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A Simple Estimate of Visceral Fat Area by Multifrequency Bioimpedance Analysis Is Associated with Multiple Biomarkers of Inflammation and Cardiometabolic Disease: A Pilot Study

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Abstract: There is a need for identifying whether simple techniques for estimating visceral fat can accurately predict inflammatory and cardiometabolic disease (CMD) biomarkers in various populations. We aimed to determine whether a simple estimate of visceral fat area by multifrequency bioelectrical impedance analysis (MFBIA) was independently associated with multiple biomarkers of inflammation and CMD. Seventy-eight men and women (mean ± SD: age 52.0 ± 10.8 y; visceral fat area 105.6 ± 55.0 cm^2) self-reported their medical histories and activity levels. Visceral fat area was estimated with MFBIA, CMD and inflammatory biomarkers were measured by fasting blood draw, and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated. Associations were assessed using multivariable linear regression. With adjustment for age, sex, height, race/ethnicity, family history of diabetes, and smoking, a 1-standard deviation (1-SD) increase in visceral fat (55 cm^2) was associated with higher levels of insulin (60.4%), triglycerides (43.6%), C-reactive protein (38.7%), interleukin-6 (33.9%), leptin (77.9%), and HOMA-IR (51.8%, $p < 0.01$ for all). These associations were attenuated but remained significant when physical activity and sedentary behavior were entered into the model ($p \leq 0.01$). These findings suggest that a simple estimate of visceral fat area by MFBIA may be a good indicator of increased CMD risk and may be useful in clinical practice.

Keywords: obesity; abdominal obesity; insulin resistance; C-reactive protein; leptin

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1. Introduction

Obesity is considered a subclinical, chronic inflammatory state associated with elevated concentrations of circulating inflammatory markers [1,2]. Accumulating experimental and epidemiological research suggests that inflammation plays an important role in the pathophysiology of cardiovascular and metabolic (cardiometabolic) disorders such as insulin resistance, diabetes, and cardiovascular disease [1–3]. Central adiposity, comprised of both visceral and subcutaneous adipose tissue, is a key source of pro- and anti-inflammatory biomarkers [1,3–5]. Compared to subcutaneous adipose tissue, visceral adipose tissue (visceral fat) has greater inflammatory activity and secretes more inflammatory biomarkers into the portal circulation [5]. Although the mechanisms responsible for the association between visceral fat and metabolic abnormalities have not been fully elucidated, evidence suggests inflammation may be a key link between visceral fat and cardiometabolic disease [2,3,6].

Previous research has shown that visceral fat is associated with biomarkers of inflammation, particularly C-reactive protein (CRP) and interleukin-6 (IL-6) [7–10]. However, few studies report on the independent associations between visceral fat and multiple biomarkers of inflammation, and to our knowledge, only two report on adiponectin and leptin [11,12]. Although most studies use computed tomography (CT), which is the gold standard for estimating visceral fat, its usefulness as a preventive health screening tool is

limited due to the expense, availability, technical expertise needed, and radiation exposure. There is a need for more practical methods, such as anthropometric or surrogate markers of visceral fat. In a recent integrative review on the associations between visceral fat and incident cancer and cardiovascular disease, Silveira et al. [13] concluded that further investigation is needed to identify the predictive capacity of different measurement techniques of visceral fat. It was also indicated that although waist circumference is widely used as a surrogate for visceral fat, the protocols used to measure waist circumference differ considerably across studies and the measurement reflects both subcutaneous and visceral adipose tissue, which may affect predictive capacity. Indeed, several studies suggest that routine clinical measurements such as body mass index (BMI) and waist circumference are less predictive of inflammation than visceral fat [7,14].

Taken together, this indicates there is a need for identifying techniques for estimating visceral fat that are simple to administer, inexpensive, and accurately predict risk factors in various populations [13,14]. Estimates of visceral fat that are predictive of multiple biomarkers of inflammation and cardiometabolic disease may be beneficial in clinical practice, particularly in low-resource settings such as rural settings, to help identify individuals at increased risk for subclinical inflammation and development of cardiometabolic disease.

