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Body fat, especially visceral fat, is associated with electrocardiographic measures of sympathetic activation

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ABSTRACT

Objective

Obesity is associated with sympathetic activation, but the role of different fat depots is unclear. The association between body fat, specifically visceral fat, and electrocardiographic measures of sympathetic activation in a population with structurally normal hearts was investigated.

Design and Methods

In this cross-sectional baseline analysis of the Netherlands Epidemiology of Obesity study, body fat percentage was assessed with BIA and abdominal subcutaneous (SAT) and visceral adipose tissue (VAT) with magnetic resonance (MR) imaging. Mean heart rate (HR) and five other electrocardiographic measures of sympathetic activation were calculated. We performed multivariate linear regression analyses.

Results

In 868 participants with a mean age (SD) of 55 (6) years, BMI of 26 (4) kg/m^2, 47% men, body fat was associated with HR and two other measures of sympathetic activation. Per sex-specific SD of total body fat, the difference in HR was 1.9 beats/min (95% CI: 1.0, 2.9; p<0.001) and per SD waist circumference 2.1 beats/min (95% CI: 1.3, 2.9; p<0.001). The difference in heart rate per SD VAT was 2.1 beats/min (95% CI: 1.3, 3.0; p<0.001).

Conclusions

Body fat, especially visceral fat, was associated with electrocardiographic measures of sympathetic activation. Our study implies that already before the onset of cardiovascular disease, excess (visceral) body fat is associated with sympathetic activation.

INTRODUCTION

Obesity is associated with altered function of the autonomic nervous system.[1-6] The autonomic nervous system controls homeostasis and regulates visceral functions by modulations in sympathetic and parasympathetic outflow. Altered autonomic function is characterized by reduced adaptive modulation and sympathetic activation. Neurohumoral activation increases the risk of both first and secondary cardiovascular events and is also associated with subclinical cardiovascular disease (CVD).[7-9]

Body fat may stimulate the sympathetic nervous system and induce autonomic dysfunction by the secretion of adipokines such as leptin, C-reactive protein, TNF-α, plasminogen activator inhitor-1 and interleukin-6. These cytokines either cross the blood-brain-barrier and directly stimulate the central sympathetic nervous system in the hypothalamus, or induce a state of low-grade inflammation that can also stimulate the sympathetic nervous system.[10]

In general, visceral fat seems stronger associated with CVD risk factors than subcutaneous fat. This may be explained by more macrophage infiltration and a higher secretion rate of pro-inflammatory cytokines.[11-14] However, subcutaneous fat is the largest fat depot in the body, accounting for approximately 90% of total body fat. Therefore this depot may still be important in absolute contribution to sympathetic activation. Most previous studies on the association of body fat with sympathetic activation did not distinguish between different abdominal fat depots.[1-4] We hypothesize that visceral fat is stronger associated with sympathetic activation than subcutaneous fat.

Electrocardiographic markers that are commonly used to study sympathetic activation are heart rate and heart rate variability.[15, 16] Three recent experimental studies showed that the sympathetic overdrive is also reflected in other changes on the standard electrocardiogram (ECG).[17-19] These changes include alterations in conduction times and ventricular repolarization. The ECG is an inexpensive, non-invasive and widely available diagnostic tool and may therefore be easy to use in large studies on sympathetic activation.

The aim of our study was to investigate to what extent overall and abdominal fat are associated with both established and novel measures of sympathetic activation in a population without underlying CVD.

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study with oversampling of individuals with a BMI ≥ 27 kg/m^2. The design and data collection of the NEO study were described previously.[20] The baseline visit was performed in the NEO study centre in the Leiden University Medical Centre. Prior to this study visit, participants fasted overnight and completed questionnaires. At the NEO study centre an extensive physical examination was performed. In a random subgroup of participants, magnetic resonance (MR) imaging scans of the abdomen and heart were performed.

The present study is a cross-sectional analysis of the baseline measurements of all participants with MR imaging of the heart and abdomen. Exclusion criteria for our study were self-reported CVD (defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease), ejection fraction (EF) <50%, left ventricular hypertrophy (LVH) (defined as >2SD deviation from the mean body surface area corrected left ventricular end-diastolic mass), prevalent diabetes mellitus (DM) (defined as use of glucose lowering drugs, fasting glucose concentration ≥7.0 mmol/L or postprandial glucose concentration ≥11.1 mmol/L), abnormal ECG or missing data on measures of body fat or sympathetic activation. For the analyses including heart rate or heart rate variability, we additionally excluded participants using beta blockers or calcium antagonists. For the analyses including QTc, Tpeak-end, spatial ventricular gradient or spatial QRS-T angle we additionally excluded participants with ventricular conduction defects. The NEO study was approved by the Medical Ethical Committee of the Leiden University Medical Centre. Before inclusion, all participants gave their informed consent.

