Abdominal Adiposity Distribution Quantified by Ultrasound Imaging and Incident Hypertension in a General Population

Ekim Seven, Betina H. Thuesen, Allan Linneberg, Jørgen L. Jeppesen

Abstract—Abdominal obesity is a major risk factor for hypertension. However, different distributions of abdominal adipose tissue may affect hypertension risk differently. The main purpose of this study was to explore the association of subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) with incident hypertension in a population-based setting. We hypothesized that VAT, rather than SAT, would be associated with incident hypertension. VAT and SAT were determined by ultrasound imaging in 3363 randomly selected Danes (mean age 49 years, 56% women, mean body mass index 25.8 kg/m²). We constructed multiple logistic regression models to compute standardized odds ratios with 95% confidence intervals per SD increase in SAT and VAT. Of the 2119 normotensive participants at baseline, 1322 were followed for a median of 5 years without any change in BMI and WC, whereas 203 had developed hypertension. In models including both VAT and SAT, the Framingham Hypertension Risk Score variables (age, sex, smoking status, family history of hypertension, and baseline blood pressure) and glycated hemoglobin, odds ratio (95% confidence interval) for incident hypertension for 1 SD increase in VAT and SAT was 1.27 (1.08–1.50, \(P=0.004\)) and 0.97 (0.81–1.15, \(P=0.70\)), respectively. Adjusting for body mass index instead of SAT attenuated the association between VAT and incident hypertension, but it was still significant (odds ratio, 1.22 [1.01–1.48, \(P=0.04\)]) for each SD increase in VAT. In conclusion, ultrasound-determined VAT, but not SAT, was associated with incident hypertension in a random sample of Danish adults. (Hypertension. 2016;68:00–00. DOI: 10.1161/HYPERTENSIONAHA.116.07306.) ● Online Data Supplement

Key Words: adipose tissue ▪ adiposity ▪ blood pressure ▪ hypertension ▪ obesity ▪ ultrasound

Abdominal fat accumulation is associated with increased risk of hypertension and cardiovascular disease (CVD). Abdominal adipose tissue is manifested in the subcutaneous and visceral tissues, with visceral adipose tissue (VAT) considered being more harmful than subcutaneous abdominal adipose tissue (SAT). VAT is highly metabolically active and produces a variety of substances, for example interleukin-6 (IL-6), which contributes to low-grade inflammation and insulin resistance. Furthermore, increased VAT has been associated with the activation of the sympathetic nervous system and lower circulating concentrations of natriuretic peptides (NPs), which could contribute to hypertension in individuals with increased VAT.

Anthropometric measurements, such as waist circumference (WC) and body mass index (BMI), are easily obtainable surrogate measures of abdominal and overall adiposity. However, as both WC and BMI do not differentiate between VAT and SAT, they are imprecise. A more accurate quantification of VAT and SAT can be acquired with the use of computed tomography (CT) or magnetic resonance imaging (MRI), but these techniques are costly, time consuming, limited by their accessibility, and have radiation issues (CT). Opposed to these modalities, ultrasound imaging is cheap, fast, and easily accessible, and it produces reproducible and valid estimates of VAT and SAT, and hence a suitable modality to use in large epidemiological studies.

A few prospective studies have shown positive associations between VAT, and to some degree SAT, and metabolic risk factors, including hypertension. However, these studies utilized CT or MRI to quantify VAT and SAT, which, as previously mentioned, does come with some limitations for clinical use. To our knowledge, no prospective population-based study has explored the associations between sonographic VAT and SAT measurements and hypertension. With that in mind, we conducted this study in a group of randomly selected Danes from the background population. We hypothesized that VAT, rather than SAT, would be associated with both prevalent and incident hypertension.

Methods
A supplementary Methods section is provided in the online-only Data Supplement, which in detail describes our study population and our abdominal ultrasound practice.
Study Population

We based this study on data from the Health2006 study, which was conducted at the Research Centre for Prevention and Health (RCPH), the Capital Region of Denmark. A total of 3471 individuals (participation rate of 44.7%) entered the Health2006 study and participated in the baseline examination between June 2006 and June 2008. Participants were further invited for a follow-up examination 5 years later, where parts of the baseline study examination were repeated.4,23

Eligible criteria for our study were a full set of blood pressure (BP) measurements, data about use of antihypertensive medication, and baseline ultrasound measurements of SAT and VAT. Exclusion criteria were prevalent CVD at baseline. On the basis of these criteria, we included 3363 individuals for the cross-sectional analysis and 2119 for the prospective analysis. Figure 1 shows a Health2006 study CONSORT diagram presenting number of subjects participating in the baseline and follow-up examinations and the reasons for nonparticipation.

