Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women\(^1\)\(^-\)\(^5\)

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ABSTRACT

Background: Obesity is associated with oxidative stress, and the oxidation of LDL is thought to play a crucial role in the generation of atherosclerotic lesions.

Objective: The objective was to describe the association of waist circumference (WC) and body mass index (BMI; in kg/m\(^2\)) with plasma circulating oxidized LDL (ox-LDL) and C-reactive protein (CRP).

Design: This cross-sectional study included data for circulating ox-LDL and CRP from a subpopulation of 586 men and women enrolled in a population-based survey conducted in 2000 in Girona, Spain. Multivariate analysis was performed to describe the independent association of WC and BMI with ox-LDL and CRP.

Results: Multivariate logistic regression analysis adjusted for lifestyle, educational level, and dietary confounders showed a direct association of WC (quartile distribution) and BMI categories with ox-LDL \((P\) for linear trend \(= 0.002)\) and CRP \((P\) for linear trend \(= 0.004)\). Subjects in the top quartile of WC and with a BMI \(> 29.9\) were at high risk of elevated circulating concentrations of ox-LDL and CRP. Further adjustment for cardiovascular disease risk factors did not substantially modify these associations. The risk of high ox-LDL concentrations in overweight (BMI \(= 25.0–29.9\)) or obese (BMI \(\geq 30\)) subjects with a WC \(< 102\) cm (men) or \(< 88\) cm (women) was not significantly different from that in normal-weight subjects with these WCs. In contrast, overweight or obese subjects with higher WCs (WC \(\geq 102\) cm for men and \(\geq 88\) cm for women) were at significantly higher risk of increased ox-LDL.

Conclusion: High WC was associated with high concentrations of ox-LDL independently of BMI in the study population. Am J Clin Nutr 2006;83:30–5.

KEY WORDS Oxidized LDL, abdominal obesity, cardiovascular disease risk factors, weight, BMI

INTRODUCTION

Obesity, defined as a body mass index (BMI; in kg/m\(^2\)) \(\geq 30\), is a chronic metabolic condition and the most prevalent metabolic disease in Western and westernized countries, with prevalence rates of 20% for males and 25% for females (1). Obesity is discussed as an independent risk factor for atherosclerosis because of its associations with oxidative stress (2) and inflammation (3). In particular, the accumulation of abdominal fat, which can be indirectly measured through waist circumference (WC), is an important coronary artery disease (CAD) risk factor. This is due to its association with a series of metabolic disorders such as diabetes mellitus, hypertension, and dyslipidemia (4).

Oxidation of LDL is a hallmark of atherosclerosis development (5). Patients with manifest CAD have elevated plasma concentration of circulating oxidized LDL (ox-LDL) (6–8), which are associated with the severity of symptoms (6, 9) and degree of CAD (7). Circulating ox-LDL has been shown to be a prognostic marker of CAD in cardiac transplant patients (10).

The few studies that used circulating ox-LDL to investigate oxidative stress induced by obesity have shown decreased circulating ox-LDL concentrations in morbidly obese patients after weight loss following surgery (11). They have also shown decreased concentrations in postmenopausal women after a 12-wk diet-induced weight loss (12). However, the results concerning the correlations between BMI and ox-LDL are controversial (11, 12). Concentrations of circulating ox-LDL and ox-LDL antibodies did not vary significantly between overweight and normal-weight subjects in a Japanese population. In this population, however, significantly higher odds ratio for high serum ox-LDL were observed in both obese females and in obese subjects with hypertension (13).

Higher serum concentrations of CRP have been found in obese patients (BMI \(> 30\)) (14) and have been shown to decrease with weight loss (15). CRP concentrations have been shown to be

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\(^2\) TW and HS contributed equally to the study, and each can be regarded as first authors of this article.

\(^3\) Neither the entire paper nor any part of its content was published previously or is being submitted to another journal.

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directly related with malondialdehyde (MDA)-modified LDL concentrations (16).

It has been shown that obesity increases oxidative stress (17). Adipose tissue is a highly metabolic active organ. Hence, it might be reasonable to assume that abdominal fat is closely involved in the production of oxidative stress. However, data on the association of abdominal fat and oxidative stress are scarce.