Data collection

Self-identified ethnicity of participants was grouped into white (reference category) and other. Level of education was grouped in low education (defined as no education, primary education or lower vocational training) versus high education (reference). Tobacco smoking was categorized into current, former or never (reference) smokers. Physical activity was expressed in MET-hours per week. Blood was sampled after an overnight fast of at least 10 hours. Two postprandial blood samples were taken 30 minutes and 2.5 hours after a liquid mixed meal (400 ml, 600 kcal). Fasting and postprandial glucose concentrations were measured with the enzymatic colorimetric method (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany). Participants were asked to bring all their medication. The heart was imaged by MRI (1.5 Tesla MRI, Philips Medical Systems, Best, Netherlands) in short-axis view using an ECG-triggered balanced turbo-field-echo sequence. A 3-dimensional 3-directional velocity-encoded MRI technique was used. End

systolic and end diastolic ventricular masses and volumes were measured. Left and right stroke volumes (SV) were defined as the difference between the end diastolic volume and the end systolic volume. Left ventricular ejection fraction was calculated by dividing left ventricular SV by the end diastolic volume. The left ventricular mass was measured end diastolic and was standardized for body surface area.[21]

Body fat

Height and weight were measured without shoes and one kilogram was subtracted to correct for the weight of clothing. Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. The circumference of the waist (WC) was measured in the middle of the distance between the lowest rib and the crista iliaca superior with a flexible steal tape. Body fat percentage was measured using a bio-impedance device (TBF-310, Tanita International Division, UK). Abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were quantified by MRI (1.5 Tesla MRI, Philips Medical Systems, Best, Netherlands) using a turbo spin echo imaging protocol. At the level of the fifth lumbar vertebra three transverse images with a slice thickness of 10 mm were obtained during a breath-hold. The fat depots were converted from the number of pixels to centimetres squared. The average of the three slices was used in the analyses.

Established and novel measures of sympathetic activation

A 12-lead ECG was obtained using a Mortara Eli-350 electrocardiograph (Mortara Instrument Inc., Best, Netherlands) after a resting period of at least ten minutes. Standard 10-second ECGs were stored in an 8-lead (I, II, II, V1-V6), 5000 sample comma-separated-value file. The Kors matrix was used to calculate vector cardiograms (VCG) from the eight independent ECG leads.[22] ECGs and VCGs were analyzed using the automatic MATLAB-based (The MathWorks, Natick, MA) program BEATS and the semi-automatic program LEADS.[23, 24]

BEATS was used to detect the timings of all QRS complexes and calculated R-R intervals (ms). All ECGs were checked for falsely identified QRS complexes or non-sinus beats, and the timings were manually adjusted. Also the complexes surrounding the non-sinus beat were removed from the mean R-R interval calculations. Mean heart rate (HR) in beats/min was calculated as 60 divided by the mean R-R interval in seconds. The coefficient of variation (CV) was expressed in percentage and calculated as the mean R-R interval divided by the standard deviation of the mean R-R interval x 100.

LEADS was used to calculate QT time (ms), QTc (corrected according to the Bazett formula) and Tpeak-end duration (ms). The QRS and T integral vectors were approximated

by calculating the numerical sum of X-Y-Z deflections (amplitudes of positive deflections are added and those of negative deflections subtracted). The spatial QRS-T angle was defined as the angle (°) between the integral QRS vector and the integral T vector. The spatial ventricular gradient (mV*ms) was calculated as the vectorial sum of these vectors.

Statistical analyses

In the NEO study, individuals with a BMI ≥ 27 kg/m^2 were oversampled. To correctly represent associations in the general population, adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made.[25] This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population. All results were based on weighted analyses.

Baseline characteristics of the study population were stratified by categories of BMI.[26] Data were expressed as mean (SD), median (25th-75th percentile) or as percentage. The CV was ln-transformed. We performed linear regression analyses to examine the association between body fat and measures of sympathetic activation. As determinants we used BMI (kg/m^2), total body fat (%), WC (cm), VAT (cm^2) and SAT (cm^2). We standardized these values to be able to compare the strength of the association between determinants. Standardization was performed separately for men and women because of the large difference in distribution. In addition, we tested for interaction between measures of body fat and sex by adding an interaction term to all linear regression models. As outcome variables we used HR (beats/min), CV (%), QTc time (ms), Tpeak-end duration (ms), the spatial ventricular gradient (mV*ms) and the spatial QRST angle (°). We calculated the difference and 95% confidence interval per SD of the determinant. For ln-transformed outcome variables, we expressed the regression coefficient as percentage difference. The crude associations were adjusted for age, sex, tobacco smoking, ethnicity, education, physical activity and fasting state (yes/no). The analyses with VAT were additionally adjusted for SAT and vice versa.