Main Outcome of Interest: Hypertension

BP was measured by trained staff at least twice with a mercury sphygmomanometer (Mercuro 300, Speidel & Keller) with appropriate cuff size, after 5 minutes of rest, in the sitting position. If systolic BP (SBP) ≥140 mm Hg or diastolic BP (DBP) ≥90 mm Hg, the measurements were repeated twice at the same visit to minimize the white coat effect with the 2 lowest values being recorded, and the average of the recorded measurements was used.

Prevalent hypertension was defined as baseline SBP ≥140 mm Hg or baseline DBP ≥90 mm Hg or use of antihypertensive medication at baseline. Incident hypertension was defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg or use of antihypertensive medication at the 5-year follow-up among individuals who were not hypertensive at baseline.

To correct for the use of antihypertensive medication in one of our quantitative BP analyses, we used the fixed Cui–Harrap adjustment criteria were prevalent CVD at baseline. On the basis of these criteria, we included 3363 individuals for the cross-sectional analysis and 2119 for the prospective analysis. Figure 1 shows a Health2006 study CONSORT diagram presenting number of subjects participating in the baseline and follow-up examinations and the reasons for nonparticipation.

Secondary Outcomes: CVD

Information on CVD was obtained from the Danish National Patient Register. The included diagnoses were the International Classification of Diseases (ICD) ICD10 and ICD8 diagnoses involving ischemic heart disease (ICD10: I20–I25 and ICD8: 410–414) and stroke (ICD10: I60–I69 and ICD8: 431, 433–434 and 436). Information about death was obtained from the Danish Register of Causes of Death.

Exposure Measurements

Participants underwent a health examination, including measurement of height, weight, and WC. Height was measured without shoes to the nearest cm; weight was measured in light clothing without shoes to the nearest 0.1 kg. WC was measured midway between the lower rib margin and the iliac crest to the nearest cm, without any pressure to the body surface and with an unstretched tape meter. BMI was calculated conventionally. Fat percentage was measured using a foot-to-foot Tanita Body Composition Analyzer (TBF-300, TANITA Corporation of America, Inc, Arlington Heights, IL). An individual was considered having a positive family history of hypertension if either parent was diagnosed with hypertension.

Fasting blood samples were drawn from a cubital vein in the supine position and analyzed, including analysis for glycated hemoglobin (HgbA1c), at the Steno Diabetes Center, Gentofte, Denmark, using standard laboratory techniques.23

The ultrasound measurements were done with the participants lying on their back. SAT was the distance in centimeters between the posterior edge of the abdominal muscles and the skin. VAT was the distance between the posterior edge of the abdominal muscles and the front of the lumbar spine.22,24 All ultrasound measures were performed after a quiet expiration applying minimal pressure without the displacement of the intra-abdominal contents as described by Stolk et al17 with an Aquila Pie Medical, Esaote Europe, Maastricht, The Netherlands. These ultrasound measures have been validated against CT and MRI elsewhere.15,17,29,30

Statistics

All analyses were performed with SAS, version 9.4 (SAS Institute, Cary, NC). VAT and SAT were normally distributed. Descriptive data are expressed as the mean±SD or proportions in percent. Both categorical (quartiles) and continuous analyses were performed. VAT and SAT were standardized by dividing each adiposity measure by its own distribution-specific SD. This was done to overcome the fact that the 2 adiposity measures are on different scales and allowed direct comparisons. Multiple logistic regression models were constructed to compute standardized odds ratios (ORs) with 95% confidence intervals for 1 SD increase for VAT and SAT and for sex-specific VAT and SAT quartiles with both prevalent and incident hypertension as outcome. We adjusted for age (and sex; model 1), model 1+ factors belonging to the Framingham Hypertension Risk Score (smoking status, parental history of hypertension, and baseline SBP and DBP [only incident model]) and HgbA1c (model 2), and finally model 2+VAT or SAT or BMI as relevant (model 3). The predictive performance of the models, with and without variables of interest, was summarized using c-statistics (area under curve) estimated from the logistic regression models by means of an SAS macro as proposed by Pencina et al.32

