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Authors: Dunet V, Feihl F, Dabiri A, Allenbach G, Waeber B, Heinzer R, Prior JO

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THORACIC FAT VOLUME IS INDEPENDENTLY ASSOCIATED WITH CORONARY VASOMOTION

Vincent Dunet, MD,* François Feihl, MD,† Amin Dabiri, MD,† Gilles Allenbach, MD,* Bernard Waeber, MD,† Raphaël Heinzer, MD,‡ John O. Prior, PhD, MD,*

*Department of Nuclear Medicine; †Department of Clinical Physiopathology; ‡Center for Investigation and Research in Sleep, Lausanne University Hospital, Lausanne, Switzerland

Address for correspondence:

John O. Prior, PhD, MD, Department of Nuclear Medicine, Lausanne University Hospital, Rue du Bugnon 46, 1011 Lausanne, Switzerland. Phone: +41 21 314 43 48. Fax: +41 21 314 43 49. E-mail: John.Prior@chuv.ch

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Abstract

Purpose

Thoracic fat has been associated with an increased risk of coronary artery disease (CAD). As endothelium-dependent vasoreactivity is a surrogate of cardiovascular events and is early impaired in atherosclerosis, we aimed at assessing the possible relationship between thoracic fat volume (TFV) and endothelium-dependent coronary vasomotion.

Methods

Fifty healthy volunteers without known CAD or major cardiovascular risk factors (CRF) prospectively underwent a ^{82}Rb cardiac PET/CT to quantify myocardial blood flow (MBF) at rest, and MBF response to cold pressor testing (CPT-MBF) and adenosine (stress-MBF). TFV was measured by a 2-D volumetric CT method and common laboratories (glucose and insulin levels, HOMA-IR, cholesterol, triglyceride, hsCRP) were checked. Relationships between CPT-MBF, TFV and other CRF were assessed using non-parametric Spearman rank correlation test and multivariate linear regression analysis.

Results

All of the 50 participants (58±10y) had normal stress-MBF (2.7±0.6 mL/min/g; 95%CI: 2.6-2.9) and myocardial flow reserve (2.8±0.8; 95%CI: 2.6-3.0) excluding underlying CAD. Univariate analysis revealed a significant inverse relation between absolute CPT-MBF and sex (ϱ=–0.47, p=0.0006), triglyceride (ϱ=–0.32,p=0.024) and insulin levels (ϱ=–0.43,p=0.0024), HOMA-IR (ϱ=–0.39,p=0.007), BMI (ϱ=–0.51,p=0.0002) and TFV (ϱ=–0.52,p=0.0001). MBF response to adenosine was also correlated with TFV (ϱ=–0.32,p=0.026). On multivariate analysis TFV emerged as the only significant predictor of MBF response to CPT (p=0.014).

Conclusions

TFV is significantly correlated with endothelium-dependent and -independent coronary vasomotion. High TF burden might negatively influence MBF response to CPT and to adenosine stress, even in persons without CAD, suggesting a link between thoracic fat and future cardiovascular events.

Keywords: thoracic fat; endothelial function; myocardial blood flow; PET

Introduction

Endothelial dysfunction is widely recognized as the key-step toward atherosclerosis and as a surrogate predictor of cardiovascular event. Numerous studies highlighted the influence of diabetes, insulin resistance, uncontrolled hypertension, smocking as well as menopause upon the impairment of endothelium-dependent vasomotion using quantitative cardiac PET/CT analysis.

Thoracic fat is defined by the sum of intra-pericardial fat derived from splanchnopleuric mesoderm plus extra-pericardial fat surrounding the pericardium within the mediastinum [1]. Recent studies reported that pericardial fat correlated with coronary plaque [2] and might promote coronary artery disease (CAD) development by influencing microvascular function [3]. In fact, although pericardial fat has been independently associated to an increase risk of CAD [4-6], the exact mechanism is not known yet. Some studies suggest a direct influence of adipokines by an outside-to-inside pathway from epicardial fat contributing to vascular inflammation and plaque progression. Two recent studies showed an association between intra-pericardial fat and hyperemic coronary blood flow measured by PET/CT [7] and between epicardial fat thickness measured by echocardiography and flow mediated dilation [8]. Nevertheless no relation between endothelium-dependent coronary vasoreactivity and thoracic fat has been demonstrated yet.

Our aim was to assess whether thoracic fat, at an early stage, may influence endothelium-dependent vasomotion in patients without CAD or detectable cardiovascular risk factor.

