EVALUATION OF RACIAL DIFFERENCES IN RESTING AND POSTPRANDIAL ENDOTHELIAL FUNCTION IN POSTMENOPAUSAL WOMEN MATCHED FOR AGE, FITNESS AND BODY COMPOSITION

Objective: We investigated endothelial function at rest and after a high-fat meal challenge in African American (AA) and Caucasian postmenopausal women matched for age, body mass index, percent fat and fitness level.

Design: Pilot study.

Setting: University of Virginia General Clinical Research Center. Participants: Eight AA and 8 Caucasian postmenopausal women.

Intervention: Participants underwent a VO2 peak treadmill protocol, percent fat assessment, and brachial artery flow-mediated dilation measurements (baseline and 4 hours following a high-fat meal).

Main outcomes measures: Baseline and postprandial flow mediated dilation (FMD) following a high-fat meal.

Results: FMD values were similar in AA (5.4%, 95% CI: 3.3, 7.4) and Caucasian women (4.0%, 95% CI: 2.0, 6.1). There was no significant change in FMD from baseline to four hours following the meal challenge within groups (AA: .9%, $P = .397$, Caucasian: 2.3%, $P = .063$) or between groups ($P = .449$), despite a significant increase in triglycerides (AA: 81.8 mg/dL, $P < .001$, Caucasian: 99.7 mg/dL, $P = .004$).

Conclusions: The present pilot study found that when postmenopausal AA and Caucasian women are matched for age, fitness and body composition, reported racial differences in resting endothelial function were not observed. Additionally, there were no racial differences in postprandial endothelial function or metabolism following a high-fat meal. (Ethn Dis. 2013; 23(1):43–48)

Key Words: Flow-mediated Dilation, Postmenopausal, Racial Differences, African American, Postprandial, Endothelial Function

INTRODUCTION

Endothelial dysfunction is believed to be an early event in atherogenesis, and is an independent risk factor for cardiovascular events and hypertension in postmenopausal women. Similar to the epidemiological data showing racial differences in cardiovascular risk, endothelial function is reduced in African American (AA) compared to Caucasian women. This risk is exacerbated by the deleterious cardiometabolic consequences of menopause and aging, both of which have been shown to further reduce endothelial function. This is likely due to the increased presence of cardiovascular disease (CVD) risk factors, reduced physical activity/exercise participation, and the decline in endogenous estrogen levels following menopause. Loehr et al found that AA postmenopausal women had lower (−.54%) flow mediated dilation (FMD) values compared to Caucasians. Based on data from a recent meta-analysis, this racial difference in FMD corresponds to approximately a 7% increased risk in CVD in AA women. However, it is possible that this difference could be confounded by aerobic fitness and obesity, as they have not been measured in previous studies finding racial differences in endothelial function. This may be an important consideration as exercise capacity is a strong predictor of mortality.

Endothelial function is associated with fitness and obesity levels, exercise training improves FMD, and racial differences in body fat have been reported in postmenopausal women.

Postprandial endothelial dysfunction may be an important component of CVD risk, as humans spend much of their time in a postprandial state. Although racial differences in fasting endothelial function have been reported previously, little data exist to our knowledge evaluating racial differences in endothelial function following a meal challenge. Since AA individuals exhibit greater oxidative stress in response to postprandial lipemia compared to Caucasians, it is plausible that following a high-fat meal, AA could have greater reduction in endothelial function. However, there are no data to our knowledge whether racial differences are present in postprandial endothelial function specifically following a high-fat meal challenge.

Since AA individuals exhibit greater oxidative stress in response to postprandial lipemia compared to Caucasians, it is plausible that following a high-fat meal, AA could have greater reduction in endothelial function.

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The purpose of our study was to obtain pilot data regarding racial differences in endothelial function at rest and 4 hours following a high-fat meal challenge in postmenopausal AA and Caucasian women who were matched for age, aerobic fitness, percent body fat, and body mass index.

