Free Fatty Acid Flux in African-American and Caucasian Adults—Effect of Sex and Race

Søren Nielsen1,2, Anne E. Sumner3, Bernard V. Miller 3rd3,4, Hana Turkova5, Samuel Klein4 and Michael D. Jensen2

Objective: Obesity, insulin resistance, and diabetes disproportionately affect African-American (AA) women. Abnormal adipose tissue free fatty acid (FFA) release is associated with these conditions. Resting energy expenditure (REE) and sex predict FFA release in Caucasians, but whether this is true in AA is unknown. The sex-specific relationships between FFA release, REE, and race was compared.

Design and Methods: 100 adults (47% AA, 50% male, age 32 ± 8 years [mean ± SD]) from three different centers underwent duplicate measures of FFA release ([U-13C] palmitate) and REE (indirect calorimetry). Body composition was determined by DXA and abdominal imaging.

Results: AA participants had lower REE, but similar FFA concentrations and flux compared with Caucasian participants. The significant predictors of palmitate release were REE, sex, and race. REE and FFA flux were correlated in both sexes and both races. In a multiple linear regression analysis with AA as the dependent variable and REE, sex, total fat mass, fat-free mass, and insulin as independent variables, REE was the only independent predictor of FFA release in men. Both REE and race predicted palmitate flux in women.

Conclusions: FFA flux is related to REE, but the relationship differs in AA and Caucasian women.

Introduction

African-Americans (AA) have a greater incidence and prevalence (1,2) of obesity and obesity-related morbidity than Caucasians (3). Some studies indicate AA women are more insulin resistant than Caucasian women (3,4). An upper-body fat distribution, specifically with increased visceral fat, predicts a greater risk of metabolic and cardiovascular complications. However, the risk for diabetes and cardiovascular disease in AA women with upper-body obesity (UBO) is not necessarily greater than similarly obese Caucasian women (3,5). In fact, a risk factor profile without major metabolic abnormalities and that is quite similar to that of lower-body obese AA women is reported (6,7), although this may be confounded by the lower fasting plasma triglyceride concentrations in AA women independent of insulin resistance (8,9). Whether this is related to differences in adipose tissue metabolism is not clear. Circulating free fatty acids (FFA), originating from adipose tissue lipolysis, mediate at least some of the metabolic risk related to UBO; many of the metabolic abnormalities associated with high-risk obesity can be reproduced by experimentally raising plasma FFA (10,11). Moreover, the possibility that abnormal adipose tissue metabolism contributes to the racial health disparities because of obesity in AA women is supported by in vitro (12,13) and in situ (microdialysis) studies that find racial differences with respect to basal and insulin suppressed lipolysis (14,15). On the contrary, there appears to be no clear racial differences in the ability of insulin to suppress systemic lipolysis in women in vivo (8); we found no studies testing for differences between AA women and men (8,16). Hence, despite more prevalent and high-risk obesity in AA women, it remains to be determined whether the characteristics of FFA metabolism are similar in AA and Caucasian men and women (17,18).

Although there are numerous approaches for analyzing FFA kinetic data (per kg body weight, per kg fat-free mass, per kg fat mass), we found that postabsorptive FFA flux in Caucasian adults is most closely related to resting energy expenditure (REE). In addition, we reported that women have significantly greater FFA release relative to REE than men (19). These findings suggest that postabsorptive FFA release is responsive to lean tissue oxidative needs and that Caucasian women have greater nonoxidative FFA disposal than Caucasian men. Given that AA adults have lower REE than Caucasian adults (20,21), in these studies we examined whether the relationship between FFA release and REE is different in AA and Caucasian women.
women and men. To address this question, we measured palmitate flux and REE in AA and Caucasian women and men in duplicate after 2 weeks of the same weight maintenance diet. The primary aim was to compare by race the sex-specific relationship between palmitate flux and REE.

Methods

One hundred nondiabetic adults (47% AA, 50% male) were recruited to participate at Clinical Research Centers (CRC) at the Mayo Clinic, Rochester, Minnesota, National Institutes of Health (NIH), Bethesda, Maryland, and Washington University at St. Louis, Missouri (Mayo 62%, NIH 25%, and Washington University at St. Louis 13%). The study protocol was approved by Institutional Review Boards at each institution. All volunteers gave informed consent. Participants were nonsmokers and were not taking any medication known to affect either FFA or glucose metabolism. All participants were weight stable for the previous 3 months and had a normal oral glucose tolerance test prior to entering the study. The women were premenopausal. Volunteers were recruited such that approximately one-half of the men and women were lean and one-half were obese and that a wide range of body fat distribution was included within the obese group. All volunteers had normal hematologic indices and liver and renal function tests. Results for the Caucasian participants from Mayo Clinic were previously published (19).