Methods

Study design

In this monocentric study, volunteers were prospectively enrolled from January 2009 to June 2009. Before inclusion, they all underwent a medical examination to screen for cardiovascular

risk factor. Inclusion criteria were (1) volunteers with no cardiovascular risk factor and (2) no medication. Participants with diabetes mellitus (fasting plasma glucose level >126mg/dL), past or present smoking, hypertension (≥140/90 mmHg), low density lipoprotein (LDL) level ≥160mg/dL, high density lipoprotein (HDL) level <30mg/dL, triglyceride (TG) level (>400mg/dL), peripheral artery disease, known coronary artery disease or myocardial infarction, cardiomyopathy, renal failure, peripheral neuropathy, systemic disease, or contraindication to adenosine (asthma, chronic obstructive bronchitis, 2nd and 3rd degree atria-ventricular bloc) were thus excluded. Weight was not a exclusion criteria.

For every participant, fasting glucose plasma, insulin plasma, LDL, HDL, TG, high-sensitivity C-reactive protein (hsCRP) levels were measured, and insulin resistance was assessed by calculating homeostasis model assessment (*HOMA-IR*) index (*HOMA-IR = fasting plasma glucose* (mmol/L) × *fasting plasma insulin* (μU/mL) / 22.5). Volunteers were refrained at least 6h from any food and ≥24h from caffeine intake before PET studies. The study was approved by the Ethic Committee of The University of Lausanne. Every participant signed a written consent form.

PET studies

All the volunteers fulfilling the inclusion criteria (n=50) underwent ^{82}Rb cardiac PET/CT measurements of myocardial blood flow (MBF). After a rest study, the participant underwent cold pressor testing (CPT) to assess MBF changes mainly due to endothelium-dependent vasomotion [9]. CPT was performed by a 2-min immersion of the left lower limb in iced water starting one minute before the administration of ^{82}Rb. Finally, a pharmacological stress was carried out by adenosine (140μg/kg/min) infusion over 6 minutes to determine myocardial blood flow increase (stress-MBF) mainly due to endothelium-independent vasomotion and myocardial

flow reserve (MFR) in order to exclude underlying coronary artery disease. For each study, after a 10-second infusion of ^{82}Rb (1450MBq) 6-min dynamic (12x8 s, 5x12 s, 1x30 s, 1x60 s, 1x120 s) cardiac PET (Discovery LS, GE Medical Systems, Milwaukee, WI, USA) was acquired. A cardiac computed tomography (CT) was also performed to correct for photon attenuation by soft tissues.

Data were reconstructed using OSEM (8 subsets, 2 iterations) and processed with the fully-automatic Flowquant 1.2.3 software using a previously described [10] two-compartment modeling approach to derive myocardial blood flow at rest, during the cold pressor test and during the pharmacological stress. Stress/rest uptake images were also analyzed in a semi-quantitative way (summed rest score and summed difference score) to exclude myocardial ischemia/scar using a 17-segment model. Before processing, good alignment between PET and CT series was verified to avoid attenuation correction artifact.

Blood pressure, heart rate and a 12-lead ECG were recorded at 1-minute intervals during each procedure. To correct for cardiac workload, rest and CPT myocardial blood flow were also normalized using the rate-pressure product (*RPP = heart rate ×systolic blood pressure*).

The radiation dose for each patients was estimated to be 3×1.8 mSv for rest, CPT and stress Rb-82 [11] and 3×0.2 mSv for the low-dose attenuation correction CT [12] resulting in a total dose of 6 mSv. The product of the CT dose index (CTDI= 0.7±0.1 mGy) and the z-axis scan coverage (18 cm) provides the dose-length product (DLP= 12.6±1.7 mGy.cm), which was subsequently multiplied by the conversion factor for the chest (k= 0.017 mSv. mGy^{-1}.cm^{-1}) to obtain the effective dose for the CT acquisitions.

Thoracic Fat Volume Quantification

CT studies were performed using an unenhanced, low-dose protocol (120 kV tube voltage, 10 mA tube current, 1.0 sec/rotation, 4 x 5mm detector configuration, 50 cm scan field-of-view, 18 cm z-axis coverage) on the 4-slice CT scanner (Light Speed Plus, GE Medical Systems, Milwaukee, WI, USA) of our PET/CT (Discovery LS; GE Medical Systems, Milwaukee, WI, USA). Data were reconstructed by filtered back projection with a 512x512 pixels matrix and slice thickness of 5 mm resulting in a spatial resolution of 1x1x5mm. Thoracic fat volume was determined using a threshold 2-D short axis based method after transferring patient imaging data on a dedicated workstation (Advantage Windows 4.4; GE Medical Systems, Milwaukee, WI, USA) [13]. Reformatted images were obtained from the raw data of axial images to the 2D short axis views with 5-mm slice thickness and 5-mm intersection gaps. Thoracic fat volume was defined as the adipose tissue from the surface of the heart to the adjacent organ into the inferior mediastinum. Thus, in a volume of interest extending from the pulmonary trunk bifurcation to the diaphragm, a CT attenuation threshold (–200 to –30 HU) was used to isolate thoracic fat. Finally thoracic fat volume in mL was automatically measured using a histogram-based analysis (Figure 1).

