Relation of Changes in Body Fat Distribution to Oxidative Stress

Heval M. Kelli, MD[a], Frank E. Corrigan III, MD[a], Robert E. Heinl, MD[b], Devinder S. Dhindsa, MD[b], Muhammad Hammadah, MD[a], Ayman Samman-Tahhan, MD[a], Pratik Sandesara, MD[a], Wesley T. O'Neal, MD[a], Ibhar Al Mheid, MD[a], Yi-An Ko, PhD[b], Viola Vaccarino, MD, PhD[a,b], Thomas R. Ziegler, R, MD[a], Laurence S. Sperling, MD[a], Kenneth Brigham, MD[c], Dean Jones, PhD[a], Greg S. Martin, MD, MSc[c], and Arshed A. Quyyumi, MD[a,*]

Android fat is a surrogate measure of visceral obesity in the truncal region. Both visceral adiposity and oxidative stress (OS) are linked to cardiometabolic risk factors and clinical cardiovascular disease. However, whether body fat distribution (android vs gynoid) is associated with OS remains unknown. We hypothesized that increased android fat will be associated with greater OS. Body fat distribution and markers of OS, including plasma levels of reduced (cysteine and glutathione) and oxidized (cystine and glutathione disulfide) aminothiols, were estimated in 711 volunteers (67% female, 23% black, mean age 48 ± 11) enrolled in the Emory Georgia Tech Predictive Health study. At 1 year, 498 subjects had repeat testing. At baseline, anthropometric and fat distribution indexes, including body mass index, waist circumference, weight/hip ratio, and android and gynoid fat mass correlated with lower plasma concentrations of glutathione and higher cystine levels indicative of higher OS. At 1 year, the change in android but not gynoid fat mass or body mass index negatively correlated with the change in the plasma glutathione level after adjustment for cardiovascular risk factors. Increased body fat, specifically android fat mass, is an independent determinant of systemic OS, and its change is associated with a simultaneous change in OS, measured as plasma glutathione. In conclusion, our findings suggest that excess android or visceral fat contributes to the development of cardiovascular disease through modulating OS. © 2017 Published by Elsevier Inc. (Am J Cardiol 2017;120:2289–2293)

Oxidative stress (OS) may be defined as the occurrence of macromolecular damage from free radicals and the disruption of thiol, leading to dysfunctional redox control.[1] OS contributes to the pathophysiology of cardiovascular disease (CVD), partly through the inactivation of nitric oxide, resulting in endothelial dysfunction. Increased OS can be estimated as lower levels of circulating glutathione, an increased level of cystine, or a higher ratio of oxidized to reduced aminothiols.[1] Although body mass index (BMI) is an often used measure of adiposity, there is substantial variation in regional fat accumulation across BMI values in individual subjects.[2] Several studies have explored cross-sectional relations between fat distribution and various measurements of OS.[3] However, whether changes in gynoid versus android fat are associated with simultaneous changes in OS over time is unknown. Our aim in the present study was to evaluate the effect on systemic OS of temporal changes in android and gynoid fat mass in a study of subjects enrolled in a lifestyle intervention study. Our hypothesis was that decreases in abdominal (android) fat mass will be associated with lowering of OS.

Methods

Working adults without recent acute illnesses (n = 711) who were largely university employees were recruited by advertisement and selected by invitation as part of the Predictive Health Initiative (*http://predictivehealth.emory.edu*) from December 2007 to December 2010. Subjects visited the Emory-Georgia Tech Center for Health Discovery and Well Being for detailed phenotyping as previously outlined.[4] At the baseline visit, each subject was assigned a health partner, a subject who was specifically trained to utilize the subjects' data profiles and develop health-related goals and a personalized action plan at each visit. Subjects with an acute illness, hospitalization within the past year, pregnant women, and subjects with poorly controlled medical conditions were excluded.

[a]Emory Clinical Cardiovascular Research Institute, Emory University, Atlanta, Georgia; [b]Rollins School of Public Health, Emory University, Atlanta, Georgia; and [c]Predictive Health Institute, Emory University, Atlanta, Georgia.
Manuscript received June 23, 2017; revised manuscript received and accepted August 17, 2017.

See page 2293 for disclosure information.

This work was supported by the Marcus and Woodruff Foundations, Atlanta, Georgia, and the Emory University/Georgia Tech Predictive Health Institute, and awards UL1 RR025008 and UL1 TR000454 from the Clinical and Translational Science Award Program, the National Institutes of Health (NIH), the National Center for Research Resources, and the National Center for Advancing Translational Sciences. Dr. Quyyumi is supported by NIH grants 5P01HL101398-02, 1P20HL113451-01, 1R56HL126558-01, 1RF1AG051633-01, R01 NS064162-01, R01 HL89650-01, HL095479-01, 1U10HL110302-01, 1DP3DK094346-01, and 2P01HL086773, and American Heart Association grant no. 0000031288. HMK has been supported by the Abraham J. and Phyllis Katz Foundation and NHLBI T32 THL130025A.

*Corresponding author: Tel: 404 727 3655; fax: 404 712 8785.
E-mail address: aquyyum@emory.edu (A.A. Quyyumi).

