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# Association of Body Fluid Volumes and Body Fat Distribution with Abnormal Ankle-Brachial Index

Liang Li, Jinjin Zhang, Lei Wang, Zhikun Zhao and Yunfeng Xia*

## Abstract

**Background:** Ankle-brachial index (ABI) is a simple, non-invasive and easy-to-obtain measure for the evaluation of atherosclerotic peripheral arterial disease (PAD). This study aimed to investigate the relationships between body fluid volumes, body composition, body fat distribution and ABI at a population perspective.

**Results:** Using the US National Health and Nutrition Examination Survey Data (NHANES) during 1999–2000, 2001–2002, and 2003–2004, adults ≥ 40 years old were eligible for inclusion. Univariate and multivariable linear and logistic regression analyses were performed to determine the associations between ABI, body fluid volume and body composition assessed by bioelectrical impedance analysis (BIA), and body fat distribution assessed by dual-energy X-ray absorptiometry (DEXA). After exclusion, the final analytic sample contained 1535 participants who were representative of totally 28,572,458 subjects in the US. After adjustments for relevant confounders, estimated fat mass was significantly and inversely associated with ABI (beta: − 0.0009, 95% CI = − 0.0015, − 0.0003). Total percent fat (beta: − 0.0024, 95% CI = − 0.0033, − 0.0014), trunk percent fat (beta: − 0.0016, 95% CI = − 0.0023, − 0.0009), and percent fat at the four limbs were also significantly and inversely associated with ABI ($p < 0.001$). In addition, subjects with higher estimated fat mass, total percent fat, trunk percent fat and higher percent fat at the four limbs were all significantly more likely to have abnormal ABI < 0.9. No significant association between parameters of body fluid volume and abnormal ABI was observed.

**Conclusions:** Estimated fat mass, total percent fat, trunk percent fat and percent fat at the four limbs were significantly and inversely associated with ABI. Subjects with abnormal ABI are more likely to have higher total percent fat, trunk percent fat and the limb fat. These findings fill the knowledge gap on the relationships between atherosclerosis and body fat distribution. Further well-designed prospective studies are needed to confirm the present findings.

**Keywords:** Ankle-Brachial index (ABI), Atherosclerosis, Peripheral artery disease (PAD), Body fluid volume, Body fat distribution

## 1 Background

Atherosclerotic cardiovascular diseases (ASCVDs) involves the build-up of cholesterol plaque in arteries and includes acute coronary syndrome such as fatal/non-fatal myocardial infarction and unstable angina, cerebrovascular accidents, and peripheral artery disease (PAD). To date, ASCVDs are predominant causes of death worldwide. An estimated 17.9 million people died from ASCVDs in 2019, representing 32% of all global deaths. Of these deaths, 85% were due to heart attack and stroke [1]. Most ASCVDs can be prevented by addressing lifestyles such as tobacco use, unhealthy diet, physical inactivity, and harmful alcohol use. It is crucial to detect

*Correspondence: yunfeng-xia@edusbm.com

Department of Geriatric Medicine, The Fourth Medical Center of PLA General Hospital, No.51 Fu Cheng Road, Haidian District, Beijing, China

ASCVDs as early as possible such that proper interventions may be started earlier to lower the subsequent morbidity and mortality [2–4].

PAD, in particular, is a prevalent but underdiagnosed manifestation of atherosclerosis. In most cases of PAD, atherosclerotic plaques narrow the arterial flow lumen which restricts blood flow to the distal extremity. Proper awareness of PAD has a significant clinical impact because PAD acts as a marker for systemic atherosclerosis. Moreover, patients with PAD have an equivalent cardiovascular risk to those with previous myocardial infarction and require aggressive risk factor modification to improve their long-term health outcomes. In clinical practice, the ankle brachial index (ABI) is widely used by a variety of specialist nurses, physicians, surgeons and podiatrists in different settings as a surrogate indicator for the severity of PAD, which is believed as a simple, non-invasive, rapid, and cheap method [5, 6].

Previously, obesity, especially central obesity, was linked to large arterial stiffness. Several dietary fats, body composition such as low skeletal muscle and abdominal visceral adiposity, and were also associated with increased risk for arterial stiffening in a list of prior study reports [7–12]. However, the potential associations between body fluid, body fat and ABI have not been demonstrated before.

To fill the current knowledge gap, in this study, we aimed to investigate whether body fluid volumes and body fat distribution is associated with ABI, using a nationally representative large cohort. We hypothesized that some measures of body fluid volumes and certain body fat distribution are independently associated with ABI.