Protocol

For two weeks prior to the study days and for the two days of the study, participants were given weight maintenance diets (40% carbohydrate, 40% fat, 20% protein) prepared in metabolic kitchens. On day 14, the volunteers were admitted to a CRC for a three-day–two night stay. The evening meal was provided at 18:00 h on the day of admission and a snack, providing approximately 10% of daily energy needs, was given at 21:00 h. A catheter was placed in an antecubital vein and kept patent with a 0.9% saline infusion.

On each of the two study days [U-13C]palmitate was administered in the antecubital vein as a continuous intravenous infusion from 06:30 h to 07:30 h as described previously (22). REE and respiratory exchange ratio (RER) were measured each morning from 07:00 h to 07:30 h before the participants arose from bed. The metabolic carts were calibrated before the measurements. The indirect calorimeters used were DeltaTrac Metabolic Cart: Sensormedics, Inc, Yorba Linda, CA (Mayo); TrueOne 2400, ParvoMedics, Sandy, Utah (NIH and Washington University). Blood samples were obtained from an arterIALIZED hand vein prior to the start of the [U-13C]palmitate infusion and at 30, 40, 50 and 60 min afterward. By collecting samples prior to the start of the tracer infusion each day, we could account for any residual tracer in the plasma FFA pool (none was found). Samples were transferred to chilled tubes, placed on ice, centrifuged within 10 min, and stored at −70°C. All samples were analyzed in the Mayo Clinic Metabolomics Core Laboratory. After the study was completed each day, the volunteer was given a protocol breakfast and allowed to resume usual activities. Participants returned to the CRC at 17:00 h, consumed the evening meal and snack as described above, and repeated the study the next morning. Because the studies at NIH and Washington University included two sequential study days rather than the four sequential days for the Mayo studies (19), we used only the first two days of the data from the Mayo studies to assure comparable data quality from each center. To balance participant burden with the goal of achieving a good estimate of average palmitate flux, we used our existing data from the group of volunteer who underwent four studies (19) to determine the added variability we would incur from performing fewer studies. We found that performing only one study would result in an average absolute difference from the mean of four studies of 19% (which was deemed unacceptable), whereas the average absolute differences for two and three studies were 9 and 4%, respectively. We therefore decided to ask the additional volunteers at NIH and Washington University to undergo only two studies. Sixty-two participants (12 AA) were studied at Mayo, 25 (22 AA) were studied at NIH, and 13 AA were studied at Washington University.

Body composition

Total body fat and fat-free mass (FFM) was measured by dual-energy X-ray absorptiometry (DXA) scanning at Mayo (Lunar Radiation Corp, Madison, WI, USA) (23), NIH, and Washington University (Hologic QDR 4500, Hologic, Inc., Bedford, MA, USA). Intra-abdominal and subcutaneous abdominal fat masses were assessed by abdominal scans; at Mayo and NIH by a single-slice computed tomography scan of the abdomen at the L2–L3 level (24), at Washington University by MRI at the L4–L5 interspace.

Analysis of samples

Insulin concentrations were measured using chemiluminescent sandwich assays (Sanofi Diagnostics Pasteur Inc., Chaska, MI, USA), and catecholamines were measured using high-pressure liquid chromatography (HPLC) with electrochemical detection. A test of identical samples revealed that norepinephrine concentrations measured at NIH were slightly greater than at Mayo but correlated well between the two sites. Therefore, plasma norepinephrine concentrations were excluded from the multiple regression analysis. Plasma triglyceride concentrations were measured using an automated analyzer and standard enzymatic technique. All analyses of 13C enrichment of plasma palmitate and palmitate concentration were performed using LC/MS (25).

Calculations

Palmitate turnover rates were calculated from the mean enrichment values and tracer infusion rates (19,22) using steady-state formulas. The average of each individual’s flux from both study days was used for statistical analysis.

Statistical analyses

Unless stated otherwise, data was presented as mean ± SD. Students t-tests and Pearson correlations were performed. Multiple regression analyses, both nonstepped and stepwise forward, were performed using log-transformed palmitate flux as the dependent variable and race, REE, fat-free mass, fat mass, and insulin as independent variables. Parameter estimates were back-transformed after statistical calculations, whereas 95% confidence intervals (CI) were calculated from the log-transformed standard error before back-transformation. Thus, parameter estimates and CI’s represent relative changes (ratio change) per unit absolute change in the independent parameters. Only pre-specified independent variables were entered into the analyses; a P-value < 0.05 was required to keep the independent variable in the model. Because epinephrine values were available for only 77% of subjects separate regression analyses were performed for this subset.
Results

