Visceral adipose tissue, adiponectin levels and insulin resistance are related to atherosclerosis as assessed by whole-body magnetic resonance angiography in an elderly population

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A c t i v e s t a t e

Objective: The principal aim of this study was to determine whether the amount of visceral adipose tissue (VAT) is more related than subcutaneous adipose tissue (SAT) to atherosclerosis assessed by whole-body MRA. A further objective was to investigate whether traditional risk factors, inflammation, or adipokines could explain the hypothesized relationship between VAT and atherosclerosis.

Methods: Men and women aged 70 were recruited from the general population into the Prospective Investigation of The Vasculature in Uppsala Seniors (PIVUS) and 306 of them underwent WBMRA in a clinical 1.5-T scanner. The arterial tree was assessed for degree of stenosis or occlusion and a total atherosclerotic score (TAS) was established. Information on risk factors and BMI and on SAT and VAT, segmented on an axial MR scan was collected. Adiponectin, leptin, and high sensitive C-reactive protein (hsCRP) were measured in serum. HOMA index was used as a marker of insulin resistance.

Results: VAT was related to TAS independently of gender, total obesity (BMI), amount of SAT, hsCRP and also to the traditional risk factors included in the Framingham risk score (FRS) in an elderly population. Adiponectin or the HOMA insulin resistance, but not leptin or VAT, together with FRS was significantly related to TAS in a multiple censored regression model.

Conclusion: Adiponectin attenuated the relationship between VAT and TAS, suggesting that adiponectin and insulin resistance is an important link between visceral adiposity and atherosclerosis.

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A r t i c l e h i s t o r y

Received 15 June 2008
Received in revised form 17 October 2008
Accepted 11 November 2008
Available online xxx

A c k n o w l e d g e m e n t

Acknowledgments.

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1. Introduction

Obesity is increasing worldwide, as well as in Sweden [1], and is related to a high caloric intake and a low physical activity. The distribution of adipose tissue seems to be more important for development of atherosclerotic diseases than does the amount of fat, and the waist-to-hip ratio is a stronger predictor of cardiovascular (CV) events than body mass index (BMI) [2]. Abdominal adipose tissue can be assessed by magnetic resonance imaging (MRI) and computed tomography (CT) and can be segmented into abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). VAT has been reported to be an independent risk factor for future myocardial infarction (MI) in elderly women [3] and to be a predictor for coronary artery disease (CAD) onset in middle-aged men [4] and of all-cause mortality in men [5].

Although associated, the exact mediators between increased fat mass in general, and VAT in particular, and atherosclerosis are yet to be determined. Increased levels of traditional CV risk factors [6] may explain part of the increased risk, but an obesity-associated inflammatory status [7] and increased levels of adipocyte-derived hormones or adipokines may contribute.

Adiponectin possesses antiatherosclerotic, anti-diabetic, and anti-inflammatory properties and plasma levels of adiponectin are inversely related to the amount of visceral fat. In previous studies an inverse relationship has been shown between adiponectin and carotid artery intima-media thickness (IMT), a marker for subclinical atherosclerosis [8], as well as between adiponectin and the presence of CAD [9] or coronary artery calcification [10]. It has also been found that low adiponectin levels predict future CV events in some [11] but not all studies [12]. In contrast, circulating levels of leptin increases with obesity and women have markedly higher levels than men. Leptin has diverse actions related to satiety and...
metabolism [13]. We have previously shown that high levels predict development of first-ever myocardial infarctions and stroke, mainly in men [12,14]. Leptin may promote atherosclerosis through several mechanisms as recently reviewed by Beltowsky [15].

With the evolving concept of whole-body magnetic resonance angiography (WBMRA) [16], it is possible to assess the whole arterial tree in one session without ionizing radiation. This minimally invasive global atherosclerotic assessment enables studies of the relation between the burdens of vascular stenosis and occlusions and various constitutional and metabolic CV risk factors.

The aim of this study was to determine whether the amount of VAT and SAT were differentially related to atherosclerosis as assessed by whole-body MRA. A further objective was to explore whether traditional risk factors, inflammation, or actions of adipokines could explain the hypothesized relationship between VAT and atherosclerosis.

2. Materials and methods

This section has previously been given in detail in different publications [17,18].

2.1. Material

Eligible were all subjects aged 70 living in the community of Uppsala, Sweden. The subjects were randomly chosen from the register of community living. 1016 subjects participated giving a participation rate of 50.1%. Of those, a random of 306 subjects was evaluated with WBMRA. The study was approved by the Ethics Committee of the University of Uppsala.

