Total adiponectin does not predict cardiovascular events in middle-aged men in a prospective, long-term follow-up study


Department of Internal Medicine, Central Hospital, 652 30 Karlstad, Sweden
Faculty of Health Sciences, University Hospital, Örebro, Sweden
The Medical Research Laboratories, Clinical Institute, Aarhus University Hospital, Aarhus, Denmark
Värmland County Research Council, Karlstad, Sweden
Department of Clinical Sciences, Lund University, University Hospital, Malmö, Sweden

Received 29 May 2009; received in revised form 15 October 2009; accepted 16 October 2009
Available online 12 February 2010

Abstract

Aim. – Plasma total adiponectin is a marker of insulin resistance, but its role in predicting cardiovascular events is unclear. We aimed to investigate the role of adiponectin as a predictor of cardiovascular risk in middle-aged men, and to describe the association between adiponectin and glucose metabolism.

Methods. – In this population-based prospective study of middle-aged men (n = 3885), total adiponectin was analyzed. All individuals had undergone an oral glucose tolerance test (OGTTs), and the mean follow-up duration was 27 years. Regression analyses were carried out for indices of glucose metabolism in relation to quintiles (Q1–Q5) of total adiponectin levels. After stratification for smoking or not, the association between total adiponectin and the first incidence of fatal or non-fatal cardiovascular disease (CVD) was analyzed, using Cox’s proportional-hazards regression model.

Results. – In a separate multiple-regression analysis and after adjusting for possible confounders, the relationship between adiponectin levels and markers of glucose metabolism were found to be significant (P < 0.05). However, adiponectin did not independently predict the risk of stroke, coronary events, or a combination of these two outcomes.

Conclusion. – Levels of total plasma adiponectin are not useful for predicting long-term cardiovascular events in middle-aged men, but are strongly associated with glucose metabolism and markers of insulin resistance.

© 2010 Elsevier Masson SAS. All rights reserved.

Keywords: Adiponectin; Cardiovascular events; Coronary events; Glucose metabolism; Stroke; Prospective study; Long-term
plasmatic d’adiponectine n’étaient pas, en revanche, un facteur prédictif indépendant de survenue des accidents vasculaires cérébraux, des événements coronaires ou d’un composite des deux.

Conclusion. – Les concentrations plasmaticques d’adiponectine totale n’ont pas de valeur prédictive à long terme des événements cardiovasculaires chez des hommes d’âge moyen, mais sont étroitement associées au métabolisme de glucose et aux marqueurs d’insulinorésistance.

© 2010 Elsevier Masson SAS. Tous droits réservés.

Mots clés : Adiponectine ; Événements cardiovasculaires ; Événements coronaires ; Metabolisme du glucose ; Accidents vasculaires cérébraux ; Étude prospective

1. Introduction

The adipocyte-derived plasma protein adiponectin has been implicated as a marker of insulin sensitivity and glucose metabolism [1–3]. Furthermore, low serum levels of adiponectin have been reported in obese compared with non-obese subjects, as well as negative correlations between adiponectin levels and body mass index (BMI) [1,4,5]. Prospective epidemiological studies have suggested that elevated adiponectin concentrations are associated with greater insulin sensitivity and a reduced risk of type 2 diabetes, independent of obesity and other potential confounders [6–8]. Thus, the development of interventions that raise adiponectin levels has been proposed as a target for improving insulin sensitivity and possibly for preventing coronary heart disease (CHD) [9]. Adiponectin has also been proposed to protect against cardiovascular disease (CVD) by other mechanisms. It has, for example, been shown that adiponectin is a strong anti-inflammatory agent and is able to downregulate adhesion molecule expression on the surface of endothelial cells [10–12].

However, according to one prospective study and associated meta-analysis, any association between adiponectin levels and future CHD is unlikely to be strong [13]. Also, several other recently published studies on the prospective association between adiponectin and CVD have shown inconsistent results [14–18]. This suggests that it is still of importance to further investigate the role of total adiponectin as a long-term prospective risk marker for CVD in middle-aged subjects classified by glucose tolerance at baseline, as glucose metabolism could be either a confounder or a mediating variable in the putative risk association. This was possible to do with a long-term follow-up analysis of middle-aged men included in the Malmö preventive project (MPP), a large-scale health-screening study carried out between 1974 and 1992 in southern Sweden [19]. These data also enabled us to examine the risk for stroke and CHD events both separately and in combination.