The hypothesis of the present study was that abdominal fat, measured indirectly through WC, is independently related to circulating ox-LDL and inflammatory markers. For this purpose, we analyzed plasma concentrations of circulating ox-LDL and CRP in a random subsample from a cross-sectional survey. In addition, we determined the independent association of circulating ox-LDL and CRP with WC and BMI.

SUBJECTS AND METHODS

Subjects

Six thousand free-living Spanish men and women aged 25–74 y were randomly selected from the general population of Girona according to the 1996 census and participated in this study from 1999 to 2000. After census errors were excluded, 4539 eligible subjects were left, of whom 3179 agreed to participate. In vivo ox-LDL was measured in a subpopulation of 308 men and 279 women (total of 587 ox-LDL samples). The study was conducted according to the guidelines of the Helsinki Declaration, and the study protocol was approved by an ethics committee. All participants gave their informed consent.

Laboratory measurements

Venous blood was obtained in the morning after the subjects had fasted for ⩾12 h and was kept frozen at −40 °C until assayed. The concentration of ox-LDL in plasma was measured with a sandwich enzyme-linked immunosorbent assay procedure by using the murine monoclonal antibody mAb-4E6 as capture antibody (bound to microtitration wells) and a peroxidase-conjugated antiapolipoprotein B antibody recognizing ox-LDL bound to the solid phase (Mercodia AB, Uppsala, Sweden). Non-high sensitive quantitative determination of C-reactive protein (CRP) was performed with an immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany) on a Cobas Mira analyzer. The sensitivity of the CRP method used was lower than that of high-sensitivity (hs)-CRP; therefore, the top quartile of the CRP distribution level was used as a cutoff for high CRP values. Serum glucose, total cholesterol (TC), HDL cholesterol, and triacylglycerol concentrations were determined by using enzymatic kits (Roche Diagnostic, Basel, Switzerland) adapted to a Cobas Mira Plus autoanalyzer (Hoffmann-La Roche, Basel, Switzerland). LDL cholesterol was calculated with the Friedewald formula.

Other measurements

A precision scale of easy calibration was used to measure weight while the subjects were in their underwear. The readings were rounded to 200 g. Height was measured in the standing position, and the measurements were rounded to 5 mm. BMI was determined as weight (kg) divided by height squared (m²). WC was measured at the level midway between the lowest rib margin and the iliac crest. Measurements were taken with a tape measure in centimeters and rounded to 0.5 cm while the subject was standing. Abdominal obesity was defined as a WC ⩾ 88 cm in women and ⩾102 cm in men (18). Blood pressure was measured with a standard mercury sphygmomanometer.

Food consumption and nutrient intakes were measured with the use of a validated food-frequency questionnaire (19), which was administered by a trained interviewer. Energy consumption and nutrient intakes were calculated from the food-frequency questionnaire by using MEDISYSTEM 2000 software (Conyecte, Madrid, Spain). Leisure-time physical activity was measured with the Minnesota physical leisure-time questionnaire, which had been previously validated for Spanish men and women (20, 21). Information on smoking and alcohol drinking habits was obtained with the use of a structured open-ended questionnaire. Participants were categorized as never smokers, former smokers, and current smokers. The latter group was asked to report the average daily amount of cigarettes smoked. The participants were asked to report their typical consumption of alcohol beverages during the past week, and the average alcohol intake was calculated. The participants were categorized as non-alcohol consumers, moderate alcohol consumers (≤20 g/d), and elevated alcohol consumers (>20 g/d). The educational status was categorized as basic education, secondary school, and university.

Statistical analyses

General linear modeling procedures (PROC GLM, version 8.0; SAS Institute Inc, Cary, NC) were used to estimate age, lifestyle, and cardiovascular disease risk factors according to weight categories and WC. Weight categories were defined as normal weight (BMI = 18.5–24.9), overweight (BMI = 25.0–29.9), and obesity (BMI ⩾ 30). Quartiles of WC were calculated separately for men and women. The prevalence of abdominal obesity (WC ⩾ 102 cm in men and 88 cm in women) corresponds to 0%, 0%, 11%, and 100% for the first, second, third, and top quartiles, respectively.

