Low levels of adiponectin predict worsening of arterial morphology and function

Stefan Stöck a,d,*, Michiel L. Bots d, Peter Angerer b, Clemens von Schacky c, Diederick E. Grobbee d, Christiane E. Angermann a, Jochen Seufert a

a Department of Medicine I/Center of Cardiovascular Medicine, University of Würzburg, Germany
b Institute and Outpatient Clinics for Occupational and Environmental Medicine, Ludwig-Maximilians University Munich, Germany
c Preventive Cardiology, Ludwig-Maximilians University Munich-Innenstadt, Germany
d Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands

Received 16 March 2006; received in revised form 24 October 2006; accepted 29 November 2006

Abstract

Adipocytokines are under investigation as mediators of cardiovascular risk. In 142 non-diabetic postmenopausal women, we investigated whether plasma levels of adiponectin and leptin are associated with changes in carotid intima-media thickness (IMT) and distensibility as assessed by high-resolution ultrasound. Adiponectin but not leptin correlated weakly with baseline measures of IMT and distensibility. After 12 months, carotid IMT showed a significant progression [0.023 mm (95% CI, 0.014–0.031 mm)] whereas stiffness was unaltered. A threshold was identified for the relation of adiponectin with both progression of IMT and stiffness. Age-adjusted adiponectin levels in the lowest quartile versus second to fourth quartile were related to progression of IMT (odds ratio, 2.99; 95% CI, 1.81–5.09) and stiffness (odds ratio, 1.71; 95% CI, 1.19–4.07). Adjustment for possible confounding factors and intermediates weakened this association only to a minor degree. No such associations were observed for leptin. We conclude that low levels of adiponectin are associated with adverse changes in morphology and function of central arteries over time independently of other cardiovascular risk factors in postmenopausal non-diabetic women.

Keywords: Adiponectin; Atherosclerosis; Carotid artery; Intima-media thickness; Distensibility; Risk factors

1. Introduction

Adipose tissue is a key endocrine organ in the clustering of metabolic abnormalities as hypertension, hyperlipidemia, insulin resistance, and obesity, all well-described risk factors/mediators for the development of atherosclerosis and cardiovascular events [1]. Adiponectin is a novel adipocyte-derived collagen-like protein, highly specific to adipose tissue, and abundantly present in plasma [2,3]. In contrast to other adipocytokines as leptin, circulating adiponectin is paradoxically decreased in obesity, type 2 diabetes, and cardiovascular disease [3–6]. An important role for adiponectin in the modulation of inflammation has been suggested from in vitro experiments in which physiological concentrations of adiponectin dose-dependently inhibited expression of endothelial adhesion molecules [7]. Moreover, anti-proliferative effects were observed in mechanically injured arteries of adiponectin knockout mice. In this model, adiponectin deficiency aggravated and adiponectin supplementation attenuated neointimal thickening. Thus, adiponectin may represent an important link between obesity and atherosclerosis and a potential anti-atherosclerotic and anti-inflammatory factor [1,8]. About 50% higher adiponectin levels have been reported in women compared with men [6,9]. In both sexes, adiponectin was inversely related to body mass index (BMI) and BMI-adjusted levels of leptin, triglycerides, and positively related to HDL.
cholesterol [4,6,9–11]. Low levels of adiponectin have been associated with hypertension [12], impaired endothelium-dependent vasodilation [13], increased risk of myocardial infarction in men regardless of the presence of diabetes [14,15], and ischemic cerebrovascular disease [16]. We recently showed, that atherosclerosis progression is substantial in postmenopausal women in the course of 1 year [17], and that this change is associated with increased carotid artery stiffness and blood pressure [18,19]. It is unknown whether adipocytokines are predictive of changes in arterial morphology and function. We therefore examined whether the plasma levels of adiponectin and leptin are associated with changes in carotid IMT and distensibility.