**METHODS**

**Participants**

Participants in our study were obtained from a larger prospective exercise training study, and were matched for age, percent fat, body mass index, and VO₂ peak. Postmenopausal healthy volunteers were recruited from the Charlottesville, Virginia area. The study protocol was approved by the institutional review board and each participant provided written informed consent. Follicle stimulating hormone (FSH) levels were measured to confirm postmenopausal status, however duration of menopause was not determined. Potential participants were excluded if they were taking ace-inhibitors, cholesterol lowering medications, estrogen replacement therapies, participating in aerobic exercise more than 2 times per week, had type 1 or 2 diabetes, were pre-menopausal, hypertensive or had other medical conditions that contraindicated treadmill exercise testing. This allowed us to match eight pairs of postmenopausal women. All participants were non-smokers, were not taking antioxidant supplements, and refrained from caffeine consumption for 2 hours prior to exercise testing and 12 hours prior to the meal challenge.

**Body Fat**

Percentage body fat was measured using air displacement plethysmography (BodPod, Life Measurement Instruments, Concord, Calif) corrected for thoracic gas volume as described previously.²⁰

**Determination of VO₂ Peak**

Each participant began walking at an initial velocity of 60 m/min with velocity increasing by 10 m/min every 3 min (the duration of each stage) until volitional exhaustion. Metabolic measures were obtained through standard open circuit spirometry (Viasys Vmax 229, Yorba Linda, Calif). VO₂ peak was determined as the highest one-min oxygen consumption value obtained.

**Baseline Measurements and Meal Challenge**

Participants were admitted to the General Clinical Research Center (GCRC) after an overnight fast and rested in bed for 15 minutes prior to obtaining a fasting blood sample and the baseline measurement of FMD. Additionally, participants refrained from acute exercise for 24 hours prior to the GCRC admission. All meal challenges were initiated between 0700 and 0800h, were prepared by the GCRC metabolic kitchen, and had a caloric density of 550 kcal. The meal consumed was a breakfast meal consisting of sausage links, orange juice, eggs (with cheese), and whole milk. The macronutrient composition of the meal was approximately 57% fat, 25% protein, and 18% carbohydrate. The fatty acid composition of the meal was approximately 49% saturated, 35% monounsaturated, and 5% polyunsaturated fatty acids. We based this meal challenge on previous data from our laboratory that showed a decrease in postprandial FMD at 4 hours with a similar meal in adults with the metabolic syndrome.²¹

**Endothelial Function**

Flow mediated dilation was evaluated at rest and 4 hours following the high-fat meal challenge. Brachial artery assessments were obtained using 2D and Doppler ultrasound measurements (HDI 5000, Philips Ultrasound, Andover, Mass). Artery diameter was assessed using Brachial Analyzer Software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa), and the brachial artery mean blood velocity was measured using a pulse-wave Doppler with on-line angle correction and analysis software. All imaging was performed in the long axis approximately 4 cm proximal from the antecubital fold in the anterior/medial plane. Arterial diameters (mm) were calculated as the mean distance between the anterior and posterior wall at the vessel-blood interface. Baseline diameters measurements were triggered to the R-wave of the cardiac cycle. Mean baseline diameter was determined by averaging diameters over 1 min (~12 images). Flow-mediated dilation measurements were defined as the percent change in vessel diameter from rest to peak dilation.²² We did not measure shear rate as data indicate that shear rate area under the curve is not related to FMD in older participants.²³ The same individual analyzed the data from all the FMD studies.

Prior to baseline measurements, participants were asked to rest in a supine position for 15 min. A blood pressure cuff was placed 4–5 cm distal from the antecubital fold and inflated to 50 mm Hg above resting systolic blood pressure for 5 min. The cuff was then rapidly deflated (reactive hyperemia) and measurements were obtained for peak blood flow velocity for approximately 15 seconds and ultrasound images were taken for an additional 1 minute 45 seconds to determine peak dilation (usually 60 to 90 s after cuff release).

