The Relationship Between Waist Circumference and Biomarkers for Diabetes and CVD in Healthy Non-Obese Women. The Pensacola Study

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Abstract

Background: Waist circumference (WC) measurement has been shown to perform as well as or better than body mass index (BMI) in identifying women at risk for diabetes and cardiovascular disease (CVD). The goal of this study was to determine the relationship between WC and the classic biomarkers of risk in healthy women.

Methods: Nondiabetic, non-obese, middle-aged women were categorized by WC quartiles. The correlation of WC to fasting plasma glucose (FPG), A1c, lipid profile, C-reactive protein (CRP) and white blood cell count (WBC) was examined.

Results: Waist circumference correlated with triglycerides, CRP, cholesterol/HDL, non-HDL, LDL, and glucose, and inversely with HDL (r = 0.465, 0.414, 0.321, 0.298, 0.267, 0.279, -0.266, respectively; P = 0.000 for all), but not A1c or WBC. There was a trend of increasing glucose, LDL, and CRP with increasing WC. Quartile 4 showed the highest glucose, CRP, LDL, triglycerides, cholesterol/HDL ratio, and non-HDL.

Conclusion: Increasing WC correlates with several biomarkers of risk for diabetes and CVD in healthy women.

Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are intimately linked,1-4 with obesity and inflammation5-8 being common threads. The prevalence of obesity in American women was 61.8% in 2004; the prevalence increases with age and is influenced by ethnicity. Timely intervention is effective in delaying and diminishing the effects of T2DM and CVD, and the American Heart Association and American Diabetes Association use public education strategies, such as online quizzes and brochures, to help individuals assess their risk.9,10 Body mass index (BMI) has been the conventional assessment of obesity and is determined by measuring height and weight, but it is a confusing measurement for the general public as it requires conversion of height and weight to meters and kilograms. Furthermore, the designation of a BMI of 25 as overweight and of 30 as obese12 does not account for individual differences in gender, muscle mass, or distribution of fat, and may not be applicable across all races.13 The National Institutes of Health has determined that abdominal fat is an independent predictor for morbidity, and that waist circumference (WC) is a clinically-acceptable measure of abdominal fat. A WC greater than 35 inches (88 cm) is considered high risk for women.14 Increased WC has recently been shown to perform as well as or better than BMI in identifying women at risk for diabetes15 and CVD.13,16,17 Waist measurement is simple to perform at home and does not add any cost to a physical examination done by a professional. Studies have been conducted that examined either the effect of WC on the risk factors for diabetes, the incidence of diabetes, risk factors of CVD, or the incidence of CVD, and usually focused on obese individuals or diabetic individuals, or both. The true laboratory values of the biomarkers are often blurred, as many times participants are taking lipid-lowering medication.

The goal of this study was to determine the relationship between increasing WC and the conventional laboratory biomarkers for T2DM and CVD in healthy subjects without the confounders of medication use, obesity, diabetes, gender, and age, and to identify a WC cutoff that could be used to identify women who might be at risk. The biomarkers measured were fasting plasma glucose (FPG), A1c, high sensitivity C-reactive protein (CRP), white blood cell count (WBC), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides.

Materials and Methods

Population

Two hundred women between the ages of 40 and 54 were recruited by newspaper, television, and radio advertisements in the Pensacola, Florida area between November 2004 and May 2005. Participants were interviewed to determine their health status, race, age, and menopause status. Three women had a previous hysterectomy and were not aware of their menopause status; these women were grouped with the postmenopausal women. Participants removed heavy clothing, belts, and shoes before measurements were made. Two pounds (0.91 kg) were subtracted to account for clothing weight. Body mass index was calculated as weight in kg/height in m2. The waist measurement was taken above the iliac crest at the natural waistline, and the hip measurement was taken at the largest area of the natural hip line. One inch was subtracted from the waist and hip measurements to account for clothing. Participants had temperature and blood pressure readings taken; readings above 135 systolic...
or 85 diastolic were repeated in the other arm. Hypertension was defined as blood pressure greater than 135 systolic or greater than 85 diastolic, or a history of hypertension whether or not treated with medication. All measurements were verified with the subjects as a double check to determine if they were expected. All of the subjects had normal body temperature readings the day of the blood collection, and all of them stated that they were healthy.

Participants were excluded if they were outside the specified age range, obese (BMI ≥ 30), had a waist circumference of 39.4 inches (100 cm) or more, were a known diabetic, had an FPG greater than or equal to 126 mg/dL (7.0 mmol/L), or if they took medication to lower their cholesterol. One hundred eighty-two women remained. All of the subjects were in good health. This study was approved by the Institutional Review Board at the University of West Florida; all subjects provided informed consent.