Demographic and metabolic characteristics are presented in Table 1. The expected sex differences in body composition were observed. In addition, AA women and men had lower REE and plasma triglycerides than Caucasian women and men, respectively. BMI was similar in AA and Caucasian men but greater in AA women than in Caucasian women. P-values for comparisons between men and women are not provided in Table 1. For both AA and Caucasians, men had more FFM, higher REEs, lesser percent body fat (all P < 0.001), and higher triglycerides (P < 0.05). Caucasian men had a larger visceral fat area than Caucasian women (P < 0.01), whereas AA women had greater abdominal subcutaneous fat area than AA men (P < 0.05). To understand whether there were significant site differences in the indirect calorimetry and body composition measurements, we examined the correlations between REE and FFM for AA and Caucasian men and women (Figure 1). Correlations were highly significant (P < 0.001) in all groups. A nonstepped multiple regression analysis with REE as the dependent variable and FFM, race, and other covariates as independent factors was performed to control for differences in body composition measurements.
site, and sex as independent variables showed that FFM and race were significant predictors of REE, whereas site and sex were not (data not shown).

Compared with Caucasian men, AA men had similar palmitate concentrations and flux (Table 1). Similar findings were observed in Caucasian women compared with AA women. Relationships between palmitate flux and REE were significant in all groups as depicted in Figure 2. Of note, RER was similar across the range of REE in each group. We tested whether site was a factor influencing the relationship between palmitate flux and REE by performing a multiple regression analysis with palmitate flux as dependent variable and REE, site, race, and sex as independent variables. The analysis revealed that REE \((P < 0.001)\), race \((P = 0.002)\), and sex \((P < 0.001)\), but not site \((P = 0.17)\), significantly predicted palmitate flux.

Because we found evidence of racial differences in the relationship between REE and FFA flux, further analyses were performed. A
Racial Differences in FFA Metabolism

Multiple linear regression analysis by sex and race: dependent variable: palmitate flux

| Variable       | Parameter estimate | 95% CI    | P-value |
|----------------|--------------------|-----------|---------|
| Caucasian and AA men |                    |           |         |
| $R^2 = 0.37$, adj $R^2 = 0.32$ |                    |           |         |
| Race           | 0.88               | 0.76–1.01 | 0.08    |
| REE            | 1.0007             | 1.0002–1.0012 | 0.005 |
| Fat-free mass  | 1.00               | 0.99–1.01 | 0.94    |
| Fat mass       | 1.00               | 1.00–1.01 | 0.33    |
| Intercept      | 26                 | 15–45    | <0.001  |
| Caucasian and AA women |                |           |         |
| $R^2 = 0.42$, adj $R^2 = 0.37$ |                |           |         |
| Race           | 0.85               | 0.69–1.05 | 0.13    |
| REE            | 1.0007             | 1.0002–1.0011 | 0.009 |
| Fat-free mass  | 1.00               | 0.98–1.02 | 0.79    |
| Fat mass       | 1.00               | 0.99–1.01 | 0.44    |
| Intercept      | 32                 | 14–72    | <0.01   |

Results of a nonstepped, multiple linear regression analysis using REE, race, fat-free mass, fat mass (or visceral mass), and insulin concentrations as potential independent predictors of palmitate flux; only significant predictors are shown.

A nonstepped multiple linear regression analysis showed that REE was a significant independent predictor of palmitate flux in men, whereas race, fat-free mass, fat mass (or visceral mass), and insulin concentrations did not enter into the final model (Table 2). A stepwise forward multiple regression analysis provided results similar to those observed with the nonstepped analysis (Table 3) and a similar analysis using data from the subset of participants ($n = 32$) in whom plasma epinephrine concentrations were available did not detect an effect of epinephrine (Table 4).

Multiple-linear regression analyses similar to those used for the data from men revealed that palmitate flux in women was significantly and independently predicted by REE in the nonstepped model.

Forward stepwise multiple regression analysis by sex and race: dependent variable: palmitate flux ($N = 32$ men/35 women)

| Variable       | Parameter estimate | 95% CI    | P-value |
|----------------|--------------------|-----------|---------|
| Caucasian and AA men |                    |           |         |
| $R^2 = 0.38$, adj $R^2 = 0.36$ |                    |           |         |
| REE            | 1.0009             | 1.0005–1.0013 | <0.001 |
| Intercept      | 16                 | 8–35     | <0.001  |
| Caucasian and AA women |                |           |         |
| $R^2 = 0.36$, adj $R^2 = 0.34$ |                |           |         |
| REE            | 1.0005             | 1.0001–1.0008 | 0.012 |
| Fat-free mass  | 1.0193             | 1.0066–1.0320 | 0.005 |
| Intercept      | 17                 | 10–31    | <0.001  |

Results of a stepwise forward multiple linear regression analysis using REE, race, fat-free mass, fat mass (or visceral mass), epinephrine and insulin concentrations as potential independent predictors of palmitate flux; only significant predictors are shown.