Statistical analysis

All statistical analyses were carried out with Stata 11.1. Continuous variables are presented as mean ± standard deviation (SD). Wilcoxon rank sum test was used to compare characteristics according to sex (male=1, female=0). Relations between variables were assessed using non-parametric Spearman's rank correlation test (ϱ). For sex, significance or non-significance of the relations was confirmed using logistic regression that is more suitable for categorical variables (data not shown). Multivariate linear regression analysis and stepwise multiple linear regression

analysis were performed using significant univariate predictors to determine independent relationship to TFV or MBF response. Before performing multivariate analysis, collinearity of variables was searched. A threshold of >0.7 for absolute correlation coefficient was considered to remove collinear variables [14]. Weight was thus judged collinear with sex and BMI, and insulin/glucose were judged collinear with HOMA-IR. Consequently, only sex, BMI and HOMA-IR were included in the multivariate analysis model. A p-value <0.05 was considered as significant.

Results

Population Characteristics

In total, 50 volunteers (19F/31M) were included. All of them successfully underwent complete PET/CT studies and laboratories. There were no unexpected side effects during adenosine infusion. All the patients had normal stress/rest perfusion study with a summed difference score and a summed rest score of zero. Baseline characteristics are summarized in Table 1. There was significant difference between men and women for weight, BMI, insulin, glucose, HDL, LDL levels, but not for hsCRP and TG (data not shown). Among baseline characteristics fasting insulin levels were highly correlated with sex (ϱ= 0.45, p=0.0012), weight (ϱ= 0.79, p<0.0001), BMI (ϱ= 0.82, p<0.0001), plasma glucose levels (ϱ= 0.62, p<0.0001), hsCRP (ϱ= 0.60, p<0.0001) and HDL (ϱ= –0.57, p<0.0001). On multivariate linear regression analysis (sex, BMI, glucose, hsCRP and HDL levels), BMI, glucose and hsCRP emerged as independently related to fasting insulin levels (p<0.03).

Thoracic Fat Volume

All of the 50 CT series were successfully processed. Mean thoracic fat volume was 250±156 mL. Men had a significantly higher TFV than women (317±154 vs. 141±83 mL, $p<0.0001$). Among clinical and biological characteristics, shown in Table 2, TFV was significantly correlated with sex ($\varrho= 0.59$, $p<0.0001$), weight ($\varrho= 0.84$, $p<0.0001$), BMI ($\varrho= 0.85$, $p<0.0001$), insulin levels ($\varrho= 0.68$, $p<0.0001$), glucose plasma levels ($\varrho= 0.52$, $p=0.0002$), HOMA-IR ($\varrho= 0.67$, $p<0.0001$), HDL levels ($\varrho= –0.61$, $p<0.0001$), triglyceride levels ($\varrho= 0.53$, $p=0.0001$) and hsCRP ($\varrho= 0.41$, $p=0.005$). Relationships between TFV and age or LDL level were not statistically significant ($p>0.4$). Including variables significantly correlated with TFV in univariate analysis (sex, BMI, HOMA-IR, HDL, TG and hsCRP), multiple linear regression analysis highlighted that sex ($p=0.005$) and BMI ($p<0.001$) were independently correlated with TFV.

Myocardial Blood Flow Response

Fifty participants significantly increased myocardial blood flow from baseline conditions (rest-MBF= 1.0±0.4 mL/min/g) in response to pharmacological stress (stress-MBF= 2.7±0.6 mL/min/g, $p<0.0001$). For all of them myocardial flow reserve was >2 and stress-MBF was > 2 mL/min/g, excluding hemodynamically significant coronary artery disease, balanced ischaemia or microvascular disease. Using non-parametric Spearman's rank correlation test, MBF response to adenosine was only associated with weight ($\varrho=–0.31$, $p=0.029$) and TFV ($\varrho=–0.32$, $p=0.026$). TFV was the only variable independently associated with MBF response to adenosine on stepwise multiple linear regression analysis ($p=0.012$).

Rate pressure product significantly increased by 28±3% during cold pressor testing compared to rest conditions (10433±2792 $mmHg.min^{-1}$ vs. 8137±1885 $mmHg.min^{-1}$, $p<0.0001$).

