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MR and CT evaluation of cardiovascular risk in metabolic syndrome

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Genetically determined prospect to become long-lived is associated with less abdominal fat and in particular less abdominal visceral fat in men

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CHAPTER 2

ABSTRACT

Objective: Familial longevity is marked by an exceptionally healthy metabolic profile and low prevalence of cardiometabolic disease observed already at middle age. We aim to investigate whether regional body fat distribution, which has previously shown to be associated with cardiometabolic risk, is different in offspring of long-lived siblings compared to controls.

Materials and Methods: Our institutional review board approved the study and all participants ($n = 344$, average age in years 65.6) gave written informed consent. Offspring ($n = 175$) of nonagenarian siblings were included. Their partners ($n = 169$) were enrolled as controls. For abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) measurements, a single-slice 8.0 mm computed tomography (CT) acquisition was planned at the level of the 5th lumbar vertebra. In addition, participants underwent prospectively electrocardiography-triggered unenhanced volumetric CT of the heart. Abdominal VAT and SAT areas (cm^2) and epicardial adipose tissue (EAT) volumes (mL) were acquired by semiautomated segmentation techniques. Linear regression analysis was performed adjusting for cardiovascular risk factors.

Results: Total abdominal fat areas were smaller in male offspring compared to controls (353.0 cm^2 versus 382.9 cm^2 , $p = 0.022$). The association between low abdominal VAT areas in male offspring (149.7 cm^2 , versus 167.0 cm^2 in controls, $p = 0.043$) attenuated after additional adjustment for diabetes ($p = 0.078$). Differences were not observed for females. EAT volumes were similar between offspring of long-lived siblings and controls.

Conclusion: Males who have genetically determined prospect to become long-lived have less abdominal fat and in particular less abdominal VAT compared to controls.

INTRODUCTION

Fat distribution plays a major role in human health (1) (2). Offspring of long-lived siblings have a lower prevalence of diabetes and metabolic syndrome at middle age, and, among individuals without diabetes, are more glucose tolerant and insulin sensitive compared to controls (3). The aim of our study was to investigate whether fat distribution as measured by computed tomography is related to longevity by comparing distribution of abdominal visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT), and epicardial adipose tissue (EAT) in offspring of nonagenarian siblings and controls.

MATERIALS AND METHODS

Study population

Our institutional review board approved the study and all participants gave informed consent. Offspring of long-lived siblings were recruited from the Leiden Longevity Study (4). In short, 421 long-lived families were enrolled in the study between 2002 and 2006 according to the following inclusion criteria: (1) there were at least two living siblings per family who fulfilled the age criteria and were willing to participate, (2) men had to be aged 89 years or older and women had to be aged 91 years or older and (3) the sib pairs had to have the same parents. In 2002, only 0.5% of long-lived men were aged ≥ 89 years, and only 0.5% of long-lived women aged ≥ 91 years. Accordingly, siblings who meet these age criteria are even rarer and are estimated to represent far less than 0.1% of the long-lived population (4). Offspring of these long-lived siblings were included as they were shown to have a 30% lower mortality rate compared to the general population (4). Their partners, who share the same geographical and socio-economic background, were enrolled as control group (4). There were no selection criteria on health or demographic characteristics. For the current study, subjects were recruited from the offspring of the long-lived siblings and their spouses. In addition, their partners, who share the same geographical and socio-economic background, were enrolled as control group (4). There were no selection criteria on health or demographic characteristics. In total, 344 subjects comprising 175 offspring and 169 controls were included. Imaging was performed between September 2009 and December 2010. Within this time frame, blood samples were taken for the determination of nonfasting serum levels of glucose, triglycerides, and high-density lipoprotein (HDL) and total cholesterol. Information on body mass index (BMI) and smoking status was obtained from the study subjects. Information on medical history including presence myocardial infarction and diabetes was requested from the participants' general practitioner (4). Hypertension was defined as use of antihypertensive medication as obtained from pharmacy records (5).