RESULTS

Descriptive statistics

In total 6673 participants were included in the NEO study, 1205 with a MRI scan of abdomen and heart. We consecutively excluded participants with pre-existent CVD (n=104), prevalent diabetes (n=106), EF <50% (n=23), LVH (n=81), abnormal ECG (n=2) and missing data (n=21). The total population of the current study consisted of 868 participants. For analyses on heart rate and the coefficient of variation we additionally

excluded participants using beta blockers or calcium antagonists (n=98), resulting in 770 included participants. For the analyses including QTc, Tpeak-end, spatial ventricular gradient and spatial QRS-T angle we additionally excluded participants with ventricular conduction defects (n=73), resulting in 795 included participants. Because of the skewed distribution, the CV was ln-transformed.

Table 1: Baseline characteristics stratified by WHO categories of BMI (26)

	BMI category (kg/m^2)		
	< 25 (43%)	**25-30 (45%)**	**> 30 (12%)**
Sex (% men)	35	54	42
Age (years)	56 (6)	55 (6)	55 (6)
Ethnicity (% Caucasian)	98	96	95
Smoking			
Current (%)	9	14	13
Former (%)	40	44	52
Education (% low)	15	22	31
Total body fat (%)			
Men	19 (3)	26 (3)	33 (4)
Women	32 (4)	39 (4)	46 (3)
Waist circumference (cm)			
Men	88 (5)	99 (6)	113 (6)
Women	78 (7)	91 (7)	105 (8)
Mean VAT (cm^2)			
Men	73 (32)	120 (48)	176 (62)
Women	47 (26)	78 (31)	125 (49)
Mean SAT (cm^2)			
Men	150 (42)	209 (49)	327 (80)
Women	194 (54)	295 (54)	431 (94)
Heart rate (beats/min)	64 (10)	65 (10)	68 (10)
CV (%)	2.8 (1.7-4.5)	2.5 (1.6-4.0)	2.6 (1.6-4.1)
QTc (ms)	408 (27)	406 (26)	416 (26)
Tpeak-end (ms)	79 (14)	81 (13)	82 (15)
Ventricular gradient (mV*ms)	66 (24)	67 (25)	58 (20)
QRS-T spatial angle (°)	51 (26)	48 (25)	56 (30)

Data are presented as mean (SD), median (25th-75th percentile) or percentage. Results were based on weighted analyses (n=868)

BMI=body mass index; low education=no education, primary education or lower vocational training; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; CV=coefficient of variation; QTc=corrected QT time

Baseline characteristics stratified by BMI categories (< 25 kg/m^2, 25-30 kg/m^2 (overweight), ≥ 30 kg/m^2 (obese)) are shown in Table 1. Participants with overweight were more often men. Obese individuals were on average less educated. Total body fat, waist circumference, visceral fat and subcutaneous fat were higher in de overweight and obese group than in the group with BMI < 25 kg/m^2.

Body fat, heart rate and coefficient of variation

The analyses on heart rate and the coefficient of variation included 770 participants. When stratified by BMI, heart rate was higher and the coefficient of variation in percentage was lower in the overweight and obese group than in the group with BMI < 25 kg/m^2 (Table 1). The interaction terms between body fat measures and sex were non-significant in all linear regression models (*p*-values ranging from 0.41 to 0.84). This suggests that although women have a higher amount of total body fat than men, the association between body fat and sympathetic activation is not essentially different between men and women. BMI,

Table 2: Difference (95% CI) in heart rate and coefficient of variation per SD of body fat

Fat measure (SD)	Model	Mean HR (beats/min)	R^2	P-value	CV (%)	R^2	P-value
BMI (m: 3 kg/m^2; w: 4 kg/m^2)	1	1.3 (0.4, 2.1)	0.01	0.004	−4 (−11, 2)	0.00	0.225
	2	1.4 (0.5, 2.2)	0.05	0.001	−5 (−12, 2)	0.04	0.162
	3	1.4 (0.6, 2.3)	0.07	0.001	−3 (−9, 3)	0.06	0.377
Total body fat (m: 5%; w: 6%)	1	1.9 (1.0, 2.9)	0.04	<0.001	−5 (−12, 2)	0.00	0.204
	2	1.9 (1.0, 2.8)	0.06	<0.001	−4 (−12, 3)	0.04	0.238
	3	1.9 (1.0, 2.9)	0.09	<0.001	−2 (−9, 4)	0.06	0.446
WC (m: 9 cm; w: 12 cm)	1	2.0 (1.1, 2.8)	0.04	<0.001	−6 (−13, 1)	0.01	0.095
	2	2.0 (1.1, 2.8)	0.06	<0.001	−6 (−13, 1)	0.04	0.087
	3	2.1 (1.3, 2.9)	0.09	<0.001	−4 (−11, 2)	0.06	0.149
VAT (m: 54 cm^2; w: 39 cm^2)	1	2.3 (1.4, 3.2)	0.05	<0.001	−8 (−16, −1)	0.01	0.034
	2	2.1 (1.3, 3.0)	0.07	<0.001	−7 (−15, 1)	0.04	0.081
	3	2.1 (1.3, 3.0)	0.09	<0.001	−4 (−12, 3)	0.06	0.216
	4	1.7 (0.6, 2.9)	0.10	0.004	−2 (−12, 7)	0.06	0.642
SAT (m: 71 cm^2; w: 98 cm^2)	1	1.4 (0.5, 2.3)	0.02	0.002	−6 (−13, 1)	0.01	0.095
	2	1.6 (0.8, 2.4)	0.05	<0.001	−7 (−14, 0)	0.04	0.048
	3	1.7 (0.8, 2.5)	0.08	<0.001	−5 (−11, 0)	0.06	0.060
	4	0.8 (−0.3, 1.9)	0.10	0.158	−4 (−12, 3)	0.06	0.248