Myocardial blood flow significantly increased from 1.0±0.4 mL/min/g to 1.2±0.3 mL/min/g (p=0.01) in response to cold pressor testing. Furthermore, absolute CPT-MBF was significantly correlated (Table 3) with sex (ϱ=–0.47, p=0.0006), weight (ϱ=–0.58, p<0.0001), BMI (ϱ=–0.51, p=0.0002), triglyceride level (ϱ=–0.32, p=0.024), fasting blood insulin (ϱ=–0.43, p=0.0024), HOMA-IR (ϱ=–0.39, p=0.007) and TFV (ϱ=–0.52, p=0.0001) (Figure 2). On stepwise multiple linear regression analysis, after exclusion of weight and fasting blood insulin, TFV emerged as the only independent predictor of MBF response to CPT (β=–0.38, p=0.014).

Discussion

To our knowledge, it is the first study reporting a direct and independent correlation between thoracic fat volume and endothelium-dependent coronary vasomotion in patients without previously known cardiovascular risk factor, medication or biological significant risk factor.

Thoracic Fat Volume Relation To Baseline Characteristics

Thoracic fat volume has recently been related to cardiovascular risk [4]. Whilst global fat burden is well known to be involved in the development of metabolic syndrome, there are increasing evidences that thoracic fat independently contributes to coronary artery disease genesis due to its function as well as its anatomical position. In accordance with previously published study [15], we found a strong correlation between weight and TFV (ϱ= 0.84, p<0.0001) as well as between BMI and TFV (ϱ= 0.85, p<0.0001). Moreover, BMI was independently correlated with thoracic fat volume (p<0.001), hence confirming the underlying relation between global fat burden and thoracic fat. As other white adipose tissue, it was reported that thoracic fat acts as an independent endocrine organ secreting hormones such as adiponectin, leptin, or resistin [16]. By secreting

adipokines, adipose tissue may promote insulin plasma production and insulin resistance [17]. Supporting this hypothesis, our analysis highlighted the correlation between insulin plasma levels (ϱ= 0.68, p<0.0001), HOMA-IR (ϱ= 0.67, p<0.0001) and TFV. Insulin resistance hence results in a increased triglyceride releasing in the peripheral circulation due to adipocyte lipolysis, as the relation between TFV and triglyceride levels might suggest (ϱ= 0.53, p=0.0001). Thoracic fat may also promote systemic inflammation by local macrophages secretion of cytokines and chemokines such as hsCRP [16]. This is supported by the correlation between hsCRP and TFV (ϱ= 0.41, p=0.005). Thus, by early contributing to chronic systemic inflammation that has extensively been reported as a key-step toward atherosclerosis [18, 19], thoracic fat may act in the development of coronary artery disease.

Thoracic fat relation to endothelium-dependent vasomotion

Endothelial function as well as myocardial blood flow has extensively been studied using cardiac PET/CT for the past few years. Endothelium-dependent vasoreactivity is a surrogate marker of cardiovascular events [20], which can be significantly impaired by smoking, uncontrolled diabetes or hypertension, dyslipidemia, or menopause. Therefore, numerous studies demonstrated the need for a control of cardiovascular risk factor to normalize response to cold pressor testing as a sign of conserved endothelium-dependent vasomotion [21-25]. In accordance with those results we report a significant inverse correlation between MBF response to CPT and triglyceride levels (ϱ=–0.32, p=0.024) fasting insulin plasma levels (ϱ=–0.43, p=0.0024) or HOMA-IR (ϱ=–0.39, p=0.007), even in a population of healthy volunteers. As reported above, numerous studies demonstrated that adiponectin plasma levels were negatively correlated with visceral fat burden and that low adiponectin contributed to insulin resistance [17, 26]. Prior et al. [27] showed that

insulin resistance had a direct negative influence upon endothelium-dependent vasoreactivity. Thus, our results suggest that an increased TFV may be related to insulin resistance and impaired endothelial response to cold pressor test, potentially by reducing adiponectin plasma levels. This point needs confirmatory studies, however.

Although we did not find any correlation between hsCRP and CPT-MBF, the relation between TFV and hsCRP (ϱ= 0.41, p=0.005) suggest that thoracic fat may contribute to chronic systemic inflammation, which results in an impairment of basal endothelial NO synthesis [18]. The indirect effect of thoracic fat upon endothelium could be mediated by an increased insulin resistance and a pro-inflammatory cytokine production. Although a direct vasocrine outside-to-inside effect of some adipokines from perivascular fat has been reported in a swine model [28], we cannot confirm its reality in humans based on our study. The independent correlation between TFV and CPT-MBF (p=0.014) found on multivariate analysis may suggest that TFV could participate in those different pathways that lead to significantly impair coronary endothelial function. However, the respective role of epicardial and paracardial fat in this process cannot be deduced from our study.