Evaluation of fitness level and postprandial FMD measurements occurred on separate days in order to eliminate potential confounding effects of acute aerobic exercise on FMD.²⁴

**Blood Sampling Procedures**

Three fasting blood samples were obtained (15 minutes apart) for analysis of baseline blood glucose, insulin and triglycerides. Adiponectin was measured from one blood sample obtained at baseline. Four hours following the high fat-meal challenge, we obtained a blood sample which was analyzed for triglycerides, glucose and insulin. Serum glucose concentrations were measured.
in duplicate using a glucose oxidase method (YSI Instruments 2300 STAT Plus, Yellow Springs, Ohio). Serum insulin concentrations were measured using a solid-phase chemiluminescent immuno- metric assay (Diagnostic Products Corporation, Immulite 2000, Los Angeles, CA); the assay sensitivity was 2 μU/mL and the mean intra- and interassay coefficients of variation were 4.1% and 5.1% for insulin. Serum adiponectin concentrations were measured using a commercially available ELISA method (Linco, St. Charles, MO); the assay sensitivity was 780 ng/mL and the mean intra- and interassay coefficients of variation were 6.7% and 13.4%. Triglycerides were measured at the University of Virginia clinical lab through a glycerol phosphate oxidase method. The homeostasis model assessment–insulin resistance (HOMA-IR: [fasting glucose (mg/dL) × fasting Insulin (μU/mL)] / 405) and quantitative insulin-sensitivity check index (QUICKI: 1/[log fasting insulin (μU/mL) + log fasting glucose (mg/dL)]) were calculated.

**Statistical Analysis**

Statistical analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC). The baseline demographic data including participant: age, body weight, body mass index, height, percent body fat, peak VO\(_2\), and blood measures (baseline insulin, glucose, adiponectin, triglycerides) were compared between the AA and Caucasian study groups with a Wilcoxon rank-sum test. Pearson correlations were used to evaluate the relationship between baseline blood analyses (triglycerides, insulin and glucose, HOMA-IR, QUICKI) with baseline FMD. In addition, we evaluated the correlation of the change in postprandial triglycerides values with change in FMD at 4 hours.

*Endothelial Function*

Brachial diameter and peak blood flow following occlusion were similar between AA and Caucasian participants (Table 1). Baseline FMD was similar between AA (5.4%, CI: 3.3, 7.4) and Caucasians participants (4.0%, CI: 2.0, 6.1). As shown in Figure 1, there was no significant change in FMD from baseline to four hours within groups in AA (9%, \(P=0.397\)) or Caucasian (2.3%, \(P=0.63\)) participants or between groups (\(P=0.449\)). Additionally, there were no significant changes in brachial artery diameter or peak blood flow velocity following the meal challenge (\(P>0.05\)) (Table 2).

**Table 1. Baseline participant characteristics. Results are presented as mean ± SD**

<table>
<thead>
<tr>
<th></th>
<th>African American (n=8)</th>
<th>Caucasian (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>55.0 ± 1.7</td>
<td>57.6 ± 5.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163.4 ± 4.1</td>
<td>162.1 ± 6.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.1 ± 16.5</td>
<td>76.7 ± 13.8</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>30.8 ± 6.2</td>
<td>29.3 ± 5.1</td>
</tr>
<tr>
<td>VO(_2) peak, mL/kg/min</td>
<td>22.1 ± 3.4</td>
<td>21.5 ± 3.4</td>
</tr>
<tr>
<td>Body Fat, %</td>
<td>43.4 ± 7.8</td>
<td>43.2 ± 7.8</td>
</tr>
<tr>
<td>Adiponectin, ng/mL</td>
<td>14556.31 ± 4561.5</td>
<td>16464.42 ± 6527.9</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/mL</td>
<td>6.92 ± 6.4</td>
<td>4.52 ± 4.4</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>92.5 ± 11.3</td>
<td>95.00 ± 10.9</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>63.8 ± 17.0</td>
<td>141.6 ± 119.0</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.61 ± 1.5</td>
<td>1.42 ± 1.3</td>
</tr>
<tr>
<td>QUICKI</td>
<td>.35 ± .1</td>
<td>.38 ± .4</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>2.9 ± .5</td>
<td>2.8 ± .5</td>
</tr>
<tr>
<td>Peak blood flow velocity, cm/sec</td>
<td>99.9 ± 29.7</td>
<td>99.9 ± 22.6</td>
</tr>
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</table>