**Laboratory Tests and Precision**

Subjects were instructed to fast for 10 to 12 hours, and blood was collected in the morning. Blood for glucose and lipid analysis was collected in serum separator tubes and centrifuged within 60 minutes of collection. Automated chemistry analysis was performed on a Dade RXL (Dade Behring, Deerfield, IL), specifically glucose by Hexokinase G-6-PD method, and triglycerides and total cholesterol by enzymatic methods. High-density lipoprotein and LDL cholesterol assays were direct homogeneous methods using accelerator selective detergent methodology. Hemoglobin A1c was collected in ethylendiaminetetraacetate (EDTA) and was performed by high-performance liquid chromatography (HPLC) on a Tosoh A1c 2.2+ (Tosoh Bioscience, San Francisco, CA). High sensitivity C-reactive protein was performed on a Beckman Coulter HMX (Beckman Coulter, Fullerton, CA). White blood cell count was performed with nephelometry on a BN ProSpec system (Dade Behring, Deerfield, IL). White blood cell count was performed on a Beckman Coulter HMX (Beckman Coulter, Fullerton, CA). All assays were performed at West Florida Hospital, Pensacola, Florida within 1 day of collection by a qualified technical staff member. The between-run imprecision (CV) for glucose, A1c, hs-CRP, total cholesterol, LDL, HDL triglycerides, and WBC were 1.7%, 2.9%, 5.8%, 2.1%, 2.7%, 4.0% 2.9%, and 2.2%, respectively. Non-HDL was calculated as the total cholesterol minus the HDL.

**Statistics**

The Statistical Package for Social Sciences (SPSS version 13.0) was used for statistical analysis of the data. Chi square analysis was performed on all dichotomous variables, and a Pearson coefficient was computed. A 0.05 level of significance was established a priori. All P values reported are 2-sided. Variables included postmenopausal status (yes/no), exercise 30 minutes per day (yes/no), current cigarette smoker (yes/no), hypertension (yes/no), and use of hormone replacement therapy (yes/no). Since most of the participants were Caucasian, race was dichotomized as Caucasian (yes/no). Continuous variables were analyzed by general linear model with Tukey as the post-hoc test.

Tests for linear trend across the quartiles of waist circumference were conducted by correlating the median of each variable to its quartile and are reported as the Pearson correlation with 2-tailed significance.

The risk factor total for diabetes was determined as the sum of these risks: age ≥ 45, BMI ≥ 25, family history of diabetes, history of gestational diabetes or birth of a baby over 9 lbs, hypertension whether treated or not, fasting triglycerides > 250 mg/dL or HDL < 35 mg/dL, race other than Caucasian, fasting glucose ≥ 100 mg/dL, and CRP > 6.1 mg/L. Proportions were compared by chi-square analysis, and a P value ≤ 0.05 was considered significant.

Correlations between waist circumference and biomarkers were computed with Pearson coefficients, and are presented as crude correlations, adjusted for menopause status (model 1), or adjusted for menopause, HRT use, exercise, smoking, and presence of hypertension (model 2).

**Results**

One hundred eighty-two middle aged women qualified for the study, with an average age of 47.6 years. They ranged in weight from 87 to 196 lbs, had a BMI between 16.5 and 29.9, and had a waist-to-hip ratio (WHR) of 53.7% to 88.8%. They were predominantly Caucasian (93.4%), with 2.2% Asian American, and 6.6% African American. Almost half reported exercising at least 30 minutes per day (45%); 40.1% were post menopausal or reported a past hysterectomy. About a quarter of them had hypertension (21.4%), 7.6% were current smokers, and 14.8% used

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**Table 1 Descriptive Statistics of Participants in Quartiles of Waist Circumference**

