

Relationship between endothelium-dependent vasodilation and fat distribution using the new “imimomics” image analysis technique

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Obesity

Abstract *Background and aims:* We investigated how vasoreactivity in the brachial artery and the forearm resistance vessels were related to fat distribution and tissue volume, using both traditional imaging analysis and a new technique, called “Imimomics”, whereby vasoreactivity was related to each of the >2M 3D image elements included in the whole-body magnetic resonance imaging (MRI).

Methods and results: In 326 subjects in the Prospective investigation of Obesity, Energy and Metabolism (POEM) study (all aged 50 years), endothelium-dependent vasodilation was measured by acetylcholine infusion in the brachial artery (EDV) and flow-mediated vasodilation (FMD). Fat distribution was evaluated by dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI). EDV, but not FMD, was significantly related to total fat mass, liver fat, subcutaneous (SAT) and visceral (VAT) adipose tissue in a negative fashion in women, but not in men. Using Imimomics, an inverse relationship was seen between EDV and a local tissue volume of SAT in both the upper part of the body, as well as the gluteo-femoral part and the medial parts of the legs in women. Also the size of the liver, heart and VAT was inversely related to EDV. In men, less pronounced relationships were seen. FMD was also significantly related to local tissue volume of upper-body SAT and liver fat in women, but less so in men.

Conclusion: EDV, and to a lesser degree also FMD, were related to liver fat, SAT and VAT in women, but less so in men. Imimomics both confirmed findings from traditional methods and resulted in new, more detailed results.

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Introduction

Obesity is a condition being associated with atherosclerosis and cardiovascular disease [1,2], and Mendelian randomization studies have established a causal effect of

general and abdominal adiposity on cardiovascular disease and cardiometabolic traits [3,4]. An early event in atherosclerosis formation is endothelial dysfunction, and obesity has been linked to a defect endothelium-dependent vasodilation [5–8]. Such an association is present already

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in childhood [9,10], and the fact that weight-loss both in children and adults is associated with an improved endothelium-dependent vasodilation [11–13] suggests that obesity is causally related to an impaired endothelium-dependent vasodilation.

Thus, although the link between obesity and a poor endothelium-dependent vasodilation is well established the link between fat distribution and endothelium-dependent vasodilation is less well studied [14]. Abdominal fat distribution seems to be more deleterious than adipose tissue located in the hip region [15,16]. Also visceral adipose tissue (VAT) is regarded as more dangerous than subcutaneous adipose tissue (SAT) [17–19], and increased liver fat has been highlighted to be a major player in the harmful effects of obesity, although it was not related to incident CVD in the MESA study [20]. Therefore, the aim of the present study was to investigate the associations between adipose tissue distribution and endothelium-dependent vasodilation, as evaluated both in the forearm resistance vessels as well as in the brachial conduit artery.

We used two kinds of evaluation of fat distribution, one based on traditional measurements by dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI), and one novel approach by which endothelium-dependent vasodilation is related to each of the >2 million 3D image elements included in the registration of a whole-body MRI, so called “Imiomics” [21]. The imiomics technique uses approximately 1 million voxels (3D elements being comparable to 2D pixels) in the MR image to create coordinates in three dimensions. Thereafter, each of these voxels are compared between each individual, and each voxel in each subject obtains a value for size and lipid content. These approximately 1 million values for size (or lipid content) could when be compared to some other non-imaging phenotype, like a blood test value, or as in

this case endothelial function. This will result in approximately 1 million correlation coefficients that could be visualized as a whole-body image in three dimensions (see Fig. 1).

We aim of the study was to investigate how vaso-reactivity in the brachial artery and the forearm resistance vessels were related to fat distribution and tissue volume, comparing traditional imaging analysis and a new technique, called “Imiomics”. The hypotheses tested were that the Imiomics technique would provide more detailed and new information on the relationship between endothelium-dependent vasodilation and fat distribution than traditional measurements, and that the associations between endothelium-dependent vasodilation and fat distribution differed between men and women.

Methods

Sample

The Prospective study on Obesity, Energy, and Metabolism (POEM) recruited 50-year-old men and women from the general population by a random invitation by mail using public population registers for the municipality of Uppsala, Sweden [22]. The participants received their invitation one month after their 50th birthday. A total of 502 individuals took part in the study, a participation rate of 25%. The study was approved by the ethics committee at Uppsala University (Approval numbers: Uppsala Log No. 2009/057 and Log No. 2012/143), and the participants gave their informed consent.

