Chapter 3

Chapter 3.1
Adiponectin serum concentrations in men with coronary artery disease: the Ludwigshafen risk and cardiovascular health (Luric) study*

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Abstract

Background: Adiponectin, the most abundant adipocytokine of adipose tissue cells, has recently been found to be decreased in coronary artery disease (CAD). Data concerning adiponectin in different stages of CAD are rare, and it was not investigated if adiponectin levels are influenced by the severity of angina pectoris.

Methods: Thus, we measured adiponectin serum levels by means of ELISA in 1626 male probands, including 273 control subjects, 367 subjects with silent CAD, 608 patients with stable, and 378 patients with unstable angina.

Results: As compared to controls (8.56; 5.85 to 12.85 μg/ml) and subjects with silent CAD (8.60; 5.99 to 12.64 μg/ml), adiponectin was significantly decreased in patients with stable (7.22; 5.06 to 10.41 μg/ml; p < 0.001 for both) and unstable angina (6.72; 4.08 to 10.08 μg/ml; p < 0.001 for both). By a logistic regression analysis, low adiponectin levels were identified as a significant independent predictor for stable and unstable angina (p < 0.001 for both). No significant differences of adiponectin were observed, neither between the stable and unstable angina group, nor between any classes of angina according to the Canadian Cardiovascular Society (CCS) Angina Score for stable angina.

Conclusions: These results suggest, that decreased adiponectin levels are indicative for symptomatic CAD, but are not further influenced by the progression of this disease.

Introduction

Adiponectin, a collagen-like adipocytokine exclusively expressed in adipose tissue, was found to be decreased in obesity and in males [1, 2]. Markedly low adiponectin levels were observed in patients with insulin resistance and diabetes [3-7], dyslipidemia [8] or in smokers [9]. There is growing evidence that adiponectin has a protective effect against atherosclerosis, due to anti-inflammatory and anti-atherogenic features [10]. This is supported by the fact that high plasma adiponectin levels are associated with a lower risk for myocardial infarction [11]. Adiponectin serum levels were observed decreased in CAD independently of common cardiovascular risk factors [12]. Patients with unstable angina and myocardial infarction (MI) showed lower adiponectin concentrations [13, 14], as well as diabetic patients with CAD compared to CAD patients without diabetes [15, 16]. These observations are derived from small numbers of probands, and investigations concerning correlations between adiponectin and severity classes of angina are still missing. This fact prompted us to investigate the role of adiponectin in a large study population with a broad spectrum of clinical and laboratory data.

Materials and methods

Study participants

The LURIC study comprised 3,309 caucasian patients hospitalized for elective diagnostic coronary angiography. Inclusion criteria beside the availability of a coronary angiogram were clinical stability (except of acute coronary syndromes [ACS]) and German ancestry. Exclusion criteria were any chronic or acute illness other than ACS, recent surgery, and a history of malignancy within the past five years. A detailed description of the LURIC study design and baseline characteristics were outlined elsewhere [17]. In order to exclude the influence of gender, we included only male subjects (n = 1626) in our present analysis. This cohort consisted of a control (n = 273), a silent CAD (n = 367), a stable (n = 608), and an unstable angina group (n = 378). Coronary artery disease was defined as the presence of a visible luminal narrowing ≥ 50% stenosis in ≥ 1 of 15 coronary segments. The control group consisted of subjects without symptoms of angina that were scheduled for coronary angiography but did not fulfil angiographic criteria for CAD. The silent CAD group comprehended patients with angiographically proven CAD without symptoms of angina. Stable and unstable angina were diagnosed by angiography, and by clinical symptoms [17]. In case of stable angina, subjects were classified according to the Canadian Cardiovascular Society (CCS) Angina Score. Myocardial infarction was excluded by electrocardiography and analysis of cardiac enzymes. We analysed all serum samples available from the LURIC study for before mentioned groups. Referring to the fact that MI patients were enrolled in LURIC some days after the onset of the MI, we did not include them in this analysis because they represent a heterogeneous population (i.e. time from onset of MI, treatment).
**Analytical methods**

Fasting blood samples were obtained by venipuncture in the early morning, before coronary angiography. Samples were stored for 3 to 6 years at –80°C until they were analysed for adiponectin concentrations in one central laboratory. Adiponectin serum levels were determined by ELISA (Biovendor Laboratory Medicine Inc., Brno, Czech Republic) according to manufacturer’s instructions. Both, the intra- and inter-assay coefficient of variation were below 10%. Other parameters were measured with standard laboratory procedures [17].