The associations of ox-LDL and CRP with the weight categories and quartiles of WC were estimated by logistic regression analysis with the use of the PROCLOGISTIC procedure of SAS (version 8.0; SAS Institute Inc). CRP showed a nonparametric distribution that was not corrected by logarithmic transformation. Hence, linear regression analysis including this variable was not feasible. Furthermore, BMI and WC showed a high colinearity (Pearson’s correlation coefficient: r = 0.72) in the present study population. For these reasons we decided to use logistical regression models instead of linear regression models.

The weight categories (normal weight, overweight, and obesity) and quartiles of WC were introduced as predictor variables for circulating ox-LDL (top tertile of plasma concentration of ox-LDL) and CRP (top quartile of plasma concentrations of CRP). The first model was controlled for age (y, continuous variable), leisure-time physical activity [metabolic equivalents (METs; continuous variable)], smoking and alcohol consumption (none; moderate: ≤20 g/d; and elevated >20 g/d), educational level (basic education, secondary school, university; categorical variable), energy consumption (kcal; continuous variable), vitamin C (mg; continuous variable), vitamin E (mg; continuous variable), and polyunsaturated fatty acids (g; continuous variable). The second model was additionally adjusted for diabetes (binary; categorical variable), high concentrations of LDL cholesterol (binary; categorical variable), and low HDL-cholesterol concentrations (binary; categorical variable).
Serious collinearity problems were observed when both variables (BMI and WC) were included in the model. When we tested the linear regression model for collinearity we found that the variance inflation factor was not so large (2.302; clearly lower than the classic value of 10) and, consequently, tolerance ([1/2.302] = 0.434) was considered acceptable. However, the 2 eigenvalues near zero (0.015 and 0.006) indicate 2 collinearities among predictors. Consequently, the condition indexes were very high (14.17 and 21.98), which suggested at least moderate collinearity. Additionally, the proportion of total variance of the predictors were high (BMI = 0.92; circumference = 0.82) and were also associated with the smallest eigenvalue. These data show that serious collinearity problems exist. Hence, it is reasonable to assume that a statistical analysis that includes both variables would lead to misleading results. Therefore, we created a new variable that we included in both logistic and linear regression models (as a dummy variable). This was carried out to explore in detail whether an independent association of WC with ox-LDL (linear and logistic regression analysis) and CRP (logistic regression analysis only) existed. For this purpose, the subjects were characterized through BMI and WC categories and grouped as follows: 1) BMI = 18.5–24.9 and WC < 102 cm in men and <88 cm in women; 2) BMI = 25.0–29.9 and WC < 102 cm in men and <88 cm in women; 3) BMI = 25.0–29.9 and WC ≥ 102 cm in men and ≥88 cm in women; 4) BMI ≥ 30 and WC < 102 cm in men and <88 cm in women; and 5) BMI ≥ 30 and WC ≥ 102 cm in men and ≥88 cm in women. Tests for the interaction of BMI categories and quartiles of WC with age and sex were performed. No significant interaction was found. Dietary supplement consumption was low in the present study population (all supplements: 5%; vitamin supplements: 1.3%) and, hence, was not included as a covariable in the linear or logistic regression analysis. Differences were considered significant if P < 0.05.

**RESULTS**

General characteristics of the study population according to weight classification and quartiles of WC are presented in Table 1. Age and plasma concentrations of ox-LDL increased across weight categories and quartiles of WC. In contrast, BMI and WC were inversely associated with energy consumption and dietary intake of polyunsaturated fat (Table 1). The prevalence of subjects with diabetes, hypertension, low concentrations of HDL cholesterol, and high concentrations of LDL cholesterol increased across weight categories and quartiles of WC. Subjects with higher BMIs and WCs smoked less and had a lower educational level than did those with a lower WC.