The participants were examined after fasting overnight. Waist and hip circumference were recorded at the umbilical and trochanter levels, respectively, and WHR was calculated. Fat and lean mass was established using DXA. Whole-body MRI and dedicated imaging of liver and

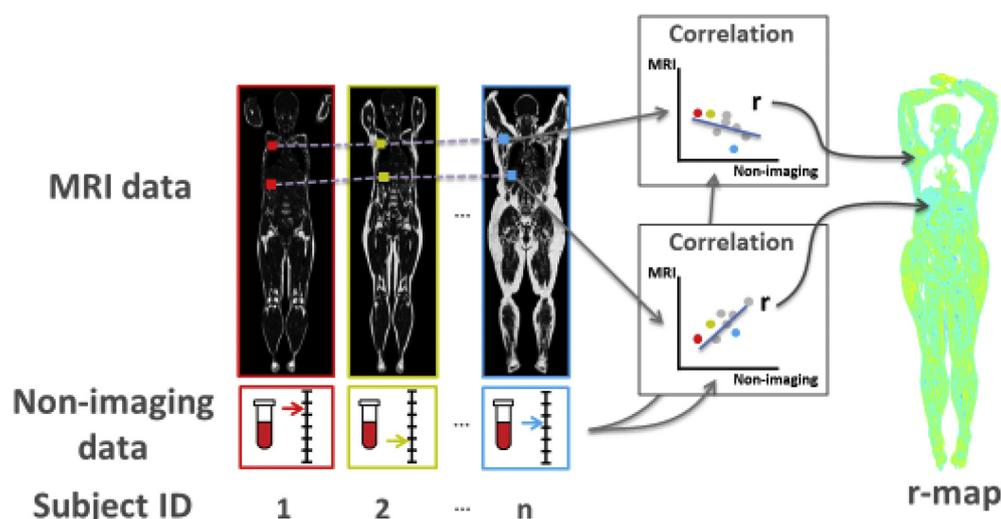


Figure 1 Illustration of the analysis enabled by Imiomics. Collected whole-body MR images are aligned to each other by use of deformable image registration. This achieves point-by-point correspondences between the images. This allows voxel wise comparison of the image data. This also allows studies of associations with non-imaging data (e.g. results from blood analysis or endothelial function) by voxel-wise correlations of MRI and non-imaging data and generation of resulting correlation (r -) maps.

pancreas were performed on those who volunteered for this part of the study. MRI was performed on a separate day, within one month from the main study visit. This study includes only the 326 subjects with a technically appropriate MRI registration.

DXA

Total and regional body fat and lean mass were estimated using the same Dual-energy X-ray absorptiometry scanner (DXA; Lunar Prodigy, GE Healthcare). To minimize potential operator bias, one experienced nurse performed all scans in the same room. The precision error of the DXA measurements in our laboratory was calculated using triple measurements in 15 subjects with repositioning according to recommendations from the International Society for Clinical Densitometry. Total fat and lean mass evinced a precision error of 1.5% and 1.0%, respectively. In the analysis, automatic edge detection was consistently employed; nevertheless, all scans were carefully checked for errors and manually corrected if necessary.

MRI

Subjects images were acquired using a 1.5T clinical MR system (Philips Achieva, Philips Healthcare, Best, Netherlands) in supine position with a continuously moving bed setup and the integrated body coil. Imaging included a whole-body water-fat imaging protocol which used a spoiled 3D multi gradient echo sequence. Scan parameters were: TR/TE1/ Δ TE = 5.9/1.36/1.87 ms, 3 unipolar echoes, flip angle 3°. Imaged field of view (FOV) 530 × 377 × 2000 mm³, reconstructed voxel size 2.07 × 2.07 × 8.0 mm³ in left-right × anterior-posterior × foot-head directions. A dedicated scan of the liver, which also included the pancreas, was undertaken for detailed analysis of fat content in the liver and pancreas. However, this liver scan was not included for the first 94 subjects in the POEM study. Scan parameters were: TR/TE1/ Δ TE = 8.66/0.92/1.32, 6 unipolar echoes, flip angle 5°. Imaged FOV 384 × 288 × 150 mm³, reconstructed voxel size 3.0 × 3.0 × 10.0 mm³. Water-fat image reconstruction was performed employing an algorithm developed inhouse. The imaging protocol and the reconstruction method have been described in more detail previously [23]. Liver and pancreas fat was quantified using manual delineation of the volume of interest using the software ImageJ (version 1.45s). A trained operator demarcated as much as possible of the volume of interest but avoided tissue borders to limit partial volume effects. Median fat contents of the volumes of interest were used as measurements of tissue fat content.

The VAT and SAT depots were quantified by deforming manually defined depots in a male and female reference subject to all other subjects by utilizing the image-registration method used for the Imiomics analysis described below. The deformed regions were further processed by thresholding operations, removing voxels with fat content <50%.

Imiomics

Image registration is a key component of the Imiomics technique. In image registration, a target image is deformed to match a (fixed) reference image by computing and applying deformation field. For each point in the reference image, the deformation field defines a corresponding point in the target image. These point-by-point correspondences are used in Imiomics analyses, in which whole-body MRI images are deformed to a reference whole-body MRI volume. The reference whole-body MRI volume constitutes a reference coordinate system, where each point has a corresponding point in all volumes in the cohort. This enables the voxel-wise statistical analysis procedure [21], in which associations between tissue volume and fat content from MRI can be related to study non-imaging data (Fig. 1).