**Statistics**

Statistical analysis was performed by SPSS version 11.5. Data were expressed as mean ± 1 standard deviation, and for adiponectin as median and interquartile range (25th to 75th percentile) because they were not normally distributed. Continuous variables were examined for skewness and curtosis, and were logarithmically transformed when appropriate, to achieve a normal distribution. Comparisons between groups were calculated by student’s t test for continuous and by chi-square test for categorical variables. Logistic regression analysis including one of the angina groups, and either the control or the silent CAD group was applied to test whether adiponectin serum levels were independent determinants of stable or unstable angina. This was performed with adiponectin, LDL, HDL, BMI, and age as continuous variables, and with gender, diabetes, hypertension, and smoking as categorical variables. Pearson correlation analysis of adiponectin and other continuous variables was applied. A value of \( p < 0.05 \) was considered statistically significant.

**Results**

For clinical and laboratory characteristics of study probands, see Table 1. As compared to the controls, adiponectin was significantly decreased in patients with angiographically proven CAD \( (p < 0.001) \). No significant difference was observed between controls \( (8.56; 5.85 \text{ to } 12.85 \mu g/ml) \), and subjects with silent CAD \( (8.60; 5.99 \text{ to } 12.64 \mu g/ml) \). Compared with each of the two previous groups (controls, silent CAD), adiponectin was significantly decreased in patients with stable \( (7.22; 5.06 \text{ to } 10.41 \mu g/ml; p < 0.001 \text{ for both}) \) and unstable angina \( (6.72; 4.80 \text{ to } 10.08 \mu g/ml; p < 0.001 \text{ for both}) \) (see Fig. 1). By a logistic regression analysis including controls and angina patients, low adiponectin serum concentrations turned out to be independent predictors for stable and unstable angina \( (p < 0.001 \text{ for both}) \). Reduced adiponectin concentrations remained highly significant independent determinants for stable and unstable angina, even by using silent CAD patients instead of controls in this logistic regression analysis \( (p = 0.001 \text{ for both}) \). There was no significant difference, neither between the stable and unstable angina group, nor between any classes of angina according to the Canadian Cardiovascular Society (CCS) Angina Score for stable angina \( \text{class I: } 7.86; 5.36 \text{ to } 10.61; \text{ class II: } 7.16; 4.95 \text{ to } 10.39; \text{ class III: } 7.01; 4.75 \text{ to } 10.11; \text{ class IV: } 6.76; 5.50 \text{ to } 10.36 \mu g/ml) \). Although adiponectin was significantly decreased in the diabetic patients as compared to subjects without diabetes \( (p = 0.03) \), adiponectin did not differ
significantly between patients affected with both CAD and diabetes compared to those affected only with CAD ($p = 0.1$). Concerning comparisons between groups, a separate analysis of all subjects without diabetes revealed similar statistic results as for the whole study group. There was only a less significant reduction of adiponectin in patients with stable ($p = 0.03$) and unstable angina ($p = 0.019$), as compared to the silent CAD group concerning $p$ values. On the other hand, an analysis exclusively with the diabetics revealed also no significant differences between the angina groups or between the control and the silent CAD group. Compared to silent CAD, adiponectin was significantly reduced in subjects with stable ($p = 0.03$) and unstable angina ($p = 0.026$). There was only a nonsignificant trend towards lower adiponectin levels in the stable ($p = 0.13$) and the unstable angina group ($p = 0.12$) as compared to the diabetic silent CAD group. In the whole study population, adiponectin was significantly reduced in smokers ($p = 0.03$), but it was not decreased in patients with hypertension, and did not correlate with systolic or diastolic blood pressure (not shown). Significant positive correlations were found between adiponectin and HDL ($p < 0.001, r = 0.22$), as well as adiponectin and apolipoprotein-A1 ($p < 0.001, r = 0.17$). The correlations were significantly negative with triglycerides ($p < 0.001, r = -0.23$), BMI ($p < 0.001, r = -0.17$), fasting glucose ($p = 0.001, r = -0.08$), and fasting insulin ($p < 0.001, r = -0.15$). CRP did not significantly correlate with adiponectin ($p = 0.52, r = 0.017$).