Multifrequency bioelectrical impedance analysis uses advanced technology to estimate lean mass, fat mass, hydration status, and visceral fat area and has been found to be accurate, reliable, safe, and easy to administer with a standardized protocol [15,16]. The estimate of visceral fat area by multifrequency bioelectrical impedance analysis has been found to be significantly correlated with that measured by CT ($r = 0.759$ and $r = 0.992$) [17,18]. Therefore, the purpose of this study was to examine whether a simple estimate of visceral fat area, using multi-frequency bioimpedance analysis, was independently associated with biomarkers of inflammation and cardiometabolic disease in a sample of men and women. We hypothesized that a simple estimate of visceral fat area by multifrequency bioelectrical impedance analysis would be positively associated with inflammatory biomarkers and cardiometabolic disease risk factors and that these associations would be independent of relevant covariates such as age, sex, smoking status, and physical activity levels. A secondary aim was to determine whether an established cut point for visceral fat area [19] was associated with increased levels of inflammatory and cardiometabolic biomarkers, when estimated by multifrequency bioelectrical impedance analysis.

2. Materials and Methods

A convenience sample of 78 adults were recruited from a university and the surrounding community to participate in this cross-sectional study that included one visit to the laboratory. Participants were recruited and all data were collected from July 2018 to February 2019. Study exclusion criteria included having a pacemaker or large metal implant fitted (i.e., full hip or knee replacement), taking diuretic medications that influence hydration status, or currently pregnant or breast feeding. Eligibility for the study was determined by a pre-screening questionnaire that was completed over the phone by a trained researcher. The University of Idaho Institutional Review Board approved the study (protocol 18-079) and all participants provided verbal and written informed consent to participate in the study. All experiments were conducted in accordance with the Declaration of Helsinki.

2.1. Questionnaires

A health history questionnaire was used to assess medical history and cigarette smoking status (current or non-smoker). Domain-specific sedentary behavior was assessed using the Sedentary Behavior Questionnaire, which has been found to be valid and reliable in adults [20]. The questionnaire assesses the average amount of time spent in nine different types of sedentary behaviors on a typical weekday and weekend day. Total time spent in each of the nine behaviors was converted to hours, summarized, and multiplied by five for weekdays and two for weekend days. Average sedentary behavior in hours per day

was calculated by summing the weekday and weekend sedentary time and dividing the amount by seven.

Physical activity was assessed with the short form of the International Physical Activity Questionnaire (IPAQ). The IPAQ asks questions about physical activity performed in bouts of at least 10 min or more, including walking, moderate, and vigorous activities. Average physical activity in minutes per day was calculated by multiplying the amount of time spent in each activity by the number of days the activity was performed throughout the last seven days and dividing this value by seven.

2.2. Anthropometric and Blood Pressure Measurements

A calibrated digital scale and stadiometer were used to measure body mass to the nearest 0.1 kg and height to the nearest 0.1 cm (Seca 220; Hamburg, Germany). Tension-regulated anthropometric tape (Alimed; Dedham, MA, USA) was used to measure waist circumference. Repeat measurements were taken at the level of the iliac crest until two measurements within 0.5 cm were obtained and averaged. Body mass index was calculated from weight and height and normal weight, overweight, and obese were defined as body mass index of 18.5 to 24.9 kg/m^2, 25.0 to 29.9 kg/m^2, and $\geq$30.0 kg/m^2, respectively. After the participant sat quietly for five minutes, blood pressure was measured using an automated monitor (Omron HEM-907XL; Kyoto, Japan). Repeat measurements were taken, two minutes apart, until two measurements within 5 mmHg were obtained and averaged.

