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Relationship between CT anthropometric measurements, adipokines and abdominal aortic calcification

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Abstract

Background—Visceral obesity and aortic calcification are both associated with cardiovascular events. The purpose of this study was to examine if visceral obesity was associated with the severity of abdominal aortic calcification.

Methods—One hundred and forty eight patients with peripheral artery disease were assessed by CT angiography. The severity of infra-renal abdominal aortic calcification was measured using a validated technique. The size of the visceral and subcutaneous compartments were estimated from anthropometric measurements made from the same CT. Calcification and anthropometric measurements were compared with Spearman's correlation and multiple logistic regression (adjusting for age, gender, hypertension, diabetes, smoking and cholesterol).

Results—The relative size of the visceral compartment estimated from CT diameter ratios was correlated with abdominal aortic calcification severity, $r=0.27$, $p=.001$ and independently associated with calcification allowing for other cardiovascular risk factors (OR 6.63, 95% CI 1.90–23.14). The relative size of the visceral compartment was associated with serum osteoprotegerin levels, suggesting a possible mechanism underlying the detrimental influence of visceral adiposity.

Conclusion—The association of visceral adiposity and arterial calcification suggests one mechanism which may contribute to the detrimental effects of central obesity.

Keywords

Aortic calcification; obesity; computer tomography; visceral

1. Introduction

Over the last decade there has been increasing interest in the identification of new cardiovascular risk factors which can be targeted to further reduce the mortality and morbidity of cardiovascular events. Arterial calcification is an example of a surrogate marker assuming greater importance as a predictor of cardiovascular risk. Both coronary and peripheral arterial

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calcification have been correlated to increased cardiovascular events and mortality [1,2]. Obesity is another example of an increasingly prevalent and important cardiovascular risk factor. Visceral deposition of adipose has been independently related to cardiovascular events [3–5].

Studies assessing abdominal aortic calcification have been more limited than those examining the coronary circulation, often utilising subjective measures such as plain X-ray [2] or not reporting the reproducibility of their technique [6]. We recently developed a reproducible technique to measure abdominal aortic calcification on CT angiography [7]. Given that both abdominal adiposity and aortic calcification are related to cardiovascular events we postulated that the two could be linked. In this study we assessed if there was any association between CT based measurements of body size and aortic calcification.

2. Methods

2.1. Patients

To investigate our hypothesis that abdominal adiposity and aortic calcification were related we carried out a prospective study. We entered consecutive patients undergoing Computer Tomography Angiogram (CTA) referred to our vascular service for investigation of peripheral artery disease. For ethical reasons we were limited to including patients who required CTA to investigate their presenting problem. Thus the patients entered with peripheral artery disease all had intermittent claudication or abdominal aortic aneurysm (AAA) since those with critical ischaemia required assessment by conventional angiography. We initially carried out a pilot study to assess the most appropriate method of assessing abdominal obesity on CT in our institution (see below). For the main study we felt a minimum significant correlation between our CT measurements of abdominal obesity and abdominal aortic calcification was a correlation coefficient of 0.25. We estimated that 140 patients would give us 90% power in detecting this correlation in comparison to no correlation (alpha 0.05). Based on previous referral of patients for CTA of approximately 10 per month we planned a 20 months recruitment period. Ethical approval for the study was granted by the local Institutional Ethics Committee. Written informed consent was obtained from all participants. Patients were recruited prospectively and consecutively from the Vascular Outpatients clinic between May 2004 and December 2006. The inclusion criteria for the study were:

- 1) A diagnosis of intermittent claudication or AAA;
- 2) CTA required to further assess peripheral artery disease according to the treating physician.

The exclusion criteria were:

- 1) previous surgical (endovascular or open) repair of AAA or aortic occlusive disease which would interfere with aortic calcification measurement;
- 2) patients deemed not suitable for a CTA by the treating physician for reasons including contrast allergy, inability to lie flat and serum creatinine $>120\mu\text{M}$.