## 2 Results

### 2.1 Study Population

A total of 31,126 subjects were identified from 1999–2000, 2001–2002, and 2003–2004 study cycles of the NHANES, and of whom 29,402 received examinations at the mobile examination center (MEC). There were 9145 participants aged ≥ 40 years. After excluding the participants who's data were missing regarding ABI measures ($n = 1573$), those who had an ABI > 1.3 ($n = 38$), and who did not have complete information on body fluid volume, body composition, body fat distribution ($n = 5964$) and dietary variables ($n = 34$), the final study population were totally 1535. Using the NHANES sample weight formulae, this analytic sample size was representative of a total of 28,572,458 subjects in the US. The flow chart of study selection process is presented as Fig. 1.

### 2.2 Characteristic of the Study Population by ABI Status

The included subjects had a median age of 44.5 years, and the majority were females (68.2% in abnormal group and 51.2% in normal group). ABI was categorized into two groups: abnormal group: < 0.90 ($n = 24$) versus normal group: 0.9–1.3 ($n = 1511$). Weighted means and proportions of body fluid volume, body composition, body fat

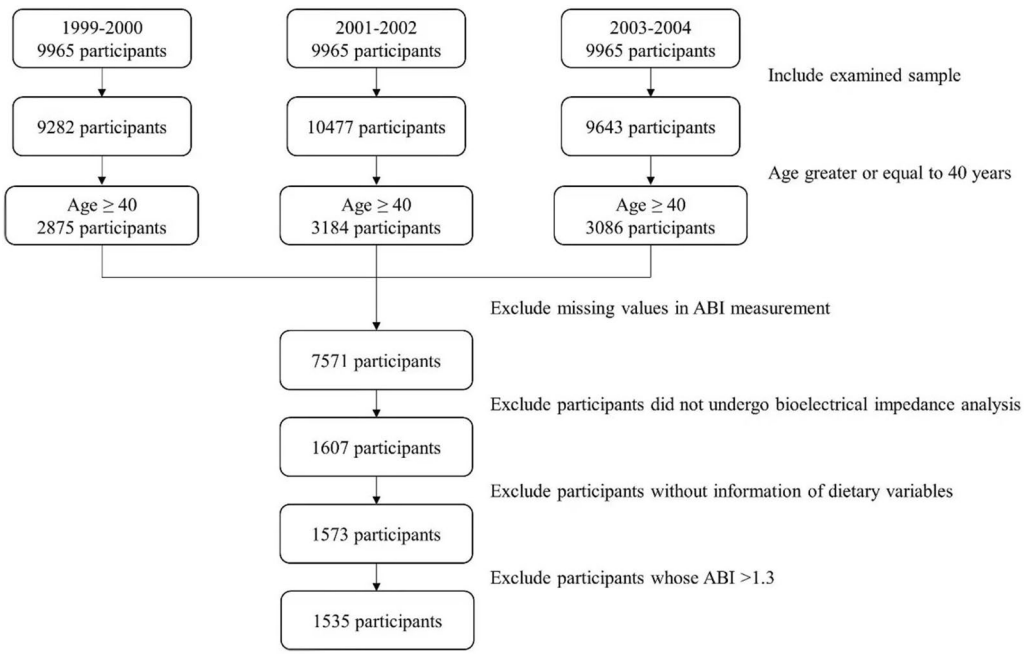


**Fig. 1** Flow diagram of study selection process

distribution, demographic characteristics, comorbidities, laboratory measurement, as well as nutrients intake by ABI groups are shown in Table 1. In specific, estimated fat mass and estimated percent body fat were significantly higher in the abnormal ABI group ($p=0.003$, $p<0.001$, respectively). The parameters of body fat distribution, including total percent fat ($p=0.003$), left arm percent fat ($p=0.010$), right arm percent fat ($p=0.008$), left leg percent fat ($p=0.007$), right leg percent fat ($p=0.008$) and trunk percent fat ($p=0.008$) were all significantly higher in the abnormal ABI group, while estimated extracellular fluid volume, intracellular fluid volume, total water body volume or fat-free mass were not significantly different between the two groups. In addition, the abnormal ABI group consisted of significantly more current smokers (52.6% vs 25.7%) but less former and never smokers (3.9% vs 24.3% and 43.5% vs 50.0%, respectively) than the normal ABI group ($p=0.017$). Subjects in the normal ABI group had a higher proportion of diabetes than the abnormal ABI group (5.2% vs 0.5%, $p=0.002$). Average SBP was higher in the abnormal ABI group. With regard to laboratory parameters, serum folate and albumin level was lower in the abnormal ABI group ($p=0.004$ and $p<0.001$, respectively), while WBC (white blood cell) count was higher in the abnormal ABI group ($p=0.026$, respectively) (Table 1).