0002-9149/© 2017 Published by Elsevier Inc.
https://doi.org/10.1016/j.amjcard.2017.08.053

Subjects were followed up with comprehensive evaluations at baseline and after 1 year (n = 498). The study was approved by the Emory University Institutional Review Board and informed consent was obtained from all subjects.[5] At the baseline visit, each subject was assigned 1 of 6 health partners, individuals who were specifically trained to utilize subjects' data profiles and to collaboratively generate a health goal and personalized action plan at each visit.[4] Details of the HP intervention are described in the supplement section (Appendix S1). BMI was calculated as weight in kilogram/(height in meter).[2] Waist and hip circumferences were measured in centimeters by 2 measurements of the circumference with the recorded measurement representing the mean of the two. Waist/hip ratio was defined as the ratio of the waist-to-hip circumference. Body composition variables were calculated by dual-energy x-ray absorptiometry (iDXA, GE Lunar Densitometry, General Electric Company, Boston, MA/USA) that is considered to be a gold standard measure for the identification of whole-body fat mass within 2% coefficient of variation. The android region included an area from the top of the iliac crest to 20% of the distance from the iliac crest to the bottom of the subject's head. The gynoid region extended from the top of the greater trochanter down a distance twice the height of the android region.[6]

Hypertension, hypercholesterolemia, and diabetes mellitus were defined according to the Joint National Committee, Adult Treatment Panel III and American Diabetes Association criteria, respectively, and smoking habits were recorded.[7–9] Tobacco use was self-reported and categorized by questionnaire on each examination. Fasting lipid profile, metabolic panel, and C-reactive protein (CRP) (Quest Diagnostics, Madison, New Jersey) levels were measured at each visit.

Plasma cysteine, its oxidized form cystine, glutathione, and its oxidized form, glutathione disulfide, were measured in all subjects using high-performance liquid chromatography-mass spectrometry as previously described.[10] Lower levels of circulating glutathione or increased levels of cystine indicate a higher OS. Briefly, venous blood was transferred immediately into preprepared Eppendorf tubes containing preservatives to retard auto-oxidation, centrifuged, and stored at −80°C for no more than 2 months before transfer to the laboratory. Analyses by high-performance liquid chromatography were performed after dansyl derivatization on a 3-aminopropyl column with fluorescence detection. Metabolites were identified by coelution with standards and were quantified by integration relative to the internal standard, with validation relative to external standards as previously described.[11] Ratios of oxidized to reduced aminothiols (cystine/glutathione) were obtained directly.

Study variables are described as the mean ± standard deviation (unless otherwise specified) for continuous variables or as counts and proportions for categorical variables. Group differences were evaluated by Student *t* tests and proportional differences by 2-proportion z tests. Multivariate linear regression models were constructed to determine relations between measurements of fat distribution and measurements of OS after adjusting for age, gender, ethnicity, tobacco use, hypertension, diabetes, high-density lipoprotein (HDL), total cholesterol, and CRP. At 1 year of follow-up, univariate analysis was performed using the Pearson correlation coefficient, in addition to multivariate analysis using linear regression models to determine the relations between the change in the measurement of fat distribution and the change in the measurement of OS. Statistical analyses were conducted with Statistical Package for Social Sciences 23 (IBM SPSS, Inc., Chicago, Illinois).

Results

The demographic and clinical characteristics of the 711 baseline cohorts and 498 who had been prospectively followed up are presented in Table 1. The sample was 66% female and 72% white, with a mean age of 48 ± 11 years; 34% had a reported history of hypertension, 16% had hyperlipidemia, and 6% were smokers. The mean BMI was 27.8 kg/m^2 and the waist/hip ratio was 0.83 (Table 1). Android and gynoid fat mass were higher in blacks and in those with hypertension and diabetes mellitus, and in those with elevated triglyceride and low-density lipoprotein levels and lower HDL levels. However, only android mass was higher with increasing age and in smokers, whereas only gynoid mass was higher in women. Android and gynoid fat mass were also highly correlated with each other and with BMI and waist circumference (Supplementary Table S1).

At baseline, all measurements of adiposity correlated negatively with glutathione and positively with cystine levels and the cystine/glutathione ratio, suggesting the presence of higher systemic OS in those with increased adiposity (Table 2). Multivariate analyses were performed to investigate whether these associations were independent of covariates, including age, gender, race, mean arterial pressure, total cholesterol, HDL, and CRP levels, history of diabetes, and smoking (Table 3). After adjustment for these cardiovascular risk factors and with gynoid and android fat mass in the same model, only android fat remained correlated negatively with glutathione and positively with the cystine/glutathione ratio, whereas gynoid fat was only positively associated with the cystine level (Table 3).

After 1 year, the 498 subjects who had returned for repeat testing had lost a mean of 1.3 kg (2.9 lb) in weight with simultaneous reductions in BMI, waist circumference, waist/hip ratio, and android and gynoid fat mass. The subjects also had lower systolic and diastolic blood pressures and lower total cholesterol and low-density lipoprotein levels. This finding was accompanied by a significant reduction in glutathione levels, indicating increased OS in the entire group after 1 year (Table 1). However, the change in BMI, android fat mass, and the android/gynoid ratio were all correlated inversely with the change in glutathione level, suggesting that reductions in android rather than gynoid fat mass were associated with a reduction in OS (Table 4). Even after adjustment for changes in BMI and gynoid fat mass at 1 year, both the changes in android fat mass and the android/gynoid ratio remained negatively correlated with changes in glutathione levels ($\beta = -0.110$, $p = 0.022$, and $\beta = -0.134$, $p = 0.005$, respectively) (Supplementary Table S2). Further adjustment for subjects who started on a statin, antihypertensive, or diabetic medication during follow-up and changes in age, tobacco use, diabetes, blood pressure, total cholesterol and HDL, and CRP at 1 year did not alter the results substantially (Supplementary Table S2). This finding indicates that decreases in android rather than gynoid fat mass were associated with the lowering of OS over time. Additional analyses