No patients who entered the study were subsequently excluded from analysis. Data sheets were completed regarding the patients' medical history and blood tests. The data was stored on a Microsoft Excel spreadsheet (Microsoft Office 2003). Intermittent claudication was defined by an appropriate history obtained by a vascular specialist, a positive Edinburgh claudication questionnaire and confirmation of significant stenosis ($>50\%$) or occlusion of lower limb arteries on CTA. AAA was defined by a maximum aortic diameter of $\geq 30\text{mm}$. Hypertension was defined by a history of high blood pressure or receiving treatment to reduce blood pressure.

Diabetes was defined by a fasting blood glucose ≥ 7.0 mM, or history of, or treatment for hyperglycaemia. Smoking status was classified into current smokers (smoked within the last month), ex-smokers (given up for more than one month) and never smokers [8].

2.2. CT measurements

Contrast enhanced CT images were obtained using a 4-slice multiscanner (MX800, Philips). The imaging and workstation protocols utilised to measure infrarenal abdominal aortic calcification have previously been validated in detail [7]. Briefly selected images were reconstructed on a workstation utilising defined thresholds (Center Hounsfield Unit (CH) level 1400 and Window width Hounsfield Unit (WH) 2000), automated function setting and image magnification. The reproducibility of repeat calcification volume measurements with this protocol has been previously assessed. Intra and inter-observer coefficient of variation were approximately 1% [7]. For consistency anthropometric measurements were made using the most cephalad slice on which the iliac crest appeared in all patients (Fig. 1). Measurements were made using electronic callipers on a workstation in mms. Two anterior-posterior diameters were measured by creating a vertical line from the anterior abdominal skin through the middle of the vertebral body to the skin of the posterior surface. AP1 represented the entire length of this line, while AP2 the distance from the linea alba to the posterior aspect of the spinous process. The anterior-posterior ratio (APR) was calculated as $AP2/AP1$. Two similar transverse measures were made by creating a horizontal line passing through the most anterior aspect of the vertebral body. T1 represented the entire length of this line, while T2 was the distance from the anterior surface of the right external oblique to that of the left. The transverse ratio (TR) was calculated as $T2/T1$. Thus a larger visceral compared to subcutaneous compartment would lead to an increase in both ratios. A further estimate of the subcutaneous fat was made by creating a vertical line from the anterior aspect of the skin to that of the external oblique at a point 50mm right of the umbilicus (SC). Intra- and inter-observer reproducibility of these measurements were assessed by repeat measurements one week apart on 15 patients. The intra-observer coefficient of variation were 0.94, 0.74 and 4.0% for APR, TR and SC, respectively. The inter-observer coefficient of variation were 2.6, 3.2 and 10% for APR, TR and SC, respectively. The mean confidence intervals for the inter-observer assessments were 0.001, 0.006 and 0.67 mm for APR, TR and SC, respectively. In a pilot study we compared our CT measurements with waist circumference, waist to hip ratio and body mass index in 38 patients presenting with intermittent claudication. AP1 and AP2 were both correlated with waist circumference ($r=0.73$, $p<.0001$; $r=0.66$, $p<.0001$), waist to hip ratio ($r=0.43$, $p=.01$; $r=0.51$, $p=.002$) and body mass index ($r=0.43$, $p=.01$; $r=0.19$, $p=.25$). T1 and T2 were also correlated with waist circumference ($r=0.62$, $p<.0001$; $r=0.66$, $p<.0001$), waist to hip ratio ($r=0.32$, $p=0.04$; $r=0.37$, $p=.03$) and body mass index ($r=0.60$, $p<.0001$; $r=0.48$, $p=.003$). Maximum abdominal aortic diameter was measured from axial CTA slices using electronic callipers.

2.3. Blood analysis

Blood was collected after an overnight fast on the morning of the CT assessment Cholesterol, triglyceride, high and low density lipoprotein, were measured by automated enzymatic methods [9]. C-reactive protein (CRP) was measured by particle-enhanced turbimetry (Roche Diagnostics, Basel, Switzerland) [9] Osteoprotegerin (OPG), osteopontin (OPN), leptin, adiponectin, resistin and matrix gamma-carboxyglutamic acid (Gla) protein (MGP) were measured with commercial ELISA (Biovendor and R&D Systems) previously validated in detail [8,10].