### 2.3 Univariate Linear Regression Analysis of the Associations Between ABI and Study Variables

Univariate linear analysis was performed to determine the associations between ABI and the parameters of body fluid volume, body composition, body fat distribution and the other covariates. Estimated fat mass (beta: − 0.0014, 95% CI = − 0.0020, − 0.0008), estimated percent body fat (beta = − 0.0018, 95% CI = − 0.0025, − 0.0011), total percent fat (beta: − 0.0026, 95%CI = − 0.0034, − 0.0018), trunk percent fat (beta: − 0.0021, 95% CI = − 0.0029, − 0.0014) as well as percent fat of the four limbs were all significantly associated with ABI. No significant association between parameters of body fluid volume and ABI was observed (Table 2).

### 2.4 Multivariable Linear Regression Analysis of the Association Between ABI and Study Variables

In multivariable linear analysis, after adjusting for relevant confounders, participants with estimated fat mass (beta: − 0.0009, 95%CI = − 0.0015, − 0.0003), estimated percent body fat (beta: − 0.0008, 95%CI = − 0.0016, 0.0000), total percent fat (beta: − 0.0024, 95%CI = − 0.0033, − 0.0014), trunk percent fat (beta: − 0.0016, 95% CI = − 0.0023, − 0.0009) and percent fat of the four limbs remained to be associated with a significantly ABI. No significant association between parameters of body fluid volume and abnormal ABI was found (Table 3).

### 2.5 Multivariable Logistic Regression Analysis of the Association Between Abnormal ABI and Study Variables

In multivariable logistic analysis, after adjusting for relevant confounders, higher estimated fat mass (OR = 1.04, 95% CI = 1.01–1.07), estimated percent body fat (OR = 1.04, 95% CI = 1.01–1.08), total percent fat (OR = 1.09, 95%CI = 1.04–1.14), trunk percent fat (OR = 1.09, 95%CI = 1.03–1.16) and higher percent fat of the four limbs were all significantly more likely to have abnormal ABI < 0.9. No significant association between parameters of body fluid volume and abnormal ABI was found (Table 4).

## 3 Discussion

To date, this is the first analysis evaluating the potential relationships between abnormal ABI, body fluid volume, body composition and body fat distribution at a large population level. In this study cohort of 1535 participants represented as 28,572,458 US subjects. The results showed that after adjusting for relevant confounders, estimated fat mass, total percent fat, trunk percent fat and percent fat of the four limbs were inversely associated with ABI. Subjects with higher estimated fat mass, total percent fat, trunk percent fat and higher percent fat of the four limbs were all significantly more likely to have abnormal ABI.

In general, these results unveiled the relationships between parameters of body fat distribution and ABI, a validated indicator of atherosclerosis.

The ABI measure is regarded as a simple and convenient method for diagnosing lower extremity PAD. During the past two decades, abnormally high ABI was also continuously associated with an increased risk of cardiovascular events, cerebrovascular events, and even death from any cause [13–17].

On the other hand, body fat distribution is also closely related to cardiovascular diseases. A previous study correlated truncal fat distribution measured using DEXA to the extent of coronary atherosclerosis in Korean patients, and concluded that truncal fat distribution were more clinically relevant to atherosclerosis compared with total body fat or BMI [18]. The percentage of visceral adipose tissue by itself had been regarded as a risk factor for both small vessel cerebrovascular disease and cerebral atherosclerosis of the large-to-medium-sized arteries [19]. Another prior study in Europe reported that visceral adipose tissue contributed beyond overall adiposity to subclinical atherosclerosis, particularly in women [20].