Data acquisition

Participants underwent prospectively electrocardiography (ECG)-triggered unenhanced volumetric CT of the heart. All examinations were performed on a 320 multidetector-row computed tomography (CT) scanner (Aquilion ONE, Toshiba Medical Systems, Otawara, Japan). The scan range was set between the carina and cardiac apex. Depending on the expected scan range, a 320×0.5 mm or 280×0.5 mm detector configuration was used. The preferred reconstruction phase was determined during a breath-hold exercise and set at 45% or 75% of the cardiac RR-cycle, depending on heart rate. Volumetric imaging was acquired during a single heartbeat during breath hold at inspiration. Scan parameters were: tube voltage 120 kV and tube current 200–400 mA, depending on participant's size and shape (200 mA for small or thin participants, 250 mA for average size participants, and 300–400 mA for large or obese participants). Rotation time was 0.35 sec. Images were reconstructed with a slice thickness of 3 mm. Estimated effective radiation dose varied between 0.8 and 2.4 mSv (mean, 1.2 mSv) for cardiac CT. For abdominal fat measurement, a single-slice 8.0 mm acquisition was planned at the level of the 5th lumbar vertebra. All examinations were performed on a 320 multidetector-row computed tomography (CT) scanner (Aquilion ONE, Toshiba Medical Systems, Otawara, Japan). Scan parameters were: tube voltage 120 kV, tube current 310 mA, Rotation time: 0.5 sec. Imaging was performed during breath hold after expiration. Estimated radiation dose was 0.3 mSv for abdominal CT.

Abdominal adipose tissue

Data were processed by two research fellows (MS, BR) under direct supervision of a radiologist with 13 years of experience in cardiac and abdominal imaging (LK). Analysis of abdominal VAT and SAT was performed with dedicated fat measurement software available at the CT scanner console. An automatic tool was used for recognizing fat. Predefined thresholds for adipose tissue were set from -150 to -30 Hounsfield Units (HU) (22). By manual outlining of the abdominal muscular wall (Figure 1), subcutaneous adipose tissue was separated from intraabdominal adipose tissue surrounding the internal abdominal organs, which includes intraperitoneal and retroperitoneal adipose tissue. Intraabdominal adipose tissue is referred to as abdominal VAT. Accordingly, total abdominal fat areas (cm^2) as well as abdominal VAT and SAT areas (cm^2) were calculated automatically. In addition, VAT / SAT ratio was calculated.

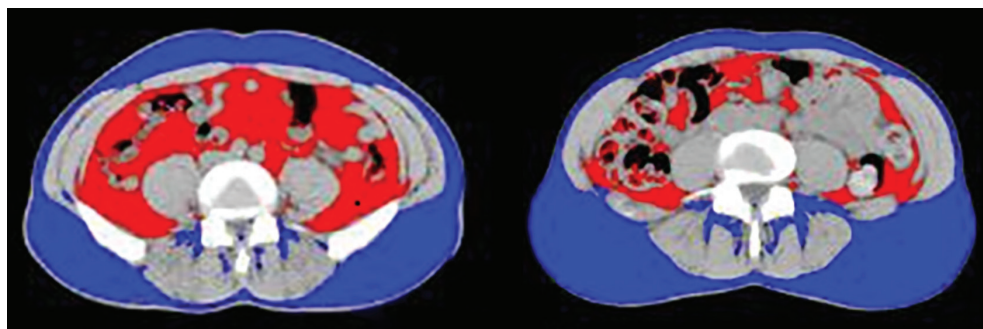


Figure 1. Computed tomography abdominal images at the level of the 5th lumbar vertebra, 8.0 mm slice thickness. The red areas represent visceral fat and the blue areas represent subcutaneous fat. Left panel: 49-year old male control participant, BMI of 26, the visceral fat area measured 119.6 cm² and the subcutaneous fat area 156.8 cm². Right panel: 50-year old female offspring participant, BMI of 20.1, the visceral fat area measured 39.6 cm² and the blue area subcutaneous fat area 150.2cm².

Epicardial adipose tissue

For analysis of epicardial adipose tissue, QMASS research software (Medis, Leiden, the Netherlands) was used. Epicardial fat was defined as all adipose tissue located within the pericardium, measured from the lowest point of the left ventricular apex to the lowest point at the pulmonary trunk at its diversion into the left and right pulmonary artery. The pericardium was manually traced within each slice to determine a region of interest (Figure 2). Within the region of interest, adipose tissue was defined as pixels within a window level of -190 to -30 HU (23). Accordingly, the area in squared centimetres that corresponds to the defined HU range was calculated automatically. The total EAT volume was then calculated by the summation of all slides multiplied by the reconstructed slice thickness in centimetres, which was 0.3 cm. This allowed manual calculation of the EAT volume in millilitres. Twenty scans were slightly incomplete, missing a maximum of one or two slices at the scan edges at either the apex or the pulmonary trunk. These scans were also included as the amount of adipose tissue in these missing slices was considered negligible.