**RESULTS**

Demographic data are displayed in Table 1. The AA group had a mean ± SD age of 55.0 ± 1.7 yr, percent fat of 43.4 ± 7.8%, and VO\(_2\) peak of 22.1 ± 3.4 mL/kg/min. The Caucasian group had a mean age of 57.6 ± 5.2 yr, a body fat of 43.2 ± 7.8% and VO\(_2\) peak of 21.5 ± 3.4 mL/kg/min. No significant differences in baseline characteristics were found between AA and Caucasian participants. However, the difference in triglycerides at baseline approached significance between AA (63.8 mg/dL) and Caucasian (141.6 mg/dL) participants (\(P=0.83\)). The correlation between baseline FMD and baseline triglycerides, insulin, adiponectin, insulin, glucose, HOMA or QUICKI values were not significant (all \(P>0.05\)).

*Endothelial Function*

Brachial diameter and peak blood flow following occlusion were similar between AA and Caucasian participants (Table 1). Baseline FMD was similar between AA (5.4%, CI: 3.3, 7.4) and Caucasians participants (4.0%, CI: 2.0, 6.1). As shown in Figure 1, there was no significant change in FMD from baseline to four hours within groups in AA (9%, \(P=0.397\)) or Caucasian (2.3%, \(P=0.63\)) participants or between groups (\(P=0.449\)). Additionally, there were no significant changes in brachial artery diameter or peak blood flow velocity following the meal challenge (\(P>0.05\)) (Table 2).

*Change in Postprandial Triglycerides, Glucose, and Insulin*

The change in triglycerides, glucose and insulin are shown in Table 2. Within group analysis showed that postprandial triglycerides were significantly elevated following the meal challenge in AA (81.8 mg/dL, \(P<0.001\)) and Caucasian (99.7 mg/dL, \(P=0.004\)) participants with no significant differences in change in triglycerides (\(P=0.579\)). The correlation between...
Fig 1. Change in FMD in AA and Caucasian postmenopausal women following a high fat meal challenge (57% fat, 25% protein, 18% carbohydrate). Results are presented as adjusted least square means with 95% confidence intervals.

change in triglycerides and change in FMD was not significant (r=-.15, P=.570). Additionally, no significant changes in insulin or glucose values within or between groups were found following the meal challenge (all P>.05).

DISCUSSION

The results of our pilot study indicate that no racial differences in baseline FMD or change in FMD following a high-fat meal were evident in postmenopausal women matched for age, fitness and body composition. Due to the fact that these are pilot data, a larger study is necessary to confirm these findings. However, to our knowledge, no studies exist evaluating the effects of a high-fat meal on endothelial function in AA compared to Caucasians individuals.

Previously reported racial differences in endothelial function have been proposed secondary to several potential etiologies. These include elevated oxidative stress, endogenous ET-1 (ET-1) and asymmetric dimethylarginine (ADMA) levels. However, many of these factors are also affected by fitness level and exercise training. Exercise training has been shown to improve antioxidant capacity, thus potentially reducing the vasoconstricting effects of oxidants on nitric oxide availability. Evidence from smaller training studies have found that exercise training improves ET-1 and ADMA levels. African American individuals have been shown to have lower fitness levels compared to Caucasians, which may be related to a lower percentage or total number of oxidative muscle fibers. Zeno et al found that in healthy adults (n=142), the majority of AA participants (57.1%) had low or fair fitness (determined through a treadmill VO2 max test) compared to Caucasians (31.4%). Recent epidemiological data suggest that exercise capacity may reduce mortality in a similar manner in AA and Caucasian individuals. Kokkinos et al found that for each metabolic equivalent increase in exercise capacity during a Bruce protocol treadmill test, there was a similar reduction in all-cause mortality risk in AA (14%) and Caucasian (12%) men. Therefore, future studies in this area should consider that the reported racial impairment in endothelial function could be due to reduced fitness.