2. Subjects and methods

2.1. Study population

We made use of a dataset collected in the Postmenopausal Hormone Replacement against Atherosclerosis (PHOREA) study, a randomized controlled observer-blind single-center trial on the effect of hormone replacement (HRT) given for 1 year on carotid atherosclerosis progression comparing three groups: standard-progestin (1 mg/day 17β-estradiol continuously plus 0.025 mg gestodene for 12 days every month) versus low-progestin (1 mg 17β-estradiol plus 0.025 mg gestodene for 12 days every third month) versus no HRT. The design, study flow, and main results of this trial have been reported [17]. In short, eligible women were clinically healthy, between 40 and 70 years of age, had >1 mm carotid IMT in at least one of the predefined segments, and had given written informed consent. This study on adipocytokines was restricted to those participants who showed a compliance of >90% as assessed by pill count, provided three sets of blood specimens at baseline, and after 6 and 12 months, of >90% as assessed by pill count, provided three sets of blood specimens at baseline, and after 6 and 12 months, were free of diabetes mellitus, and showed no signs and symptoms of infection at clinical examination at respective visits. The study was approved by the local ethics committee of the faculty of medicine of the University of Munich. It was conducted according to the International Conference for Harmonisation-Guidelines for Good Clinical Practice (ICH-GCP). Body mass index (BMI) was calculated as body weight (kg) divided by squared height (metres).

2.2. Carotid artery assessment

Detailed description of the carotid IMT measurement and its reproducibility is given elsewhere [17]. In brief, IMT was measured from non-diseased intimal and medial far wall layers of the carotid artery on both sides, visualized by high-resolution 7.5 MHz ultrasound (Apogee CX Color, ATL). Three segments were defined: the distal 10 mm of the common carotid artery, the carotid bifurcation from the widening of the artery up to the flow divider, and the proximal 10 mm of the internal carotid artery. Baseline and follow-up scans were performed at the same optimal longitudinal view of the maximum IMT. The mean maximum IMT value for each was calculated by averaging values from left and right sides per subject. Change in CIMT was calculated as follow-up value minus baseline value. As reported earlier [17], the reproducibility [mean difference (S.D.)] of carotid IMT scans in 30 postmenopausal women was 0.004 (0.102) mm for mean maximum thickness corresponding with a 99% confidence interval (CI) of −0.045 to +0.049 mm. Hence, progression of IMT was defined as a difference between follow-up and baseline value larger than 0.049 mm.

Carotid stiffness was assessed with the same transducer at the same visits after a supine rest of at least 20 min, as described elsewhere [18]. In brief, M-mode scans of the left and right common carotid artery 10 mm proximal to the carotid bifurcation were recorded simultaneously with an ECG for 20 s on S-VHS videotape. Blood pressure was measured by a semiautomatic device (Dinamap, Johnson & Johnson Medical, Arlington, TX) at the brachial artery three times during the examination, and the averages were reported. Digitized images of all scans were evaluated in random order by a reader blinded to the temporal order of scans. Distensibility was calculated according to the formula:

\[
DC = \frac{2 \times \Delta D}{\Delta P},
\]

where DC is the distensibility coefficient, \(\Delta D\) the difference between systolic and diastolic diameter, \(D_d\) the diastolic diameter, and \(\Delta P\) is the pulse pressure. The averaged distensibility coefficient of the left and right common carotid artery was calculated. As reported earlier [18], the reproducibility [mean difference (S.D.)] of distensibility scans in 23 postmenopausal women was 1.9 (7.2) × 10⁻³ kPa⁻¹, corresponding with a 99% CI of −1.8 to +5.6 × 10⁻³ kPa⁻¹. Worsening of distensibility was therefore defined as a difference between follow-up and baseline value smaller than −1.8 × 10⁻³ kPa⁻¹.