The Imiomics image registration method employs a tissue-specific handling of bone, lean tissue and adipose tissue. The degree of elasticity of the deformation required to align two images tends to differ across these different tissue types. This prior knowledge is made use of by performing the image registration of the different tissues sequentially, applying registration parameters appropriate to each tissue. The Imiomics image registration method comprises the following three steps: 1) Articulated, piecewise affine, registration of bone sections; 2) Registration of water images with constraints on bone; 3) Registration of fat images with constraints on bone and water. This image registration method has been presented and evaluated [21] on MRI water-fat image data and attains image registration results appropriate for Imiomics analyses.

In the Imiomics statistical analysis procedure [21] correlations between imaging and non-imaging parameters are calculated in the reference coordinate system. This study considered the global, non-imaging parameters EDV and FMD and the point-wise imaging parameters local tissue volume and fat content. The Pearson correlation coefficient between the non-imaging parameters and the imaging parameters was computed for each voxel in the reference coordinate system.

The invasive forearm technique

Forearm blood flow (FBF) was measured by venous occlusion plethysmography (Elektromedicin, Kullavik, Sweden). A mercury in-silastic strain gauge was placed at the upper third of the forearm, which was resting comfortably just above the level of the heart. The strain gauge was connected to a calibrated plethysmograph. Venous occlusion was attained by a blood-pressure cuff applied proximal to the elbow and inflated to 50 mm Hg by a rapid cuff inflator. Evaluations of FBF were made by computing the mean of at least five consecutive recordings.

An arterial cannula was placed in the brachial artery. Only one attempt to insert the cannula in each arm was permitted. Resting FBF was measured 30 min following cannula insertion. After evaluation of resting FBF, local intra-arterial drug infusions were administered for 5 min

for each dose with a 20-min washout period between the drugs. The infused dosages were 25 and 50 $\mu\text{g}/\text{minute}$ for Acetylcholine (Clin-Alpha, Switzerland) to evaluate EDV. The dosages of these drugs were selected in order to produce FBFs on the steep part of the dose-response curve without causing systemic effects.

The present study only used data from the highest doses of Acetylcholine. EDV was defined as FBF during infusion of 50 $\mu\text{g}/\text{min}$ of Acetylcholine minus resting FBF divided by resting FBF.

We have previously shown that the reproducibility (coefficient of variation, CV) for EDV is 10% [24].

The brachial artery ultrasound technique

The brachial artery was assessed by external B-mode ultrasound imaging.

2–3 cm above the elbow (Acuson XP128 with a 10 MHz linear transducer, Acuson, Mountain View, CA). Settings for depths and gains were optimized to identify the lumen to vessel wall interface. The subject rested in the supine position for at least 30 min prior to the first scan and remained supine throughout the evaluation. Blood-flow increase was induced by inflation of a pneumatic cuff placed around the forearm to a pressure at least 50 mmHg above systolic blood pressure. When the cuff was rapidly deflated five minutes later, the artery was scanned continuously for 90 s and recorded on a super-VHS videotape for subsequent analysis of the diameter in end-diastole. FMD was defined as the maximal brachial artery diameter registered between 30 and 90 s after cuff release minus diameter at rest divided by the diameter at rest.

We have previously shown that the reproducibility (CV) is 3% for baseline brachial artery diameter and 29% for FMD [25].

Statistics

Linear regression analysis (OLS) was used to evaluate the relationships between traditional measurements of anthropology and fat distribution and EDV and FMD. The relationships are presented as correlation coefficients. Variables that were skewed to the right were ln-transformed to achieve a normal distribution (liver fat, pancreatic fat, VAT, SAT, EDV, FMD). STATA 14 was employed for these computations (Stata Corp, College Station, TX).

An interaction term between BMI and the traditional adipose tissue variables were included in the regression models with either EDV or FMD as dependent variables.

For the Imiomics analyses, linear regression was applied to generate the r - and p -value maps for all parameters. MATLAB (version 2016b, The MathWorks Inc., Natick, MA) was employed for these calculations. The p -values are visualized by the colormap *jet* inverted, which represents low p -values (near zero) as dark red.

For the Imiomics part we also performed interaction analysis between BMI and volume and BMI and lipid content. We also performed Imiomics correlation analysis

(Pearson correlations) stratified by dividing the subjects in groups based on their body mass index BMI. The cutoff values were chosen as the median value in the full cohort; 26.1 (male) and 25.4 (female).

The Imiomics results were analyzed visually. To minimize the number of false positive findings from the large number of tests performed, we report only associations found in many image elements within a certain anatomical structure as dark red in the p -value maps, corresponding to p -values far below 0.05.

Results

The relevant characteristics of the cohort is summarized in Table 1.