2.4. Statistical analysis

The relationship between APR and TR with leptin adjusting for conventional risk factors were examined with multiple linear regression. Infrarenal abdominal aortic calcification values were not normally distributed even after log transformation. We therefore used non-parametric tests in all univariate analyses and ordinal logistic regression analysis in multivariate assessments. The relationships between anthropometric measurements, serum cytokines and aortic calcification volumes were assessed with Spearman's correlation. Tertiles of anthropometric diameters were compared by Kruskal Wallis test. Similar to studies of coronary artery calcification we used ordinal multiple logistic regression analysis to assess the independent relationship between anthropometric measurements and aortic calcification adjusting for other cardiovascular risk factors [11]. Continuous variables were converted to tertiles and along with nominal variables were examined for their ability to predict upper compared to lower tertiles of aortic calcification. They were reported as odds ratios (OR) with 95% confidence intervals (95% CI).

3. Results

3.1 Characteristics of the cohort

CTA revealed that 68 of the 148 patients had an AAA of whom 16 also had symptoms of intermittent claudication. The characteristics of the patients in relation to whether they had an AAA are illustrated in Table I. As shown patients were receiving a variety of different medications although none were prescribed warfarin.

3.2 Relationship between CT anthropometric measurements and abdominal aortic calcification

Abdominal aortic calcification was positively correlated with APR ($r=0.27$, $p=.001$) and TR ($r=0.26$, $p=.001$), but negatively correlated with SC ($r=-0.28$, $p=.001$). Tertiles of APR and TR were associated with aortic calcification volume, serum leptin and OPG concentrations (Table II and Fig. 2). Leptin was independently associated with APR and TR adjusting for age, diabetes, hypertension, smoking and cholesterol (For APR $\beta=-0.39$, $t=-4.6$, $p<.0001$; For TR $\beta=-0.47$, $t=-5.7$, $p<.0001$). As previously reported, aortic calcification was correlated with serum OPG ($r=0.36$, $p<.0001$) but not with leptin ($r=-.042$, $p=.64$) [10]. Adjusting for traditional risk factors (age gender, smoking history, diabetes, hypertension and cholesterol) APR (OR 3.53, 95% CI 1.12–11.13, $p=.03$) and TR (OR 6.63, 95% CI 1.90–24.14, $p=.003$) were independently associated with aortic calcification volume (see Table III for models).

4. Discussion

Evidence from a number of sources supports the detrimental effects of visceral compared to peripheral subcutaneous adipose deposition. Waist circumference has been associated with increased cardiovascular events in a range of populations [3–5,12–14]. In a smaller number of studies CT measured visceral adipose volume was found to be a stronger predictor of cardiovascular events than other measures of obesity [14,15]. The importance of central obesity has been appreciated in making waist circumference an essential component of the definition of metabolic syndrome [16].

In the present study we have identified an association between a surrogate measure of visceral adipose and aortic calcification (Table II and Table III, Fig. 2). We chose to use CT diameters of the visceral compartment rather than adipose estimates based on workstation thresholds because in a pilot study the reproducibility of the latter technique was noted to be poor, with coefficient of variation of >10%. Intra and inter-observer coefficient of variation for APR and TR were all less than 5% and provided a very simple assessment method. In addition we found

our CT measurements to be correlated with anthropometric measures in a sub-set of 38 patients in a pilot study ($r=0.32$ to 0.72 , see methods). It is to be expected that these two different techniques of assessing adiposity would not be completely correlated for a number of reasons. For example, anthropometric measurements are made with the patient standing and are circumferential while CT measurements are made with a patient lying supine on a table and are diameters (Fig. 1). Other studies reporting more complicated estimates of visceral adipose deposition have often not reported measurement reproducibility [14,15]. The associations between APR and TR with abdominal aortic mineralisation were noted to be independent of other factors previously associated with calcification, including age, gender, hypertension and smoking [6,17–19]. Despite the good reproducibility of our CT measurements of adiposity more detailed assessment of the relationship between these and other measures of obesity are required in future studies.

Traditional risk factors such as hypercholesterolaemia, diabetes and hypertension were not associated with calcification (Table III). This may relate to the concurrent treatment of these conditions. Over 50% of patients were receiving statins and approximately one-third of patients were prescribed angiotensin II modifying medication, beta blockers and calcium channel blockers. One-fifth of patients were receiving oral hypoglycaemics. To truly assess whether concurrent therapy accounts for the lack of association of traditional risk factors would have required long term assessment of the control of these risk factors or assessments of drug compliance over many years. In contrast to older investigations other more recent studies have failed to find an association between traditional risk factors and aortic calcification supporting that increased use of therapy may be responsible for this effect [19,20].