2.3. Laboratory analysis

Serum samples were taken non-fasting, processed immediately, and stored at −80°C until analysis. All analyses on adiponectin, leptin, and inflammatory and endothelial markers were performed in duplicates by analytical personnel, blinded to treatment allocation. Commercially available high-sensitivity immunoassays were used according to the manufacturer’s protocol, and each subject’s samples were run concurrently. Adipocytokines were measured from plasma. For adiponectin, an ELISA (B-Bridge International Inc.) with intraassay variability (coefficient of variation) <6% and interassay variability <7.5% was used. Leptin was determined by a human specific leptin radioimmunoassay (LINCO Research Inc., St. Charles, MO, USA) with a sensitivity of >0.5 ng/ml, <3.4% intraassay and <3.6% interassay variability. ICAM-1, VCAM-1, and E-selectin (R&D Systems Inc., Minneapolis, MN, USA) and high-sensitivity

Please cite this article in press as: Störk S, et al., Low levels of adiponectin predict worsening of arterial morphology and function, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2006.11.044

ATH-9767; No. of Pages 7
CRP (Immundiagnostik GmbH, Bensheim, Germany) were assayed by enzyme-linked immunosorbent assay kits. All coefficients of variation for these assays were below 8.5%. All other metabolic markers reported herein and FSH were measured as part of the clinical routine in our clinical laboratory.

2.4. Data analysis

Distributions of baseline characteristics were described using mean (S.D.) or median (interquartile range) as appropriate. If used in linear regression analysis, skewed variables were log normalized. Changes over time were compared by t-tests for paired data. Linear and logistic regression was used to examine the association between change in IMT and distensibility (used as dependent variables, respectively) and adipocytokines (independent variable). Age was forced into all models. First, outcome variables on a linear scale and log adipocytokines were entered into linear regression models. First, outcome variables on a linear scale and log adipocytokines were entered into linear regression models and first quartile of adiponectin (first quartile) and IMT progression or worsening of stiffness, adiponectin was entered in further modeling steps as a dichotomized variable (first versus second to fourth quartile). Among potential effect modifiers (see below), collinearity diagnostics were run using the eigenvalue criterion and standardized residual plots were inspected. Third, logistic regression was used to quantify the effect of adipocytokines on progression of atherosclerosis or worsening of stiffness by use of odds ratios (OR) and 95% CI. For these analyses, dichotomized dependent variables were computed applying the progression criteria for CIMA and stiffness as described above. Adjustments were made in four steps. Basic models were adjusted for age, baseline levels of CIMA or distensibility, respectively, and mean arterial pressure for distensibility models only (i.e., model 1). Then, the following possible confounders were considered: years since menopause, smoking, family history of coronary heart disease (CHD) before age 60, hours of leisure time activity per week, intake of antihypertensive medication, intake of lipid lowering medication (i.e., model 2). To assess the effect of possible intermediates, adjustment was made for BMI, CRP, total cholesterol, HDL cholesterol (i.e., model 3). Blood pressure variables, glucose, uric acid, LDL cholesterol and triglycerides were excluded from model 3 due to collinearity. Finally, a model was built which comprised all factors included in models 1–3 (i.e., model 4). In order to avoid the bias potentially introduced by combining carotid distension with brachial pulse pressure we repeated all respective analyses with the (unadjusted) variable pulsatile carotid diameter change as an approximation for distensibility. To ease interpretation and comparison of OR’s for IMT progression and increase in stiffness, regression coefficients for distensibility were recomputed after multiplication with −1 (as in Table 4). All tests were performed two-sided. For data analysis SPSS statistical software (version 12.0.5) was used.