**Table 1** Characteristics of study population by ABI ($n = 1535$)

| Study variables | Total ($n = 1535$) | ABI | | *p* value |
|---|---|---|---|---|
| | | Abnormal (< 0.9) ($n = 24$) | Normal (0.9–1.3) ($n = 1511$) | |
| Body fluid volume | | | | |
| Estimated extracellular fluid volume (L) | 17.4 (17.1,17.6) | 18.1 (16.1,20.0) | 17.4 (17.1,17.6) | 0.657 |
| Estimated intracellular fluid volume (L) | 23.1 (22.7,23.5) | 23.2 (20.7,25.7) | 23.1 (22.7,23.5) | 0.946 |
| Estimated total water body volume (L) | 40.5 (39.8,41.1) | 41.3 (36.9,45.7) | 40.5 (39.8,41.1) | 0.803 |
| Body composition | | | | |
| Estimated fat mass (kg) | 27.5 (26.8,28.3) | 34.7 (32.7,36.7) | 27.4 (26.7,28.2) | **0.003** |
| Estimated fat-free mass (kg) | 54.3 (53.5,55.2) | 55.3 (49.4,61.1) | 54.3 (53.5,55.2) | 0.828 |
| Estimated percent body fat (kg) | 33.4 (32.9,34.0) | 38.6 (35.6,41.5) | 33.4 (32.8,33.9) | **< 0.001** |
| Body fat distribution | | | | |
| Total percent fat | 34.0 (33.4,34.6) | 40.5 (39.1,41.8) | 33.9 (33.3,34.5) | **0.003** |
| Left arm percent fat | 34.4 (33.6,35.1) | 41.1 (39.5,42.8) | 34.3 (33.5,35.0) | **0.010** |
| Right arm percent fat | 34.4 (33.6,35.1) | 41.2 (40.3,42.0) | 34.3 (33.5,35.0) | **0.008** |
| Left leg percent fat | 35.4 (34.7,36.2) | 41.8 (40.1,43.6) | 35.3 (34.6,36.1) | **0.007** |
| Right leg percent fat | 35.8 (35.1,36.5) | 41.8 (40.3,43.3) | 35.8 (35.0,36.5) | **0.008** |
| Trunk percent fat | 33.8 (33.2,34.4) | 40.7 (38.6,42.7) | 33.7 (33.1,34.3) | **0.008** |
| Demography | | | | |
| Age | 44.5 (44.3,44.7) | 45.5 (44.3,46.7) | 44.5 (44.3,44.7) | 0.290 |
| Gender | | | | 0.282 |
| Male | 765 (48.6) | 7 (31.8) | 758 (48.8) | |
| Female | 770 (51.4) | 17 (68.2) | 753 (51.2) | |
| Race/ethnicity | | | | 0.068 |
| White | 683 (77.1) | 7 (29.2) | 676 (46.0) | |
| Hispanic | 464 (11.3) | 5 (11.6) | 459 (11.3) | |
| Black | 348 (11.6) | 12 (29.3) | 336 (11.4) | |
| Others[a] | 40 | 0 | 40 | |
| SBP | 121.2 (120.0,122.4) | 133.1 (122.7,143.5) | 121.0 (119.8,122.3) | **0.004** |
| DBP | 76.2 (75.4,77.0) | 75.3 (73.0,77.5) | 76.2 (75.4,77.0) | 0.611 |
| Smoking status | | | | **0.017** |
| Never | 773 (49.9) | 12 (43.5) | 761 (50.0) | |
| Former | 332 (24.1) | 2 (3.9) | 330 (24.3) | |
| Current | 430 (26.0) | 10 (52.6) | 420 (25.7) | |
| Comorbidity | | | | |
| Diabetes | 101 (5.1) | 1 (0.5) | 100 (5.2) | **0.002** |
| Arthritis | 255 (18.7) | 6 (27.1) | 249 (18.6) | 0.417 |
| Hypertension | 293 (19.0) | 13 (34.6) | 280 (18.9) | 0.138 |
| Laboratory measurement | | | | |
| Serum folate (nmol/L) | 31.0 (29.5,32.5) | 24.1 (21.4,26.7) | 31.1 (29.5,32.6) | **0.004** |
| Serum albumin (g/dL) | 43.5 (43.2,43.8) | 41.8 (40.8,42.7) | 43.5 (43.3,43.8) | **< 0.001** |
| CRP (mg/dl) | 0.4 (0.3,0.4) | 0.5 (0.4,0.6) | 0.4 (0.3,0.4) | 0.342 |
| WBC count (SI) | 7.2 (7.0,7.3) | 8.6 (7.0,10.2) | 7.2 (7.0,7.3) | **0.026** |
| Serum total bilirubin (umol/L) | 11.9 (11.6,12.3) | 10.5 (7.9,13.2) | 12.0 (11.6,12.3) | 0.295 |
| Total cholesterol (mmol/L) | 5.3 (5.3,5.4) | 5.3 (4.9,5.7) | 5.3 (5.3,5.4) | 0.832 |
| Triglycerides (mmol/L) | 1.7 (1.6,1.8) | 2.4 (0.0,4.8) | 1.7 (1.6,1.8) | 0.225 |
| Hemoglobin (g/dl) | 14.5 (14.4,14.7) | 14.9 (13.7,16.1) | 14.5 (14.4,14.7) | 0.602 |
| Serum vitamin B12 (pmol/L) | 373.5 (355.5,391.4) | 407.8 (330.4,485.1) | 373.1 (355.2,390.9) | 0.308 |
| Homocysteine (umol/L) | 8.4 (8.2,8.7) | 7.8 (6.8,8.9) | 8.4 (8.2,8.7) | 0.221 |