2.3. Body Composition

Body composition was measured using a multi-frequency bioimpedance analysis (InBody 770, Seoul, Republic of Korea), after the participant had been in the standing position for at least 10 min. This analyzer uses a tetrapolar eight-point tactile electrode system which separately measures impedance of five segments of the body—legs, arms, and trunk—using six different frequencies (1, 5, 50, 250, 500, and 1000 kHz). The six frequencies are used to predict intracellular and extracellular water compartments of the total body water in the different body segments. Lean body mass, fat mass, and visceral fat area are then calculated based on the segmental analysis. The edema index is defined as the ratio of extracellular water to total body water. Calibration and testing were done according to the manufacturer's instructions. In brief, participants wore light clothing and wiped the palms of their hands and soles of their feet with a saline tissue prior to testing. Participants were instructed to stand barefoot, with feet covering the electrodes on the foot platform, while holding the handles with hands covering the electrodes and arms abducted.

2.4. Biomarkers of Inflammation and Cardiometabolic Disease

Venipuncture blood draws were performed on each participant during the morning hours after a 12 h fast for the determination of serum glucose, insulin, lipids, and inflammatory biomarkers. Blood samples were centrifuged and serum was frozen at -80 °C for future analysis. Frozen samples for were sent to a National Institutes of Health-funded laboratory for analysis. Insulin was measured on a TOSOH Biosciences 900AIA analyzer (South San Francisco, CA, USA) using the immunofluorescence method. This assay had a minimum sensitivity of 0.5 uU/mL and an intra-assay CV of 1.5%. Glucose was measured using the glucose oxidase method (Stanbio Laboratory, Boerne, TX, USA) with a minimum sensitivity of 2 mg/dL and intra-assay CV of 1.3%. Triglycerides, high-density lipoprotein (HDL) cholesterol, and total cholesterol were measured on a Stanbio Laboratory analyzer (Boerne, TX, USA) and had a minimum sensitivity of 2, 5, and 5 mg/dL, respectively, and an inter-assay CV of 1.1%, 6.1%, and 1.3%, respectively. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. CRP was measured using Pointe Scientific turbidometric reagent (Canton, MI, USA) on a Stanbio analyzer (Boerne, TX, USA) with a minimum sensitivity of 0.5 mg/L and an intra-assay CV of 7.5%. Adiponectin and leptin were measured using Millipore RIA kits (EMD Millipore, Billerica, MA, USA) with a minimum sensitivity of 2.5 mg/mL and 1.9 ng/mL, respectively, and an intra-assay CV of

6.5% and 5.3%, respectively. IL-6 and tumor necrosis factor alpha (TNFα) were measured using MesoScale Discovery (Rockville, MD, USA) Human Proinflammatory Panel kits using chemiluminescence with minimum sensitivities of 0.067 pg/mL and 0.09 pg/mL, respectively. IL-6 and TNFα had inter-assay CV of 4.4% and 3.4%, respectively.

Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as (glucose × insulin)/405 and homeostasis model assessment for β cell function (HOMA-β) was calculated as (360 × insulin)/(glucose − 63).

2.5. Sensitivity Power Analysis

We performed a post-hoc sensitivity power analysis using GPower (Version 3.1.9.2, Universität Kiel, Germany) for linear multiple regression. Given our sample size of 78 and 8 predictors in our final linear regression model, our designed study has 90% power to detect effects (f^2) of at least 0.273 [21].

2.6. Statistical Analyses

All data were examined for normal distribution using Q-Q and box plots and the Shapiro–Wilk test of normality. Data that were non-normally distributed were natural log transformed and rechecked for normality. Natural log transformation normalized all variables. Analysis of covariance was used to determine the difference between means of biomarkers of inflammation and cardiometabolic disease by low- versus high-risk groups for visceral fat (high risk $\geq$ 100 cm^2 [19], while controlling for non-modifiable risk factors (age, sex, and race/ethnicity). Simple correlations were used to assess associations between variables.