Relationship between EDV and FMD and traditional measurements of obesity

EDV was significantly related to a number of traditional anthropometric measurements of obesity in women, but not in men. EDV was furthermore related to total fat mass at DXA and fat mass at the trunk and arm, but not leg in women. These relations were not present in men.

For measurements like waist/hip-ratio (WHR), trunk fat at DXA and VAT and SAT at MRI, the degree of explanation (R^2) for relationships vs EDV was in the order of 6–8% in women, but as low as 0.8–1.7% in men (Table 2).

EDV was also related to liver fat, SAT and VAT in women, but not in men.

FMD was not significantly related to any of the traditional indices of obesity in any of the sexes (Table 2).

Table 1 Basic characteristics and markers of fat distribution and endothelium-dependent vasodilation as measured by acetylcholine infusion in the brachial artery (EDV) and flow-mediated vasodilation (FMD) in the men and women in the sample.

Variable	Women		Men	
	N	Mean (SD)	N	Mean (SD)
Height (cm)	171	166.5 (6.8)	155	179.3 (6.3)
Weight (kg)	171	72 (12.7)	155	85.9 (11.8)
Waist circumference (cm)	171	89.6 (11)	155	94.6 (10)
Hip circumference (cm)	171	103.1 (8.4)	155	101.1 (6.2)
BMI (kg/m^2)	171	26 (4.5)	155	26.7 (3.6)
Waist/hip-ratio	171	0.87 (0.06)	155	0.93 (0.06)
Total fat mass at DXA (kg)	160	26 (9.8)	147	22 (8.8)
Total lean mass (kg)	160	42 (5.5)	147	60 (5.7)
Fat mass at trunk (kg)	160	13 (5.4)	147	13 (5.7)
Fat mass at leg (kg)	160	9.4 (3.5)	147	5.7 (2.3)
Lean mass at leg (kg)	160	13 (2.0)	147	20 (2.2)
Fat mass at arm (kg)	160	2.7 (1.2)	147	1.9 (0.9)
Lean mass at arm (kg)	160	4.6 (0.8)	147	7.8 (1.0)
Liver fat (%)	142	3.5 (5.7)	117	5.0 (5.6)
Pancreas fat (%)	142	4.4 (3.4)	116	7.0 (6.3)
Visceral adipose tissue (L)	171	2.5 (1.5)	155	4.4 (2.2)
Subcutaneous adipose tissue (L)	171	7.5 (3.4)	155	5.7 (2.8)
EDV (%)	117	632.3 (358.9)	128	503.5 (319.2)
FMD (%)	159	7.1 (4.7)	138	6.0 (4.6)

Table 2 Pearson correlation coefficient is given for relationships between endothelium-dependent vasodilation as measured by acetylcholine infusion in the brachial artery (EDV) and flow-mediated vasodilation (FMD) and multiple indices of body composition in men and women separately. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Variable	EDV		FMD	
	Women	Men	Women	Men
Height	-0.04	0.12	-0.02	-0.05
Weight	-0.25**	-0.03	0.10	-0.02
Waist circumference	-0.28***	-0.09	0.02	0.01
Hip circumference	-0.19*	-0.03	0.09	-0.07
BMI	-0.26**	-0.12	0.11	0.01
Waist/hip-ratio	-0.26**	-0.13	-0.05	0.07
Total fat mass at DXA	-0.24**	-0.07	0.08	0.03
Total lean mass	-0.13	0.02	0.08	-0.13
Fat mass at trunk	-0.28***	-0.09	0.09	0.03
Fat mass at leg	-0.12	-0.01	0.07	0.03
Lean mass at leg	-0.12	0.01	0.07	-0.15
Fat mass at arm	-0.27***	-0.07	0.06	0.03
Lean mass at arm	-0.10	0.01	0.08	-0.13
Liver fat	-0.22*	-0.18	-0.01	0.07
Pancreas fat	-0.10	-0.09	0.05	-0.02
Visceral adipose tissue	-0.25**	-0.11	0.11	0.06
Subcutaneous adipose tissue	-0.25**	-0.13	0.09	0.03

Following adjustment for the Framingham score (a unified score for the estimation of cardiovascular risk, including blood pressure, diabetes, HDL and LDL-cholesterol and smoking) (suppl table 1), the reported correlation coefficients for the relationships between EDV (or FMD) and the fat distribution variables changed very little compared to unadjusted relationships and none of the previously noted significant relationships lost in significance following adjustment.

When the interaction term between BMI and the traditional adipose tissue variables were included in the regression models with either EDV or FMD as dependent variables, in no case the interaction term showed a p -value < 0.05 (details are given in Suppl Table 2). This indicates that the reported relationships between the traditional adipose tissue variables and EDV or FMD are not significantly influenced by the degree of BMI and therefore no stratification by BMI is needed in this context.