Basic characteristics of the total sample and the WBMRA subsample are given in Table 1. Abdominal and hip circumferences were recorded. During blood pressure measurements and blood sampling, the subjects lay supine in a quiet room. Blood pressure was measured with a calibrated mercury sphygmnomanometer after at least 30 min rest and the average of three recordings was used. Hypertension was defined as systolic blood pressure over 140 mm Hg and/or diastolic blood pressure over 90 mm Hg or on antihypertensive treatment. Lipid variables and fasting blood glucose were measured by standard laboratory techniques. Diabetes was defined as fasting blood glucose level over 6.2 mmol/l or that the subject were on anti diabetic treatment. From these data, the Framingham risk score was calculated for each individual subject in accordance with the score sheet available at http://www.nhlbi.nih.gov/about/framingham/. The subjects were asked how many times a week they performed exercise for at least 30 min.

Leptin and adiponectin were analysed with double-antibody radioimmunoassays (RIA) (Linco Res., St. Louis, MO, USA). Total coefficient of variation (CV) for leptin was 4.7% at both low (2–4 ng/ml) and high (10–15 ng/ml) levels, and for adiponectin the total CV was 15.2% at low (2–4 μg/ml) and 8.8% at high (26–54 μg/ml) levels. High sensitive C-reactive protein (hsCRP) was measured in human serum by an ultrasensitive particle enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20 autoanalyser (Thermo Clinical Labystems, Espoo, Finland). The inter-assay coefficient of variation was 3.2%. Serum insulin was measured by an enzymatic-immunological assay (Boehringer Mannheim), HOMA insulin resistance index was defined as: fasting blood glucose × serum insulin/22.5 [19], not evaluated in subjects on insulin treatment.

2.2. Whole-body MRA

Imaging was performed on a 1.5-Tesla MRI system (Gyroscan Intera, Philips Medical Systems, Best, The Netherlands) with a 30 mT/m gradient system, using the standard quadrature body coil. The whole body was scanned in the supine position using a 3D RF-spoiled T1-weighted gradient echo sequence before and after injection of 40 ml Gd-DTPA-BMA (Omniscan, GE Healthcare, Oslo, Norway) at a rate of 0.6 ml/s. The acquired slice thickness was 4 mm with a resolution of 1.76 mm × 1.76 mm. Imaging did not include the coronary arteries.

The arterial tree was categorized into five territories: (1) the carotids including internal carotid artery (ICA) and common carotid artery, (2) the aorta including both the thoracic and abdominal part, (3) the renal arteries, (4) the pelvic/upper limbs including common iliac artery, external iliac artery (EIA), common femoral artery (CFA), superficial femoral artery (SFA) and popliteal artery (POP), (5) the lower legs including tibio-peroneal trunk (TPT), anterior tibial artery (ATA), peroneal artery (PA) and posterior tibial artery (PTA).

In order to obtain a comparable graded number reflecting the atherosclerosis in each territory, an atherosclerotic score (AS) was calculated for each territory. A normal vessel segment received null points, less than 50% stenosis was given one point and 50% reduction or more of the vessel diameter including occlusions was given two points. The points for the vessel segments in a territory were summarized. That sum was then divided with the maximum sum that would be achieved if all included segments had a more than 50% stenosis or occlusion.

A global total atherosclerosis score (TAS) was defined as the sum of AS for the five territories, the maximum TAS thus being 500 points.

2.3. Segmentation of adipose tissue

The segmentation was based on one MR image in each of 286 subjects, exclusion of the remaining subjects was due to missing information on a technical basis. An axial Balanced Fast Field Echo (B-FFE) sequence, with the parameters 10-mm slice thickness, 450-mm rectangular field of view (RFOV), 256 × 256 matrix size, and 70% scan percentage, was used both as a scout view to plan the acquisitions and for segmenting the distribution of visceral and subcutaneous adipose tissue at the L4–L5 level. The distribution for each of the two adipose tissues was identified and manually contoured with a region of interest (ROI) on one axial image in each subject, using the software package ImageJ.

Please cite this article in press as: Hansen T, et al. Visceral adipose tissue, adiponectin levels and insulin resistance are related to atherosclerosis as assessed by whole-body magnetic resonance angiography in an elderly population. Atherosclerosis (2008), doi:10.1016/j.atherosclerosis.2008.11.007
(http://rsb.info.nih.gov/ij/). The number of pixels in the ROI were then converted to an area (cm²). In VAT smaller vessels in the abdomen were included, with the exclusion of the aorta, the inferior vena cava and the common iliac vessels. The parenchymal organs and bowel were excluded. Fat despaired in the muscles was excluded from SAT. Imaging was not done on the same occasion as the baseline investigation and no restrictions in terms of fasting or time of day were applied.