Thoracic fat relation to endothelium-independent vasomotion

Bucci et al. [7] recently reported that both intra-pericardial fat and extra-pericardial fat was significantly associated with hyperemic MBF (R=–0.36 and R=–0.44, respectively; p<0.0005). Supporting the direct influence of TFV upon endothelium-independent vasomotion, we found a negative correlation between TFV and hyperemic MBF (ϱ=–0.32, p= 0.026). Bucci et al. [7] concluded that the independent predictive value of intra-pericardial fat volume upon hyperemic MBF support the hypothesis of an outside-to-inside direct paracrine/vasocrine effect. It has also

been reported that perivascular fat could stimulate smooth muscle cell proliferation [29]. Though we were not able to discriminate respective contribution of epicardial and paracardial fat, our data suggest that thoracic fat could early contribute to atherosclerosis by impairing endothelial function and modifying smooth vessel musculature reactivity. Moreover, a direct effect of thoracic fat upon coronary wall from the intima to the adventice could contribute to the formation of arterial plaque, and promote plaque inflammation as recently suggested by Saam et al. [30]. From endothelial dysfunction to coronary artery disease, mounting evidences thus suggest that thoracic fat is a crucial actor of coronary atherosclerosis due to his pro-inflammatory and vasoactive role.

Study limitations

Although our study demonstrated an independent correlation between TFV and MBF response to CPT as well as between TFV and MBF response to adenosine, we recognize some limitations.

For our volunteers had no cardiovascular risk factor, and for everyone had thoracic fat, it was not possible to use a normal control population for comparison. Nevertheless, a prospective study assessing the evolution of TFV and CPT-MBF could be planed to sustain the results we showed by taking the person as his own control. In the same time, by prospectively measuring adipokine plasma changes over time, the exact effect of this cytokine upon endothelium-dependent vasomotion could be specified.

Moreover, because both intra-pericardial fat and extra-pericardial fat have different embryologic origin, they may have different contribution to atherosclerosis process. Due to ungated acquisition and low-dose parameters of CT acquisition, image spatial resolution was not sufficient to separately measure epicardial (or intra-pericardial) and paracardial (or extra-

pericardial) fat volume, hence its relation to CPT-MBF. Nevertheless, it may be difficult to distinguish their exact influence, since both can contribute to systemic inflammation and insulin resistance leading to impair baseline endothelial function. To avoid misinterpretation on the nature of our data, we strictly compared our results to previous studies that used the same definition for thoracic fat or that reported results applying to both epicardial and paracardial fat. As spatial resolution was different from Cheng et al. [1], a comparison with TFV normal value previously published was not possible.

Finally, as TFV could easily be quantified, it could be easily implemented in daily practice. While endothelium-dependent coronary vasomotion is a surrogate of future cardiovascular events, the prognostic value of TFV as compared to CPT-MBF remains unknown. The clinical relevance of initial TFV quantification should therefore be investigated, especially in patients with low likelihood of developing cardiovascular event, as well as the impact of TFV decrease on cardiac event occurrence. TFV monitoring could thus constitute an attractive complementary tool to assess patients' cardiovascular risk.

Conclusions

TFV significantly correlated with both endothelium-dependent and independent coronary vasomotion, even in persons with normal hyperemic myocardial perfusion imaging. Since endothelium-dependent vasomotion has been recognized as a surrogate marker for cardiovascular events, our results suggest a responsibility of thoracic fat toward future cardiovascular events. While outside-to-inside adipokines secretion through the arterial wall has been described, our results might suggest an effect upon NO-dependent and -independent vasodilatation, as well as an

influence of insulin resistance and chronic systemic inflammation mediated by thoracic fat. Further studies are needed to elucidate the putative mechanism.