Results were based on weighted analyses (n=770)
Model 1: Crude; 2: Adjusted for age and sex; 3: Adjusted for age, sex, smoking, ethnicity, education, physical activity and fasting state; 4: Model 3 + SAT (in VAT analyses) or Model 3 + VAT (in SAT analyses)
BMI=body mass index; WC=waist circumference; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; HR=heart rate; CV=coefficient of variation

total body fat percentage and waist circumference were positively associated with mean heart rate (Table 2). After adjustment for potential confounders, the association was the strongest for waist circumference. Per SD waist circumference (men: 9 cm; women: 12 cm), the difference in heart rate was 2.1 beats/min (95% CI: 1.3, 2.9; $p<0.001$). There were no associations between body fat and the CV in the adjusted analyses.

As is shown in Table 2, VAT and SAT were both associated with heart rate after adjustments for confounders. After adjustment for SAT, VAT remained independently associated with heart rate: per SD VAT the change in heart rate was 1.7 beats/min (95% CI: 0.6, 2.9; $p=0.004$). However, the association between SAT and heart rate attenuated after adjustment for VAT to a difference of 0.8 beats/min (95% CI: −0.3, 1.9; $p=0.158$).

Body fat, QTc time and Tpeak-end

The analyses on QTc time and Tpeak-end included 795 participants. There was no significant interaction between body fat measures and sex in the linear regression analyses. Waist circumference was stronger associated with QTc than BMI or total body fat percentage. Per SD (men: 9 cm; women: 12 cm) increase in waist circumference, QTc increased with 4.5 ms (95% CI: 2.4, 6.6; $p<0.001$). No associations were observed with Tpeak-end (Table 3).

VAT was independently associated with QTc. After adjustment for all confounders and SAT, the change in QTc per SD VAT was 4.3 ms (95% CI: 1.8, 6.8; $p=0.001$). After adjusting for confounders and VAT, the difference in QTc per SD SAT was 1.0 ms (95% CI: −1.5, 3.4; $p=0.448$).

Body fat, spatial ventricular gradient magnitude and spatial QRS-T angle

Table 3 shows the results of the linear regression analyses between body fat and the spatial ventricular gradient and spatial QRS-T angle. There was no significant interaction between body fat measures and sex. BMI, total body fat and waist circumference were inversely associated with the ventricular gradient magnitude. Waist circumference was most strongly associated with the ventricular gradient magnitude with a difference of −3.4 mV*ms (95% CI: −5.6, −1.2; $p=0.003$) per SD waist circumference (9cm/12cm). After adjustment for potential confounders, BMI, total body fat and waist circumference were not associated with the spatial QRS-T angle.

After adjusting for confounders and SAT, the difference in the ventricular gradient per SD VAT was −4.4 mV*ms (95% CI: −7.0, −1.7; $p=0.001$). SAT was not associated with the ventricular gradient magnitude after adjustment for VAT. After adjustment for confounders and SAT, the difference in QRS-T angle per SD VAT was 3.2° (95% CI: 0.0, 6.3; $p=0.052$).

Table 3: Difference (95% CI) in QTc, Tpeak-end, ventricular gradient and QRS-T angle per SD of body fat

