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Original article

The plasma leptin/adiponectin ratio predicts first cardiovascular event in men: A prospective nested case–control study

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Objective: The plasma leptin/adiponectin (L/A) ratio has been proposed as a preferential marker of atherosclerosis susceptibility compared to leptin and adiponectin alone. We determined the extent to which the L/A ratio predicts incident cardiovascular disease (CVD) taking account of clinical risk factors, microalbuminuria, the total cholesterol/HDL cholesterol (TC/HDL-C ratio), triglycerides, high sensitive C-reactive protein (hs-CRP) and insulin sensitivity (homeostasis model assessment (HOMAβ)).

Methods: A community-based prospective nested case–control study was carried out in 103 non-diabetic men who developed a first cardiovascular event (cases) and 106 male control subjects (no clinically manifest CVD and no lipid lowering drug use at baseline; median follow-up of 3.0 and 10.5 years, respectively). Plasma leptin, adiponectin, the leptin/adiponectin (L/A) ratio, as well as hs-CRP, HOMAβ and the TC/HDL-C ratio were determined at baseline.

Results: Plasma leptin levels and the L/A ratio were higher in cases vs. controls (p=0.002 for both), but the difference in adiponectin was not significant (p=0.10). Age-adjusted incident CVD was associated with plasma leptin (p=0.045 to p=0.001). The relationships of incident CVD with plasma leptin (p=0.19) and adiponectin (p=0.073) lost statistical significance after additional adjustment for smoking, waist circumference, hypertension, microalbuminuria, the TC/HDL-C ratio, hs-CRP and HOMAβ. In this fully adjusted analysis, the L/A ratio remained predictive of incident CVD (hazard ratio: 1.40 (95% CI 1.05–1.87), p=0.024).

Conclusion: This study suggests that the L/A ratio may be a preferential marker of a first cardiovascular event in men compared to plasma leptin and adiponectin levels alone.

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1. Introduction

Leptin and adiponectin are secreted by adipose tissue, and represent the most abundant adipokines in human plasma [1–5]. Despite intensive study, the relevance of leptin and adiponectin in cardiovascular disease (CVD) prediction is still unsettled. Although high plasma leptin levels predict incident CVD, this relationship is to a considerable extent attenuated by obesity [6]. A meta-analysis revealed that higher plasma adiponectin levels confer only a modest cardioprotective effect [7]. Intriguingly, pro-atherogenic effects of leptin and anti-atherogenic effects of adiponectin could paradoxically disappear in people who are already suffering from advanced atherosclerosis [8,9].
and insulin sensitivity when evaluating the relationship of cardiovascular outcome with the L/A ratio.

In the present study, we determined the extent to which incident CVD is associated with the L/A ratio. To this end a prospective nested case–control study was carried out among participants of the community-based Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort. The setup of this study enabled us to take account of conventional clinical and non-clinical risk factors, microalbuminuria, hs-CRP and insulin sensitivity.

2. Subjects and methods

2.1. Study population and design

The local medical ethics committee approved the study, and all participants gave written informed consent. The present prospective nested case–control study was carried out among participants of the PREVEND cohort. Details of the PREVEND study have been published elsewhere (www.PREVEND.org, [21]). The PREVEND study comprises a prospective study in a predominantly Caucasian population that was started in 1997. To this end all inhabitants of the city of Groningen, the Netherlands, aged 28 to 75 years were sent a questionnaire and a vial to collect a first-morning-void urine sample (prescreening). Of these subjects, 40,856 responded (47.8%) and returned a vial to a central laboratory for urinary albumin and creatinine assessment. After exclusion of patients with insulin-treated diabetes mellitus and pregnant women, all subjects with a urinary albumin concentration of ≥10 mg/L (n=7768) were invited of which 6000 participated. Furthermore, 3394 randomly selected subjects with a urinary albumin concentration of <10 mg/L were invited of which 2592 participated. These 8592 subjects participated in the baseline screening and constitute the actual PREVEND cohort.