Data on the association of weight categories and quartiles of WC with circulating ox-LDL are shown in Table 2. BMI and WC were directly associated with high concentrations of circulating ox-LDL after control for sex, age, total energy consumption, leisure-time physical activity, smoking and alcohol drinking status, and dietary intakes of vitamin C, vitamin E, β-carotene, and polyunsaturated fatty acids (Table 2, model 1). The strength of these associations was not substantially affected after additional adjustment for other cardiovascular disease risk factors (Table 2, model 2).

The associations of elevated concentrations of CRP with weight categories and quartiles of WC are shown in Table 3. High concentrations of CRP were directly associated with WC and BMI (Table 3, model 1). The association of CRP with BMI, but not with WC, was attenuated after further control for cardiovascular disease risk factors (Table 3, model 2).

Multiple logistic and linear regression analyses showed that a high WC (>102 cm in men and >88 cm in women) in overweight (BMI = 25.0–29.9) and obese (BMI ≥ 30) subjects was directly associated with ox-LDL (Tables 4 and 5) and CRP (Table 4) compared with subjects with normal weight (BMI = 18.5–24.9) and a normal WC (<102 cm in men and <88 cm in women).
and obesity (BMI (categorical: 0–0.60 mg/dL), and obesity (BMI ≥30).

A direct relation was previously found between general obesity and markers of oxidative stress and the susceptibility of lipids to oxidative modification in humans independently of other coronary heart disease risk factors (15, 22–26). Furthermore, Holvoet et al (27) showed that BMI is one of the strongest predictors of circulating ox-LDL concentrations. In addition, studies that have investigated the reduction of total body fat have shown reductions in plasma markers of oxidative stress after weight loss induced by diet (12, 15, 23, 28, 29), surgery (11), and pharmacologic agents (30). Reactive oxygen species generation by isolated leukocytes was also reduced after a 4-wk diet-induced weight loss (23). Furthermore, 2 population-based studies have shown significant correlations of urinary isoprostanes (2) and weight loss induced by diet (12, 15, 23, 28, 29), surgery (11), and other coronary heart disease risk factors (15, 22–26). Furthermore, 2 population-based studies have shown significant correlations of urinary isoprostanes (2) and weight loss induced by diet (12, 15, 23, 28, 29), surgery (11), and other coronary heart disease risk factors (15, 22–26).

**DISCUSSION**

The main finding of the present study was that high plasma concentrations of circulating ox-LDL and CRP were directly associated with WC (quartile distribution) and BMI categories. Most importantly, an increased amount of abdominal fat (defined by WC) was associated, independently of BMI and other confounder variables, with these biomarkers of oxidative stress and inflammation, which are known risk factors of atherosclerosis development.

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The emphasis on abdominal fat has been suggested because of the strong adverse health effects associated with excessive abdominal fat. An important finding of the present study was that

**TABLE 2**

<table>
<thead>
<tr>
<th>Weight category</th>
<th>OR (95% CI)</th>
<th>P for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>1</td>
<td>0.024</td>
</tr>
<tr>
<td>Overweight</td>
<td>1.67 (1.07, 2.62)</td>
<td>1.59 (0.99, 2.55)</td>
</tr>
<tr>
<td>Obesity</td>
<td>2.30 (1.37, 3.85)</td>
<td>1.89 (1.08, 3.28)</td>
</tr>
</tbody>
</table>

1 The top tertile of ox-LDL for men was >74.0 U/L and that for women was >69.4 U/L.
2 Adjusted for sex, age, energy consumption, educational level, leisure-time physical activity, smoking and alcohol drinking status, and dietary intakes of vitamin C, vitamin E, β-carotene, and polyunsaturated fatty acids.
3 Adjusted for the variables in model 1 and diabetes, HDL cholesterol (categorical: 0: >40 mg/dL for men or >50 mg/dL for women), and 0 ≤50 mg/dL for women and 1 = <40 mg/dL for men and <50 mg/dL for women), and LDL cholesterol (categorical: 0: <160 mg/dL and 1: >160 mg/dL).
4 Normal weight (BMI = 18.5–24.9), overweight (BMI = 25.0–29.9), and obesity (BMI ≥30).
5 Men: quartile 1, ≤87.9 cm; quartile 2, 88.0–94.9 cm; quartile 3, 95.0–102.9 cm; quartile 4, ≥103.0 cm. Women: quartile 1, ≤72.9 cm; quartile 2, 73.0–81.9 cm; quartile 3, 82.0–91.9 cm; quartile 4, ≥92.0 cm.