The mechanisms underlying the more detrimental effects of visceral compared to peripheral and subcutaneous adipose are presently not defined. It has been postulated that visceral adipose releases different quantities of adipokines, such as leptin and inflammatory cytokines, such as C-reactive protein [21–23]. In the present study we found an association between serum leptin and OPG with APR and TR. Serum leptin was strongly negatively correlated with increase in APR and TR (Table II, Fig. 2). This correlation supports data demonstrating a greater association of leptin with subcutaneous compared to visceral adipose, i.e. as the ratio between subcutaneous to visceral adipose increases so does leptin production [23–26]. Circulating leptin concentrations have been independently correlated with the area of subcutaneous rather than visceral adipose tissue [24]. The concentration of leptin has been demonstrated to be higher in subcutaneous compared to visceral adipose biopsies [25,26]. Furthermore the expression of leptin in subcutaneous adipocytes has been negatively correlated to visceral fat volume [23]. It should be noted however that in some studies leptin has also been associated with visceral fat volume. For example, a prospective study of Japanese subjects carried out by Tong and colleagues reported that higher circulating leptin levels at baseline predicted greater deposition of visceral adipose at 5 years [27].

The role of leptin in ossification and calcification is controversial [28]. Leptin has been implicated in both promotion and inhibition of bone formation in animal models [28]. *In vitro* leptin enhances calcification by bovine smooth muscle cells [29]. Plasma leptin concentrations have been positively correlated with coronary calcification in asymptomatic subjects and patients with diabetes [30,31]. In the present study we found no association between circulating leptin concentration and infrarenal abdominal aortic calcification. These findings may reflect different mechanisms underlying peripheral arterial mineralisation or the fact we studied patients with peripheral artery disease, which was not the case in the two previous studies [30,31].

We have previously demonstrated an association between circulating concentrations of the bone cytokine OPG and abdominal aortic calcification [10]. We confirmed this finding in this

study which involved a larger number of patients than previously presented. An association between the visceral compartment size and serum OPG levels is also reported in the present study (Table II). This finding could explain the association between APR/ TR and aortic calcification. Circulating concentrations of OPG have been associated with arterial calcification at a number of sites and also prospectively correlated with cardiovascular events [32,33]. It has been demonstrated that adipose contains stem cells capable of differentiating into osteoblasts *in vitro* and that similar control pathways modulate bone and adipocyte differentiation [34,35]. It is thus possible that components of visceral adipose tissue are able to produce OPG or other cytokines, which can control the development of arterial mineralisation and atherosclerosis. This hypothesis requires further investigation.

The association of visceral obesity and aortic calcification could also relate to the influence of peripheral artery disease on mobility of patients. Over half of our patients had intermittent claudication which limited their exercise capacity (Table I). Impaired gut perfusion and intestinal angina due to mesenteric artery atherosclerosis can be associated with weight loss, however, none of our patients had these symptoms.

In conclusion this study demonstrates an association between the size of the visceral compartment and the severity of abdominal aortic calcification. We have also found a correlation between the visceral compartment size and circulating OPG concentrations, suggesting a mechanism which may contribute to the poor prognosis of visceral adipose deposition. These findings require confirmation in larger cohorts.

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Figure One.

a) Illustration of anthropometric measurements made from CT images. The anterior-posterior ratio (APR) was calculated by dividing the measurement illustrated with the pink line (AP1) by the distance represented by the blue line (AP2). The transverse ratio (TR) was calculated by dividing the measurement illustrated with the red line (T1) by the distance represented by the green line (T2). b) The subcutaneous fat depth was calculated by measuring the distance represented by the pink line. See methods for more details.

Figure Two.

a) Box plots illustrating median, inter-quartile range and range of abdominal aortic calcification for patients with different tertiles of transverse ratio. b) Box plots illustrating median, inter-quartile range and range of serum leptin concentrations for patients with different tertiles of transverse ratio.