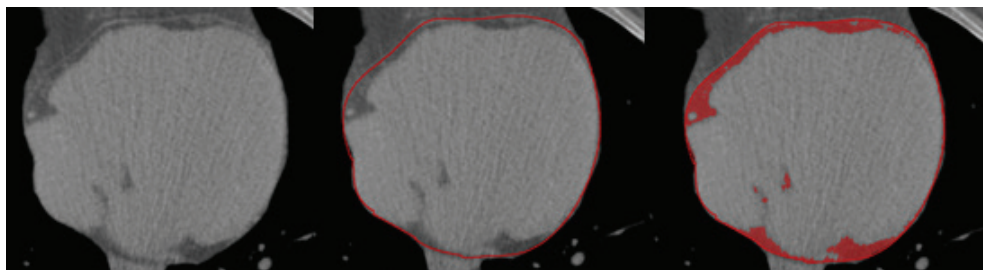


Figure 2. Computed tomography image of the heart, 3mm slice thickness. Left panel: the heart with the pericardium. Middle panel: On each slice level, contours were manually drawn at the pericardium. Right panel: Within the region of interest, the area in squared centimetres that corresponds to the defined Hounsfield unit range was calculated automatically (area in red). The total epicardial adipose tissue volume was then calculated by the summation of all slides multiplied by the reconstructed slice thickness in centimetres, which was 0.3 cm. In this participant, the amount of epicardial fat on this slice was 8.52 cm². By multiplying with slice thickness of 0.3cm, representing 2.56 cm³ (or mL) of fat. Total epicardial fat volume by multiplying by numbers of slices was 99.38 mL.

Statistical analysis

All statistical analyses were performed separately for sexes. Differences in subject characteristics between offspring and controls were assessed using student's t-test, Pearson chi-squared test, and linear regression analysis adjusting for age and BMI. Differences in regional adipose tissue distribution in offspring of long-lived and control subjects were assessed with linear regression analysis, adjusting for age, BMI, smoking, hypertension, diabetes and serum levels of glucose, high-density lipoprotein (HDL) cholesterol, and triglycerides. Analyses were repeated after adjusting for age, BMI, smoking, and use of specific type of antihypertensive medication (diuretics, beta-blockers, ace-inhibitors, calcium antagonists). Difference in VAT/SAT ratio between sexes was assessed using the same models. Continuous variables were tested for normality and, if appropriate, logarithmically transformed and used in calculations (LnTriglycerides). Data are presented as geometric means. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS), version 20.0. $P < 0.05$ was considered statistically significant.

RESULTS

Subject characteristics are shown in table 1. Regional adipose tissue distribution in offspring of long-lived and control subjects is shown in table 2. In men, total abdominal fat areas were smaller in offspring compared to controls (353.0 cm² versus 382.9 cm², respectively, $p = 0.022$ in the fully adjusted model). Abdominal SAT areas were smaller in male offspring compared to controls, however the difference between the two groups did not reach statistical significance ($p = 0.070$). Abdominal VAT areas were smaller in male offspring compared to controls after ad-

justing for age, BMI, smoking, hypertension, and serum levels of glucose, high-density lipoprotein (HDL) cholesterol, and triglycerides (149.7 cm² versus 167.0 cm², respectively, $p = 0.043$) but after additionally adjusting for diabetes the association attenuated ($p = 0.078$). In subjects without diabetes ($n = 326$), abdominal VAT areas were smaller in male offspring compared to controls (149.6 cm² versus 166.6 cm², respectively, $p = 0.042$). Mean VAT/SAT ratio was 0.778 in males versus 0.483 in females ($p < 0.001$ in the fully adjusted model). There was no difference in VAT/SAT ratio between offspring and controls. EAT volume were similar in offspring of long-lived siblings and controls, in both males and females (table 2). Results did not change materially after adjusting analyses for use of specific type of antihypertensive medication (data not shown).