Avoid not applicable due to one cell with zero count, not include the analysis

$p < 0.05$ are shown in bold. Continuous variables are shown as weighted mean and 95% CI; categorical variables are shown as unweighted counts (weighted %)

*ABI* ankle brachial index; *CRP* C-reactive protein; *DBP* diastolic blood pressure; *SBP* systolic blood pressure; SI, standard unit; *WBC* white blood cell

**Table 2** Univariate linear regression analysis of the associations between ABI and study variables

| | Estimate (95% CI)[a] | *p* value |
|---|---|---|
| Body fluid volume | | |
| Estimated extracellular fluid volume (L) | 0.0026 (0.0011, 0.0041) | **< 0.001** |
| Estimated intracellular fluid volume (L) | 0.0015 (0.0006, 0.0024) | **0.002** |
| Estimated total water body volume (L) | 0.0010 (0.0004, 0.0016) | **< 0.001** |
| Body composition | | |
| Estimated fat mass (kg) | − 0.0014 (− 0.0020, − 0.0008) | **< 0.001** |
| Estimated fat-free mass (kg) | 0.0007 (0.0003, 0.0012) | **< 0.001** |
| Estimated percent body fat (kg) | − 0.0018 (− 0.0025, − 0.0011) | **< 0.001** |
| Body fat distribution | | |
| Total percent fat | − 0.0026 (− 0.0034, − 0.0018) | **< 0.001** |
| Left arm percent fat | − 0.0018 (− 0.0024, − 0.0012) | **< 0.001** |
| Right arm percent fat | − 0.0019 (− 0.0025, − 0.0013) | **< 0.001** |
| Left leg percent fat | − 0.0022 (− 0.0029, − 0.0016) | **< 0.001** |
| Right leg percent fat | − 0.0021 (− 0.0027, − 0.0015) | **< 0.001** |
| Trunk percent fat | − 0.0021 (− 0.0029, − 0.0014) | **< 0.001** |
| Demography | | |
| Age | − 0.0005 (− 0.0029, 0.0020) | 0.715 |
| Gender | | |
| Male | Ref | |
| Female | − 0.0351 (− 0.0488, − 0.0213) | **< 0.001** |
| Race/ethnicity | | |
| White | Ref | |
| Hispanic | − 0.0081 (− 0.0239, 0.0078) | 0.309 |
| Black | − 0.0505 (− 0.0623, − 0.0388) | **< 0.001** |
| SBP | − 0.0009 (− 0.0014, − 0.0004) | **< 0.001** |
| DBP | − 0.0005 (− 0.0012, 0.0002) | 0.132 |
| Smoking status | | |
| Never | Ref | |
| Former | 0.0179 (0.0069, 0.0290) | **0.002** |
| Current | − 0.0289 (− 0.0450, − 0.0127) | **< 0.001** |
| Comorbidity | | |
| Diabetes | − 0.0226 (− 0.0504, 0.0051) | 0.108 |
| Arthritis | − 0.0153 (− 0.0359, 0.0054) | 0.143 |
| Hypertension | − 0.0166 (− 0.0306, − 0.0026) | **0.022** |
| Laboratory measurement | | |
| Serum folate (nmol/L) | 0.0003 (− 0.0001, 0.0006) | 0.099 |
| Serum albumin (g/dL) | 0.0045 (0.0026, 0.0064) | **< 0.001** |
| CRP (mg/dl) | − 0.0282 (− 0.0424, − 0.0140) | **< 0.001** |
| WBC count (SI) | − 0.0064 (− 0.0092, − 0.0037) | **< 0.001** |
| Serum total bilirubin (umol/L) | 0.0022 (0.0010, 0.0034) | **< 0.001** |
| Total cholesterol (mmol/L) | − 0.0043 (− 0.0099, 0.0014) | 0.135 |
| Triglycerides (mmol/L) | − 0.0017 (− 0.0042, 0.0008) | 0.182 |
| Hemoglobin level (g/dl) | 0.0030 (− 0.0014, 0.0075) | 0.180 |
| Serum Vitamin B12 (pmol/L) | 0.0000 (0.0000, 0.0000) | 0.676 |
| Homocysteine (umol/L) | − 0.0006 (− 0.0026, 0.0014) | 0.575 |

**Table 2 (coninted)**

*P* < 0.05 are shown in bold

*ABI* ankle brachial index; *CRP* C-reactive protein; *CI* confidence interval; *DBP* diastolic blood pressure; *OR* odds ratio; *SBP* systolic blood pressure; *SI* standard unit; *WBC* white blood cell

[a] Unstandardized beta coefficients are reported

Visceral fat, but not subcutaneous fat, is significantly associated with increased risk for CVD in a multi-ethnic cohort [21]. These studies together imply a specific role of body fat distribution in the early development of atherosclerosis.