3. Results

The general characteristics of the study population and their correlation with adipocytokines are shown in Table 1. Correlations (ordered by strength of association, high to low) were found between adiponectin and HDL cholesterol, triglycerides, BMI, LDL cholesterol, leptin, family history of CHD, total cholesterol, glucose, CRP, pulse pressure (Table 1). For leptin, correlations were found with BMI, uric acid, CRP, family history of CHD, HDL cholesterol, adiponectin, triglycerides, E-selectin, diastolic blood pressure. All associations were in the expected direction. No association was found between leptin and baseline measures of carotid IMT or distensibility (Table 1). The correlation between log adiponectin and log CRP was weak (r = −0.20; P = 0.017), and was abolished after adjustment for BMI or triglycerides or HDL cholesterol (Pearson’s partial correlation coefficient −0.10 to −0.14, all P > 0.20). As expected, CRP correlated with these factors at the level of ≥0.30 (all P<0.01). No correlation was present between adiponectin and adhesion molecules.

After 12 months of follow-up, mean levels of adiponectin and leptin were unaltered (Table 2) as was distensibility and change in pulsatile carotid diameter change. Progression in stiffness (see Section 2) was observed in 25% of participants. By contrast, carotid IMT showed a significant progression of 0.023 mm (95% CI, 0.014–0.031 mm) after 1 year, and 29% of participants showed progression. No marked differences were found between progressors and non-progressors with respect to variables listed in Table 1 except for plasma adiponectin levels (detailed data not shown). Systolic blood pressure and pulse pressure values were reduced after 6 and 12 months whereas diastolic blood pressure was reduced at 6 months but unchanged after 12 months.

In linear regression analysis, there was a trend for an association between log levels of plasma adiponectin and age-adjusted change in carotid IMT on a linear scale (β = −0.013 ± 0.009, P = 0.053) and also age-adjusted change in distensibility (β = 0.37 ± 0.28, P = 0.069). ANCOVA identified the presence of a threshold for the relation of both progression of IMT (Fig. 1, top left) and stiffness (Fig. 1, bottom left) to age-adjusted adiponectin levels. Levels within the first adiponectin quartile (low to 5.1 μg/ml) were associated with worsened arterial morphology and function compared to second to fourth quartile. Therefore, in final logistic regression models (Tables 3 and 4), a dichotomized adiponectin variable was used with >5.1 μg/ml as cutoff value. In these models the association between low levels of adiponectin (first quartile) and IMT progression or worsen-
The main finding of this study was that low adiponectin levels independently predicted progression of atherosclerosis and worsening of stiffness in large central arteries of postmenopausal women in the course over 1 year. The association for both outcome variables to a minor degree. By contrast, leptin was not predictive of change in IMT or distensibility, resulting in very similar OR’s and P-values (detailed data not shown). No effect of hormone replacement intake became evident in any analyses.

4. Discussion

The main finding of this study was that low adiponectin levels independently predicted progression of atherosclerosis and worsening of stiffness in large central arteries of postmenopausal women in the course over 1 year. The association
between adiponectin and adverse change in arterial morphology and function was unaffected by traditional cardiovascular risk factors and was only partially explained by possible intermediates as metabolic and inflammatory markers. By contrast, no such associations were observed for leptin.

Our results are in agreement with observational studies describing correlations in the same direction and of similar magnitude for adiponectin with BMI and lipid profile [4,9,20], and CRP [5,10,11,14,15,21]. However, the association between adiponectin and CRP was in large parts explained by BMI and/or lipid profile. By contrast, no relationship between adiponectin and other mediators of an inflammatory state as vascular adhesion molecules was found and the correlation with E-selectin was weak (−0.15, P = 0.076). Although adiponectin inhibited the expression of vascular adhesion molecules in endothelial cells in vitro [7] this has not been confirmed in humans. Even in obese Pima Indians, a population prone to obesity and diabetes, adiponectin only weakly correlated with a variety of adhesion molecules [22]. Thus, the adverse effects of low levels of plasma adiponectin might not be substantively mediated by adhesion molecules. The relation of adiponectin with baseline measures of IMT and distensibility was low.