Linear trends were assessed by using the SAS CONTRAST statement. The validity of the multiple logistic regression models was checked by Hosmer and Lemeshow (HL) goodness-of-fit test, and the results of this test are reported as HL test equals relevant values. In the logistic regression models, the linearity assumption was checked by adding the term squared and cubed to the model and checking for significance. Because we found no sex-based differences in the relationship between VAT and SAT and both prevalent and incident hypertension (P≥0.12 for interaction), we pooled all the data together to increase power in our outcome analyses. A P value of less than 0.05 was considered significant.

Results

A Results section is provided in the online-only Data Supplement.

Characteristic of Participants

General characteristics of the Health2006 study for participants included in the cross-sectional analysis and the prospective analysis are summarized in Table 1. Compared with men, women had significantly less VAT (cross-sectional population (mean±SD): 5.6±2.1 cm versus 7.6±2.5 cm; prospective
population: 5.0±1.6 cm versus 6.7±2.0 cm, \( P<0.001 \) and more SAT (cross-sectional population: 3.2±1.3 cm versus 2.8±1.1 cm; prospective population: 2.9±1.3 versus 2.7±1.0, \( P<0.001 \)). Of the 3363 participants included in the cross-sectional analysis, 1244 were categorized being hypertensive, including 434 participants (34.9%) taking antihypertensive medication.

Cross-Sectional Analysis

Table 2 presents trends for variables across sex-specific SAT and VAT quartiles. Both more SAT and VAT was associated with higher age, higher BMI, larger WC, higher fat percentage, higher SBP and DBP, and a higher frequency of antihypertensive treatment, and for both SBP and DBP and frequency of hypertensive treatment.}

Table 1. General Characteristics of the Health2006 Cohort for Participants Included in the Cross-Sectional Analysis and the Prospective Analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cross-Sectional Analysis (n=3,363)</th>
<th>Prospective Analysis (n=1,432)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49±13</td>
<td>46±12</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>1,870 (56)</td>
<td>864 (60)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.8±4.6</td>
<td>24.7±3.9</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>88±13</td>
<td>84±12</td>
</tr>
<tr>
<td>Fat percentage, %</td>
<td>30±9</td>
<td>28±9</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue, cm</td>
<td>3.0±1.2</td>
<td>2.8±1.2</td>
</tr>
<tr>
<td>Visceral adipose tissue, cm</td>
<td>6.5±2.5</td>
<td>5.7±2.0</td>
</tr>
<tr>
<td>Family history of hypertension, %</td>
<td>51.4</td>
<td>52.4</td>
</tr>
<tr>
<td>Active smoker, %</td>
<td>24.2</td>
<td>21.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130±18</td>
<td>120±10</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82±11</td>
<td>77±7</td>
</tr>
<tr>
<td>Prevalent hypertension, n (%)</td>
<td>1,244 (37.0)</td>
<td>0</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>434 (12.9)</td>
<td>0</td>
</tr>
<tr>
<td>Self-reported diabetes mellitus, n (%)</td>
<td>55 (1.6)</td>
<td>10 (0.7)</td>
</tr>
<tr>
<td>HgbA1c, %</td>
<td>5.4±0.5</td>
<td>5.3±0.4</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD and frequency in percent for categorical variables. HgbA1c indicates glycated hemoglobin.
antihypertensive treatment, the trend was stronger across VAT quartiles \((P<0.001)\).

The associations between SAT, VAT, and prevalent hypertension are shown in Table S1. Adjusted for age, HgbA1c, smoking status, parental history of hypertension, and SAT, the risk of prevalent hypertension increased robustly with higher sex-specific VAT quartiles (\(>280\% \text{ increase in OR from the 1st to the 4th sex-specific VAT quartile}\)), and when VAT was entered as a continuous variable in the adjusted model above, including BMI instead of SAT, VAT was still significantly associated with prevalent hypertension (OR \([95\% \text{ confidence interval}] 1.38 [1.22–1.55]\) for 1 SD increase in VAT, \(P<0.001, \text{HL test}=0.13\)). In contrast, although the risk of prevalent hypertension increased with higher sex-specific SAT quartiles, adjusted for age, HgbA1c, smoking status, parental history of hypertension, and VAT, the strength of the association was overall weak (\(<40\% \text{ increase in OR from the 1st to the 4th sex-specific SAT quartile}\)), and when SAT was entered as a continuous variable in the adjusted model above, including BMI instead of VAT, SAT was not significantly associated with prevalent hypertension (\(P=0.98\)). Finally, as shown in Figure 2, within each weight class (normal weight, overweight, and obese), both SBP and DBP increased with increasing VAT tertile, whereas regarding SAT, this increase was only seen in the lowest SAT tertile and not at all in the 2 highest SAT tertiles.