Relationship between EDV and FMD and local tissue volume by imiomics

Displayed in the p -value maps from the coronal slice, a highly significant relationship was observed between EDV and a local tissue volume of the SAT in the upper part of the body, as well as the gluteo-femoral part and the medial parts of the legs in women. As could be seen from the r -value maps, these associations were inverse. Moreover, also the size of the liver and heart were inversely related to EDV. The transversal slices further disclose inverse relationships between EDV and size of the heart, VAT and SAT located at the ventral part of the upper leg in women (Fig. 1).

Regarding FMD, this measure of endothelial function was inversely related to local tissue volume of SAT in

especially the abdominal region and in the arms in women. Moreover, also the size of the liver and VAT were inversely related to FMD (Fig. 1).

In men, a less clear picture was seen regarding EDV, with inverse relationships vs local volume of SAT only detected in the abdominal region and only parts of the liver. Also the inverse relationship between EDV and volume of VAT seen in women was less pronounced in males (Fig. 2).

Regarding FMD, inverse relationships were seen mainly with volume of the ventral part of abdominally located SAT, as well as parts of the quadriceps muscle. Furthermore, inverse relationships between FMD and volume of the trochanter region and the ileac crest were seen in men (Fig. 2). The described associations are summarized in Table 3.

A highly significant interaction between BMI and volume regarding EDV was mainly seen in women in the SAT part of the thigh region (Suppl Fig. 3). When the distribution of BMI split into a high and low group according to the sex-specific median, the relationship between EDV and SAT thigh volume was mainly seen in the lower BMI group (Suppl. Fig 4).

A similar significant interaction as for women was seen in men between the SAT volume of the thigh region and BMI regarding EDV (Suppl. Fig 3). When the distribution of BMI split into a high and low group according to the sex-specific median, the relationship between FMD and this SAT volume was mainly seen in the lower BMI group (Suppl. Fig 4). A highly significant interaction between BMI and volume regarding FMD was mainly seen in men in the skeletal muscle part of the upper and lower parts of the legs. When the distribution of BMI split into a high and low group according to the sex-specific median, the relationship between FMD and the skeletal muscle volume was mainly seen in the higher BMI group (Suppl. Fig 5).

Relationship between EDV and FMD and lipid content by imiomics

In women, an inverse relationship between EDV and the lipid content of SAT in the upper part of the body was found, with a clear division into a superficial and deep part of the SAT. Also an inverse relationship between EDV and lipid content in the liver and parts of the epi/pericardium was noted in women. No convincing relationships between FMD and lipid content were disclosed in women (Fig. 3).

Also in men, an inverse relationship between EDV and the lipid content of SAT in the upper part of the body was found, although the distinct division into a superficial and deep part of the SAT was not noted. No convincing pattern between FMD and lipid content were disclosed in men (Fig. 3).

The described associations are summarized in Table 3.

Like for the relationship between EDV (or FMD) and the traditional adipose tissue measurements, adjustment for the Framingham score did not cause any important change