Smoking is a well-established risk factor for CVD, and is also associated with chronic inflammation, lower adiponectin levels [20] and impaired glucose metabolism. This is why it was relevant to not only adjust for smoking, but also to stratify by smoking habits.

The primary objective of the present long-term follow-up study was to investigate the role of adiponectin as a predictor of risk for the first fatal or non-fatal cardiovascular event in a large population-based cohort of middle-aged men, before and after adjusting for conventional risk factors for CVD. The secondary objective was to investigate the cross-sectional associations between adiponectin levels and markers of glucose metabolism based on detailed data from oral glucose tolerance tests (OGTTs).

2. Methods

2.1. Participants

The preventative case-finding programme known as the MPP was started in 1974 in the department of preventive medicine of the University Hospital Malmö in Sweden [19]. The intention was to screen large strata of the adult population in the city of Malmö to identify high-risk individuals for preventative interventions. Subjects were invited to participate in health screening, including physical examination, a questionnaire on lifestyle habits and laboratory tests. Between 1974 and 1992, a total of 22,444 men and 10,902 women attended the screening programme, giving an overall attendance rate of 71% of all those invited. Blood samples were drawn and stored in a biobank at –80 °C. Total adiponectin levels (mg/L) were recently analyzed in a subgroup of 3885 middle-aged men born in specified years and living in Malmö but, otherwise, not selected in any other way. The rationale behind choosing this subset of MPP participants was that all of these subjects had undergone an OGTT. The inclusion period for this subgroup was limited to approximately 3 years to ensure that no drift in mean BMI and adiponectin values was likely to occur over time.

2.2. Measurements

Clinical examination included measurement of height (m), weight (kg) and supine blood pressure (mmHg) after 10 minutes of rest, using a cuff of appropriate size, as described elsewhere [19]. BMI was calculated as kg/m². Blood samples were obtained at the baseline screening investigation, and were immediately separated for plasma.

Methods for measuring total cholesterol and triglycerides (all in mmol/L) have also been previously described [21]. As part of the health-screening programme, an OGTT (75 g of glucose, and multiple determinations of blood glucose levels at 0, 20, 40, 60, 90 and 120 minutes) was performed to obtain an accurate measurement of glucose tolerance, which was then calculated as the area under the curve (AUC) for glucose during OGTT.

Blood glucose was measured using a hexokinase method [19], and plasma insulin levels measured in mIU/L (mIU/L = mIU/L × 7.175/1000) using a non-specific radioimmunoassay [22]. Intra- and interassay coefficients of variation were 5 and 8%, respectively. The homoeostasis model assessment of insulin resistance (HOMA-IR) index was
calculated for each individual (fasting glucose × insulin/22.5) as an indicator of insulin resistance [23].

Plasma adiponectin (mg/L) was determined, as previously described, by an in-house (Aarhus, Denmark) time-resolved immunofluorometric assay (TR-IFMA), based on a method using two monoclonal antibodies and recombinant human adiponectin (R&D Systems, Abingdon, UK) [24]. The adiponectin molecule is known to form a range of polymers, of which the predominant polymers are trimers, hexamers and highly congregated multimers [25]. Western blot assays have demonstrated that both monoclonal antibodies used in the TR-IFMA assay are able to detect several adiponectin polymers in serum, including the three major molecular forms:

- high-molecular-weight (HMW);
- medium-molecular-weight (MMW);
- low-molecular-weight (LMW).

All standards and unknown samples were analyzed in duplicate, with the exception of non-specific binding (NSB), which was analyzed in quadruplicate. The intra-assay coefficient of variation (CV) was less than 5% and the interassay CV was less than 10%.

2.3. Outcome assessment

All 3885 subjects were followed-up until the end of 2004 in national registries for fatal and non-fatal coronary events and stroke (International Classification of Disease [ICD]; 8th and 9th versions 410–414 and 430–436, and ICD 10th versions: I20–I25 and I60–I64). All national-registry data were made available by the Swedish National Board of Health and Welfare.

2.4. Statistical methods

Available and established risk factors for prospective analyses of CVD risk were selected. The data were divided into five quintiles (Q1–Q5), wherein subjects in Q1 had the lowest adiponectin levels and those in Q5 had the highest. Baseline characteristics and tests for the linear trend across quintiles using regression analysis are described for each quintile (Table 1). Linear regression was used for continuous covariates, and logistic regression by the Cochran–Armitage test was used for binary factors.