### Prospective Analysis

With a median follow-up of 4.8 years, 1432 normotensive participants at baseline attended the follow-up examination and were included in the prospective analysis. A total of 203 individuals had developed hypertension between baseline and follow-up, including 54 individuals (26.6\%) taking antihypertensive medication.

Table 3 shows associations between SAT, VAT, and incident hypertension. SAT, neither expressed as sex-specific SAT quartiles nor expressed as a continuous variable, was significantly associated with incident hypertension in any of the models \((P>0.34)\). In contrast, VAT, either expressed as sex-specific VAT quartiles or expressed as a continuous variable, was significantly associated with incident hypertension in all of the models, including adjustment for either SAT or BMI. Thus, VAT was associated with a 53\% increase in OR from the 1st to the 4th sex-specific VAT quartile adjusted for age, HgbA1c, smoking status, parental history of hypertension, BMI, and baseline SBP and DBP, and with an OR (95\% confidence interval) for incident hypertension for 1 SD increase in VAT of 1.22 (1.01–1.48), \(P=0.041, \text{HL test}=0.38\), adjusted for sex, age, HgbA1c, smoking status, parental history of hypertension, BMI, and baseline SBP and DBP. Furthermore, adjusting for fat percentage, instead of SAT, produced a similar result (OR, 1.25 [1.04–1.50], \(P=0.016, \text{HL test}=0.25\) for each SD increase in VAT).

Finally, to turn things around, we calculated ORs for incident hypertension for sex- and age-adjusted BMI quartiles with subsequent adjustments for SAT and VAT (Figure 3). We saw a strong association between BMI and incident hypertension; however, this association was completely abolished when we adjusted for VAT and remained basically unchanged when we adjusted for SAT.

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### Table 2. Clinical Characteristics Presented by Sex-Specific Subcutaneous Adipose Tissue and Visceral Adipose Tissue Quartiles

