to postmenopausal women or to men. In addition, the only races that are included are African-Americans and Caucasians. It is important to note that our cross-sectional data reflect correlations and not cause and effect relationships. In conclusion, we present the first evidence that in vivo adipose kinetics (TG replacement rates and DNL) in women with obesity vary by ethnicity, with higher rates in Caucasians versus African-Americans. Importantly, our data suggest that the scFEM AT may have a higher capacity for TG storage and be more lipolytically active relative to the scABD depot. 

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