Interestingly, a previous study had reported that the higher the fat mass of the legs compared to the arms, fat-free mass of the arms compared to the legs, and fat mass or fat-free mass of the limbs compared to the trunk, the lower the prevalence of CVD-risk factors [22].

Despite the relationships of body fat distribution and CVDs were demonstrated, no previous study has yet directly associated ABI measures and body fat distribution. In the present analysis we attempted to determine the links between DEXA-measured body fat distribution and ABI measures, and found that both truncal fat and limb fat were associated with abnormal ABI. Although directly comparisons between the findings of ours and the prior studies cannot be made, the results seem consistent with the previous ones that linked truncal fat with CVD risks.

In addition to trunk fat, the present study also found significant associations between higher limb fat and abnormal ABI. As mentioned above, ABI < 0.9 is a good indicator for PAD in the primary care settings, and PAD is a condition where a build-up of fatty deposits in the

**Table 3** Multivariable linear regression analysis of the associations between ABI and body fluid volume, body composition and body fat distribution

| | Estimate (95% CI) | *p* value |
|---|---|---|
| Body fluid volume | | |
| Estimated extracellular fluid volume (L) | − 0.0015 (− 0.0041, 0.0010) | 0.227 |
| Estimated intracellular fluid volume (L) | − 0.0011 (− 0.0025, 0.0002) | 0.099 |
| Estimated total water body volume (L) | − 0.0007 (− 0.0017, 0.0002) | 0.121 |
| Body composition | | |
| Estimated fat mass (kg) | − 0.0009 (− 0.0015, − 0.0003) | **0.002** |
| Estimated fat-free mass (kg) | − 0.0005 (− 0.0012, 0.0001) | 0.115 |
| Estimated percent body fat (kg) | − 0.0008 (− 0.0016, > 0.0001) | 0.053 |
| Body fat distribution | | |
| Total percent fat | − 0.0024 (− 0.0033, − 0.0014) | **< 0.001** |
| Left arm percent fat | − 0.0019 (− 0.0028, − 0.0010) | **< 0.001** |
| Right arm percent fat | − 0.0019 (− 0.0027, − 0.0011) | **< 0.001** |
| Left leg percent fat | − 0.0026 (− 0.0036, − 0.0015) | **< 0.001** |
| Right leg percent fat | − 0.0022 (− 0.0032, − 0.0012) | **< 0.001** |
| Trunk percent fat | − 0.0016 (− 0.0023, − 0.0009) | **< 0.001** |

Each measure of body fluid and fat distribution was performed in separate multivariable model using linear regression, adjusted for gender, race, smoking status and SBP and unstandardized beta coefficients are reported

$p < 0.05$ are shown in bold

*aOR* adjusted odds ratio; *CI* confidence interval

**Table 4** Multivariable logistic regression analysis of the associations between abnormal ABI (< 0.9) and body fluid volume, body composition and body fat distribution

| | aOR (95% CI)[a] | *p* value |
|---|---|---|
| Body fluid volume | | |
| Estimated extracellular fluid volume (L) | 1.05 (0.82,1.35) | 0.669 |
| Estimated intracellular fluid volume (L) | 1.00 (0.91,1.11) | 0.938 |
| Estimated total water body volume (L) | 1.01 (0.94,1.09) | 0.812 |
| Body composition | | |
| Estimated fat mass (kg) | 1.04 (1.01,1.07) | **0.018** |
| Estimated fat-free mass (kg) | 1.01 (0.95,1.06) | 0.836 |
| Estimated percent body fat (kg) | 1.04 (1.01,1.08) | **0.025** |
| Body fat distribution | | |
| Total percent fat | 1.09 (1.04,1.14) | **< 0.001** |
| Left arm percent fat | 1.05 (1.01,1.09) | **0.010** |
| Right arm percent fat | 1.05 (1.01,1.09) | **0.009** |
| Left leg percent fat | 1.07 (1.02,1.12) | **0.009** |
| Right leg percent fat | 1.06 (1.01,1.11) | **0.009** |
| Trunk percent fat | 1.09 (1.03,1.16) | **0.003** |