Men without a history of myocardial infarction, major ischemia, percutaneous transluminal coronary angioplasty or coronary artery bypass graft at baseline were eligible for the present nested case–control study, as described [21]. Diabetes mellitus, use of lipid lowering drugs and urinary albumin excretion >300 mg/24 h at baseline were additional exclusion criteria. Initial selection of cases was men who experienced an event during follow-up as defined below. The original time frame of observation included the period from enrolment (1997) until 31 December 2003 or 31 December 2002 until which date information regarding specific causes of death follow up information was available [23]. For the present study an extended follow-up, censored at 31 December 2008 was used. Control subjects were randomly selected from the baseline sample applying similar exclusion criteria as for the cases [21]. The current report includes 103 cases and 106 controls in whom in addition to serum lipids, apolipoproteins (apo), insulin and hs-CRP, plasma adiponectin, leptin measurements were available.

Since the matching variable may not be the confounding factor, but may make part of a causal pathway linking exposure to outcome, cases were not individually matched with control subjects with respect to age and other clinical variables, thereby following the methodological approach as proposed by O.S. Miettinen [22]. As a consequence, we controlled for confounding and effect modification using multivariable analyses.

The combined end-point was incident cardiovascular disease (CVD), defined as death from CVD, hospitalization for myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass grafting [21]. Verification of the vital status, causes of death and of cardiovascular end-point was performed as described [21]. Body mass index (BMI) was calculated as the ratio between weight and height squared (in kg/m²). Waist circumference was measured on bare skin between the 10th rib and the iliac crest. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or the use of antihypertensive drugs. Insulin resistance was estimated using homeostasis model assessment (HOMA), and was calculated with the equation: fasting plasma insulin (mU/L) × glucose (mmol/L)/22.5. Microalbuminuria was defined as urinary albumin excretion between 30 and 300 mg/24 h based on two 24 h urine collections.

2.2. Laboratory analyses

Serum and ethylenediaminetetraacetic acid-anticoagulated plasma samples were stored at −80°C until analysis. Plasma glucose was measured shortly after blood collection. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides and glucose were measured as described [21,23]. Non-HDL cholesterol (non-HDL-C) was calculated as the difference between plasma total cholesterol and HDL-C. Apolipoprotein (apo) B and apoA-I were determined by nephelometry using commercially available reagents [23]. Plasma leptin was analyzed using Luminex xMAP technology (Lincos Research Inc, St. Charles, MO, USA, Lincarex panel B (HADK-2-61K-B). Validation experiments have shown that leptin levels measured with this technology are strongly correlated with assay results obtained by enzyme-linked immunosorbent assay (ELISA) obtained from Lincor Inc. ([24]; data provided by the manufacturer). Total adiponectin was measured by enzyme-linked immunosorbent assay (Linco Research Inc., St. Charles, MO, USA, cat no EZHADP-61K). The withins assay coefficients of variation for leptin and adiponectin were 8.9% and 6.4%, respectively. Insulin was measured by microparticle enzyme immunoassay (AxSYM Insulin assay; Abbott Laboratories, Abbott Park II, USA). hs-CRP was measured by nephelometry with a threshold of 0.18 mg/L [23].

2.3. Statistical analyses

SPSS 18 was used for data analysis. Two-sided p-values ≤0.05 were considered significant.

Data are expressed as mean±SD or in median (interquartile range). Differences in variables between cases and controls were compared with unpaired Student’s t-tests. Between group differences in dichotomous variables were compared by χ²-analysis. Because of skewed distribution, logarthimically transformed values of leptin, adiponectin, the L/A ratio, triglycerides, hs-CRP, insulin and HOMA were used in the analysis. Between group differences in leptin, adiponectin and the L/A ratio were also determined after controlling for age. Univariate relationships were assessed by Pearson’s correlation coefficients.

Associations between incident CVD and plasma levels of leptin, adiponectin and the L/A ratio were determined using age-adjusted Cox proportional hazards analyses. The date of a first event was used to calculate duration of follow-up. The proportional hazards assumption was not violated in any of the models. Hazard ratios (HRs) are reported per SD change with 95% confidence interval (95% CI). HRs of adipokines and the L/A ratio were also determined after further adjustment for clinical variables, the TC/HDL-C ratio, triglycerides and microalbuminuria, as well as after additional adjustment for HOMA and hs-CRP. We used Cox proportional hazard analyses, because some controls were lost to follow-up before census date.