Fig. 1. Compared to controls (8.56; 5.85 to 12.85 μg/ml) and subjects with silent CAD (8.60; 5.99 to 10.41 μg/ml), adiponectin serum concentrations were significantly reduced in patients with stable (SA) (7.22; 5.06 to 10.41 μg/ml; $p < 0.001$ for both) and unstable angina (UA) (6.72; 4.08 to 10.08 μg/ml; $p < 0.001$ for both)
<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 273)</th>
<th>Silent CAD (n = 367)</th>
<th>Stable angina (n = 608)</th>
<th>Unstable angina (n = 378)</th>
<th>Silent CAD vs. SA (p value)</th>
<th>SA vs. UA (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (μmol/l)</td>
<td>8.56 (5.85–12.85)</td>
<td>8.60 (5.99–12.64)</td>
<td>7.22 (5.06–10.41)</td>
<td>6.72 (4.08–10.08)</td>
<td>0.000</td>
<td>0.217</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.64 ± 12.64</td>
<td>64.29 ± 9.49</td>
<td>62.74 ± 9.35</td>
<td>63.99 ± 9.48</td>
<td>0.020</td>
<td>0.052</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.85 ± 4.16</td>
<td>27.26 ± 3.64</td>
<td>27.72 ± 3.84</td>
<td>27.83 ± 3.84</td>
<td>0.065</td>
<td>0.621</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>136.96 ± 21.27</td>
<td>142.53 ± 25.98</td>
<td>147.55 ± 23.67</td>
<td>142.80 ± 22.35</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.63 ± 12.89</td>
<td>82.20 ± 12.05</td>
<td>83.11 ± 11.97</td>
<td>80.73 ± 11.19</td>
<td>0.232</td>
<td>0.003</td>
</tr>
<tr>
<td>Systemic hypertension (%)</td>
<td>48</td>
<td>53</td>
<td>65</td>
<td>55</td>
<td>0.000</td>
<td>0.003</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.46 ± 1.16</td>
<td>5.18 ± 1.12</td>
<td>5.44 ± 1.12</td>
<td>5.20 ± 1.06</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>Total triglycerides (mmol/l)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01 ± 1.88</td>
<td>1.95 ± 1.21</td>
<td>1.99 ± 1.36</td>
<td>2.13 ± 1.51</td>
<td>0.626</td>
<td>0.030</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.03 ± 0.83</td>
<td>2.86 ± 0.82</td>
<td>3.04 ± 0.90</td>
<td>2.87 ± 0.83</td>
<td>0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97 ± 0.24</td>
<td>0.91 ± 0.24</td>
<td>0.94 ± 0.24</td>
<td>0.91 ± 0.24</td>
<td>0.018</td>
<td>0.032</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.31 ± 1.72</td>
<td>5.74 ± 1.90</td>
<td>5.67 ± 1.77</td>
<td>5.68 ± 1.67</td>
<td>0.717</td>
<td>0.764</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>23</td>
<td>41</td>
<td>30</td>
<td>38</td>
<td>0.003</td>
<td>0.034</td>
</tr>
<tr>
<td>Smoker (former and current, %)</td>
<td>66</td>
<td>76</td>
<td>79</td>
<td>78</td>
<td>0.260</td>
<td>0.619</td>
</tr>
<tr>
<td>Statines (CSE-inhibitors) (%)</td>
<td>14</td>
<td>57</td>
<td>53</td>
<td>62</td>
<td>0.190</td>
<td>0.003</td>
</tr>
<tr>
<td>Oral antidiabetic medication (%)</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>12</td>
<td>0.558</td>
<td>0.219</td>
</tr>
</tbody>
</table>

BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

<sup>a</sup> In case of history of hypertension, treated with antihypertensives for treatment of blood pressure.