$p < 0.05$ are shown in bold

*aOR* adjusted odds ratio; *CI* confidence interval

[a] Each measure of body fluid and fat distribution was performed in separate multivariable model using logistic regression, adjusted for race and SBP

arteries restricts blood supply to leg muscles. Therefore, it is not surprising that subjects with an abnormal ABI had a greater percent limb fat. Moreover, it was previously reported each local fat depot can be considered an independent endocrine organ that actively produces biologically active molecules, such as pro- and anti-inflammatory cytokines and adipokines [23]. Consequently, accumulating evidence suggests the development of CVD may be mediated through the regional distribution of adipose tissue [23]. Importantly, the pro-inflammatory effect of excessively deposited body adipose tissue partly explains the relationships between higher trunk fat and limb fat determined by BIA and abnormal ABI in the present study, although no difference of traditional markers of atherosclerosis such as serum lipids was observed between the two groups.

The major strengths of this study were the usage of the nationally representative database with a large multi-ethnic population sample, with a number of important socio-demographic, behavioral and laboratory parameters being adjusted. However, this study has several limitations. First, the NHANES is a cross-sectional dataset, and thus no causal inference could be made. Second, information regarding duration and severity of the comorbidities were lacking. Third, some variables included in the analyses were based on the interview (questionnaire) data and are subject to potential recall bias or misunderstanding of the question.

## 4 Conclusion

US adults with abnormal ABI are more likely to have higher total percent fat or trunk percent fat but not the limb fat or body fluid volume. These findings fill the knowledge gap on the relationships between atherosclerosis and body fat distribution. Further well-designed prospective studies are needed to confirm the present findings.

## 5 Materials and Methods

### 5.1 Data Source

This study was a secondary analysis of data from The National Health and Nutrition Examination Survey (NHANES) database, which was collected by the Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS) in the USA. (http://www.cdc.gov/nchs/nhanes/). The NHANES program began in the United States in the early 1960s, and has been conducted as a series of surveys focusing on different population groups and health topics. Samples for the NHANES surveys are selected to represent the United States population of all ages. NHANES used a multi-stage, stratified, clustered, probability sampling design to identify a nationally representative sample of non-institutionalized civilians in the US Weights are created in NHANES to account for the complex survey design (including oversampling), survey non-response, and post-stratification adjustment to match total population counts from the Census Bureau. When a sample is weighted in NHANES, it is representative of the US civilian non-institutionalized resident population. A sample weight is assigned to each sample person. Further information about background, design, and protocols of the NHANES are available on the NHANES website (http://wwwn.cdc.gov/nchs/nhanes).

### 5.2 Ethics Statement

NHANES was reviewed and approved through the NCHS Research Ethics Review Board, and informed consent was provided by each participant. Please check the NHANES website for NCHS Research Ethics Review Board Approval (https://www.cdc.gov/nchs/nhanes/irba98.htm). Since all of the NHANES data are de-identified, the analysis of the data does not require Institutional Review Board approval (IRB) or further informed consent.

### 5.3 Study Population

Data of adults $\geq 40$ years old in the NHANES database between 1999 and 2004 were extracted. The participants with incomplete data for ABI measures and other main study variables were excluded from the study cohort.

### 5.4 Assessment of ABI

ABI is the ratio of the blood pressure at the ankle to the blood pressure in the upper arm (brachium). It is usually regarded as an indicator for PAD in asymptomatic individuals. In the NHANES, the ABI exam was performed by trained health technicians in a specially equipped room in the mobile examination center (MEC). Participants lied supine on the exam table during the exam. Systolic pressure is measured on the right arm (brachial artery) and both ankles (posterior tibial arteries). Systolic blood pressure is measured twice at each site for participants aged 40–59 years and once at each site for participants aged 60 years and older. Participants are excluded from the exam if they have a bilateral amputation or weigh over 400 pounds (due to equipment limitations). Participants was categorized into two groups by ABI measures: abnormal group (ABI $< 0.9$) and normal group (ABI 0.9–1.3) for further comparison.

### 5.5 Assessment of Body Fluid Volume, Body Composition and Body Fat Distribution

In this NHANES database, body fluid measures including extracellular fluid volumes, intracellular fluid volumes, total water body volumes, and body fat including estimated fat mass, fat-free mass and percent body fat were determined by Bioelectrical impedance analysis (BIA). The NHANES bio-impedance spectroscopy (BIS) multi-frequency measurements were collected in the BIA examination. A small alternating current was passed through surface electrodes placed on the right hand and foot and the impedance to the current flow was measured by different electrodes placed adjacent to the injection electrodes. The voltage drop between electrodes provided a measure of impedance, or opposition to the flow of the electric current.