We tested for significant differences in the magnitude of associations between visceral fat area and the individual biomarkers of inflammation and cardiometabolic disease by sex using multiplicative interaction terms. There were no significant or robust interactions by sex. Therefore, we present data for the entire sample. Multivariable linear regression, using the enter method, was used to determine the independent associations between visceral fat area and individual biomarkers of inflammation (CRP, IL-6, TNFα, adiponectin, and leptin) and cardiometabolic disease (glucose, lipids, insulin, HOMA-IR, HOMA-β). The initial model (model 1) was adjusted for age, sex, height, race/ethnicity, family history of type 2 diabetes, and smoking. Model 2 included model 1 plus sedentary behavior and moderate-to-vigorous physical activity. Total body fat, BMI, and waist circumference were highly correlated with visceral fat area, causing multicollinearity issues within the regression model, so they were not used as covariates (i.e., tolerance and variance inflation factor). Regression diagnostics indicated there were no problems with multicollinearity among independent variables in the final models. IBM SPSS Statistics (IBM SPSS Statistics 24, Armonk, NY, USA) was used for all analyses with a p value of 0.05 used to determine statistical significance.

3. Results

The study cohort characteristics are presented in Table 1. Overall, the mean age of participants was 52 years and the majority of participants were women (65.4%) and nonsmokers (89.7%). Most participants were non-Hispanic white (81%), with 15% reporting Hispanic or Latino and less than 5% reporting Asian or African American race or ethnicity. On average, participants were classified as overweight, with a BMI of 25.9 kg/m^2, body fat percentage of 22.3%, and visceral fat area of 105.6 cm^2. Overall, participants met the national physical activity guidelines, with an average of 48 min/d of moderate-to-vigorous physical activity. The average total sedentary time was 7.5 h/day, which is consistent with national data [22].

Table 1. Participant Characteristics (n = 78).

Variable	Mean (SD)/Freq (%)	Range
Female	51 (65.4)	
Caucasian	63 (80.8)	
Hispanic	12 (15.4)	
Asian	1 (1.3)	
African American	2 (2.6)	
Family history of type 2 diabetes	31 (39.8)	
Current smoker	8 (10.3)	
Age (years)	52.0 (10.8)	35–79
Body mass (kg)	75.4 (16.3)	45.4–112.5
BMI (kg/m^2)	25.9 (4.4)	17.3–37.0
Body fat (%)	29.2 (10.0)	10.4–50.6
Body fat (kg)	22.3 (10.3)	6.7–49.9
Visceral fat (cm^2)	105.6 (55.0)	23.5–232.6
Lean body mass (kg)	52.4 (11.5)	36.1–80.5
Skeletal muscle index (kg/m^2)	7.4 (1.1)	5.5–10.3
Total body water (L)	38.4 (8.4)	26.5–59.1
Edema index	0.38 (0.01)	0.37–0.40
Waist (cm)	89.6 (12.9)	65.1–114.8
Moderate-to-vigorous PA (min/day)	48.1 (46.6)	0–180
Sedentary behavior (min/day)	450.2 (151.9)	66.4–1103.6
Systolic blood pressure (mmHg)	120 (13.9)	89–159
Diastolic blood pressure (mmHg)	74 (10.1)	54–108
Glucose (mmol/L) [a]	5.8 (0.6)	4.6–10.8
Insulin (uU/mL) [a]	5.9 (4.8)	1.1–40.5
Total cholesterol (mmol/L)	5.5 (1.0)	3.2–9.1
HDL cholesterol (mmol/L)	1.8 (0.4)	1.0–2.7
LDL cholesterol (mmol/L)	3.1 (0.8)	1.0–5.4
Triglycerides (mmol/L) [a]	1.1 (0.5)	0.4–4.5
C-reactive protein (mmol/L) [a]	6.1 (12.6)	3.8–221.0
Interleukin-6 (pg/mL) [a]	0.49 (0.38)	0.12–6.86
Adiponectin (µg/mL)	18.5 (9.2)	8.7–42.7
Leptin (ng/mL) [a]	17.7 (29.4)	2.8–139.5
Tumor necrosis factor alpha (pg/mL)	2.8 (0.8)	1.4–5.6
HOMA-IR	2.0 (1.9)	0.2–10.5
HOMA-β	76.4 (64.9)	7.9–388.6

BMI, body mass index; PA, physical activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β cell function; [a], data presented as median and interquartile range.