**TABLE 3**

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</tr>
</thead>
<tbody>
<tr>
<td>Normal weight</td>
<td>1</td>
<td>0.024</td>
</tr>
<tr>
<td>Overweight</td>
<td>1.53 (0.81, 2.78)</td>
<td>1.40 (0.74, 2.66)</td>
</tr>
<tr>
<td>Obesity</td>
<td>2.22 (1.07, 4.60)</td>
<td>1.86 (0.88, 3.93)</td>
</tr>
</tbody>
</table>

1 The top quartile of C-reactive protein was >0.60 mg/dL.
2 Adjusted for sex, age, energy consumption, educational level, leisure-time physical activity, smoking and alcohol drinking status, and dietary intakes of vitamin C, vitamin E, β-carotene, and polyunsaturated fatty acids.
3 Adjusted for the variables in model 1 and diabetes, HDL cholesterol (categorical: 0: >40 mg/dL for men or >50 mg/dL for women, and 1 = <40 mg/dL for men and <50 mg/dL for women), and LDL cholesterol (categorical: 0: <160 mg/dL and 1: >160 mg/dL).

**TABLE 4**

<table>
<thead>
<tr>
<th>Classification</th>
<th>ox-LDL</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal weight, normal WC (n = 198)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Overweight, normal WC (n = 187)</td>
<td>1.35 (0.82, 2.25)</td>
<td>1.41 (0.70, 2.83)</td>
</tr>
<tr>
<td>Obesity, normal WC (n = 20)</td>
<td>2.92 (1.41, 5.06)</td>
<td>2.66 (1.02, 6.96)</td>
</tr>
</tbody>
</table>

1 Values adjusted for sex, age, energy consumption, educational level, leisure-time physical activity, smoking status, alcohol consumption (continuous), diabetes, LDL cholesterol (categorical: 0: <160 mg/dL and 1: >160 mg/dL), HDL cholesterol (categorical: 0: >40 mg/dL for men and 50 mg/dL for women and 1 = <40 mg/dL for men and 50 mg/dL for women), and dietary intakes of vitamin C, vitamin E, β-carotene, and polyunsaturated fatty acids.
2 The top tertile for men was >74.0 U/L and for women was >69.4 U/L. Overall P = 0.025.
3 The top quartile was >0.60 mg/dL. Overall P = 0.207.
4 BMI = 18.5–24.9 and WC < 102 cm in men and <88 cm in women.
5 BMI = 25.0–29.9 and WC < 102 cm in men and <88 cm in women.
6 BMI = 25.0–29.9 and WC ≥102 cm in men and ≥88 cm in women.
7 BMI ≥30 and WC < 102 cm in men and <88 cm in women.
8 BMI ≥30 and WC ≥102 cm in men and ≥88 cm in women.
abdominal fat and enhanced lipid peroxidation, analyzed by urinary isoprostanes, in women. In contrast, Myara et al (24) showed no correlation between lag time for LDL oxidation and waist-to-hip ratio in nondiabetic, normotensive women. However, lag time is an in vitro test and, hence, data from lag time determinations should be carefully interpreted when compared with in vivo analysis such as plasma concentrations of circulating ox-LDL. The association of WC with ox-LDL was strong and consistent in the present study. Even after adjustment for lifestyle, cardiovascular disease risk factors, and BMI, we found a 256% higher risk in subjects in the top quartile of WC.