<table>
<thead>
<tr>
<th>Variables</th>
<th>1st (n=845)</th>
<th>2nd (n=839)</th>
<th>3rd (n=841)</th>
<th>4th (n=838)</th>
<th>(P) Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subcutaneous adipose tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>46±14</td>
<td>49±13</td>
<td>51±12</td>
<td>50±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.7±3.5</td>
<td>24.5±3.3</td>
<td>26.5±3.6</td>
<td>29.5±4.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC, cm</td>
<td>80±12</td>
<td>85±11</td>
<td>91±11</td>
<td>98±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage, %</td>
<td>23.9±7.4</td>
<td>28.4±7.7</td>
<td>31.5±8.2</td>
<td>36.4±9.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>126±18</td>
<td>129±17</td>
<td>131±17</td>
<td>134±17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79±11</td>
<td>81±10</td>
<td>82±11</td>
<td>85±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>80 (9.5)</td>
<td>103 (12.3)</td>
<td>118 (14.0)</td>
<td>133 (15.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Visceral adipose tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>44±14</td>
<td>47±13</td>
<td>51±12</td>
<td>55±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.7±2.8</td>
<td>24.1±2.9</td>
<td>26.1±3.5</td>
<td>30.2±4.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC, cm</td>
<td>79±9</td>
<td>84±9</td>
<td>89±11</td>
<td>101±12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat percentage, %</td>
<td>24.4±8.0</td>
<td>27.3±7.5</td>
<td>30.9±7.5</td>
<td>36.7±8.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>124±17</td>
<td>126±16</td>
<td>131±17</td>
<td>139±17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>78±10</td>
<td>79±10</td>
<td>83±10</td>
<td>86±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive medication, n (%)</td>
<td>51 (6.0)</td>
<td>74 (8.8)</td>
<td>101 (12.0)</td>
<td>208 (24.8)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or as frequency in percent. Cut points for SAT: Men: ≤2.1, >2.1 and ≤2.6, >2.6 and ≤3.4, >3.4 cm; Women: ≤2.2, >2.2 and ≤3.0, >3.0 and ≤4.0, >4.0 cm. Cut points for VAT: Men: ≤5.7, >5.7 and ≤7.1, >7.1 and ≤8.9, >8.9 cm; Women: ≤4.1, >4.1 and ≤5.0, >5.0 and ≤6.4, >6.4 cm. BMI indicates body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; and WC, waist circumference.
Supplementary Analyses
Because a substantial number of participants did not show up for the follow-up examination, we also examined whether VAT would be associated with hard CVD events among the 2119 individuals who were eligible to be included in the prospective analysis. During the 5-year follow-up, 64 participants experienced an ischemic heart disease event and 45 participants had a stroke. Four participants experienced both an ischemic heart disease event and a stroke. Accordingly, we constructed a combined CVD event composed of ischemic heart disease or stroke whatever came first (n=105). Of the 105 CVD events, 9 were fatal events. In a model, adjusting for age, sex, smoking status, HgbA1c, and SAT, OR (95% confidence interval) for incident CVD for 1 SD increase in VAT was 1.47 (1.25–1.76, P<0.001, HL test=0.23). Adjusting for BMI instead of SAT attenuated the association between VAT and incident CVD, but it was still significant (OR, 1.25 [1.00–1.55, P=0.046, HL test=0.29] for each SD increase in VAT). We did not include BP in the outcome analyses above, because higher BP, our variable of main interest, is a candidate pathophysiological intermediary between VAT and CVD.33 SAT was not associated with incident CVD (P=0.36).

Finally, as presented in detail in the online-only Data Supplement, adding VAT to a model, including sex, age, smoking status, baseline BP, family history of hypertension, and BMI, did not significantly improve c-statistics (P=0.34).

Discussion
In this cross-sectional and prospective population-based study, we have demonstrated that sonographic VAT measurements...
were associated with both prevalent and incident hypertension. This observation was independent of traditional risk factors for hypertension, including BMI and total body fat percentage. SAT was only weakly associated with prevalent hypertension (and in fact not after adjustment for BMI), but not incident hypertension. Furthermore, the association between VAT and hypertension was independent of weight classes, age, and sex. Our data provide some evidence that much of the BMI-related risk for hypertension is mediated through VAT because adjustment for VAT basically eliminated the BMI-associated hypertension risk.

Our results are supported by several prospective epidemiological studies. In 2004, Hayashi et al. showed using CT that intra-abdominal fat area was significantly associated with incident hypertension, even after adjustment for subcutaneous fat, in a cohort of 300 Americans with Japanese ancestry. A decade later, Chandra et al. using MRI data from the Dallas Heart Study, demonstrated that VAT associated robustly with incident hypertension, opposed to SAT or total adiposity, among 903 participants with diverse ethnicity. Finally, in 2015, Sullivan et al. showed using CT that changes in intra-abdominal fat were related to the development of hypertension among 286 Japanese Americans, suggesting a potential causal relationship.

In a pathophysiological perspective, there is evidence that supports the concept of VAT being more harmful than SAT, also about the development of hypertension. In 2004, Klein et al. showed that liposuction, which leads to substantial reduction in SAT (but not VAT), did not significantly improve obesity-associated metabolic abnormalities, including BP, which can be seen with weight loss. In contrast, omentectomy (which primarily consist of VAT removal) together with adjustable gastric banding, had significant positive and long-term effects on the metabolic profile in obese individuals, compared with adjustable gastric banding alone. However, no treatment stratified BP data were reported. Furthermore, Öhman et al. showed that visceral fat transplantation in mice accelerated atherosclerosis significantly more than sham-operated or subcutaneous fat transplanted mice. Finally, VAT, but not SAT, was found to be associated with incident CVD after adjustment for clinical risk factors and generalized adiposity in the Framingham Heart Study.