3.1. Biomarkers of Inflammation and Cardiometabolic Disease by Low versus High Visceral Fat

After adjusting for age, sex, and race/ethnicity, mean levels of insulin, triglycerides, CRP, IL-6, leptin, TNFα, HOMA-IR, and HOMA-β were significantly higher and HDL cholesterol was significantly lower in those with a visceral fat area at or above 100 cm^2 compared to those with a visceral fat area below this threshold (Table 2, $p < 0.05$). There were no differences between groups for mean levels of glucose, LDL cholesterol, total cholesterol, or adiponectin ($p > 0.05$).

3.2. Simple and Multivariable Linear Associations between Visceral Fat and Biomarkers of Inflammation and Cardiometabolic Disease

Visceral fat area was significantly correlated with natural log (Ln) CRP (r = 0.43, $p < 0.001$), Ln IL-6 (r = 0.40, $p < 0.001$), Ln leptin (r = 0.84, $p < 0.001$), TNFα (r = 0.26, $p = 0.02$), HOMA-IR (r = 0.42, $p < 0.001$), HOMA-β (r = 0.42, $p < 0.001$), insulin (0.42, $p < 0.001$), Ln triglycerides (r = 0.41, $p < 0.001$), waist circumference (r = 0.73, $p < 0.001$), BMI (r = 0.86, $p < 0.001$) and total body fat (r = 0.99, $p < 0.001$) but not adiponectin, glucose, HDL, or LDL cholesterol ($p > 0.05$).

Table 2. Adjusted mean characteristics by established high-risk cut point for visceral fat area.

Variable	Visceral Fat (cm^2)		
	Low Risk (<100 cm^2) (*n* = 42)	**High Risk (≥100 cm^2) (*n* = 34)**	***p***
Glucose (mmol/L)	5.7 (0.1)	5.6 (0.1)	0.49
Insulin (uU/mL)	4.9 (1.0)	11.7 (1.1)	<0.001
HDL cholesterol (mmol/L)	1.9 (0.04)	1.7 (0.1)	0.04
LDL cholesterol (mmol/L)	3.1 (0.1)	3.1 (0.1)	0.78
Total cholesterol (mmol/L)	5.4 (0.2)	5.6 (0.2)	0.61
Triglycerides (mmol/)	1.0 (0.1)	1.5 (0.1)	<0.001
C-reactive protein (mmol/L)	11.4 (4.8)	24.8 (5.7)	0.001
Interleukin-6 (pg/mL)	0.45 (0.13)	0.91 (0.14)	0.02
Adiponectin (μg/mL)	19.4 (1.3)	17.5 (1.4)	0.32
Leptin (ng/mL)	13.7 (2.3)	40.4 (2.6)	<0.001
TNFα (pg/mL)	2.6 (0.1)	3.0 (0.1)	0.04
HOMA-IR	1.2 (0.3)	3.0 (0.3)	<0.001
HOMA-β	47.6 (8.9)	111.7 (10.0)	<0.001

Age, sex, and race/ethnicity were used as covariates. Data are presented as marginal means (SE). HDL, high-density lipoprotein; LDL, low-density lipoprotein; TNFα, tumor necrosis factor alpha; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β cell function.