<table>
<thead>
<tr>
<th>Waist Range</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inches</td>
<td>22.0–26.5</td>
<td>27.0–28.0</td>
<td>28.5–32.0</td>
<td>32.5–36.0</td>
<td></td>
</tr>
<tr>
<td>(Centimeters)</td>
<td>(55.9–67.3)</td>
<td>(68.6–71.0)</td>
<td>(72.4–81.3)</td>
<td>(82.6–91.4)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>47.1 (4.5)</td>
<td>47.5 (4.1)</td>
<td>47.6 (3.8)</td>
<td>48.3 (4.0)</td>
<td>0.584</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>34.7%</td>
<td>39.2%</td>
<td>40.9%</td>
<td>53.6%</td>
<td>0.326</td>
</tr>
<tr>
<td>BMI</td>
<td>20.7 (1.9)</td>
<td>22.2 (1.8)</td>
<td>24.1 (1.9)</td>
<td>27.2 (1.7)</td>
<td>0.000†</td>
</tr>
<tr>
<td>WHR %</td>
<td>70.3 (4.6)</td>
<td>73.4 (4.2)</td>
<td>76.5 (4.6)</td>
<td>81.3 (3.8)</td>
<td>0.000†</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>93.5%</td>
<td>94.1%</td>
<td>95.4%</td>
<td>90.2%</td>
<td>0.799</td>
</tr>
<tr>
<td>% Exercise</td>
<td>54.5%</td>
<td>34.1%</td>
<td>31.7%</td>
<td>4.9%</td>
<td>0.023*</td>
</tr>
<tr>
<td>% Smoker</td>
<td>10.9%</td>
<td>7.8%</td>
<td>6.8%</td>
<td>4.9%</td>
<td>0.763</td>
</tr>
<tr>
<td>% Hypertension</td>
<td>3.1%</td>
<td>21.6%</td>
<td>18.2%</td>
<td>41.4%</td>
<td>0.001†</td>
</tr>
<tr>
<td>% HRT</td>
<td>8.7%</td>
<td>13.7%</td>
<td>22.7%</td>
<td>14.6%</td>
<td>0.309</td>
</tr>
<tr>
<td>n</td>
<td>46</td>
<td>51</td>
<td>44</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

*Shown as average (SD).

* P<0.05.

†P<0.01.
some form of hormone replacement therapy (HRT). None of the subjects were pregnant at the time of the study. Waist circumference ranged from 22 to 36 inches (55.9 to 91.4 cm). Waist circumference correlated strongly to BMI ($r = 0.827$, $P = 0.000$).

Participants were categorized by quartile of WC as shown in Table 1. The WC quartiles were significantly different for BMI, WHR, the percent that exercise regularly, and the presence of hypertension. They were similar with respect to age, menopausal status, race, smoking status, and the use of HRT.

### Risk Factors for Diabetes

Participants were tested for biochemical markers of glycemic status and inflammation, shown in Figure 1. Fasting plasma glucose showed a steady increase across the quartiles ($P$ for trend 0.008). Hemoglobin A1c showed no difference between the groups ($P = 0.122$). High-sensitivity CRP increased across the quartiles ($P$ for trend 0.03), and all other quartiles were significantly different than Q4. White blood cell count was not different between the groups ($P = 0.833$).

Waist circumference correlated with glucose and with CRP (shown in Table 2), and the correlation remained significant after adjustment for menopause alone (model 1) or in combination with the use of HRT, exercise, smoking status, and the presence of hypertension (model 2). Waist circumference did not correlate with hemoglobin A1c or WBC. The WC quartiles showed a statistically-significant difference with respect to the total risk factors for diabetes, shown in Figure 2 ($\chi^2 = 69.146$, $P = 0.000$). At least half of the subjects in quartiles 1, 2, and 3 were at low risk (total of 0 to 1) for diabetes, but only 5% of the subjects in quartile 4 were low risk.

### Risk Factors for Cardiovascular Disease

Triglycerides, total cholesterol, LDL, and HDL were measured directly and each showed a statistically-significant difference between the WC quartiles (Figure 3). Quartile 4 was significantly different from every other quartile for triglycerides level, and almost double the value of quartile 1. The average cholesterol level was below the recommended level of 200 mg/dL only for quartile 1. While quartiles 2 and 3 showed similar total cholesterol values, the composition of that cholesterol was different. Low-density lipoprotein increased with increasing WC quartiles (P for trend 0.034). High-
density lipoprotein cholesterol was significantly lower for quartile 3 compared with quartile 2, which resulted in a 23% difference in the average cholesterol/HDL ratio between quartiles 2 and 3. Quartile 4 had the lowest HDL of all of the quartiles and the highest results for the remaining lipids and calculations. 

The correlation between waist circumference and lipids was strong and did not change significantly when adjusted for menopause or other confounders (Table 2). The strongest correlations were found between WC and triglycerides (r = 0.465, P = 0.000) and between WC and cholesterol/HDL (r = 0.321, P = 0.000).