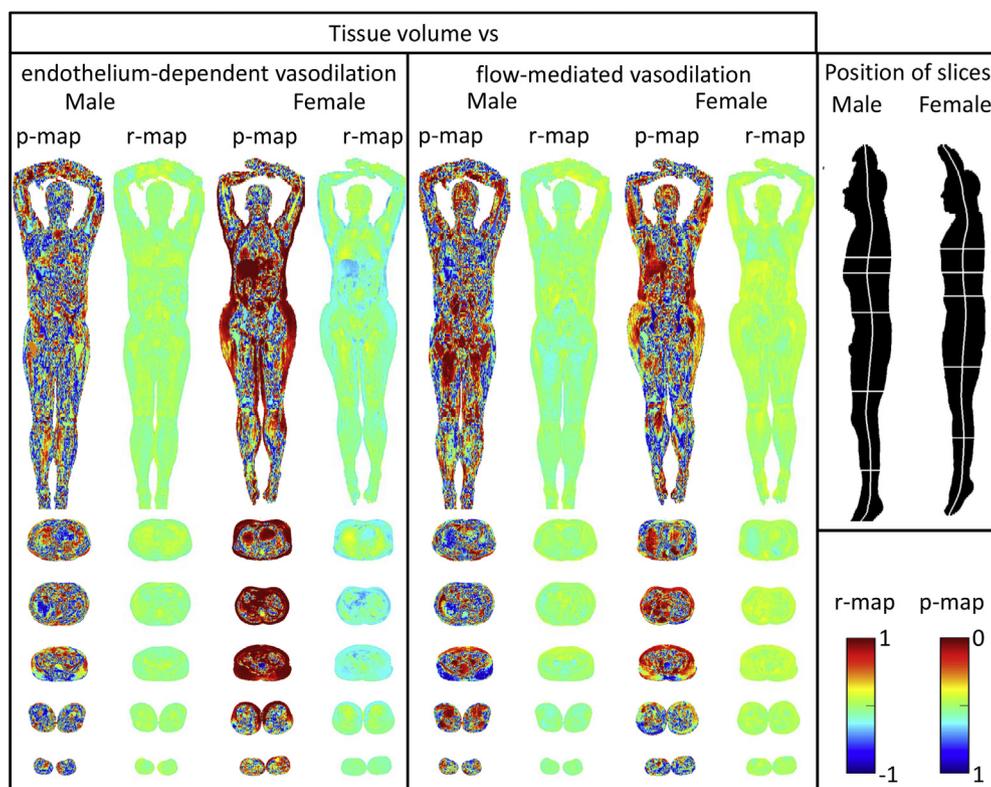


Figure 2 Imiomics maps of voxel-wise correlations (r-map) and the respective p-values (p-map) for the relationships between tissue volume and endothelium-dependent vasodilation in the fore arm resistance vessels (EDV) and flow-mediated vasodilation in the brachial artery (FMD). All maps are derived from Pearson correlation coefficients. For the r-maps, positive associations are shown in warm colors (yellow – red) and negative associations ($p \leq 0.05$) are shown in cool colors (green – blue), see the color bars. For p-values, the more red/brown, the lower p-value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3 Summary of relationships found between endothelium-dependent vasodilation as measured by acetylcholine infusion in the brachial artery (EDV) and flow-mediated vasodilation (FMD) and local tissue volumes or lipid content at the Imiomics evaluation divided in women and men. Negative denotes a significant negative association. Negative in parenthesis denotes a significant, but rather weak negative association.

	EDV		FMD	
	Woman	Men	Woman	Men
Volume				
Upper body SAT	Negative	(Negative)	Negative	(Negative)
Gluteo-femoral SAT	Negative			
Medial parts of the leg	Negative			
Ventral parts of the leg	Negative			
VAT	Negative	(Negative)	Negative	
Heart	Negative			
Liver	Negative	(Negative)	Negative	
Epi/pericardium				
Lipid content				
Upper body SAT	Negative			(Negative)
Gluteo-femoral SAT				
Medial parts of the leg				
Ventral parts of the leg				
VAT				
Heart				
Liver	Negative			
Epi/pericardium	Negative			

in the local tissue volume or lipid relationships, as could be seen in [Suppl Figures 1 and 2](#).

Furthermore, no significant interactions between BMI and lipid content regarding EDV (or FMD) were found (results not shown).

Discussion

The main result in the present study was that measurements of fat distribution were related to EDV and FMD in a more powerful way in women than in men. This was seen using both classical anthropology measurements, traditional measurements obtained by DXA or MRI, as well as using the more detailed information given by the Imiomics technique. In addition, the Imiomics analysis disclosed not previously known relationships between vasoreactivity and fat distribution.

A second aim of this study was to compare analyses using Imiomics and traditional techniques. These results are not directly comparable, e.g. Imiomics gives more detailed and new information about fat content and distribution ([Tables 2 and 3](#)). Nevertheless, those parameters that are comparable like VAT and SAT volumes and liver fat content gave similar results for EDV in woman while Imiomics found more correlations for these parameters in men for EDV and in both woman and men for FMD.