Data of body fat distribution in the present analysis were obtained from Dual-energy x-ray absorptiometry (DXA), which is the most widely accepted method of measuring body composition, due in part to its speed, ease of use, and low radiation exposure. The whole body DXA scans were administered in the NHANES MEC. In particular, DEXA scans were administered to eligible survey participants 8 years of age and older. Pregnant females, self-reported history of radiographic contrast material use in past 7 days, nuclear medicine studies in the past 3 days, and weight over 300 pounds or height over 6'5" were excluded from the examination. Total percent fat, percent fat of the limbs and truck were included in the analysis.

### 5.6 Demographic and Socioeconomic Status

The Family and Sample Person Demographics questionnaires were collected in the participants' homes by trained interviewers using the Computer-Assisted Personal Interviewing (CAPI) system. Age, sex, and race/ethnicity were recorded using interviewer-administered questionnaires.

### 5.7 Laboratory Measurement

Blood specimens were collected at NHANES Mobile Examination Centers (MECs). Whole blood specimens were processed, stored, and shipped to the Division of Laboratory Sciences, National Center for Environmental Health, and Centers for Disease Control and Prevention for analysis. Complete descriptions of the collection and analytical methods are available in the Laboratory data section of NHANES database. Individual's laboratory data such as serum albumin, total bilirubin, hemoglobin level, C-reactive protein (CRP), homocysteine, folate and vitamin B12, level of total cholesterol and triglycerides, as well as white blood cell counts were identified and included in the analysis.

### 5.8 Statistical Analysis

To take complex sampling design of NHANES data into account, all analyses were performed using SAS survey analysis procedures to generate nationally representative estimates (SAS Institute Inc., Cary, NC, USA). Weighted mean and 95% confidence interval (CI) were presented for continuous variables; unweighted number and weighted proportion were presented for categorical variables. Since three cycles of data were combined in the current study, sample weights across survey cycles were constructed according to analytic guidelines published by National Center for Health Statistics.

Differences in means between groups of ankle brachial index (ABI) were compared using SURVEYREG procedure for continuous variables, while Rao-Scott chi-square test was performed to examine difference in the proportions between ABI groups using SURVEYFREQ procedure for categorical variables. Linear regression analysis and binary logistic regression analysis were performed to evaluate the association of ABI with body fluid and fat, as well as potential covariates such as socioeconomic status, biomarkers, comorbidity, behaviors, and intake of nutrients. Probabilities modeled are cumulated over the lower Ordered Values. Variables with p-value less than 0.05 in univariate analysis were considered as potential confounding factors. Multivariable models were then constructed by adding significant covariate pertaining to socioeconomic status, biomarkers/comorbidity/examination and behavior/nutrients intake sequentially. Each measure of body fluid and fat was performed in separate multivariable model. Since fat was measured by dual energy X-ray absorptiometry in which multiple imputation was performed to deal with missing data, all analyses in terms of fat distribution were performed separately by each of the five imputed datasets and then combined using MIANALYZE procedure to provide accurate estimates of standard error.

#### Abbreviations

ABI: Ankle-brachial index; PAD: Peripheral arterial disease; NHANES: National Health and Nutrition Examination Survey Data; BIA: Bioelectrical impedance analysis; DEXA: Dual-energy X-ray absorptiometry; ASCVDs: Atherosclerotic cardiovascular diseases; MEC: Mobile examination center.

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#### Author Contributions

LL: acquisition of data; analysis and interpretation of data; drafting of the manuscript; guarantor of integrity of the entire study. JZ: acquisition of data; drafting of the manuscript; statistical analysis. LW: acquisition of data; critical revision of the manuscript; statistical analysis. ZZ: analysis and interpretation of data; critical revision of the manuscript. YX: conception and design; critical revision of the manuscript; guarantor of integrity of the entire study. All authors read and approved the final manuscript.

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None.

#### Availability of Data and Materials

The datasets used during the current study are available from the corresponding author on reasonable request.

## Declarations

#### Conflict of Interest

The authors declare that they have no competing interests.

#### Ethical Approval and Consent to Participate

NHANES was reviewed and approved through the NCHS Research Ethics Review Board, and informed consent was provided by each participant. Please check the NHANES website for NCHS Research Ethics Review Board Approval (https://www.cdc.gov/nchs/nhanes/irba98.htm). Since all of the NHANES data are de-identified, the analysis of the data does not require Institutional Review Board approval (IRB) or further informed consent.

#### Consent for Publication

Not applicable.

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