The association between baseline adiponectin and CVD risk was analyzed by Cox’s proportional-hazards regression. The first fatal or non-fatal cardiovascular event was used and, as smoking may be an important confounder, the data were stratified into smokers and non-smokers. Three different statistical models were used for the analyses. Regressions were performed first for stroke and CHD separately, and then combined as CVD. Regression analyses were performed (in a stepwise manner) starting with adiponectin and age, then adding potential covariates in the following order: BMI; systolic blood pressure (SBP); total fasting serum cholesterol; fasting serum triglycerides (log-transformed); and AUC for glucose (AUC_{glucose}) during OGTT.

For all Cox regressions, the Q5 (highest) quintile was used as the reference group. P values for trends across quintiles were analyzed by Cox regression using quintile means for adiponectin. All variables were normally distributed except for triglycerides, which were therefore log-transformed. Kaplan–Meier plots were drawn for the associations between adiponectin quintiles and the risk of cardiovascular events after fully adjusting for covariates.

The association between adiponectin and glucose metabolism (glucose at 120 minutes during OGTT, AUC_{glucose} and HOMA-IR index) was analyzed separately using multiple-regression analyses. Models were adjusted for BMI, SBP, and total fasting cholesterol and triglyceride levels.

Throughout the analysis, two-sided tests were used with a significance level of 0.05. All analyses were carried out using SPSS (version 15.0.1) statistical software (SPSS Inc., Chicago, IL, USA).

3. Results

The baseline characteristics of the entire study population are presented in Table 1. Mean values of age, weight, BMI, diastolic blood pressure (DBP), fasting serum triglycerides and different markers of glucose metabolism decreased significantly from adiponectin Q1 to Q5. The same pattern was observed for SBP, although the mean value was higher in Q5 than in Q4.

A substantial proportion of the study population were smokers (range: 48–56%).

3.1. Associations between adiponectin and markers of glucose metabolism

To examine the cross-sectional association between adiponectin and glucose metabolism separately, multiple-regression analyses were carried out, using fasting blood glucose, glucose at 120 minutes during OGTT, AUC_{glucose} and HOMA-IR index as dependent variables. After adjusting for BMI, SBP, and total cholesterol and triglycerides, there remained significant relationships between adiponectin and all measures of glucose metabolism (fasting blood glucose, glucose at 120 minutes during OGTT, AUC_{glucose} and HOMA-IR index).

3.2. Risk of cardiovascular events in relation to adiponectin levels

To analyze the association between baseline adiponectin and the first fatal or non-fatal CVD event using Cox’s proportional-hazards regression, the study participants were stratified into smokers and non-smokers, and stepwise adjustments for relevant covariates were also made. In the first regression model for non-smokers, no significant relationship was observed across the quintiles in the prediction of stroke, coronary events or total cardiovascular events. In the three corresponding analyses in smokers, the only significant decrease in risk was observed for stroke in Q4 compared with Q5. The data for stroke and total cardiovascular events are presented in Tables 2 and 3. In general, there appeared to be a U-shaped risk relationship.
Table 1
Baseline characteristics of study subjects according to quintiles of adiponectin levels (Q1–Q5).