**Fig. 1.** Age-adjusted relation between plasma adiponectin and leptin concentrations in quartiles (expressed as mean ± S.E.) and progression of mean maximum IMT of the carotid artery (upper panel) and change in distensibility (lower panel). Probability values are shown for trend test over quartiles. Limits in adiponectin levels (µg/ml) for different quartiles (Q) were: Q1, 0.5–5.1; Q2, 5.2–8.2; Q3, 8.3–10.7; Q4, 10.8–26.5. Limits in leptin levels (ng/ml) for different quartiles were: Q1, 1.5–8.4; Q2, 8.5–13.9; Q3, 14.0–25.6; Q4, 25.7–53.6.

**Table 3**
Association of low levels of adiponectin with progression of carotid intima-media thickness (dependent variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.21</td>
<td>2.01–5.14</td>
<td>0.008</td>
</tr>
<tr>
<td>2</td>
<td>4.22</td>
<td>1.98–7.00</td>
<td>0.010</td>
</tr>
<tr>
<td>3</td>
<td>2.88</td>
<td>1.65–4.10</td>
<td>0.021</td>
</tr>
<tr>
<td>4</td>
<td>2.95</td>
<td>1.62–4.15</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age and baseline CIMT values; model 2, model 1 with additional adjustment for potential confounders: years since menopause, smoking, family history for coronary heart disease before age 60, hours of leisure time activity per week, intake of antihypertensive medication, intake of lipid lowering medication; model 3, model 1 with additional adjustment for potential intermediates: BMI, hs-CRP, total cholesterol, HDL cholesterol; model 4, full adjustment for all variables from models 1–3.

**Table 4**
Association of low levels of adiponectin with worsening in stiffness (i.e., decrease in distensibility; dependent variable)

<table>
<thead>
<tr>
<th>Model</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.87</td>
<td>1.29–4.02</td>
<td>0.019</td>
</tr>
<tr>
<td>2</td>
<td>1.91</td>
<td>1.18–4.55</td>
<td>0.032</td>
</tr>
<tr>
<td>3</td>
<td>1.50</td>
<td>1.09–4.73</td>
<td>0.041</td>
</tr>
<tr>
<td>4</td>
<td>1.68</td>
<td>1.08–5.10</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Model 1, adjusted for age, baseline distensibility values, mean arterial pressure; model 2, model 1 with additional adjustment for potential confounders: years since menopause, smoking, family history for coronary heart disease before age 60, hours of leisure time activity per week, intake of antihypertensive medication, intake of lipid lowering medication; model 3, model 1 with additional adjustment for potential intermediates: BMI, hs-CRP, total cholesterol, HDL cholesterol; model 4, full adjustment for all variables from models 1–3.

First quartile vs. second to fourth quartile; limits of first quartile were 0.5–5.1 µg/ml; second to fourth quartile, 5.2–26.5 µg/ml.

Please cite this article in press as: Störk S, et al., Low levels of adiponectin predict worsening of arterial morphology and function, Atherosclerosis (2007), doi:10.1016/j.atherosclerosis.2006.11.044
probably owing to the threshold effect as discussed below. The relationship of leptin with various cardiovascular risk factors/mediators was in the same direction and of similar magnitude as reported previously [20,23,24]. In healthy humans, leptin and CRP were independently associated [24], thus linking metabolic and inflammatory cardiovascular disease mechanisms. This relationship was also evident in our data, but no correlation was found between leptin and arterial morphology and function and their changes. Thus, the adipocytokines adiponectin and leptin may exert their effects through different pathophysiological signalling pathways.