Table 1: Characteristics of the study population

	Male (n = 167)			Female (n = 177)		
	Offspring (n = 96)	Control (n = 71)	P-value	Offspring (n = 79)	Control (n = 98)	P-value
Age†	66.0 (0.62)	67.8 (0.87)	0.098	65.6 (0.67)	63.7 (0.66)	0.047*
BMI†	26.9 (0.31)	26.5 (0.37)	0.478	26.0 (0.53)	26.7 (0.45)	0.319
Hypertension, n (%)‡	16 (17%)	21 (29%)	0.047*	18 (23%)	18 (18%)	0.468
Myocard infarct, n (%)‡	1 (1%)	3 (2%)	0.195	0 (0%)	0 (0%)	N/A
Diabetes, n (%)‡	3 (3%)	8 (11%)	0.038*	2 (3%)	5 (5%)	0.402
Glucose	5.76 ± 0.11	5.97 ± 0.14	0.239	5.70 ± 0.14	5.93 ± 0.12	0.197
Triglycerides	1.78 ± 1.1	1.72 ± 1.1	0.703	1.32 ± 1.1	1.43 ± 1.1	0.288
HDL-cholesterol	1.30 ± 0.04	1.22 ± 0.04	0.140	1.63 ± 0.05	1.55 ± 0.04	0.271
Total cholesterol	5.57 ± 0.12	5.46 ± 0.13	0.549	5.67 ± 0.15	5.67 ± 0.12	0.991
Diuretics, n (%)‡	6 (6%)	3 (4%)	0.567	5 (6%)	2 (2%)	0.146
Beta blockers, n (%)‡	5 (5%)	10 (14%)	0.047*	7 (9%)	13 (13%)	0.358
Ace-inhibitors, n (%)‡	7 (7%)	15 (21%)	0.009*	9 (11%)	9 (9%)	0.629
Calcium antagonists, n (%)‡	2 (2%)	7 (10%)	0.028*	10 (13%)	4 (4%)	0.036*

Values are mean±standard error. P values are from student's t-test (†) and Pearson chi-square test (‡). For the variables glucose, triglycerides (mmol/L), HDL-cholesterol (mmol/L), and total cholesterol (mmol/L), linear regression analysis was performed, correcting for age, sex, and BMI. BMI, body mass index. HDL: high-density lipoprotein. * P-value < 0.05

Table 2: Adipose tissue distribution in offspring of long-lived and control subjects stratified to gender

	Male			Female		
	Offspring	Control	P-value	Offspring	Control	P-value
TFA (cm ²)						
Model 1	351.0 ± 12.6	371.0 ± 14.7	0.304	371.7 ± 15.7	410.5 ± 14.1	0.069
Model 2	353.8 ± 10.3	386.5 ± 11.2	0.008 *	375.4 ± 11.5	394 ± 10.3	0.120
Model 3	353.0 ± 17.3	382.9 ± 16.8	0.022 *	399.2 ± 20.3	414.0 ± 19.8	0.228
SAT (cm ²)						
Model 1	204.2 ± 8.1	211.0 ± 9.4	0.584	264.0 ± 11.3	287.5 ± 10.1	0.124
Model 2	200.8 ± 7.3	215.2 ± 8.0	0.102	262.0 ± 8.8	272.3 ± 7.8	0.268
Model 3	184.1 ± 12.2	200.8 ± 11.9	0.070	255.5 ± 15.7	264.5 ± 15.4	0.344
VAT (cm ²)						
Model 1	146.8 ± 6.5	160.0 ± 7.6	0.188	107.7 ± 6.1	123.0 ± 5.4	0.064
Model 2	153.0 ± 6.5	171.3 ± 7.1	0.020 *	113.4 ± 6.0	122.1 ± 5.3	0.169
Model 3	167.8 ± 10.7	182.1 ± 10.4	0.078	143.8 ± 10.1	149.6 ± 9.9	0.343
EAT (mL)						
Model 1	110.0 ± 5.0	111.8 ± 5.8	0.920	95.7 ± 4.8	96.6 ± 4.3	0.889
Model 2	116.5 ± 5.6	120.2 ± 6.1	0.588	101.3 ± 5.6	98.1 ± 5.0	0.581
Model 3	127.4 ± 9.4	128.2 ± 9.1	0.907	110.2 ± 9.7	108.0 ± 9.4	0.714

Values are means ± standard error. P-values are from linear regression analysis, using different models.