Fat measure (SD)	Model	QTc (ms)	R^2	P-value	Tpeak-end (ms)	R^2	P-value	Ventricular gradient magnitude (mV*ms)	R^2	P-value	QRS-T angle (°)	R^2	P-value
BMI (m: 3 kg/m^2; w: 4 kg/m^2)	1	3.0 (0.7, 5.2)	0.01	0.009	0.4 (−0.8, 1.6)	0.00	0.521	−3.5 (−5.5, −1.4)	0.02	0.001	0.8 (−1.3, 2.9)	0.00	0.453
	2	3.5 (1.4, 5.5)	0.12	0.001	0.5 (−0.8, 1.7)	0.04	0.460	−3.9 (−5.8, −1.9)	0.14	<0.001	0.9 (−1.1, 3.0)	0.04	0.369
	3	3.5 (1.3, 5.7)	0.15	0.002	0.7 (−0.4, 1.8)	0.08	0.197	−3.3 (−5.2, −1.4)	0.16	0.001	0.6 (−1.6, 2.9)	0.05	0.586
Total body fat (m: 5%; w: 6%)	1	4.1 (1.8, 6.5)	0.02	0.001	0.5 (−0.6, 1.6)	0.00	0.392	−3.7 (−6.2, −1.1)	0.02	0.005	−0.2 (−2.4, 1.9)	0.00	0.843
	2	4.1 (1.9, 6.3)	0.13	<0.001	0.4 (−0.7, 1.6)	0.05	0.462	−3.7 (−6.0, −1.4)	0.14	0.002	−0.3 (−2.6, 1.9)	0.04	0.757
	3	4.2 (1.9, 6.5)	0.16	<0.001	0.5 (−0.6, 1.7)	0.08	0.337	−3.1 (−5.4, −0.7)	0.15	0.011	−0.6 (−3.0, 1.8)	0.05	0.614
WC (m: 9 cm; w: 12 cm)	1	4.1 (1.9, 6.4)	0.02	<0.001	0.5 (−0.6, 1.6)	0.00	0.365	−3.7 (−6.1, −1.3)	0.02	0.002	0.0 (−2.2, 2.2)	0.00	0.997
	2	4.3 (2.2, 6.4)	0.13	<0.001	0.5 (−0.6, 1.6)	0.05	0.371	−3.8 (−6.1, −1.6)	0.14	0.001	0.0 (−2.2, 2.2)	0.04	0.983
	3	4.5 (2.4, 6.6)	0.16	<0.001	0.7 (−0.4, 1.7)	0.08	0.198	−3.4 (−5.6, −1.2)	0.16	0.003	−0.2 (−2.6, 2.1)	0.05	0.838
VAT (m: 55 cm^2; w: 40 cm^2)	1	5.4 (3.0, 7.7)	0.04	<0.001	0.9 (−0.3, 2.2)	0.00	0.135	−5.0 (−7.4, −2.7)	0.04	<0.001	1.3 (−0.8, 3.5)	0.00	0.230
	2	4.8 (2.7, 7.0)	0.14	<0.001	0.7 (−0.5, 1.9)	0.04	0.267	−4.7 (−7.1, −2.4)	0.15	<0.001	0.8 (−1.4, 3.0)	0.04	0.485
	3	4.7 (2.6, 6.9)	0.17	<0.001	0.8 (−0.3, 2.0)	0.08	0.159	−4.0 (−6.3, −1.8)	0.16	0.001	1.0 (−1.5, 3.5)	0.05	0.436
	4	4.3 (1.8, 6.8)	0.17	0.001	1.0 (−0.4, 2.3)	0.08	0.167	−4.4 (−7.0, −1.7)	0.16	0.001	3.2 (0.0, 6.3)	0.06	0.052
SAT (m: 72 cm^2; w: 100 cm^2)	1	2.4 (0.1, 4.8)	0.01	0.041	0.1 (−1.0, 1.2)	0.00	0.828	−2.1 (−4.4, 0.1)	0.01	0.060	−1.8 (−3.9, 0.3)	0.00	0.100
	2	3.2 (1.0, 5.3)	0.12	0.004	0.3 (−0.9, 1.4)	0.04	0.657	−2.7 (−4.9, −0.6)	0.13	0.013	−1.5 (−3.6, 0.6)	0.04	0.164
	3	3.3 (1.2, 5.4)	0.15	0.002	0.4 (−0.7, 1.5)	0.08	0.478	−2.2 (−4.3, 0.0)	0.15	0.046	−1.9 (−4.1, 0.3)	0.05	0.086
	4	1.0 (−1.5, 3.4)	0.17	0.448	−0.2 (−1.4, 1.0)	0.08	0.757	0.2 (−2.3, 2.7)	0.17	0.857	−3.5 (−6.2, −0.9)	0.06	0.009

Results were based on weighted analyses (n=795) Model 1: Crude; 2: Adjusted for age and sex; 3: Adjusted for age, sex, smoking, ethnicity, education, physical activity and fasting state; 4: Model 3 + SAT (in VAT analyses) or Model 3 + VAT (in SAT analyses)

BMI=body mass index; WC=waist circumference; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; QTc=correct QT time

Per SD SAT the change in QRS-T angle was −3.5° (95% CI: −6.2, −0.9; *p*=0.009) after all adjustments (Table 3).

DISCUSSION

In the present study, we observed that body fat (BMI, total body fat percentage) and abdominal body fat (waist circumference, VAT, SAT) were associated with mean HR, QTc and the ventricular gradient magnitude. No associations were observed of body fat with the CV and Tpeak-end duration. VAT was positively associated with the spatial QRS-T angle, independent of SAT. There was an inverse association between SAT and the spatial QRS-T angle after adjustment for VAT. The associations between waist circumference and sympathetic activation were stronger than the associations between total body fat and sympathetic activation, suggesting that abdominal fat may be more important for autonomic function than overall fat. All associations were strongest for VAT and associations between SAT and sympathetic activation attenuated after adjustment for VAT. This indicates that different fat depots within the abdomen do not only differ in localization but also in function and that the visceral fat depot is most important with regard to sympathetic activation.