3. Statistics

Censored regression was used for statistical calculations since 33% of the subjects showed no stenoses. The calculations were based on 282 subjects on account of missing values. First, separate regression models were used to correlate TAS to VAT, to SAT and to BMI, with adjustment for gender. Thereafter, using TAS as dependent variable and VAT and SAT as independent variables together with gender, the traditional risk factors included in the FRS, as well as BMI, were introduced as independent variables into the model. Subsequently, hsCRP, adiponectin, and leptin were added as independent variables to the model. A SD score transformation was performed on ln-transformed values of leptin and adiponectin due to the large difference in median value between females and males. This approach has previously been found to improve the relationship between these adipokines and measures of obesity, such as BMI. Other skewed independent variables, such as hsCRP, were log transformed. A negative regression coefficient implies an inverse relation. The statistical program package STATA 8.0 (TX, USA). The Tobit procedure was used for the calculations.

4. Results

4.1. Relations between TAS and fat distribution

In different separate regression models, VAT showed a relationship to TAS (p = 0.005) when adjusted for gender, while neither SAT nor BMI was related to TAS (p = 0.491 and p = 0.23, respectively).

When the traditional cardiovascular risk factors included in FRS (gender, blood pressure, smoking, diabetes, HDL-cholesterol, and LDL-cholesterol) were introduced as independent variables together with VAT, SAT, and BMI in a multiple regression model with TAS as dependent variable, VAT was still significantly related to TAS (p = 0.049, Table 2).

Exercise habits (median 0 times of exercise per week, range 0–8, quartile range 0–1) were related to TAS (p = 0.005), but the relationship between TAS and VAT was still significant (p = 0.019) also after adjustment for exercise habits.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>TAS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAT</td>
<td>0.15</td>
</tr>
<tr>
<td>SAT</td>
<td>0.40</td>
</tr>
<tr>
<td>BMI</td>
<td>0.22</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.16</td>
</tr>
<tr>
<td>FRS</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.18</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.020</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 2

A multiple censored regression model including measures of adiposity distribution together with traditional cardiovascular risk factors as independent variables, with TAS as dependent variable.

TAS = total atherosclerotic score, VAT = visceral adipose tissue, SAT = subcutaneous adipose tissue, BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein.

4.2. Relations between adipokines, TAS and fat distribution

Adiponectin and leptin levels were inversely related following gender adjustment (p < 0.001).

Both adiponectin and leptin were related to TAS following gender adjustment (p < 0.001 and p = 0.016, respectively). Furthermore, both adiponectin and leptin were related to VAT (p < 0.0001 for both adiponectin and leptin), but only leptin was significantly related to SAT (p = 0.26 for adiponectin and p < 0.0001 for leptin), following gender adjustment. In all those reported relationships, adiponectin was inversely related, while leptin was directly related.

4.3. Addition of hsCRP, adipokines and the HOMA insulin resistance index to regression models examining the relationship between TAS and fat distribution

When hsCRP were added as independent variables to the model presented in Table 2 (with FRS as substitute for HDL-cholesterol, LDL-cholesterol, diabetes and smoking to reduce the number of independent variables), FRS (p < 0.0001) and VAT (p = 0.025), but not hsCRP (p = 0.125), were significantly related to TAS.

However, when also adiponectin and leptin were added to the regression model, the relationship between VAT and TAS was no longer significant (p = 0.152). Now adiponectin (p = 0.020), but not leptin, together with FRS (p < 0.0001) was significantly related to TAS (Table 3).

The median value for the HOMA insulin resistance index was 1.69 mmol/l × mU/l (10th and 90th percentile 0.86 and 4.01). If HOMA index was also added to the model, only the HOMA index (p = 0.03) and FRS (p < 0.0001), but not adiponectin, were significantly related to TAS.

5. Discussion

In the present study, a relationship between VAT measured by MRI and TAS measured by WBMRA was found. This relationship was still significant after adjustment for gender, SAT, BMI, hsCRP, and traditional CV risk factors. However, the relation between VAT and TAS became non-significant when adiponectin was introduced into the multiple regression models.
Furthermore, if also insulin resistance was added to the model, this variable removed the effect of adiponectin. This indicates that the relationship between VAT and TAS, at least in part, can be explained by the fact that adiponectin levels mirror insulin resistance. Thus, adiponectin and insulin resistance could be an important pathophysiological link between visceral obesity and atherosclerosis. To our knowledge, this is the first study that has included adipose tissue segmentation, adipocytokines and assessment of subclinical atherosclerosis in a cohort comprising both sexes.