Table I**Table I Characteristics of patients in relation to AAA.**

	AAA	No AAA	Overall
Number	68	80	148
Male	49	57	106 (72%)
Age (years)	71.4±7.7	64.7±9.7	67.7±9.4
Diabetes	13	25	38 (26%)
Hypertension	49	48	97 (66%)
Current smoking	20	37	57 (39%)
Ex smoker	25	29	54 (36%)
Intermittent claudication	16	80	96 (65%)
Cholesterol (mM)	4.75±1.39	5.01±1.94	4.89±1.71
Triglyceride (mM)	1.79±0.88	2.11±1.43	1.96±1.21
LDL (mM)	2.74±1.29	2.86±1.90	2.80±1.64
HDL (mM)	1.24±0.32	1.30±0.35	1.27±0.34
Aspirin	44	52	96 (65%)
Statin	36	42	78 (53%)
Angiotensin II inhibitor*	28	25	53 (36%)
Beta blockers	24	20	44 (30%)
Calcium channel blockers	23	26	49 (33%)
Oral hypoglycaemics	8	16	24 (16%)

* Angiotensin converting enzyme inhibitor or Angiotensin II receptor blocker; AAA = Abdominal aortic aneurysm; LDL = Low density lipoprotein; HDL = High density lipoprotein. Data are numbers and percentages for nominal variables and mean ± standard deviation for continuous variables.

Table II

Association of APR tertiles with calcification, lipids and cytokine concentrations.

	APR tertile 1	APR tertile 2	APR tertile 3	P value
Number	50	49	49	
APR	0.73±0.05	0.84±0.02	0.91±0.02	<.0001
TPR	0.86±0.07	0.91±0.04	0.94±0.03	<.0001
SC (mm)	34.9±10.9	23.9±6.1	16.0±6.6	<.0001
Aortic calcification volume (mm ³)	1256±1184	1871±1762	2266±1820	.01
HDL (mM)	1.27±0.27	1.25±0.32	1.29±0.41	.87
LDL (mM)	2.96±2.17	2.93±1.53	2.49±0.89	.34
CRP (mg/L)	9.52±19.50	17.07±14.5	8.86±16.3	.93
Adiponectin (µg/ml)	8.33±4.36	9.49±4.48	10.26±5.09	.25
Leptin (ng/ml)	36.22±28.79	21.00±25.56	12.04±13.62	<.0001
Resistin (ng/ml)	25.69±19.97	24.69±13.20	25.45±10.74	.45
OPG (pM)	6.48±2.60	6.49±2.48	7.71±2.83	.01
OPN (ng/ml)	26.0±19.1	24.5±18.5	24.6±18.2	.89
MGP (nM)	24.9±18.6	25.7±14.6	29.8±18.7	.20

Numbers are mean ± standard deviation. APR = Anterior-posterior ratio; TPR = Transverse ratio; SC = Subcutaneous adipose depth; HDL = High density lipoprotein; LDL = Low density lipoprotein; CRP = C-reactive protein; OPG = Osteoprotegerin; OPN = Osteopontin; MGP = Matrix Gla protein.

Table III
Variables associated with infrarenal abdominal aortic calcification by logistic regression.

Variable	Model 1 $\chi^2=35.8$ P<.0001			Model 2 $\chi^2=40.1$ P<.0001		
	OR	95% CI	P value	OR	95% CI	P value
Age*	7.43	2.07–26.73	.002	6.94	1.91–25.25	.003
Hypertension	1.78	0.66–4.78	.25	2.14	0.77–5.96	.14
Current smoking	2.45	0.73–8.20	.15	2.82	0.81–9.78	.10
Diabetes	0.81	.28–2.34	.89	0.84	0.29–2.44	.74
Cholesterol*	1.03	.35–3.03	.96	0.97	0.31–2.97	.95
APR*	3.53	1.12–11.13	.03			
TR*				6.63	1.90–23.14	.003

Ability of models incorporating these six variables to determine patients with aortic calcification of the upper compared to the lower tertile. Odds ratios (OR) for nominal variables refer to the effect of having the risk factor compared to not. Odds ratios for continuous variables (*) refer to the effect of being in the upper compared to the lower tertile. 95% CI= 95% confidence intervals.