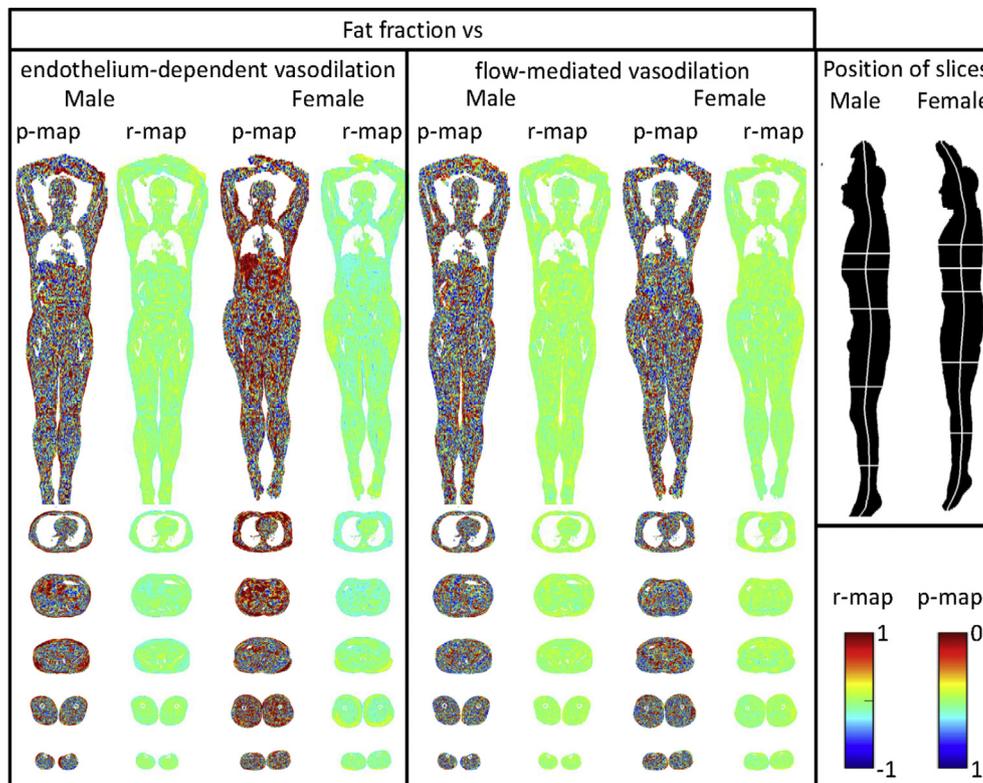


Figure 3 Imiomics maps of voxel-wise correlations (r-map) and the respective p-values (p-map) for the relationships between the fat fraction and endothelium-dependent vasodilation in the fore arm resistance vessels (EDV) and flow-mediated vasodilation in the brachial artery (FMD). All maps are derived from Pearson correlation coefficients. For the r-maps, positive associations are shown in warm colors (yellow – red) and negative associations ($p \leq 0.05$) are shown in cool colors (green – blue), see the color bars. For p-values, the more red/brown, the lower p-value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

It has previously been shown that both EDV and FMD are related to obesity [5–10] and that weight loss is associated with an improvement in endothelium-dependent vasodilation [11–13]. However, less is known about fat distribution and endothelial function. Since fat distribution is an important characteristic being related to CVD [26], it is of value to study how fat distribution might affect endothelial function, an early step in atherosclerosis development. Furthermore, since fat distribution is very different in men and women, it is essential to study sex-differences in this respect.

Indeed, the strength of the associations between EDV, and a lesser degree also FMD, and measurements of fat distribution differed substantially between men and women regardless of the sophistication of the measurements. Also the Imiomics analysis showed similar sex-differences. Our results are in line with a previous study in which EDV was impaired in obese women, but not in obese men [27].

The reason for this sex-difference is not known, especially since it was found that both markers of general obesity, like total fat mass and BMI, as well as for markers being specific for fat distribution, like waist/hip-ratio, trunk fat, VAT and SAT, were related to EDV. Thus, the differences in fat distribution normally seen between men and women cannot explain the sex-differences in the strength of associations vs EDV. The only marker of fat

distribution that showed a similar strength of association vs EDV in both men and women was liver fat.

It has previously been shown that overt non-alcoholic fatty liver disease (NAFLD) is linked to a poor FMD [28,29]. It has also been pointed out that increased levels of the hepatokine fetuin-A are linked to a poor FMD in NAFLD subjects [30]. Thus, it has to be further investigated which specific role fat accumulation in the liver and fetuin-A plays in relation to endothelial function.

Differences in sex hormones could well play a role in the different relationships between fat distribution and endothelial function between men and women, since it is well known that estrogen could have an impact on both fat distribution and endothelial function [31,32]. Adipose tissue could in itself also convert androgens to estrogen metabolites [33], which makes the interplay between estrogen, fat distribution and endothelial function very intricate as estrogen could be both a confounder and a mediator in the fat distribution/endothelial function relationship.

The mechanisms linking obesity with an impaired EDV are not known. In one study showing impaired EDV in obese subjects, co-infusion with vitamin C and also indomethacin improved EDV, suggesting that oxidative stress is one factor linking obesity with a poor endothelial function [5]. Also increased levels of asymmetric dimethylarginine (ADMA), an endogenous blocker of nitric oxide synthesis

has been described in obesity together with decreased expression of the enzyme responsible for breakdown of ADMA, dimethylarginine dimethylaminohydrolase (DDAH) [34]. In addition, upregulation of arginase 2, an enzyme involved in the breakdown of arginine, was found, and arginase inhibition improved endothelial vasodilatation, further pointing out substrate availability and inhibition in nitric oxide synthesis to be of importance in this respect. Furthermore, an altered sensitivity to the potent vasoconstrictor endothelin-1 might be of importance in this respect. In obese subjects, an impaired EDV was seen, but selective blockade of endothelin 1 receptors by BQ-123 improved EDV in the obese [8]. It has also been suggested that ghrelin might be involved in a poor EDV in the obese, since ghrelin levels were decreased when obese and lean individuals were compared and infusion of ghrelin restored the blunted EDV seen in the obese [35]. Low-grade inflammation might be another mediator linking obesity to a poor EDV, since obesity is a proinflammatory state [36] and markers of inflammation, as well as acute induced inflammation are linked to a poor EDV [37,38].

As could be seen in Table 2, EDV was related to both markers of general obesity, as well as markers of fat distribution with similar strength in women. In that case, imiomics analysis was very valuable, since detailed analysis showed that local volume of SAT both the upper and lower parts of the body were related to EDV, but that the magnitude of local volume was more pronounced in the upper parts of the body, especially in men. This Imiomics finding was further strengthened by data from DXA showing that arm fat content was more closely related with EDV than leg fat content in women. This is plausible finding, since it previously has been shown that lower body SAT is less closely related to a number of CV risk factors and shows a different gene expression profile than upper body SAT [39–41], as well as being less closely related to proinflammatory activation, another factor that could impair EDV.