Discussion

Risk of Diabetes Mellitus

The risk of diabetes increases with age, BMI, hypertension, physical inactivity, dyslipidemia (low HDL or high triglycerides, or both), and inflammation, and is higher in non-Caucasians. In this study, women were similar in age and normal to overweight, and most of them were Caucasian. Women in the highest quartile of waist circumference had the highest BMI, the highest percent with hypertension, and the lowest percent that exercised. They also had the highest triglycerides, lowest HDL, and highest CRP of all of the groups. Five of the women had fasting glucose ≥ 100 mg/dL, the cutoff for prediabetes, and 13 had CRP > 3 mg/L. There were trends of increasing glucose and CRP across the WC quartiles. Other studies have found a correlation of increased WC with insulin resistance7,18,19 and found that women with a WC between 32.3 and 35.6 inches (82 and 90.5 cm) had about 4 times the risk of becoming diabetic as women with a WC less than 29.5 inches (75 cm).15 High CRP levels are associated with an increased risk for diabetes, independent of BMI, fasting insulin, and A1c.5,6

White blood cell count is another marker for inflammation, but it was not significantly different between the quartiles. Hemoglobin A1c is a marker of long term glycemic status, but it was not significantly different between the quartiles. It may be that the small difference in glucose levels in the study group was insufficient to affect a change in A1c. Furthermore, the accuracy of A1c values is influenced in opposite directions by iron deficiency and anemia of blood loss,20,23 conditions found at a high prevalence in child-bearing and perimenopausal women.24,26

Risk of Cardiovascular Disease

The classic risk factors for CVD overlap those of diabetes, and include age, BMI, hypertension, physical inactivity, high cholesterol, diabetes, and inflammation. The prevalence of CVD is higher in non-Caucasians, smokers, and males. Waist circumference cutoffs of 32.7 and 36.6 inches, respectively (83 cm and 93 cm), were shown to be equivalent to a BMI of 24 or 30 with respect to the risk for CVD in white and black women.16,17 The women in WC quartile 4 had waist measurements between 32.5 and 38.0 inches, putting most of them between the 2 cutoffs. Biomarkers of interest are the lipids and their ratios. Quartile 4 had the highest triglycerides, total cholesterol, and LDL, and the lowest HDL of all participants.

Ridker and associates followed a large cohort of healthy, middle-aged to elderly women over a 10-year period. They found that the 3 laboratory parameters with the highest predictive value for future cardiovascular events were non-HDL cholesterol, cholesterol/HDL, and CRP, with the hazard ratio for the women in the highest quintile of each parameter being 3.08 for non-HDL, 1.53 for cholesterol/HDL, and 1.86 for CRP.27 Interestingly, the CRP was independent of the lipids, a finding consistent with other published works.28 In the current study, women in Q4 had the highest median cholesterol/HDL ratio, the highest median non-HDL, and the highest median CRP. Women in Q3 had waist measurements between 28.5 and 32.0 inches (72.4 and 81.3 cm), and this quartile also showed significant differences in LDL, HDL, and cholesterol/HDL, though not to the extent of Q4.

The Value of WC Measurement

This study focused on healthy, non-obese, middle-aged women and found that those with the largest WC were significantly different than the other groups with respect to biomarkers that have customarily been used to predict future diabetes and cardiovascular events. Menopausal status did not alter these results.

Strengths of this study are that it was specific for females and middle age, and that the lipid and glucose results are true and not influenced by lipid- or glucose-lowering medication. A limitation is that the percentage of non-Caucasians was low. Race is a risk for both diabetes and CVD, and these results may be different in a more diverse study group. Data on exercise and health status are self reported. Three women were not aware of their menopausal status due to hysterectomy. They were classified as postmenopausal to account for the effect of menstrual bleeding on A1c. It is unlikely that this small number of participants would affect the results if they were misclassified, since none of the correlation coefficients between WC and the laboratory biomarkers changed significantly after adjustment for menopause. Women in this study volunteered to participate in response to advertising, which presumes that they are actively involved in their health. The study group, therefore, is biased toward educated health-care consumers, which may have some effect on the lipid and glucose values found. As a cross-sectional study, their actual progression to diabetes or CVD cannot be ascertained.

The goal in preventive health care is to identify subjects with increased risk and do something about it. Several studies have shown the success of intervention strategies, including weight loss, increased physical activity, and modifications in fat
consumption\textsuperscript{7,29,30} as well as drug therapy\textsuperscript{3,30} in preventing or delaying T2DM and heart disease. A WC measurement done in a clinical setting along with BMI provides information about abdominal fat and is a measurement the general public is familiar with. It is a measurement that can be done easily at home. Furthermore, women are cognizant of weight gain around the middle, even without actually measuring it. In other studies, a WC greater than 83 cm showed a 1.56 odds ratio for incident CVD independent of BMI in white and black women\textsuperscript{16,17,31} and 4 times the risk for incident T2DM.\textsuperscript{15} In the present study, subjects with a WC greater than 32.5 inches (82.6 cm) showed significant changes in the conventional biomarkers for diabetes and cardiovascular disease, reaffirming WC as an appropriate clinical action threshold.\textsuperscript{14}

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