The whole PREVEND cohort consists of a random sample of subjects with urinary albumin concentration <10 mg/L and all subjects with urinary albumin concentration ≥10 mg/L [25]. Since enrichment for subjects with higher degrees of albuminuria could induce bias due to oversampling of subjects with urinary albumin concentration ≥10 mg/L, we also estimated risk in a sensitivity analysis. This sensitivity analysis was done among those subjects included in the present nested case–control study who made part of the so-called “random sample” of the PREVEND cohort, i.e. all subjects with urinary albumin concentration <10 mg/L and a subset of subjects with urinary albumin concentration ≥10 mg/L, exactly as described previously [25].
3. Results

Median follow-up was 3.0 (10th to 90th percentile, 0.9–5.8) years in cases and 10.5 (10th to 90th percentile, 9.1–11.1) years in control subjects. Cases were older, more obese, smoked more frequently, had a higher prevalence of hypertension and microalbuminuria, had higher hs-CRP levels and were more insulin resistant compared to control subjects (Table 1). Plasma TC, non-HDL-C, the TC/HDL-C ratio and triglycerides were higher, whereas HDL-C was lower in cases. Likewise, apoB and the apoB/apoA-I ratio was increased, whereas apoA-I was decreased in cases. Plasma leptin levels and the L/A ratio were elevated in cases, although the difference in adiponectin was not significant (Table 1). In age-adjusted analyses, plasma adiponectin (p = 0.013) and the L/A ratio (p = 0.006) were different in cases compared to controls, but the difference in plasma leptin did not reach significance (p = 0.065).

In all subjects combined, leptin and the L/A ratio were correlated positively with waist, HOMA_2, hs-CRP, albuminuria, the TC/HDL-C ratio, the apoB/apoA-I ratio and triglycerides (Table 2). Adiponectin was correlated inversely with waist, HOMA_2, the TC/HDL-C ratio, the apoB/apoA-I ratio and triglycerides. Comparable relationships of the leptin, adiponectin and the L/A ratio with these variables were observed in cases and control subjects separately (Table 2). The L/A ratio was neither significantly correlated with age in all subjects combined (r = 0.108, p = 0.121), nor in cases (r = 0.082, p = 0.423) and in control subjects (r = 0.092, p = 0.346) separately.

As shown in Table 2 (models 1), age-adjusted incident CVD was associated positively with leptin and inversely with adiponectin. Incident CVD was also positively predicted by the L/A ratio with the point estimate of the hazard ratio for CVD being slightly higher than that of plasma leptin alone. After additional adjustment for smoking, waist circumference, hypertension, microalbuminuria, the total cholesterol/HDL cholesterol ratio and triglycerides (models 2), the relationships of incident CVD with leptin, adiponectin and the L/A ratio remained significant (models 2). The relationships of incident CVD with leptin and adiponectin were, however, no longer statistically significant (models 2). The relationships of incident CVD with leptin, adiponectin and the L/A ratio may predict incident CVD. Indeed, in fully adjusted analyses with the apoB/apoA-I ratio instead of the TC/HDL-C ratio (cf. models 3) the relationships of incident CVD with plasma leptin (HR 1.21 (95% CI, 0.91–1.61), p = 0.19) and adiponectin (HR 0.82 (95% CI, 0.60–1.02), p = 0.073) were again not significant, whereas the L/A ratio still predicted CVD (HR 1.38 (95% CI, 1.03–1.85), p = 0.03) (Table 3).

A sensitivity analysis was carried out among subjects making part of the so-called random sample of the PREVEND cohort. This analysis again revealed that CVD risk was associated with the L/A ratio (model 1: HR 1.35 (95% CI 1.08–1.70), p = 0.009; model 2: HR 1.49 (95% CI 1.11–2.01), p = 0.009; model 3: HR 1.37 (95% CI 1.09–1.90), p = 0.062). An analysis of the KORA Augsburg studies that comprised middle-aged men and women who were initially free of clinically manifest coronary heart disease. In that report, no independent effect of the L/A ratio on incident CHD could be demonstrated [16]. Although the precise reasons for the apparent discrepancy remain to be clarified, there are several methodological differences. The MONICA/KORA Augsburg report had a case–cohort design and included higher numbers of men and women, whereas recruitment of subjects was carried out at multiple surveys. In the PREVEND project, in contrast, subject recruitment was carried out within a limited time frame. Furthermore, the current study had a nested case–control design, making that the number of participants in our study was smaller. In addition, we adjusted for hypertension and the presence of microalbuminuria. The MONICA/KORA Augsburg report took account of physical activity and alcohol consumption. On the other hand, both studies used essentially similar clinical cardiovascular end-points, and accounted for lipoprotein-related risk primarily using the TC/HDL-C ratio, which has emerged as a