<sup>b</sup> Includes patients, treated with antihypertensives for treatment of blood pressure.
Discussion

Adiponectin was recently found to be reduced in patients with coronary artery disease [12], and it has been implicated that adiponectin exerts antiatherosclerotic properties [10]. Adiponectin suppresses foam cell formation by macrophages [18], decreases the expression of endothelial adhesion molecules [19], and inhibits vascular smooth muscle migration [20]. Furthermore, adiponectin modulates inflammatory processes by inhibition of endothelial nuclear transcription factor NF-κB signaling that mediates the effects of several cytokines [21]. Animal studies showed that adiponectin knock out mice developed atherosclerotic lesions [22]. Adiponectin reduced the development of atherosclerosis in apolipoprotein E-deficient mice [23], and it was detected in injured vascular walls [24]. Adiponectin is also involved in glucose and lipid metabolism, by stimulating glucose utilization and fatty-acid oxidation [25]. Towards metabolism, our results confirm recent findings that adiponectin correlates with fasting glucose and fasting insulin levels, and is decreased in patients with diabetes [3-7]. The positive correlations between adiponectin, HDL-cholesterol and serum apolipoprotein-A1, and the negative correlation with triglycerides underline the close interaction between adiponectin and dyslipidemia [8]. Our data are also in line with studies reporting low adiponectin in smokers [9], and in obese subjects [2].

The main target of our work was to investigate the role of adiponectin in different stages of CAD. As reported previously [12, 13], we found adiponectin serum levels significantly decreased in patients with angiographically proven CAD. In accordance with a recent study [13], adiponectin was reduced independently of other common cardiovascular risk factors in patients with unstable angina as compared to controls. Our findings that adiponectin was significantly decreased in the stable angina group as compared to the controls, and the fact that adiponectin levels did not differ between the stable and the unstable angina group, are not in line with Nakamura et al. [13]. The small number of investigated probands in the study of Nakamura et al. (controls: n = 17, stable angina: n = 36, unstable angina: n = 28) may account for this.

To the best of our knowledge, we were the first who investigated adiponectin serum levels in different subclasses of angina pectoris in a large, well defined cohort. Significant different serum levels would identify adiponectin as a versatile biomarker towards diagnosis of critical perpetuation of CAD. However, we did not find significant differences between the subclasses of stable angina or between unstable and stable angina, indicating no association between adiponectin and the severity of angina pectoris. This missing correlation might suggest that adiponectin, although reduced in early stages of CAD, is not very important for further progression of this disease. Definite conclusions towards a substantial pathologic role of adiponection can only be drawn from longitudinal studies. Towards this it was implicated that high plasma adiponectin concentrations are associated with a lower risk of MI in males [11], and that increased adiponectin concentrations are prospectively associated with a lower risk of CAD in type 1 diabetics [26]. However, in a very recently published study among American Indians, adiponectin concentrations were not predictive for CAD [27]. These nonconcordant results may reflect underlying differences in the study populations. Nevertheless, our results suggest that the decrease of adipo-
nectin might occur in a relatively early stage of CAD, as recently shown in obese juveniles [28]. Thus, more longitudinal studies are warranted to analyse the association between adiponectin and the initiation and perpetuation of CAD.

Our results can not confirm observations of significantly reduced adiponectin in patients with diabetes and CAD as compared to CAD patients without diabetes [15, 16]. Thiazolidinediones, which increase adiponectin concentrations [29, 30], were not administered to our study subjects.

Underlying pathophysiological mechanisms of hypoadiponectinemia associated with CAD, may be single nucleotide polymorphisms (SNP) of adiponectin, that in case of the +276G>T polymorphism were recently shown to be associated with early onset of CAD and low adiponectin levels [31]. Further investigations of the LURIC probands concerning this genetic variant will clarify this issue.

Limitations of our work are that we did not include patients with acute MI, and that we measured only the total amount of adiponectin but not the high and low molecular forms, which exert different metabolic bioactivities [32, 33]. The role of these isoforms still remains to be clarified.

Taken together, our data suggest that reduced adiponectin serum concentrations are indicative for symptomatic CAD, but are not further reduced with the progression of this disease.

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