One strength of our study is that we excluded participants not only with pre-existent CVD but also with cardiac abnormalities diagnosed by MR imaging. Abnormalities in cardiovascular structure and function are strongly associated with autonomic function.[7-9] The exclusion of participants with abnormal hearts reduced the possibility of reverse causation because of undetected CVD in our results. Another strength of our study is the availability of visceral and subcutaneous fat measured by MRI, in addition to BMI, waist circumference and total body fat percentage. This enabled us to study the association of the different abdominal fat depots with sympathetic activation.

A limitation of our study is the absence of a direct measure of sympathetic activation. In large epidemiological studies as ours, it is often not feasible to invasively or pharmacologically measure sympathetic activity and we therefore used surrogate markers of sympathetic activation. However, heart rate and heart rate variability are well-established parameters of autonomic function.[15, 16] Heart rate is influenced by sympathetic and parasympathetic outflow to the sinus node, and dominant sympathetic activation over parasympathetic activation results in a higher heart rate and less variability in heart rate. In addition, we investigated four parameters of ventricular repolarization as novel markers of sympathetic activation. These parameters have not been validated as a markers of sympathetic activation. However, the influence of the sympathetic nervous system on

ventricular repolarization has long been recognized.[27] In addition, three recent studies in which the sympathetic nervous system was experimentally stimulated (one in pigs and two in humans) have indicated that our study parameters are strongly influenced by the sympathetic nervous system.[17-19] Because our study is an observational study, the indices of ventricular repolarization in our analyses may be influenced by factors other than sympathetic outflow, such as age, sex, physical activity and smoking. However, by adjusting for these factors, we aimed to minimize their influence on the associations between body fat and sympathetic activation. Another limitation is the use of a single ECG for the measurement of sympathetic activation, because previous studies have indicated that multiple ECGs give a more reliable estimate.[28] However, any random measurement error in the ECG measures would have diluted the true associations, leading to an underestimation in our results. A final limitation is the measurement of confounding variables by questionnaire (smoking, ethnicity, education and physical activity), which may have resulted in some misclassification. Because of this potential misclassification and the fact that this is an observational study, we cannot exclude the presence of residual confounding in our analyses.

The results of our study extend the knowledge from previous papers on body fat and sympathetic activation, by showing that abdominal fat, especially visceral fat, was stronger associated with sympathetic activation than fat mass in general. Previous studies observed an association between BMI and sympathetic activation.[1, 2, 29] However, BMI may not be a valid measure to describe body composition because it does not distinguish between body fat and lean body mass. Total body fat percentage and waist circumference reflect body fat and to some extent fat distribution. In earlier papers, total body fat has been associated with different measurements of the autonomic nervous system, as has waist circumference.[3-6, 30] In our study we measured BMI, total body fat percentage and waist circumference and because we used standardized values for these measures we were able to compare the strength of the associations with sympathetic activation.

Only a few studies were able to differentiate between visceral and subcutaneous fat depots. Three studies reported an association between VAT and sympathetic activation.[31-33] Two of these papers also investigated SAT, and reported that VAT was stronger associated with sympathetic activation than SAT.[32, 33] Another study reported that sympathetic activation in individuals with high subcutaneous fat was not different from non-obese individuals.[34] We were able to extend these observations by showing that VAT was also associated with measures of sympathetic activation on the electrocardiogram (heart rate, QTc and the ventricular gradient) after adjustment for SAT, while the association of SAT with these measures attenuated after adjustment for VAT. Finally, in

line with our results, a recent paper observed an association of BMI with QTc, but not with Tpeak-end.[35] We showed the same results for other body fat measures.

Our results suggest that subcutaneous fat is not associated with sympathetic activation, or that the association between SAT and sympathetic activation is mediated by VAT. Several mechanisms could underlie these associations. Adipocytes, or macrophages invading the adipose tissue, secrete a number of adipokines.[10, 36] Distribution of the adipose tissue is a key determinant of this adipokine secretion. The secretion rate of pro-inflammatory cytokines is higher in visceral fat than in subcutaneous fat. Furthermore, visceral fat inhibits the secretion of anti-inflammatory factors.[11-14] Some pro-inflammatory cytokines, such as leptin, can cross the blood-brain-barrier and directly stimulate the central sympathetic nervous system in the hypothalamus.[10, 37] Other cytokines, for example C-reactive protein, TNF-α, plasminogen activator inhitor-1 and interleukin-6, cause a state of low-grade inflammation that can also stimulate the sympathetic nervous system.[10, 13] Therefore, altered cytokine production may underlie the strong association between visceral fat and sympathetic activation. However, some studies have shown that leptin is more related to total or subcutaneous fat rather than to visceral fat.[32, 34] If this is true, direct stimulation of the central nervous system by leptin could not explain our finding that visceral fat is strongest associated with sympathetic activation.