With respect to pathophysiological connections between VAT and human hypertension, increased VAT has been associated with increased sympathetic nervous system activity and decreased activity in the NP system. Using gold standard microneurography to determine sympathetic nervous system activity, Alvarez et al. showed in healthy younger individuals that VAT was more closely associated with muscle sympathetic nerve activity ($r=0.65, P<0.05$) than SAT ($r=0.27, P=0.05$). Importantly, the relationship between VAT and muscle sympathetic nerve activity was found to be independent of total fat mass ($r=0.61, P<0.05$). Therefore, Alvarez et al. concluded that abdominal visceral fat is an important adipose tissue depot that links obesity with sympathetic neural activation. Another biological plausible mechanism linking VAT to human hypertension involves decreased activity of the NP system. It is well known that obese individuals have lower circulating NP concentrations, but studies from both the Dallas Heart Study and the Framingham Heart Study showed that increased VAT, determined by MRI or CT, in contrast to SAT, was significantly negatively associated with circulating concentrations of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide after multivariate adjustment. On the basis of these findings, it is reasonable to suggest that the lower amount of circulating B-type natriuretic peptide associated with increased VAT, resulting in diminished vasodilation and natriuresis, could be involved in the pathogenesis of increased VAT-related hypertension in early stages of this disease. Finally, although increased visceral fat has been related to the increased activation of the components in the renin-angiotensin-aldosterone system (RAAS), O’Seaghdha et al. found no association between VAT and circulating measures of RAAS activity in the Framingham Heart Study, and therefore, it was concluded that further studies are required to determine whether adipocyte-derived RAAS components contribute to systemic RAAS activity in humans.

In this study, we did not attempt to adjust for adipocytokines or measures of insulin resistance because previous studies from the Region of Copenhagen, conducted by our research group, have not found strong evidence to support that these substances are predictors of overweight-related incident hypertension or overweight-related incident CVD in Danish cohort studies.

**Strengths and limitations**

The strengths of our study include its population based and prospective nature, a decent number of participants and incident cases, a long follow-up period and finally data on potential confounders for multivariable adjustment. We believe that our study population represents the general population in a modern Western country setting, allowing our results to be more widespread. Finally, it is also a strength that we could show that VAT was associated with incident CVD.

Our primary limitation is the low participation rate and the relative high in study drop-out rate, which could have biased our results. The use of office BP on a single visit, instead of, for example, 24-hour BP measurements, is an important limitation. Inclusion of measures of sympathetic nervous system, RAAS, and NP system activity could have been interesting. Furthermore, it is a limitation that we have no information on menopause status and oral estrogen use because this may affect VAT. Finally, this study was observational, and hence, causality cannot be established, although several biological plausible mechanisms linking increased VAT to hypertension have been presented.

**Perspective**

Although other prospective studies have shown similar associations between VAT and hypertension, these studies used CT or MRI for the quantification of fat distribution, modalities that are limited for widespread use, as previously mentioned. This study is unique in the use of abdominal ultrasonography on a randomly selected population-based cohort. Our findings support the idea to use abdominal ultrasonography in larger epidemiological studies and other populations for that matter to gain mechanistic understanding of abnormal BP regulation, whereas the use of abdominal ultrasound in clinical settings to
pinpoint individuals in great risk of becoming hypertensive in the near future cannot be recommended at this stage.

Sources of Funding
The Health2006 Study was financially supported by The Danish Lung Society, The Danish Board of Health, The Danish Environmental Protection Agency, The Copenhagen County Research Foundation, Aase and Einar Danielsens Foundation, The Velux Foundation, ALK-Abello A/S, Denmark, and The Danish Scientific Research Council.

Disclosures
None.

References


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**What Is New?**

- Increased visceral fat determined by ultrasound imaging is associated with an increased risk of new-onset hypertension, whereas increased abdominal subcutaneous fat is not.

**What Is Relevant?**

- The risk of hypertension associated with abdominal obesity is mediated by increased visceral fat and not by increased abdominal subcutaneous fat.