The mechanisms by which abdominal adiposity per se could induce increased oxidative stress are not well defined. One hypothesis that has been suggested is that oxidative stress could be induced by low-grade systemic inflammation (16), mainly characterized by higher concentrations of serum CRP and plasma interleukin 6 (32). Several authors have suggested that a low degree of inflammation in obese persons is caused by a high secretion of proinflammatory cytokines, such as tumor necrosis factor α (33, 34). This in turn induces the production of interleukin 6. Interleukin 6 is a prime regulator of CRP synthesis in the liver (35), which leads to a low-grade inflammatory state that induces the production of free radicals and leads to increased lipid peroxidation (14, 15). In the present study, we showed that elevated CRP concentrations were related to increased abdominal fat, indirectly measured by WC. This finding is in line with the findings of previous studies, ie, higher CRP concentrations with increasing levels of abdominal fat (3, 14, 16). In the mixed model we found a tendency for a high risk of high CRP concentrations in obese subjects with normal WCs. However, this association was not statistically significant, which may have been due in part to the low number of subjects in this group. Hence, these data suggest that the association of CRP with WC was not independent of BMI. We could not show a change in the direction and magnitude of the association between WC and ox-LDL after CRP was controlled for. This result might indicate that ox-LDL concentrations increase independently of CRP in subjects with high WCs. Further studies, however, are needed to examine the relation between several markers of oxidative stress and inflammation.

Another trigger of increased oxidative stress and increased inflammation, other than cytokines, is leptin—a plasma protein secreted by adipocytes. Plasma leptin concentrations are elevated in obese humans and correlate positively with body fat mass in lean and obese subjects (36). It has been shown that leptin might exert an atherogenic effect through the generation of oxidative stress in endothelial cells (37) and the induction of the production of interleukin 6, CRP, and other acute phase reactants. This would contribute to the maintenance of a chronic low-grade inflammation state (38). Porreca et al (12) showed significant correlations between circulating ox-LDL and leptin in postmenopausal women without coronary heart disease risk factors. The authors suggested that leptin had a stronger effect on oxidative modification of LDL than of CRP. This was because the association of ox-LDL with CRP disappeared in a multivariable model, and the change in ox-LDL concentrations, from weight loss, was predicted only by changes in leptin.

Other factors that have been discussed in relation to increased oxidative stress in obesity are insulin resistance (2) and lipoprotein abnormalities (39). Insulin resistance has been shown to be induced by tumor necrosis factor α (40) and has shown direct associations with lipid peroxidation (41–43). Ho et al (44) found only weak associations between insulin resistance and circulating ox-LDL. In conclusion, a high WC is associated with high concentrations of circulating ox-LDL and CRP, independently of BMI. We appreciate the English revision made by Stephanie Lonsdale.

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REFERENCES


TABLE 5

Multiple linear regression analysis of the association of circulating oxidized LDL (ox-LDL) concentrations with waist circumference (WC) and BMI

<table>
<thead>
<tr>
<th></th>
<th>β Coefficient</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>Overweight, normal WC</td>
<td>3.366</td>
<td>−1.531, 8.264</td>
<td>0.178</td>
</tr>
<tr>
<td>Overweight, high WC</td>
<td>9.813</td>
<td>2.319, 17.306</td>
<td>0.010</td>
</tr>
<tr>
<td>Obesity, normal WC</td>
<td>4.163</td>
<td>−6.681, 15.005</td>
<td>0.475</td>
</tr>
<tr>
<td>Obesity, high WC</td>
<td>7.814</td>
<td>1.740, 13.889</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Values adjusted for sex, age, energy consumption, educational level, leisure-time physical activity, smoking, alcohol consumption (continuous), glycemia, LDL cholesterol (continuous), and HDL cholesterol (continuous), and dietary intakes of vitamin C, vitamin E, β-carotene, and polysaturated fatty acids. Reference categories: BMI (18.5–24.9) and WC (<102 cm in men and <88 cm in women.) Overall P for ox-LDL = 0.051.

BMI = 25.0–29.9 and WC < 102 cm in men and < 88 cm in women.

BMI = 25.0–29.9 and WC ≥ 102 cm in men and ≥ 88 cm in women.

BMI ≥ 30 and WC < 102 cm in men and < 88 cm in women.

BMI ≥ 30 and WC ≥ 102 cm in men and ≥ 88 cm in women.
ASSOCIATION OF ABDOMINAL OBESITY WITH OXIDIZED LDL