Similarly, we found no racial differences in postprandial endothelial function between AA and Caucasian participants following a high-fat meal challenge. Our results support the data of Muniyappa et al who found no racial differences in postprandial endothelial function following a meal challenge (20% protein, 40% fat, and 40% carbohydrate), despite lower insulin sensitivity in AA participants. However, it should be noted that contrary to previous findings, we did not observe a significant impairment in postprandial endothelial function in either group despite a similar increase in triglyceride levels in both groups. The total caloric content of the meal challenges in previous studies evaluating postprandial FMD have ranged from 596–1480 kcals and from 30–99% fat with those studies providing >45% fat kcal resulting in a decline in FMD at 4 hours. Since the size and fat content of our meal (550 kcal; 35 g fat) were less than previous reports this may have resulted in an insufficient fat load. A critical kcal load, or fat content of the meal, may be necessary to induce postprandial impairment in FMD. Strey et al, for example, reported no

### Table 2. Change in blood analyses and vascular function measurements following the high fat meal challenge. ANCOVA was adjusted for baseline value

<table>
<thead>
<tr>
<th></th>
<th>African American</th>
<th>Caucasian</th>
<th>P (Comparison Between Groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Glucose (mg/dL)</td>
<td>1.11</td>
<td>-1.98</td>
<td>.340</td>
</tr>
<tr>
<td>Δ Insulin (mg/dL)</td>
<td>1.00</td>
<td>1.34</td>
<td>.908</td>
</tr>
<tr>
<td>Δ Triglyceride (mg/dL)</td>
<td>81.78a</td>
<td>99.80a</td>
<td>.579</td>
</tr>
<tr>
<td>Δ Peak blood flow velocity (cm/sec)</td>
<td>9.81</td>
<td>13.05</td>
<td>.763</td>
</tr>
</tbody>
</table>

*a Denotes significant change within groups. A P<.05 was used as the criterion for significance.*
Correction in endothelial function in women with type 2 diabetes after consuming a 660-kcal meal containing 40 g fat. In contrast, Steer et al reported significant impairment in endothelium-dependent vasodilation in women and men after consuming a meal containing only 26–34 g fat (although total energy content of the meal was 700–900 kcal). Additionally, other factors such as the diurnal rhythm of endothelial function, and the FMD procedure itself may have counteracted the potential impairment of our high-fat meal in the present study. Thus, our pilot data suggest no racial differences in the change in FMD or other blood measures (triglycerides, glucose or insulin) following the meal challenge, and no racial differences in postprandial endothelial function in participants matched for age, body composition and fitness level.

It is important to note that there are several limitations in this investigation. Due to the pilot nature of this investigation, we had a small sample of postmenopausal women (n = 16). In addition, we did not have a fasting control condition that would have allowed us to determine the magnitude of the diurnal rhythm in FMD, and whether this differed by race. Secondly, we had a wide range of baseline triglyceride levels in the Caucasian participants compared to the AA participants (approached significance between groups), which may affect racial differences in FMD at baseline. Since the reported triglyceride levels at baseline were the average value of three samples obtained prior to the meal challenge, it is unlikely that the wider distribution seen in the Caucasian group was due to measurement error. Lastly, FMD values were not corrected for shear rate area under the curve. However, there is evidence that shear stress normalization may only be appropriate for young adults, as Thijsen et al observed no significant correlation between FMD and various methods of shear stress correction in older adults. Additionally, previous studies have observed significant relationships between FMD uncorrected for shear and cardiovascular disease risk.

CONCLUSIONS

The results of the present pilot study indicate that baseline and postprandial endothelial function is similar in AA and Caucasian postmenopausal women matched for age, fitness and body composition. Our results may suggest that fitness and body composition should be taken into account when examining differences in endothelial function between AA and Caucasians individuals. Future research should investigate the possible racial differences following meals composed of other macronutrient compositions, and the effect of previous FMD measurements on postprandial endothelial dysfunction.

ACKNOWLEDGMENTS

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REFERENCES

RACE AND POSTPRANDIAL ENDOTHELIAL FUNCTION - Swift et al