Multivariable-adjusted linear regression models were used to determine the independent associations between visceral fat and biomarkers of inflammation and cardiometabolic disease (Table 3). With adjustment for age, sex, height, race/ethnicity, family history of type 2 diabetes, and smoking (model 1), a 1-standard deviation (1-SD) increase in visceral fat (55 cm^2) was associated with higher levels of insulin (60.4%), triglycerides (43.6%), CRP (38.7%), IL-6 (33.9%), leptin (77.9%), HOMA-IR (51.8%), and HOMA-β (43.9%). These associations were slightly attenuated but remained significant when sedentary behavior and moderate-to-vigorous physical activity were entered into the model (model 2, $p \leq 0.01$). There were no associations between visceral fat and glucose, HDL cholesterol, LDL cholesterol, total cholesterol, or TNFα ($p > 0.05$).

Table 3. Multivariable regression analyses on the associations between visceral fat area and markers of cardiometabolic disease and inflammation.

Model	Visceral Fat Area		
	B [95% CI]	**St β**	***p***
Ln glucose (1 SD = 0.8 mmol/L)			
1	0.0002 [0.000, 0.001]	0.109	0.410
2	0.0002 [0.000, 0.001]	0.112	0.419
Ln insulin (1 SD = 7.1 uU/mL)			
1	0.007 [0.005, 0.010]	0.604	<0.001
2	0.007 [0.004, 0.010]	0.563	<0.001
HDL cholesterol (1 SD = 0.4 mmol/L)			
1	−0.051 [−0.106, 0.004]	−0.194	0.071
2	−0.044 [−0.102, 0.014]	−0.169	0.132
LDL cholesterol (1 SD = 0.8 mmol/L)			
1	0.033 [−0.111, 0.178]	0.059	0.645
2	0.053 [−0.097, 0.203]	0.094	0.483
Total cholesterol (1 SD = 1.0 mmol/L)			
1	0.054 [−0.117, 0.226]	0.080	0.530
2	0.074 [−0.105, 0.253]	0.109	0.412
Ln triglycerides (1 SD = 0.6 mmol/L)			
1	0.003 [0.002, 0.005]	0.436	<0.001
2	0.003 [0.001, 0.005]	0.407	0.001

Table 3. *Cont.*

	Visceral Fat Area		
Model	**B [95% CI]**	**St β**	***p***
Ln CRP (1 SD = 31.4 mmol/L)			
1	0.007 [0.003, 0.011]	0.387	0.002
2	0.008 [0.003, 0.012]	0.432	0.001
Ln interleukin 6 (1 SD = 0.87 pg/mL)			
1	0.004 [0.001, 0.006]	0.339	0.007
2	0.004 [0.001, 0.007]	0.338	0.010
Adiponectin (1 SD = 9.2 µg/mL)			
1	−0.018 [−0.053, 0.018]	−0.105	0.326
2	−0.017 [−0.054, 0.020]	−0.101	0.368
Ln leptin (1 SD = 24.5 ng/mL)			
1	0.013 [0.011, 0.015]	0.779	<0.001
2	0.012 [0.010, 0.014]	0.753	<0.001
TNFα (1 SD = 0.8 pg/mL)			
1	0.003 [−0.001, 0.006]	0.184	0.131
2	0.003 [−0.001, 0.006]	0.172	0.173
HOMA-IR (1 SD = 1.9)			
1	0.018 [0.010, 0.025]	0.518	<0.001
2	0.017 [0.009, 0.025]	0.494	<0.001
HOMA-β (1 SD = 64.9)			
1	0.585 [0.324, 0.846]	0.493	<0.001
2	0.550 [0.279, 0.820]	0.463	<0.001

B, slope; CI, confidence interval; St β, standardized beta; Ln, natural log transformed; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment for insulin resistance; HOMA-β, homeostasis model assessment of β cell function. Model 1: age, sex, race/ethnicity, family history of type 2 diabetes, smoking, height; Model 2: Model 1 + sedentary behavior and moderate-to-vigorous physical activity.