The relationships described in the present study might be different in lean and obese subjects. In both women and men, we found a positive relationship between volume expansion and EDV to be more powerful in subcutaneous part of the thigh region in the subjects with a low BMI. It may well be that extra fat deposition in this location in lean subjects could play a more important role than extra fat deposition in those already being obese.

In men, the stratification in high and low BMI disclose a novel finding that FMD was related to skeletal muscle mass in the leg mainly in the subjects with a high BMI. The role of skeletal muscle mass in endothelial function has not previously been well investigated and based on this finding deserves further investigation. Since we cannot evaluate skeletal muscle mass properly in the upper limb, where FMD is measured, we must regard leg muscle mass as a proxy for arm muscle mass in this setting, but future studies should be devoted on the role of the musculature in the arm on the measurement of FMD.

We have previously presented data on the relationships between EDV and abdominally located SAT and VAT at MRI

based on data collected in an elderly sample [14]. In that study, EDV was slightly more closely related to VAT than to SAT, but the differences in the relations between these two abdominal fat depots and EDV were not striking. In the present study, EDV was related to VAT and abdominally located SAT in a similar fashion.

Using the collection of data on lipid content at MRI, the Imiomics analysis using this modality disclosed that EDV was related to both one layer of upper-body superficial SAT and even more powerful to the deep part of the SAT in women. These two layers of SAT are separated by fascia lata, and it has been shown that these two SAT compartments differ in gene expression and that the deep SAT more resembles VAT in characteristics [42].

An impaired EDV was furthermore correlated with heart size at the Imiomics analysis. A relationship between EDV and left ventricular mass measured by echocardiography has previously been presented, both in the general population and in hypertensive patients [43,44]. The present Imiomics analysis is not performed as an ECG-gated image collection and therefore the right and left ventricle could not be adequately separated due to the movement of the heart.

In the present study, EDV was more closely related to indices of obesity than FMD. A similar finding has been published in a group of obese subjects [27]. We have also previously published the same findings in two population-based samples [22,45], while others have described a relationship between obesity and FMD, especially being evident in children and young adults [46]. Moderate weight loss kg did not change FMD [47,48], while a substantial reduction in weight induced by bariatric surgery improved FMD [49]. Thus, the association between obesity and FMD might be dependent both on the age of the investigated subjects, as well as on the magnitude of obesity. FMD was originally developed for use in children and young adults. With ageing the artery becomes less compliant and in a stiff artery no major vasodilation is seen even if the endothelium is intact. Therefore, the measurement of FMD is heavily affected by arterial stiffness in mid-age and in the elderly [50], making FMD less reliant as a marker of endothelium-dependent vasodilation with ageing. In addition, the measurement of FMD has an almost three-fold higher CV compared with EDV, and this will impair precision in the statistical analysis.

The strength of the present study is the detailed characterization of indices of obesity and fat distribution by both DXA and MRI and the use of Imiomics analysis, and that endothelium-dependent vasodilation was evaluated in both a conduit artery and resistance vessels. Amongst the weaknesses of the study is the lack of replication cohort, since we performed a large number of tests using the Imiomics technology and therefore false positive findings could occur. To minimize such findings, we have applied a conservative approach in the interpretation of the p-value maps in that we only report associations being seen in many image elements within a certain anatomical structure being dark red in the p-value maps, corresponding to p-values well below 0.05.

Both EDV and FMD were measured in the circulation of the arm and the main fat depots are not in the arm. Thus, a limitation of the present study is that we cannot evaluate the potential effects of perivascular and local fat on endothelial function. Perivascular fat is regarded as having important local effects on the endothelium, including interaction with NO synthesis and transformation by free oxygen species, altered prostanoid synthesis by COX1 and COX2, dysfunction of potassium channels, generation of pro-inflammatory cytokines as well as local hypoxia, as reviewed by Zeborska et al. [51]. There is currently no way to study the effects of perivascular fat in detail in vivo in humans. It could however be seen in the present study that the amount of fat in the arm measured by DXA was related to EDV with a similar strength as truncal fat in women, and stronger than leg fat. If this represent local effect in the arm or not has to be clarified.

In conclusion, EDV, and to a lesser degree also FMD, were related to liver fat, SAT and VAT in women, but less so in men. Imiomics both confirmed the findings from traditional methods and resulted in new, more detailed findings regarding relationships between vasoreactivity and fat distribution.

Conflicts of interest

Joel Kullberg and Håkan Ahlström are cofounders, co-owners of and part time employees Antaros Medical AB, BioVenture Hub, Mölndal, Sweden. A patent application, P1318PC00, by Robin Strand, Joel Kullberg and Håkan Ahlström describing the image registration method used in this manuscript is currently under review. Antaros Medical is currently holding the rights to the patent application.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2019.06.017>.

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