Because our study is cross-sectional, we cannot make any statements on the direction of the association. Therefore, a pathophysiological mechanism in which sympathetic activation promotes the accumulation of adipose tissue in the visceral compartment, could also explain our findings. Previous studies have shown that chronic activation of the sympathetic nervous system, caused by chronic stress or lack of physical activity could favour the accumulation of visceral fat.[38, 39] This line of reasoning would also explain the inverse association we observed between SAT and the spatial QRS-T angle; less sympathetic activation, reflected in a smaller (more favourable) QRS-T angle, could stimulate the storage of fat in the subcutaneous instead of the visceral compartment.

The association between body fat and sympathetic activation may have clinical implications. A recent study in middle aged and elderly persons without manifest heart disease showed that each increment of 5 beats/min resulted in an 11% higher risk all-cause mortality (95% CI: 1-22; p=0.033) and a 12% higher rate of cardiovascular events (95% CI: 0-26; p=0.044) after 76 months of follow up.[40] In our study, approximately 50 cm^2 of visceral adipose tissue was associated with an increase in resting heart rate of 2.1 beats/min (95% CI: 1.3, 3.0; p<0.001). The explained variance was modest. However, because our population was free of CVD and diabetes, had structurally normal hearts and we adjusted for many factors that increase the risk of cardiovascular disease, our

results still suggest that body fat, especially visceral fat, may be important in the risk of cardiovascular events and mortality.

In conclusion, we observed that body fat, especially abdominal fat, is associated with sympathetic activation. Furthermore, visceral fat seems more important than subcutaneous fat with regard to sympathetic activation. As participants in our study had structurally normal hearts, our results imply that already before the onset of CVD excess (abdominal) body fat is associated with sympathetic activation. Future prospective studies should investigate whether sympathetic activation is a mediator in the association between body fat and CVD.

REFERENCE LIST

1. Grassi G, Seravalle G, Cattaneo BM et al. Sympathetic activation in obese normotensive subjects. Hypertension 1995;25:560-563.
2. Skrapari I, Tentolouris N, Perrea D, Bakoyiannis C, Papazafiropoulou A, Katsilambros N. Baroreflex sensitivity in obesity: relationship with cardiac autonomic nervous system activity. Obesity (Silver Spring) 2007;15:1685-1693.
3. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E, Nicod P. Body fat and sympathetic nerve activity in healthy subjects. Circulation 1994;89:2634-2640.
4. Peterson HR, Rothschild M, Weinberg CR, Fell RD, McLeish KR, Pfeifer MA. Body fat and the activity of the autonomic nervous system. N Engl J Med 1988;318:1077-1083.
5. Poehlman ET, Gardner AW, Goran MI et al. Sympathetic nervous system activity, body fatness, and body fat distribution in younger and older males. J Appl Physiol 1995;78:802-806.
6. Young JB, Troisi RJ, Weiss ST, Parker DR, Sparrow D, Landsberg L. Relationship of catecholamine excretion to body size, obesity, and nutrient intake in middle-aged and elderly men. Am J Clin Nutr 1992;56:827-834.
7. Manfrini O, Pizzi C, Viecca M, Bugiardini R. Abnormalities of cardiac autonomic nervous activity correlate with expansive coronary artery remodeling. Atherosclerosis 2008;197:183-189.
8. La Rovere MT, Bigger JT Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998;351:478-484.
9. Hillebrand S, Gast KB, de Mutsert R et al. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. Europace 2013;15:742-749.
10. Smith MM, Minson CT. Obesity and adipokines: effects on sympathetic overactivity. J Physiol 2012;590:1787-1801.
11. Lemieux I, Pascot A, Prud'homme D et al. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol 2001;21:961-967.
12. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847-850.
13. He G, Pedersen SB, Bruun JM, Lihn AS, Jensen PF, Richelsen B. Differences in plasminogen activator inhibitor 1 in subcutaneous versus omental adipose tissue in non-obese and obese subjects. Horm Metab Res 2003;35:178-182.
14. Good M, Newell FM, Haupt LM, Whitehead JP, Hutley LJ, Prins JB. TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue--influence of BMI and adipose distribution. Diab Vasc Dis Res 2006;3:26-33.
15. Bootsma M, Swenne CA, Van Bolhuis HH, Chang PC, Cats VM, Bruschke AV. Heart rate and heart rate variability as indexes of sympathovagal balance. Am J Physiol 1994;266:H1565-H1571.
16. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17:354-381.
17. Ramirez RJ, Ajijola OA, Zhou W et al. A new electrocardiographic marker for sympathetic nerve stimulation: modulation of repolarization by stimulation of stellate ganglia. J Electrocardiol 2011; 44:694-699.