The action of adiponectin consists of a potential inhibition of several major atherosclerotic mechanisms, e.g., expression of adhesion molecules on the surface of endothelial cells and subsequent adhesion and migration of inflammatory cells in the vascular wall; uptake of oxidized LDL-cholesterol by scavenger receptors on the surface of foam cells; and migration of smooth muscle cells into the intima layer of the vessel wall, attracted by various growth factors [20]. Furthermore, it is well known that adiponectin levels are low in subjects with insulin resistance [21].

Leptin levels have previously been shown to predict CV events [22] and to relate to increased IMT in the carotid artery [23]. In the present study leptin was related to TAS in the univariate analysis, but did not affect the relationship between VAT and TAS and lost its significance after addition of adiponectin to the multiple regression models, suggesting a less important role for this adipokine in atherosclerosis development.

Another plausible explanation for the increased atherosclerosis observed in association with visceral fat accumulation could be actions of other inflammatory components such as CRP which has been linked to CV events [24], but not to subclinical atherosclerosis [25]. In the present study, hsCRP were not related to TAS.

One previous study, the only one to our knowledge, included measures of adipose tissue distribution assessed by CT, adipocytokines and assessment of subclinical atherosclerosis by IMT in a cohort of young apparently CV healthy women [26]. In that study, hypoadiponectinemia and subcutaneous, but not visceral, abdominal adipose tissue were related to increased IMT. In our study, SAT was not found to be related to TAS, which could indicate that the effect of the adipose tissue distribution on the amount of atherosclerosis is influenced by gender and age.

We have previously shown that in this PIVUS cohort, only VAT associated independently with dysfunctional endothelium assessed by the invasive forearm technique with acetylated (EDV) [27]. There is thus a reduced endothelium-dependent vasodilatation in elderly subjects with an increased visceral adipose tissue mass which might contribute to the increased atherosclerosis seen in association with VAT. This indicates that VAT not only alters the vascular morphology as evaluated by WBMRA but also affects the function of the endothelium as assessed by EDV.

It has previously been shown that waist-hip-ratio (WHR) was better related with myocardial infarction than BMI [2]. In conformity with these results, BMI was not significantly related to TAS in the present study. We have also shown that BMI and VAT were only moderately related, indicating that BMI and VAT carry different information.

A limitation of the current study is that the distribution of adipose tissue in the abdomen is measured in a single slice at the traditional level of the L4–L5 intervertebral space. If the whole abdomen were included in the measurements of VAT and SAT, the results might be different. A study aimed at identifying the slice with the highest association with obesity-related health risk indicators showed that a slice 10 cm above L4–L5 in men and 5 cm above in females had an association with risk indicators that was similar to or stronger than that of the total VAT volume [28]. However, when the VAT areas at L4–L5, 5 cm above L4–L5, and at the L3–L4 level were each compared with the total VAT mass, the abilities of these three slices to predict total VAT mass were comparable [29]. Other limitations of our study are the relatively low spatial resolution of WBMRA with the technique used for this study; due to technical improvements the spatial resolution that could be acquired today would be higher. When this study was conducted, the presence of nephrogenic systemic fibrosis (NSF) was not known. Afterwards, when NSF became a factor to account for, we checked the serum creatine levels and estimated glomerular filtration rate (eGFR) levels for all 306 subjects. Only one subject had a eGFR below 30 which is the limit for contraindication of contrast agent. So far there has been no report of NSF in this group. Also the fact that the present sample only consisted of Caucasians aged 70 preventing us from conclusions in other age groups and ethnicities. The relationships between VAT, TAS and adiponectin reported here could also have been influenced by other factors not evaluated in the present study, such as alcohol abuse, sleep apnea, other inflammatory pathways than covered by CRP and retinol binding protein 4 (RBP4). However, the strength of this large population-based sample should be emphasized.

From the present findings, we conclude that VAT is related to TAS independently of gender, total obesity (BMI), a marker for inflammation (hsCRP); as well as of the traditional risk factors included in FRS in an elderly population. However, adiponectin and insulin resistance attenuated the relationship between VAT and TAS, suggesting adiponectin and insulin resistance as an important link between visceral adiposity and atherosclerosis.

Acknowledgements

We wish to thank the Swedish Scientific Council for financial support (grant no. VR-K2006-71X-0676-24-3) and GE Healthcare for providing gadodiamide (OmniscanTM). We are also thankful to AstraZeneca and Uppsala and Umeå universities for financial support.

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Please cite this article in press as: Hansen T, et al. Visceral adipose tissue, adiponectin levels and insulin resistance are related to atherosclerosis as assessed by whole-body magnetic resonance angiography in an elderly population. Atherosclerosis (2008), doi:10.1016/j.atherosclerosis.2008.11.007

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