4. Discussion

This cross-sectional study evaluated whether a simple estimate of visceral fat area by multifrequency bioelectrical impedance analysis was associated with individual biomarkers of inflammation and cardiometabolic disease in a sample of adults. We found that this simple estimate of visceral fat area was positively associated with insulin, triglycerides, CRP, IL-6, leptin, HOMA-IR, and HOMA-β. Notably, these associations were independent of relevant covariates including family history of type 2 diabetes, smoking status, sedentary behavior, and moderate-to-vigorous physical activity. Furthermore, we found that insulin, triglycerides, CRP, IL-6, leptin, TNFα, HOMA-IR, and HOMA-β were significantly higher and HDL cholesterol significantly lower in those with a visceral fat area at or above 100 cm^2 compared with those with a lower visceral fat area. These findings suggest that a simple estimate of visceral fat area by multifrequency bioimpedance analysis may be a significant predictor of inflammation and may be a good indicator of risk factors for cardiometabolic disease. Overall, we extend the findings of others by describing these associations using a simple measure of visceral fat area via multifrequency bioimpedance analysis, including inflammatory markers that are associated with cardiometabolic disease but have not been extensively studied in this context (leptin and adiponectin), and controlling for important covariates that may mediate these associations (e.g., physical activity [23]).

The specific role of visceral fat and its contribution to cardiometabolic disease is not well understood; however, pro-inflammatory cytokines secreted by visceral adipocytes may be one explanation, as evidenced by in-vitro experiments [24]. Our findings suggest that a simple estimate of visceral fat area is independently associated with biomarkers of inflammation including CRP and IL-6 and are consistent with other studies that have assessed CT-derived visceral adipose tissue and these markers. For example, several studies using simple correlation analyses suggest CT-derived visceral adipose tissue is positively correlated with CRP [8,9,25] and IL-6 [8] in middle-aged men [9] and post-menopausal women [8]. Using more detailed analyses, Sam et al. [10] demonstrated

CT-derived visceral adipose tissue was independently associated with CRP but not IL-6, in men and women with obesity and type 2 diabetes, after controlling for relevant risk factors. Somewhat similarly, Pou et al. [7] reported CT-derived visceral adipose tissue was significantly associated with CRP and IL-6 in older adults (mean age 60 years) from the Framingham Offspring Cohort. In the present study, the associations of a simple estimate of visceral fat area with CRP and IL-6 were independent of all covariates. It has been shown that both visceral and subcutaneous fat depots contribute to circulating levels of IL-6 [6]. Although IL-6 has various physiological functions, chronically increased circulating levels of IL-6 are associated with increased levels of CRP, though hepatic biogenesis of CRP, and increased risk for development of type 2 diabetes and cardiovascular disease [26,27]. Moreover, the production of CRP in the liver is thought to be largely regulated by IL-6 [2,28]. CRP is a major acute-phase protein associated with chronic systemic inflammation and has been suggested to predict coronary heart disease risk beyond traditional risk factors. Accumulating evidence suggests visceral adipose tissue is an important source for obesity-induced increases in the expression and circulating levels of IL-6, and in turn CRP [29]. Our findings extend the findings of others by showing robust associations between a simple estimate of visceral fat area with IL-6 and CRP.