- Ultrasound imaging is a suitable alternative to magnetic resonance imaging and computer tomographic imaging with respect to the quantification of abdominal fat distribution in prospective studies.
Abdominal Adiposity Distribution Quantified by Ultrasound Imaging and Incident Hypertension in a General Population

Short title: Seven el al. Abdominal Ultrasound and Hypertension

Ekim Seven, Betina H. Thuesen, Allan Linneberg, Jørgen L. Jeppesen

Form Research Centre for Prevention and Health (E.S., B.H.T., A.L.), the Capital Region of Denmark, Copenhagen, Denmark; Department of Internal Medicine (E.S., J.L.J.), Hvidovre Hospital Glostrup, University of Copenhagen, Glostrup, Denmark; Department of Clinical Experimental Research (A.L.), Rigshospitalet, Denmark; Department of Clinical Medicine A.L., J.L.J.), Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

Corresponding author: Ekim Seven, MD, PhD, Department of Internal Medicine, Hvidovre Hospital Glostrup, University of Copenhagen, Nordre Ringvej 57, 2600, Glostrup, Denmark.

Email: ekim_seven@hotmail.com
Supplementary Method Section Online-Only Data Supplement

Study Population

We based the current study on data from the Health2006 study, which was conducted at the Research Centre for Prevention and Health (RCPH) in the Capital Region of Denmark. A detailed description of the Health2006 study design and methods, including characteristics of responders and non-responders, have previously been published.\(^1\) In brief, the purpose of the Health2006 study was to address research questions dealing with lifestyle-related chronic diseases, such as cardiovascular disease (CVD), diabetes, musculoskeletal disorders, asthma, allergy, chronic lung diseases, and mental disorders. The participants in the Health2006 cohort were drawn as a random sample from the background population aged 18–69 years, living in 11 municipalities in the south-western part of the greater Copenhagen area. In February 2006, a sample of 7931 persons with Danish citizenship and born in Denmark was obtained from the Danish Central Personal Register, Ministry of Internal Affairs. Of the 7931 persons in the sample, 161 were not eligible for invitation because of, for example, death or emigration, and hence 7770 persons were invited by mail to participate in the study. A total of 3.471 persons (participation rate of 44.7\%) responded and entered the Health2006 study and participated in the baseline examination between June 2006 and June 2008. Participants were further invited for a follow-up examination 5 years later, where parts of the baseline study examination were repeated.\(^2,3\)

Eligible criteria for our study were a full set of blood pressure measurements, data about use of antihypertensive medication, and baseline ultrasound measurements of SAT and VAT. Exclusion criteria were prevalent CVD at baseline based on register information. Based on these criteria, we included 3.363 persons for the cross-sectional analysis and 2.119 for the prospective analysis. Figure 1 shows a CONSORT diagram presenting number of subjects participating in the baseline and follow-up examinations and the reasons for non-participation.

All participants gave written informed consent before taking part in the study. The Health2006 study was approved by the local ethics committee (KA20060011) and was conducted in accordance with the declaration of Helsinki.

Abdominal Ultrasound Practice

The ultrasound measurements were performed by 5 trained study nurses who strictly followed the abdominal ultrasound protocol described by Stolk et al.\(^4\) This protocol describes in detail tip and tricks, training, certification, and quality control measures. The initial ultrasound training was done in collaboration with researchers from Steno Diabetes Center, Gentofte, Denmark, who had great experience in abdominal ultrasound measurements.\(^5,6\) The abdominal ultrasound measurements were done with the participants lying on their back. Subcutaneous adipose tissue (SAT) was the distance in centimeters with two decimals between the front edge of the abdominal muscles and the skin. Visceral adipose tissue (VAT) was the distance between the posterior edge of the abdominal muscles and the front of the lumbar spine.\(^7,8\) All ultrasound measures were performed after a quiet expiration applying minimal pressure without displacement of the intraabdominal contents as described by Stolk et al.\(^4\) with an Aquila Pie Medical, Esaote Europe, Maastricht, the Netherlands.
The study nurses, who performed the ultrasound measurements, were not necessarily blinded to the hypertension status of the participants, but they were not aware that their VAT and SAT measurements were going to be used in a hypertension study.

Regarding assessment of inter-observer variability with ultrasound measurements in Health2006, 20 participants were all examined by 4 of our sonographers. In this subset of 20 Health2006 participants, the coefficients of variations between the 4 sonographers was 10.3 % regarding VAT measurements and 9.7 % regarding SAT measurements. In the Health2006 study, intra-observer variability was not tested.