Model 1: adjusted for age

Model 2: adjusted for age, BMI, and smoking

Model 3: model 2 + hypertension, diabetes, and serum levels of glucose, HDL cholesterol, and triglycerides.

TFA: total abdominal fat area; SAT: abdominal subcutaneous adipose tissue; VAT: abdominal visceral adipose tissue; EAT: epicardial adipose tissue. * P-value < 0.05

DISCUSSION

Our data show that males with genetically determined prospect to become long-lived have less abdominal fat and less abdominal VAT in particular compared to controls.

Increased deposition of fat in the abdominal visceral compartment may cause dysregulation of adipokine production (6) which in turn may contribute to the initiation and progression of metabolic and cardiovascular complications of obesity including hypertension, metabolic syndrome and type 2 diabetes (2,6,7). Consistent with a potential link between abdominal VAT and health outcomes, in our study population, prevalence of hypertension (17% versus 29%) as well as type 2 diabetes (3% versus 11%) was significantly lower in male offspring of long-lived siblings compared to controls which is in accordance with previous findings in familial nonagenarians (3). Of note, after adjusting for diabetes we found that the association between low abdominal VAT areas and familial longevity attenuated. On the other hand, male offspring without diabetes were characterized by significantly lower abdominal VAT areas compared to controls. Offspring of long-lived siblings may thus be protected against development of type 2 diabetes by relatively low abdominal VAT deposition.

Fat distribution changes during life and there is a known gender difference in body fat distribution (8,9). One recent study used dual-energy X-ray absorptiometry to assess body composition in 250 healthy subjects aged 18 to 70 years. A slow increase in the amount of abdominal visceral fat, which was higher in males of all age groups compared to females, was observed until 51 years old in both sexes; in older decades, males grew progressively in the amount of abdominal visceral fat, while in females the amount of visceral fat remained steady (10). Increasing amounts of abdominal VAT with age have been related to age-linked deterioration in cardiometabolic risk profile (7,11). Our findings suggest that male offspring of long-lived siblings may have an advantageous “younger” fat distribution as would be expected by their chronological age, when compared to partners from the general population that served as controls.

In contrast to our observed difference in abdominal fat distribution in male offspring compared to controls, we found no differences in total abdominal fat and abdominal VAT between female offspring and controls. Abdominal VAT depots are relatively small in women compared to men (7,12-14) and it has been suggested that the tendency of men to accumulate abdominal VAT may be a key factor in predicting why obesity is much more hazardous in men than in women (7).

We found similar abdominal VAT/SAT ratios between offspring of long-lived siblings and controls. Although it has been suggested that VAT/SAT ratio may be a correlate of cardiometabolic risk independent of absolute fat volumes (15,16), others have shown that abdominal VAT is a stronger correlate of cardiometabolic risk than the VAT/SAT ratio (15,17). It has been noted that

abdominal VAT may be a marker of fat deposition at undesired sites such as the liver (6,7). One recent study showed that the extent of liver steatosis, assessed by liver enzymes and computed tomography, is similar between offspring of long-lived siblings and controls (18). In line with this notion, it has been suggested that familial longevity is marked by enhanced peripheral but not hepatic insulin sensitivity (19).

We found no differences in EAT deposition between offspring and controls which may indicate that abdominal VAT rather than EAT has systemic metabolic effects associated with prospect to become long-lived (20).

Because of the known differences in fat distribution between men and women, analysis were separately performed for sexes. Therefore, offspring of long-lived siblings could not be directly compared to their own partners who served as controls. However, the overall study group was environmentally matched, which is unique in study design. While single-slice images for abdominal VAT measurements are often used in research studies to limit radiation exposure, it should be noted that they may be less accurate than volumetric analysis (21). Due to the observational nature it is not possible to infer causality from our study.

In conclusion, males who have genetically determined prospect to become long-lived have less abdominal fat and less abdominal VAT in particular compared to controls.

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