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Conflicts of interest

VD, FF, AD, GA, BW and RH declare that they have no conflict of interest. John O. Prior has received a scientific grant support for this project from Bracco Diagnostics Inc., P.O. Box 5225, Princeton, NJ 08543-5225, the manufacturer of the Cardiogen-82®, the ^{82}Rb generator used in this study for performing the PET/CT examinations.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Cheng VY, Dey D, Tamarappoo B, Nakazato R, Gransar H, Miranda-Peats R, et al. Pericardial fat burden on ECG-gated noncontrast CT in asymptomatic patients who subsequently experience adverse cardiovascular events. JACC Cardiovasc Imaging. 2010;3:352-60.
2. Konishi M, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Matsubara J, et al. Association of pericardial fat accumulation rather than abdominal obesity with coronary atherosclerotic plaque formation in patients with suspected coronary artery disease. Atherosclerosis. 2010;209:573-8.
3. Sade LE, Eroglu S, Bozbas H, Ozbicer S, Hayran M, Haberal A, et al. Relation between epicardial fat thickness and coronary flow reserve in women with chest pain and angiographically normal coronary arteries. Atherosclerosis. 2009;204:580-5.
4. Greif M, Becker A, von Ziegler F, Lebherz C, Lehrke M, Broedl UC, et al. Pericardial Adipose Tissue Determined by Dual Source CT Is a Risk Factor for Coronary Atherosclerosis. Arterioscler Thromb Vasc Biol. 2009;29:781-6.
5. Kim TH, Yu SH, Choi SH, Yoon JW, Kang SM, Chun EJ, et al. Pericardial Fat Amount Is an Independent Risk Factor of Coronary Artery Stenosis Assessed by Multidetector-Row Computed Tomography: The Korean Atherosclerosis Study 2. Obesity. 2010;19:1028-34.
6. Jeong JW, Jeong MH, Yun KH, Oh SK, Park EM, Kim YK, et al. Echocardiographic epicardial fat thickness and coronary artery disease. Circulation journal : official journal of the Japanese Circulation Society. 2007;71:536-9.
7. Bucci M, Joutsiniemi E, Saraste A, Kajander S, Ukkonen H, Saraste M, et al. Intrapericardial, But Not Extrapericardial, Fat Is an Independent Predictor of Impaired Hyperemic Coronary Perfusion in Coronary Artery Disease. Arterioscler Thromb Vasc Biol. 2011;31:211-8.
8. Aydin H, Toprak A, Deyneli O, Yazici D, Tarcin O, Sancak S, et al. Epicardial fat tissue thickness correlates with endothelial dysfunction and other cardiovascular risk factors in patients with metabolic syndrome. Metab Syndr Relat Disord. 2010;8:229-34.
9. Yoshinaga K, Manabe O, Katoh C, Chen L, Klein R, Naya M, et al. Quantitative analysis of coronary endothelial function with generator-produced (82)Rb PET: comparison with (15)O-labelled water PET. Eur J Nucl Med Mol Imaging. 2010;37:2233-41.

10. Lortie M, Beanlands RS, Yoshinaga K, Klein R, Dasilva JN, DeKemp RA. Quantification of myocardial blood flow with 82Rb dynamic PET imaging. Eur J Nucl Med Mol Imaging. 2007;34:1765-74.
11. Senthamizhchelvan S, Bravo PE, Esaias C, Lodge MA, Merrill J, Hobbs RF, et al. Human biodistribution and radiation dosimetry of 82Rb. J Nucl Med. 2010;51:1592-9.
12. Einstein AJ, Moser KW, Thompson RC, Cerqueira MD, Henzlova MJ. Radiation dose to patients from cardiac diagnostic imaging. Circulation. 2007;116:1290-305.
13. Park MJ, Jung JI, Oh YS, Youn HJ. Assessment of Epicardial Fat Volume With Threshold-Based 3-Dimensional Segmentation in CT: Comparison With the 2-Dimensional Short Axis-Based Method. Korean Circ J. 2010;40:328-33.
14. Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carre G, et al. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography. 2013;36:027-46.
15. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. Circulation. 2008;117:605-13.
16. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm. 2006;74:443-77.
17. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med. 2008;14:741-51.
18. Cleland SJ, Sattar N, Petrie JR, Forouhi NG, Elliott HL, Connell JM. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. Clin Sci (Lond). 2000;98:531-5.
19. Devaraj S, Valleggi S, Siegel D, Jialal I. Role of C-reactive protein in contributing to increased cardiovascular risk in metabolic syndrome. Curr Atheroscler Rep. 2010;12:110-8.
20. Schindler TH, Hornig B, Buser PT, Olschewski M, Magosaki N, Pfisterer M, et al. Prognostic value of abnormal vasoreactivity of epicardial coronary arteries to sympathetic stimulation in patients with normal coronary angiograms. Arterioscler Thromb Vasc Biol. 2003;23:495-501.

21. Campisi R, Czernin J, Schoder H, Sayre JW, Schelbert HR. L-Arginine normalizes coronary vasomotion in long-term smokers. Circulation. 1999;99:491-7.
22. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. Lancet. 1991;338:1546-50.
23. Morita K, Tsukamoto T, Naya M, Noriyasu K, Inubushi M, Shiga T, et al. Smoking cessation normalizes coronary endothelial vasomotor response assessed with 15O-water and PET in healthy young smokers. J Nucl Med. 2006;47:1914-20.
24. Schindler TH, Campisi R, Dorsey D, Prior JO, Olschewski M, Sayre J, et al. Effect of hormone replacement therapy on vasomotor function of the coronary microcirculation in post-menopausal women with medically treated cardiovascular risk factors. Eur Heart J. 2009;30:978-86.
25. Schindler TH, Facta AD, Prior JO, Cadenas J, Hsueh WA, Quinones MJ, et al. Improvement in coronary vascular dysfunction produced with euglycaemic control in patients with type 2 diabetes. Heart. 2007;93:345-9.
26. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia. 2003;46:459-69.
27. Prior JO, Quinones MJ, Hernandez-Pampaloni M, Facta AD, Schindler TH, Sayre JW, et al. Coronary circulatory dysfunction in insulin resistance, impaired glucose tolerance, and type 2 diabetes mellitus. Circulation. 2005;111:2291-8.
28. Payne GA, Borbouse L, Kumar S, Neeb Z, Alloosh M, Sturek M, et al. Epicardial Perivascular Adipose-Derived Leptin Exacerbates Coronary Endothelial Dysfunction in Metabolic Syndrome via a Protein Kinase C-{beta} Pathway. Arterioscler Thromb Vasc Biol. 2010;30:1711-7.
29. Barandier C, Montani JP, Yang Z. Mature adipocytes and perivascular adipose tissue stimulate vascular smooth muscle cell proliferation: effects of aging and obesity. Am J Physiol Heart Circ Physiol. 2005;289:H1807-13.
30. Saam T, Rominger A, Wolpers S, Nikolaou K, Rist C, Greif M, et al. Association of inflammation of the left anterior descending coronary artery with cardiovascular risk factors,