4. Discussion

This prospective nested case–control study demonstrates that in age-adjusted analyses incident CVD is positively related to plasma leptin and the L/A ratio, and inversely to plasma adiponectin. Thus, in this study among men without clinically manifest atherosclerosis at baseline there was no evidence for a putative reverse epidemiology phenomenon, which has been implicated in paradoxically opposite relationships of these adipokines with CVD in elderly people and in subjects with advanced manifestations of atherosclerotic disease [8,9,26]. Moreover, after additional adjustment for clinical risk factors, as well as for plasma lipids and microalbuminuria, the association of incident CVD with all these adipokine measures remained statistically significant. Of note, in fully adjusted models in which we also accounted for chronic subclinical inflammation and insulin sensitivity, only the relationship of incident CVD with the L/A ratio remained statistically significant. The current study, therefore, suggests that the L/A ratio may be a preferential marker of adipokine-associated first cardiovascular events in predominantly middle-aged men compared to plasma leptin and adiponectin levels alone.

It is likely that leptin and adiponectin contribute to the pathogenesis of (obesity-related) atherosclerosis via several interrelated metabolic pathways, including effects on inflammatory processes and modulation of insulin sensitivity [1–5]. In addition, adiponectin may directly affect the synthesis of apoA-I, the major HDL apolipoprotein, as well the metabolism of triglyceride-rich lipoproteins [27,28]. As expected, the L/A ratio was strongly correlated with the waist circumference, hs-CRP, HOMA_2, the TC/HDL-C ratio and triglycerides [17–20]. We also observed a correlation with higher urinary albumin excretion, which is considered a cardiovascular risk marker as well [29,30]. These robust relationships underscore the necessity to take account of all these factors when evaluating the extent to which leptin, adiponectin and the L/A ratio may predict incident CVD. Indeed, we found no statistically independent effects of plasma leptin and adiponectin levels alone on incident CVD in fully adjusted analyses. The currently documented independent contribution of the L/A ratio to first cardiovascular events even in fully adjusted analysis needs to be compared with the results of the prospective MONICA/KORA Augsburg studies that comprised middle aged men and women who were initially free of clinically manifest coronary heart disease. In that report, no independent effect of the L/A ratio on incident CHD could be demonstrated [16]. Although the precise reasons for the apparent discrepancy remain to be clarified, there are several methodological differences. The MONICA/KORA Augsburg report had a case–cohort design and included higher numbers of men and women, whereas recruitment of subjects was carried out at multiple surveys. In the PREVEND project, in contrast, subject recruitment was carried out within a limited time frame. Furthermore, the current study had a nested case–control design, making that the number of participants in our study was smaller. In addition, we adjusted for hypertension and the presence of microalbuminuria. The MONICA/KORA Augsburg report took account of physical activity and alcohol consumption. On the other hand, both studies used essentially similar clinical cardiovascular end-points, and accounted for lipoprotein-related risk primarily using the TC/HDL-C ratio, which has emerged as a
stronger lipid risk factor than single pro- and anti-atherogenic lipoprotein measures [23,31]. In view of the relationship of the L/A ratio with plasma triglycerides, we controlled for triglycerides as well. Of additional relevance, the apoB/apoA-I ratio provides an at least equally strong lipoprotein risk factor compared to the TC/HDL-C ratio [23,32,33]. Therefore, analyses with this apolipoprotein ratio instead of the TC/HDL-C ratio were also carried out. In the current study, the L/A ratio was an independent CVD risk predictor in apoB/apoA-I ratio-adjusted analysis as well.

Several other methodological aspects of our study require consideration. We included men only in the expectation that too few events would occur in women to allow for meaningful sex-specific analysis, as is required in view of the marked sexual dimorphism of leptin and adiponectin in post-pubertal subjects [24]. Furthermore, case definition was confined to cardiac outcomes in order to reduce heterogeneity among cardiovascular end-points [21]. Thus, our results do not necessarily hold true for women or for other cardiovascular risk domains. Furthermore, it should be appreciated that the PREVEND cohort is enriched with microalbuminuric subjects [25]. A sensitivity analysis among the subset of subjects derived from the so-called random sample of the PREVEND cohort, i.e. that part of the cohort that is randomly selected from the Groningen city population [25], revealed essentially similar point estimates of the L/A ratio-associated hazard in age-adjusted as well as in fully adjusted analyses compared to the hazard ratios obtained in the whole current study group. Important bias attributable to preferential participation of microalbuminuric subjects is, therefore, unlikely. Finally, it should be noted that we assayed plasma total adiponectin, although several isoforms with different biological activity are present in the circulation [2].