(Table 2). The stepwise forward regression analysis gave similar results, but revealed that race was also a significant predictor of palmitate flux (Table 3). The stepwise forward multiple regression analysis of data from the participants ($n = 35$) in whom plasma epinephrine concentrations were available did not indicate that epinephrine was a significant predictor of palmitate flux in women.

Discussion

These studies were undertaken to determine whether the relationship between adipose tissue lipolysis and REE differs between AA and Caucasian women and men. To accomplish this we performed duplicate measurements of postabsorptive palmitate flux and REE in combination with measurements of body composition, plasma insulin, and catecholamine concentrations. The novel findings from this study are 1) FFA flux is related to REE in both AA and Caucasian men and women; 2) the relationship between FFA flux and REE is significantly different in AA and Caucasian women. Within the ranges of BMI we studied, body fat (total and visceral), FFM, and fasting insulin concentrations did not relate to FFA flux when REE was taken into account. These results should help to improve the understanding of differences in metabolic risk of obese AA women compared with Caucasian women.

Our results are consistent with previous reports showing significantly lower REE (20,21) and plasma triglycerides (3,9,26) in AA women and men compared with their Caucasian counterparts. By expanding the measurements to include measures of FFA flux, we were able to test one of the major functions of adipocytes—the export of lipid fuel via lipolysis. Very few studies have examined differences in systemic lipolysis between AA and Caucasians. Albu et al. (8) found similar basal and insulin suppressed FFA turnover in viscerally obese AA and Caucasian women, whereas Racette et al. (16) found lower basal and epinephrine-stimulated systemic lipolysis in abdominally obese AA women compared with abdominally obese Caucasian women. Neither of these results seems to explain why we...
found AA women differed from Caucasian women with respect to the REE and FFA flux relationship.

African-American women suffer disproportionately from obesity-associated conditions such as type 2 diabetes mellitus/insulin resistance, cardiovascular disease, and hypertension (3,7), but at a higher BMI level than Caucasian women (6). The AA women participating in this study had significantly lower REE relative to FFM, and those with the lowest REEs had somewhat greater palmitate flux relative to REE than Caucasian women (Figure 2, panel B). To the extent that greater fasting FFA availability may contribute to metabolic dysfunction, our data seem to indicate AA women with the lowest REEs are at higher risk. It is worth noting that despite greater palmitate flux relative to REE in some AA women, their plasma triglycerides were significantly lower than Caucasian women. We found no trend for AA women with lower REEs to have higher plasma triglyceride concentrations (data not shown), suggesting that the statistically significant, but modest differences in FFA flux between AA and Caucasian women is not driving hepatic triglyceride synthesis and secretion in the former group.

One explanation for the differences in palmitate flux in the AA women could be that these participants were slightly underfed during the 2 week feeding period prior to the study (27). However, we find this unlikely because RER was similar over the entire range of REE and the RER was not reduced compared with that of Caucasian women. Moreover, there was no weight loss in the AA women during the 2 weeks preceding the study where meals were provided for the participants. Therefore, factors other than REE may be increasing lipolysis slightly in the AA women. Importantly, our results were not affected by omitting the subgroup of AA women with the greatest BMI from the analysis. We did not find that plasma catecholamine concentrations explained the different relationship between REE and palmitate flux in the AA and Caucasian women, but assay differences between NIH and Mayo may have made it more difficult to detect such a relationship.

The study has some limitations. First, the experiments were conducted at three different sites and between-site differences in some of the experimental approaches or on-site measures might be confounding. We find this unlikely because participants of different sex and race—irrespective of site—fell well within the overall groups in the various regression analyses performed. In addition, the expected relationships between FFM and REE were found and site did not predict the relationships we examined in a multivariate regression analysis. Second, the study does not allow one to derive a cause-effect mechanism between REE and FFA flux. We have previously speculated on how and why REE may be so well linked to FFA flux (19) and noted that neither increasing (11) or decreasing (28) circulating FFA experimentally has effects on REE. Thus, although in AA men and Caucasian women and men REE is the factor that best relates to overnight postabsorptive FFA release from adipose tissue, we do not have additional insights or data that might explain this relationship beyond the possibility that greater REE (FFA consumption) might lower ambient FFA concentrations, calling into play the effects of FFA concentrations on lipolytic and anti-lipolytic hormones in a counter-regulatory manner.

In conclusion, our results imply significant differences in the relationship between energy needs and mobilization of FFA between Caucasian and AA women, but not men. The previously described significance of REE as an important determinant of lipolysis was confirmed and extended to include AA men and women. These findings are of importance to our understanding of abnormalities of lipid metabolism among sexes and races, especially when AA women are included.

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