plaque burden and pericardial fat volume: a PET/CT study. Eur J Nucl Med Mol Imaging. 2010;37:1203-12.

Figure titles and legends

Fig. 1. CT images processing for thoracic fat volume.

(**A**) Axial 2D images; (**B**) Drawing of a ROI from myocardium to adjacent organs for each slice from the pulmonary trunk bifurcation to the diaphragm; (**C**) Selection of the threshold (from –200 HU to –30 HU) leading to isolate fat in green in the predefined VOI; (**B**) Histogram based analysis to determine thoracic fat volume (473 mm^3 in this case). HU= Hounsfield Unit; ROI= region of interest; VOI= volume of interest.

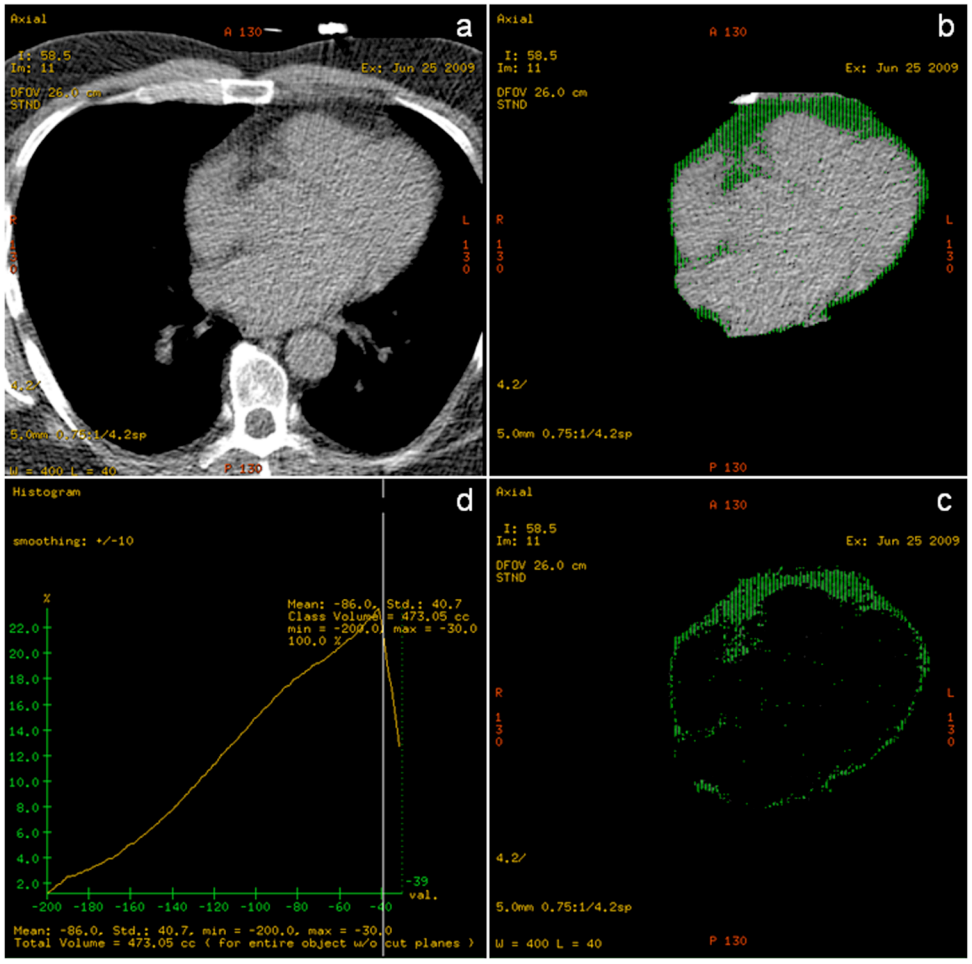
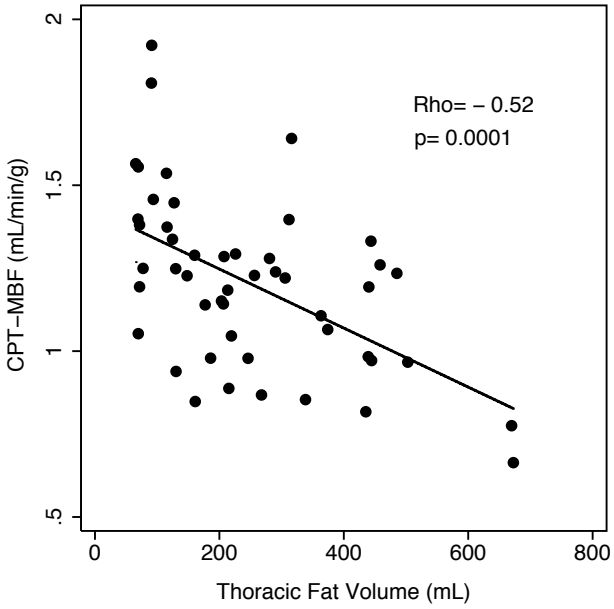