<table>
<thead>
<tr>
<th>Quintile</th>
<th>n</th>
<th>Adiponectin (mg/L)</th>
<th>Age (years) Mean ± SD</th>
<th>Weight (kg) Mean ± SD</th>
<th>BMI (kg/m²) Mean ± SD</th>
<th>SBP (mmHg) Mean ± SD</th>
<th>DBP (mmHg) Mean ± SD</th>
<th>Cholesterol (mmol/L) Mean ± SD</th>
<th>Triglyceride (mmol/L) Mean ± SD</th>
<th>Glucose (mmol/L) Mean ± SD</th>
<th>HOMA-IR Mean ± SD</th>
<th>Smoking (%)</th>
<th>Coronary events (n)</th>
<th>Stroke (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>781</td>
<td>0.87–3.86</td>
<td>47.19 ± 2.95</td>
<td>80.53 ± 11.01</td>
<td>25.91 ± 3.14</td>
<td>132.95 ± 16.46</td>
<td>89.54 ± 10.18</td>
<td>5.81 ± 1.10</td>
<td>1.99 ± 1.51</td>
<td>5.57 ± 0.96</td>
<td>1043.96 ± 221.47</td>
<td>3.11 ± 2.98</td>
<td>163</td>
<td>81</td>
</tr>
<tr>
<td>Q2</td>
<td>780</td>
<td>3.87–5.06</td>
<td>47.31 ± 2.68</td>
<td>78.20 ± 11.42</td>
<td>25.18 ± 3.19</td>
<td>131.28 ± 16.02</td>
<td>88.31 ± 10.20</td>
<td>5.81 ± 1.03</td>
<td>1.69 ± 0.81</td>
<td>5.41 ± 0.65</td>
<td>988.69 ± 171.83</td>
<td>6.67 ± 2.27</td>
<td>146</td>
<td>68</td>
</tr>
<tr>
<td>Q3</td>
<td>772</td>
<td>5.07–6.37</td>
<td>47.32 ± 2.90</td>
<td>78.11 ± 11.37</td>
<td>24.99 ± 3.29</td>
<td>130.35 ± 15.22</td>
<td>87.36 ± 9.85</td>
<td>5.89 ± 1.03</td>
<td>1.64 ± 0.92</td>
<td>5.32 ± 0.70</td>
<td>963.15 ± 182.02</td>
<td>6.19 ± 1.65</td>
<td>127</td>
<td>75</td>
</tr>
<tr>
<td>Q4</td>
<td>776</td>
<td>6.38–8.21</td>
<td>47.62 ± 2.31</td>
<td>76.42 ± 11.59</td>
<td>24.60 ± 3.25</td>
<td>128.90 ± 15.60</td>
<td>87.45 ± 9.93</td>
<td>5.75 ± 1.04</td>
<td>1.46 ± 0.92</td>
<td>5.24 ± 0.59</td>
<td>947.14 ± 176.23</td>
<td>5.90 ± 1.64</td>
<td>147</td>
<td>62</td>
</tr>
<tr>
<td>Q5</td>
<td>776</td>
<td>8.22–24.92</td>
<td>47.70 ± 2.07</td>
<td>73.35 ± 11.45</td>
<td>23.54 ± 3.23</td>
<td>129.90 ± 15.57</td>
<td>87.54 ± 10.09</td>
<td>5.79 ± 1.02</td>
<td>1.35 ± 0.63</td>
<td>5.15 ± 0.62</td>
<td>950.09 ± 172.29</td>
<td>5.83 ± 1.78</td>
<td>132</td>
<td>87</td>
</tr>
</tbody>
</table>

P value*: <0.001

Data are expressed as means ± standard deviation (SD) of continuous variables unless otherwise specified.

* Adjusted for body mass index (BMI), systolic blood pressure (SBP), cholesterol and triglycerides; multiple linear regression was used for all variables except smoking (logistic regression), and age was not adjusted for.

within the quintiles, with lower risks in Q1 and Q5 than in Q2–Q4.

The Kaplan–Meier plot for non-smokers (Fig. 1) shows no significant differences in cardiovascular-event-free survival across quintiles of adiponectin. A similar pattern was seen for smokers.

4. Discussion

The most important finding of the present prospective, population-based cohort study with long-term follow-up was that total adiponectin cannot be used as an independent predictor of CVD events in healthy middle-aged men. Surprisingly, the only significant decrease in risk was observed for stroke in smokers in Q4 (with lower adiponectin) compared with Q5. The U-shaped relationship in risk within the quintiles, with lower risk in Q1 and Q5, has previously been observed [26–28], and it has been hypothesized that, in established CVD, a counterregulatory increase in adiponectin occurs, representing the body’s physiological defence mechanism against cardiovascular alterations and the pro-inflammatory state associated with CVD.
Table 2
Hazards ratios (HR) with 95% confidence intervals (CI) for stroke risk in quintiles (Q1–Q5) of adiponectin after stepwise adjustment for covariates in smokers.

<table>
<thead>
<tr>
<th>Adiponectin plus</th>
<th>Q1 (95% CI)</th>
<th>Q2 (95% CI)</th>
<th>Q3 (95% CI)</th>
<th>Q4 (95% CI)</th>
<th>Q5 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P</td>
<td>HR</td>
<td>P</td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.68–1.53</td>
<td>0.86</td>
<td>0.56–1.31</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI</td>
<td>0.86</td>
<td>0.57–1.31</td>
<td>0.77</td>
<td>0.50–1.18</td>
<td>0.75</td>
</tr>
<tr>
<td>SBP</td>
<td>0.88</td>
<td>0.58–1.34</td>
<td>0.77</td>
<td>0.50–1.20</td>
<td>0.77</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.88</td>
<td>0.58–1.34</td>
<td>0.75</td>
<td>0.50–1.20</td>
<td>0.75</td>
</tr>
<tr>
<td>Triglycerides (log-transformed)</td>
<td>0.86</td>
<td>0.56–1.32</td>
<td>0.76</td>
<td>0.49–1.19</td>
<td>0.75</td>
</tr>
<tr>
<td>AUC_{glucose}</td>
<td>0.82</td>
<td>0.53–1.27</td>
<td>0.76</td>
<td>0.49–1.19</td>
<td>0.75</td>
</tr>
</tbody>
</table>

BMI: body mass index; SBP: systolic blood pressure.