In this study, we did not correlate our ultrasound measurements directly with gold-standard magnetic resonance imaging (MRI) or computed tomography (CT), but our ultrasound determined SAT and VAT measurements have been shown to have overall very similar standardized regression coefficients (β) to those determined by MRI in Germany with serum 25-hydroxy vitamin D as outcome variable. Furthermore, in the hands of others, SAT and VAT determined by ultrasound have been validated against CT and MRI, and the results of these validation studies have shown that abdominal ultrasound is a reliable and reproducible method to assess the amount of intra-abdominal adipose tissue and to diagnose intra-abdominal obesity. Therefore, based on the medical literature and our own findings, abdominal ultrasound can be used as a reasonably reliable alternative to CT or MRI to quantify subcutaneous and visceral fat deposits.

Supplementary Results Section Online-Only Data Supplement
Furthermore, although we showed a significant association between VAT and incident hypertension, we also thought it of interest to find out whether VAT would add prognostic ability for incident hypertension over and above other classical and easy measurable risk factors (sex, age, smoking status, baseline BP, family history of hypertension, and BMI) in our study population. Adding VAT to a model including the aforementioned risk factors increased the area under curve (AUC) from 0.7824 to 0.7844 (HL test=0.35), but the improvement in AUC=0.002 was non-significant (95% CI -0.0021 to 0.0061, P=0.34). Thus, adding VAT to the basic model did not significantly improve c-statistics.

References


Table S1. Odds Ratios and 95% Confidence Intervals for Prevalent Hypertension According to Sex-Specific Subcutaneous Adipose Tissue and Visceral Adipose Tissue Quartiles and According to Subcutaneous Adipose Tissue and Visceral Adipose Tissue as Continuous Variables

<table>
<thead>
<tr>
<th>Models</th>
<th>1(^{st})</th>
<th>2(^{nd})</th>
<th>3(^{rd})</th>
<th>4(^{th})</th>
<th>P trend</th>
<th>Hosmer and Lemeshow test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases/controls</td>
<td>228/608</td>
<td>284/550</td>
<td>340/509</td>
<td>392/452</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.14</td>
<td>1.39</td>
<td>2.18</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>(0.91–1.45)</td>
<td>(1.11–1.75)</td>
<td>(1.74–2.74)</td>
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<td>Model 2</td>
<td>1.00</td>
<td>1.15</td>
<td>1.37</td>
<td>2.04</td>
<td>&lt;0.001</td>
<td>0.18</td>
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<tr>
<td></td>
<td>(0.91–1.45)</td>
<td>(1.09–1.72)</td>
<td>(1.62–2.57)</td>
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<td></td>
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<tr>
<td>Model 3</td>
<td>1.00</td>
<td>1.05</td>
<td>1.08</td>
<td>1.39</td>
<td>0.018</td>
<td>0.10</td>
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<td>Cases/controls</td>
<td>191/655</td>
<td>214/625</td>
<td>336/509</td>
<td>503/330</td>
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</tr>
<tr>
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<td>1.03</td>
<td>1.74</td>
<td>3.27</td>
<td>&lt;0.001</td>
<td>0.07</td>
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<td>(0.81–1.32)</td>
<td>(1.38–2.20)</td>
<td>(2.59–4.13)</td>
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<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.02</td>
<td>1.69</td>
<td>3.09</td>
<td>&lt;0.001</td>
<td>0.15</td>
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<td></td>
<td>(0.79–1.30)</td>
<td>(1.33–2.13)</td>
<td>(2.43–3.92)</td>
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<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>1.00</td>
<td>0.98</td>
<td>1.58</td>
<td>2.81</td>
<td>&lt;0.001</td>
<td>0.11</td>
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<tr>
<td></td>
<td>(0.77–1.26)</td>
<td>(1.24–2.02)</td>
<td>(2.18–3.62)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>Per SD increase</td>
<td>95% CI</td>
<td>p-value</td>
<td>Adjusted for</td>
<td></td>
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<td>3*</td>
<td>1.64 (1.49–1.80)</td>
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<tr>
<td>3†</td>
<td>1.38 (1.22–1.55)</td>
<td>&lt;0.001</td>
<td>0.13</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Model 1: Adjusted for age.

Model 2: Model 1 + HbA1C, smoking status, parental history of hypertension.

Model 3: Model 2 + subcutaneous adipose tissue or visceral adipose tissue.

*Also adjusted for sex.

†Adjusted for body mass index instead of subcutaneous adipose tissue or visceral adipose tissue.