18. Vahedi F, Haney MF, Jensen SM, Naslund U, Bergfeldt L. Effect of heart rate on ventricular repolarization in healthy individuals applying vectorcardiographic T vector and T vector loop analysis. Ann Noninvasive Electrocardiol 2011;16:287-294.
19. Vahedi F, Odenstedt J, Hartford M, Gilljam T, Bergfeldt L. Vectorcardiography analysis of the repolarization response to pharmacologically induced autonomic nervous system modulation in healthy subjects. J Appl Physiol 2012;113:368-376.
20. de Mutsert R, den Heijer M, Rabelink TJ et al. The Netherlands Epidemiology of Obesity (NEO) study: study design and data collection. Eur J Epidemiol 2013;28:513-523.
21. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987;317:1098.
22. Kors JA, van HG, Sittig AC, van Bemmel JH. Reconstruction of the Frank vectorcardiogram from standard electrocardiographic leads: diagnostic comparison of different methods. Eur Heart J 1990;11:1083-1092.
23. Man SC, Van der Wall EE, Schalij MJ, Swenne CA. Beats: An interactive research oriented ECG analysis system. Computing in Cardiology 2010;1007-1010.
24. Draisma HH, Swenne CA, Van de Vooren H, Maan AC. LEADS: an interactive research oriented ECG/VCG analysis system. Computing in Cardiology 2005;32:515-518.
25. Korn EL, Graubard BI. Epidemiologic studies utilizing surveys: accounting for the sampling design. Am J Public Health 1991;81:1166-1173.
26. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. Am J Clin Nutr 1998;68:899-917.
27. Abildskov JA. Neural mechanisms involved in the regulation of ventricular repolarization. Eur Heart J 1985;6 Suppl D:31-39.
28. Schroeder EB, Whitsel EA, Evans GW, Prineas RJ, Chambless LE, Heiss G. Repeatability of heart rate variability measures. J Electrocardiol 2004;37:163-172.
29. Antelmi I, de Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. Am J Cardiol 2004;93:381-385.
30. Beske SD, Alvarez GE, Ballard TP, Davy KP. Reduced cardiovagal baroreflex gain in visceral obesity: implications for the metabolic syndrome. Am J Physiol Heart Circ Physiol 2002;282:H630-H635.
31. Poliakova N, Despres JP, Bergeron J, Almeras N, Tremblay A, Poirier P. Influence of obesity indices, metabolic parameters and age on cardiac autonomic function in abdominally obese men. Metabolism 2012;61:1270-1279.
32. Alvarez GE, Beske SD, Ballard TP, Davy KP. Sympathetic neural activation in visceral obesity. Circulation 2002;106:2533-2536.
33. Gao YY, Lovejoy JC, Sparti A, Bray GA, Keys LK, Partington C. Autonomic activity assessed by heart rate spectral analysis varies with fat distribution in obese women. Obes Res 1996;4:55-63.
34. Alvarez GE, Ballard TP, Beske SD, Davy KP. Subcutaneous obesity is not associated with sympathetic neural activation. Am J Physiol Heart Circ Physiol 2004;287:H414-H418.
35. Braschi A, Abrignani MG, Francavilla VC, Francavilla G. Novel electrocardiographic parameters of altered repolarization in uncomplicated overweight and obesity. Obesity (Silver Spring) 2011;19:875-881.
36. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85-97.
37. Ahima RS, Osei SY. Leptin signaling. Physiol Behav 2004;81(2):223-241.

38. Kyrou I, Tsigos C. Stress hormones: physiological stress and regulation of metabolism. Curr Opin Pharmacol 2009;9:787-793.
39. Mueller PJ. Physical (in)activity-dependent alterations at the rostral ventrolateral medulla: influence on sympathetic nervous system regulation. Am J Physiol Regul Integr Comp Physiol 2010; 298:R1468-R1474.
40. Johansen CD, Olsen RH, Pedersen LR et al. Resting, night-time, and 24 h heart rate as markers of cardiovascular risk in middle-aged and elderly men and women with no apparent heart disease. Eur Heart J 2013;34:1732-1739.

Supplementary table: Correlation matrix between measures of body fat and electrocardiographic measures of sympathetic activation

	Mean HR (beats/min)	P-value	CV (%)	P-value	QTc (ms)	P-value	Tpeak-end (ms)	P-value	Ventricular gradient magnitude (mV*ms)	P-value	QRS-T angle (°)	P-value
BMI (kg/m^2)	0.11	0.002	−0.05	0.120	0.08	0.024	0.05	0.196	−0.10	0.006	0.04	0.211
Total body fat (%)	0.19	<0.001	0.03	0.349	0.28	<0.001	−0.11	0.002	−0.31	<0.001	−0.13	<0.001
WC (cm)	0.13	<0.001	−0.07	0.068	0.03	0.401	0.11	0.002	0.01	0.867	0.07	0.055
VAT (cm^2)	0.17	<0.001	−0.06	0.095	0.09	0.017	0.15	<0.001	−0.07	0.045	0.11	0.002
SAT (cm^2)	0.15	<0.001	−0.03	0.463	0.16	<0.001	−0.05	0.130	−0.16	<0.001	−0.12	0.001

Results were based on weighted analyses

BMI=body mass index; WC=waist circumference; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; HR=heart rate; CV=coefficient of variation; QTc=correct QT time