To the best of our knowledge, the present study is the first to estimate the independent effect of adipocytokines on IMT progression and worsening of stiffness in central arteries. Of note, traditional cardiovascular risk factors did not weaken the association; rather, the association between adiponectin and IMT progression was enhanced after adjustment for potential confounders. Further, we considered several potential intermediates and found that only a minor part of the observed effect was explained by them. Thus, the adverse vascular effect mediated by low plasma adiponectin levels may not only be a cardiovascular risk marker but also a causal risk factor. Our findings compare favourably with a recent case-control study investigating coronary calcification progression measured by electron-beam CT [25]. In this study, low adiponectin plasma levels were independently associated with calcification progression in both diabetic and non-diabetic subjects. It is difficult to estimate the clinical importance of the magnitude of change associated with low adiponectin levels. However, women who had adiponectin levels in the lowest quartile showed an IMT progression, which was three times higher compared to women with adiponectin in the other quartiles. The presence of such a threshold has not been described so far in healthy individuals but non-linear associations were also found for risk of CHD and type 2 diabetes [5,26]. Moreover, in a recent analysis of the Health Professionals Follow-up Study, subjects with plasma adiponectin within the lowest quintile also had a substantially higher risk of myocardial infarction whereas risk plateaued in quintiles two to four [14]. However, no marked differences were seen between groups of IMT progressors and non-progressors regarding the clinical, lipid profile and inflammation parameters. We interpret this as an indication that other pathophysiological factors, which were not measured in this study favour IMT progression to a larger degree than classical risk factors. A further explanation for the non-linear associations between adiponectin and changes in vascular morphology and function may be that the clinically relevant activation of adiponectin receptors requires adiponectin concentrations above a certain threshold. In this regard, the importance of the well-described sexual dimorphism of adiponectin levels in men and women [6,9] needs to be investigated in further studies.

Thickening and stiffening of the carotid wall has been associated with increased risk of myocardial infarction and stroke across various age groups and in both sexes [27–30]. It has been estimated, that, on average, a healthy person reaches a carotid IMT of 0.78 mm at the age of 76 years [31]. In our study, we observed an annual IMT progression rate of 0.023 (95% CI, 0.014–0.031). This is in good agreement with a pooled analysis, which estimated IMT progression using control groups from published randomized controlled trials [32]. Because stringent criteria as how to define progression of IMT or stiffness are lacking, we chose a conservative approach and defined changes outside the 99% CI of reproducibility measurements as progression. According to these criteria, 25% of the cohort showed progression in stiffness and 29% in carotid IMT, regardless of concomitant treatment. Our carotid evaluation approach followed current recommendations [32]. The change in distensibility in the whole cohort pointed towards a modest (non-significant) decrease in stiffness (Table 2) probably owing to the risk management during the trial, which allowed for the modification of antihypertensive treatment. Consistently, blood pressure variables also showed modest reductions. Thus, we believe to have observed reliably measured and clinically significant changes in arterial morphology and function.

Certain aspects of the study need to be considered when interpreting our findings. We studied postmenopausal non-diabetic women with increased carotid IMT. This pre-selection may affect the generalizability of our results. Therefore, the unadjusted approach was chosen. Calculating stiffness parameters from brachial rather than carotid pulse pressure may have introduced a bias [33,34], which is, however, known to depend on risk factors [35,36]. Thus, we may have overestimated pulse pressure in individuals with more risk factors, and may have correctly estimated or underestimated pulse pressure in those with less risk factors. Given that risk factor status relates to risk of disease progression this reasoning results in an overestimation of the true association of stiffness to disease progression. In our analyses, this relation was very modest, whereas in a biased situation one would expect a positive relationship. Thus, a bias as mentioned above, albeit potentially important, is not likely to have invalidated our findings. Furthermore, it is an unresolved issue whether to adjust in progression studies for baseline levels of the outcome variable. It has been argued that baseline adjustments will lead to an overestimation of results and at least to biased results [37].

In summary, we observed a three-fold increased risk of carotid IMT progression and a 1.7-fold increased risk of arterial stiffness progression associated with low levels of adiponectin over the course of 1 year in postmenopausal women. No such association was evident for leptin. The role of adiponectin as a predictor and potential therapeutic target for vascular disease prevention merits further investigation.

Acknowledgments

This study was supported by a grant from Münchener Universitätsgesellschaft, München, and by the Deutsche
Forschungsgemeinschaft. The authors are indebted to Rosemarie Kieß and Jeanette Roller for expert laboratory diagnostics.

References