Similarly, the present study findings of the relationship between total adiponectin and markers of glucose metabolism are consistent with those of previous studies, but are here based on more detailed OGTT data [6,29,30]. However, the results of studies evaluating the association between adiponectin and cardiovascular events are as yet inconclusive [14,15,17,31], while the number of studies evaluating the association between adiponectin and stroke is limited. In a nested case-control study from Japan, Matsumoto et al. [32] showed that adiponectin levels are not independently associated with stroke.

In a population-based cohort study from the Netherlands, including both men and women with an average age greater than 60 years, Dekker et al. [26] showed that higher adiponectin levels reduced the risk of non-fatal CVD in women and men, but not the risk of all-cause or CVD mortality. In fact, higher adiponectin levels were significant predictors of all-cause and CVD mortality in both genders, and were also associated with

Table 3
Hazards ratios (HR) with 95% confidence intervals (CI) for first cardiovascular event risk in quintiles (Q1–Q5) of adiponectin after stepwise adjustment for covariates in smokers.

<table>
<thead>
<tr>
<th>Adiponectin plus</th>
<th>Q1 (95% CI)</th>
<th>Q2 (95% CI)</th>
<th>Q3 (95% CI)</th>
<th>Q4 (95% CI)</th>
<th>Q5 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>P</td>
<td>HR</td>
<td>P</td>
<td>HR</td>
</tr>
<tr>
<td>Age</td>
<td>1.09</td>
<td>0.86–1.40</td>
<td>1.05</td>
<td>0.82–1.34</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI</td>
<td>0.91</td>
<td>0.71–1.17</td>
<td>0.93</td>
<td>0.72–1.19</td>
<td>0.79</td>
</tr>
<tr>
<td>SBP</td>
<td>0.92</td>
<td>0.72–1.18</td>
<td>0.93</td>
<td>0.73–1.20</td>
<td>0.81</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.91</td>
<td>0.70–1.16</td>
<td>0.93</td>
<td>0.73–1.20</td>
<td>0.77</td>
</tr>
<tr>
<td>Triglycerides (log-transformed)</td>
<td>0.85</td>
<td>0.66–1.10</td>
<td>0.90</td>
<td>0.70–1.16</td>
<td>0.76</td>
</tr>
<tr>
<td>AUC_{glucose}</td>
<td>0.84</td>
<td>0.65–1.09</td>
<td>0.90</td>
<td>0.70–1.16</td>
<td>0.76</td>
</tr>
</tbody>
</table>

BMI: body mass index; SBP: systolic blood pressure.
of stopping smoking, the overall health may have shifted after rates [19]. Also, given the effects of modern medications and also increased the differences in long-term cardiovascular event participants (70%) compared with non-participants (30%), which health of the screened population was somewhat better in the par-
middle-aged.

Another prospective study from Sweden reported that low ing adiponectin levels and markers of glucose metabolism and and independent cross-sectional association between circulat-
ional and metabolic risk factors. There was, however, an inverse stroke in middle-aged men, after fully adjusting for conven-
trations into the risks of complications related to hyperglycaemia itself. This aspect requires further investigation.

In conclusion, we were unable to show any independent role of plasma adiponectin levels in predicting coronary events or stroke in middle-aged men, after fully adjusting for conventional and metabolic risk factors. There was, however, an inverse and independent cross-sectional association between circulating adiponectin levels and markers of glucose metabolism and insulin sensitivity in middle-aged men, independent of age and BMI. This relationship calls for further, more detailed, investigations into the risks of complications related to hyperglycaemia and adiponectin levels.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The present study was supported by the Swedish Heart-Lung Foundation, and the Ernhold Lundström Foundation and Värm-
land County Research Council in Sweden, and also the Danish Medical Research Council and Danish Diabetes Association. The authors also thank Dr Jan Cederholm, Uppsala, for the French translation of the abstract.
References