In conclusion, the present prospective nested case–control study suggests that the L/A ratio may be a preferential marker of adipokine-associated first cardiovascular events in predominantly middle aged men compared to plasma leptin and adiponectin levels alone.

Learning points

- The plasma leptin/adiponectin (L/A) ratio has been proposed as a preferential marker of adipokine-related atherosclerosis susceptibility.
- This community-based prospective nested-case control study among predominantly middle-aged men who were initially free of clinically manifest cardiovascular disease demonstrates that the L/A ratio remains predictive of first cardiovascular events after controlling of clinical and non-clinical risk markers, including chronic subclinical inflammation and insulin sensitivity.
- With plasma leptin and adiponectin levels alone no such independent relationships with incident cardiovascular disease were observed.
- This study suggests that the L/A ratio may be a preferential marker of adipokine-associated first cardiovascular events in men.

Conflict of interest

The authors of this manuscript do not have any conflicts of interest to declare.

Acknowledgment

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Table 2

Pearson’s correlation coefficients of leptin, adiponectin and the leptin/adiponectin (L/A) ratio with waist circumference, insulin sensitivity (homeostasis model assessment (HOMA), high sensitive C-reactive protein (hs-CRP), urinary albumin excretion (UAE), the total cholesterol/HDL cholesterol (TC/HDL-C) ratio and triglycerides in cases and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 209)</th>
<th>Cases (n = 103)</th>
<th>Controls (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leptin</td>
<td>Adiponectin</td>
<td>L/A ratio</td>
</tr>
<tr>
<td>Waist</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>0.597†</td>
<td>−0.285†</td>
<td>0.586†</td>
</tr>
<tr>
<td>HOMA&lt;sub&gt;r&lt;/sub&gt;</td>
<td>0.627†</td>
<td>−0.211†</td>
<td>0.564†</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.273‡</td>
<td>−0.113‡</td>
<td>0.258‡</td>
</tr>
<tr>
<td>UAE</td>
<td>0.191‡</td>
<td>−0.082‡</td>
<td>0.183‡</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>0.273‡</td>
<td>−0.247‡</td>
<td>0.337‡</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.286‡</td>
<td>−0.263‡</td>
<td>0.355‡</td>
</tr>
<tr>
<td>ApoB/ApoA-I ratio</td>
<td>0.237‡</td>
<td>−0.309‡</td>
<td>0.309‡</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficients are shown. UAE, hs-CRP, HOMA<sub>r</sub>, triglycerides, leptin, adiponectin and L/A values are logarithmically transformed.

* p ≤ 0.05.
† p ≤ 0.01.
‡ p ≤ 0.001.

Table 3

Hazard ratios (HRs with 95% confidence intervals, CI) of leptin, adiponectin and the leptin/adiponectin (L/A) ratio for incident cardiovascular disease by Cox proportional hazard analysis. HRs are given per 1 SD increase.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Leptin</td>
<td>1.25 (1.01–1.55)</td>
<td>0.045</td>
<td>1.32 (1.02–1.71)</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.74 (0.62–0.89)</td>
<td>0.001</td>
<td>0.80 (0.65–1.00)</td>
</tr>
<tr>
<td>L/A ratio</td>
<td>1.40 (1.14–1.71)</td>
<td>0.001</td>
<td>1.50 (1.15–1.97)</td>
</tr>
</tbody>
</table>

Leptin, adiponectin and the L/A ratio values are logarithmically transformed.

Models 1: adjusted for age.
Models 2: adjusted for age, smoking, waist circumference, hypertension, microalbuminuria, the total cholesterol/HDL cholesterol ratio and triglycerides.
Models 3: adjusted for age, smoking, waist circumference, hypertension, microalbuminuria, the total cholesterol/HDL cholesterol ratio, triglycerides, high sensitive C-reactive protein and insulin sensitivity (homeostasis model assessment).
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