We found significant and robust associations between a simple estimate of visceral fat area and leptin but not adiponectin. Leptin and adiponectin are secreted primarily by adipose tissue, with the expression of these biomarkers shown to vary across adipose tissue depots [2]. Production of these adipokines is also dependent on the energy status of the adipose tissue depot [2]. We show a strong, positive association between visceral fat area and leptin, which is consistent with recent findings in women who were obese that show elevated circulating levels, as well as mRNA and protein expression of leptin in visceral adipose tissue [30]. Our data, although in a small sample, provide further evidence for the importance of the contribution of visceral fat to overall leptin levels, independent of relevant covariates. Adiponectin is associated with improved insulin sensitivity and is considered an anti-inflammatory adipokine, with circulating levels inversely correlated with overall adiposity [31]. Although the secretion of adiponectin is thought to decrease with increased visceral and overall adiposity [32], aging is associated with higher levels of adiponectin [31,33] despite the increase in visceral adiposity and insulin resistance that typically occurs with age [31]. Further, preserved secretion of adiponectin from subcutaneous fat has also been shown to contribute to higher levels of adiponectin in some individuals affected by obesity [32]. These findings may partially explain why we did not see a significant association between visceral adiposity and adiponectin, but future research is needed in larger and more diverse samples to understand the contributions of the different fat depots to circulating levels of leptin and adiponectin in obesity.

Finally, although production of TNFα is upregulated in adipose tissue of individuals with obesity [1], other studies, such as ours, have demonstrated no association between circulating TNFα and visceral fat [6,7]. It may be that TNFα acts primarily locally and only small amounts are released into the systemic circulation [6].

Notably, we show that a simple estimate of visceral fat area was associated with markers of insulin resistance, independent of cardiometabolic disease risk factors, sedentary behavior, and moderate-to-vigorous physical activity. Moreover, when participants were categorized into low versus high-risk groups for visceral fat area, those in the high-risk group had more than double the levels for fasting insulin, HOMA-IR and HOMA-β, compared to the low-risk group. Although the size of the visceral fat depot is small compared to other fat depots, such as subcutaneous fat, it has been suggested that visceral fat is independently associated with insulin resistance [34]. However, this finding has not been consistent across studies. Differences in findings across studies may be due to differing measures of abdominal visceral and subcutaneous adipose tissue as well as different methods for quantifying insulin resistance.

Our findings also show that individuals with a visceral fat area at or above 100 cm^2 had higher levels of inflammatory markers and worse cardiometabolic profile than those

with a visceral fat area less than 100 cm^2. Notably, levels of triglycerides, CRP, IL-6, and leptin were 1.5 to 2.9 times higher in those with a visceral fat area at or above 100 cm^2. Using a similar cut point, Despres and Lamarsh [19] reported a CT-derived visceral fat area at or above 100 cm^2 was associated with a moderate but significant change in the metabolic risk profile in men and women (mean age 35 years). Using a slightly higher cut point, Nicklas et al. [14] reported an elevated risk for dyslipidemia and hyperinsulinemia in middle-aged to older women with a visceral fat area above 105 cm^2 compared to those with a lower visceral fat area. Our data provide support for an increased cardiometabolic disease risk with a visceral fat area at or above 100 cm^2, using a simple estimate of visceral fat area, but this finding should be confirmed with larger, more diverse samples.

Our study has several strengths including assessing a variety of inflammatory and cardiometabolic biomarkers that were analyzed at a central laboratory with a high level of reproducibility and including important covariates in the statistical analyses. However, our covariates included self-reported measures of physical activity and sedentary behavior, which may be less accurate than objective measures and subject to recall bias. We recognize our sample of participants was small and mainly non-Hispanic white, which may limit the generalizability of the findings. The study was cross-sectional which does not provide information on a possible causal association between a simple estimate of visceral fat area and biomarkers of inflammation and cardiometabolic disease. Given this, prospective studies with large samples sizes are needed to determine associations of simple measures of visceral fat area and change in biomarkers over time.

In summary, a simple estimate of visceral fat area by multifrequency bioimpedance analysis was positively associated with insulin, triglycerides, CRP, IL-6, leptin, HOMA-IR, and HOMA-β, independent of relevant covariates including smoking, sedentary behavior, and moderate-to-vigorous physical activity. These findings suggest that a simple measure of visceral fat area, as estimated by multifrequency bioimpedance analysis, may be a good indicator of risk factors for cardiometabolic disease in adults and may be useful in clinical practice.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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