Fig. 2. Relationship between myocardial blood flow response to cold pressor testing and thoracic fat volume.

CPT= cold pressor test; MBF= myocardial blood flow.



Tables

Table 1. Study population characteristics.

Abbreviations: BMI= body mass index; CPT= cold pressor test; HDL= high-density lipoprotein; HOMA-IR= homeostasis model assessment-insulin resistance; hsCRP= high-sensitivity C-reactive protein; IQR= interquartile range; LDL= low-density lipoprotein; MBF= myocardial blood flow; MFR= myocardial flow reserve; TFV= thoracic fat volume; SD= standard deviation; TG= triglyceride levels.

Characteristics n= 50	Mean±SD or Median (IQR)
Age (years)	58±10
Weight (kg)	75.5±15.9
BMI (kg/m^2)	25.7±4.9
Insulin (mUI/L)	11.3 (7.4–14.8)
Glucose (mg/dL)	103 (95–108)
HOMA-IR (1)	2.9 (1.5–4.0)
LDL (mg/dL)	132±43
HDL (mg/dL)	62±16
TG (mg/dL)	105 (79–132)
hsCRP (mg/L)	1.9 (0.9–3.8)
TFV (mL)	214 (125–338)
Rest MBF (mL/min/g)	1.0±0.4
Stress MBF (mL/min/g)	2.7±0.6
MFR (1)	2.8±0.8
CPT-MBF (mL/min/g)	1.2±0.3

Table 2. Univariate (ϱ) and Multivariate (β) correlations between TFV and population characteristics.

Abbreviations: As in Table 1.

Characteristics	ϱ	*p*-value	β	*p*-value
Sex	0.59	<0.0001	0.35	0.005
Age (years)	0.10	0.4		
Weight (kg)	0.84	0.0001	*	
BMI (kg/m^2)	0.85	<0.0001	0.76	<0.001
Insulin (mUI/L)	0.68	<0.0001	*	
Glucose (mmol/L)	0.52	0.0002	*	
HOMA-IR (1)	0.67	<0.0001	—	0.09
LDL (mmol/L)	– 0.04	0.8		
HDL (mmol/L)	– 0.61	<0.0001	—	0.4
TG (mmol/L)	0.53	0.0001	—	0.7
hsCRP (mg/L)	0.41	0.005	—	0.3

* Although significant at the univariate level, these variables have not been included in the multivariate analysis because of collinearity (between weight and BMI, as well as between insulin, glucose and HOMA-IR).

Table 3. Univariate (ϱ) and Multivariate (β) correlations between cold pressor testing myocardial blood flow (CPT-MBF) and population characteristics.

Abbreviations: As in Table 1.

Characteristics	ϱ	*p*-value	β	*p*-value
Sex	– 0.47	0.0006	—	0.06
Age (years)	0.09	0.6		
Weight (kg)	– 0.58	<0.0001	*	
BMI (kg/m^2)	– 0.51	0.0002	—	0.2
Insulin (mU/L)	– 0.43	0.0024	*	
Glucose (mmol/L)	– 0.24	0.1		
HOMA-IR (1)	– 0.39	0.007	—	0.9
LDL (mmol/L)	0.09	0.5		
HDL (mmol/L)	0.27	0.06		
TG (mmol/L)	– 0.32	0.024	—	0.8
hsCRP (mg/L)	– 0.10	0.5		
TFV (mL)	– 0.52	0.0001	–0.38	0.014

* Although significant at the univariate level, these variables have not been included in the multivariate analysis because of collinearity (between weight and BMI, as well as between insulin, glucose and HOMA-IR).