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Adiponectin multimer distribution in patients with familial combined hyperlipidemia

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ABSTRACT

Adiponectin is secreted from adipocytes in different multimers, of which the high molecular weight (HMW) form is supposed to mediate favorable metabolic and anti-atherogenic effects. We determined adiponectin multimers in 29 female and 22 male patients with familial combined hyperlipidemia (FCH) and 51 age-, gender-, and BMI-matched controls in relation to cardiovascular disease (CVD). We observed a clear sexual dimorphism of total adiponectin and its multimers. Female, but not male, FCH patients had significant lower total adiponectin and both HMW and low molecular weight (LMW) adiponectin than controls. The adiponectin sensitivity index (ASI), reflected by HMW/total adiponectin, and the LMW/HMW adiponectin ratio did not differ significantly between FCH females and control females. However, FCH females with CVD exhibited significantly lower ASI (34.2 ± 10.1% vs 46.0 ± 7.1%) and higher LMW/HMW ratio (1.5 ± 0.8 vs 0.7 ± 0.3) compared to FCH females without CVD, reflecting a more atherogenic adiponectin multimer distribution.

Adipose tissue is an active endocrine organ secreting many biologically active substances (adipokines) [1]. Adiponectin is an adipokine which is adipocyte-specific and is abundantly present in the circulation [2]. After post-translational modifications, adiponectin is secreted into the circulation in three different multimers: a low molecular weight (LMW) form, a middle molecular weight (MMW) form and a high molecular weight (HMW) form [3,4]. Previous studies reported that circulating levels of total adiponectin are decreased in disorders associated with obesity, dyslipidemia, insulin resistance and inflammation [5–9]. In the last years, many studies have also described an association of adiponectin deficiency with increased incidence of coronary heart disease [10,11]. This is in agreement with the observation that high levels of adiponectin are associated with a reduced risk of atherosclerotic plaque formation [12]. Furthermore, experimental data suggest that adiponectin is involved in prevention of foam cell formation, down regulation of adhesion molecules, inhibition of endothelial dysfunction, and smooth muscle cell proliferation and migration [13–16]. Therefore, adiponectin is supposed to be protective against cardiovascular diseases (CVD).

Recently, we reported that total plasma adiponectin is decreased in patients with familial combined hyperlipidemia (FCH), even after adjustment for body adiposity and degree of insulin resistance [17]. FCH is the most common heritable, multifactorial lipid disorder with a prevalence of 1–5% in the general population. The disturbed lipid profile seen in patients with FCH is characterized by elevated levels of total cholesterol (TC), triglycerides (TG), and apolipoprotein B (apoB). Other characteristic features are increased levels of low-density lipoprotein cholesterol (LDLc), decreased levels of high-density lipoprotein cholesterol (HDLc) and the presence of small dense LDL (sdLDL) [18–20]. In addition, FCH patients are often obese and insulin resistant [21]. So, FCH patients are exposed to several cardiovascular risk factors which contribute to the 2- to 5-fold increased risk to develop CVD before the age of 60 years [22,23]. The pathophysiology of this lipid disorder is still unknown, but the finding of reduced plasma adiponectin levels in patients with FCH supports the hypothesis that a disturbed adipose tissue metabolism may contribute to FCH [17].

Recently tools became available to specifically determine LMW, MMW, and HMW forms of adiponectin, and researchers started to focus on these different multimers [3,4,24]. In this way, it was revealed that the favorable metabolic effects of adiponectin are attributed to the HMW multimer. Plasma levels of the HMW form show a higher correlation with glucose tolerance than total level of adiponectin and this multimer is selectively suppressed in coronary artery disease (CAD) and elevated during weight loss [25–27]. Furthermore, Pajvani et al. described that the ratio of HMW

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to total adiponectin, also called the adiponectin sensitivity index (ASI), correlates stronger with insulin sensitivity than just total adiponectin in patients with diabetes type 2 [28].

At the moment no data are present about the adiponectin multimer distribution in patients with FCH. Therefore, the first purpose of this study was to investigate whether a reduced plasma level of total adiponectin in FCH is associated with an altered distribution of the adiponectin multimers. Secondly, we evaluated the associations of adiponectin multimers with the presence of CVD in FCH.

Methods

Study population. In this study, 51 patients with FCH and 51 controls were included. The patients with FCH were derived from a cohort consisting of 37 FCH families [29]. The control subjects were obtained from the Nijmegen Biomedical Study (NBS), comprising a random sample of the total population surrounding Nijmegen [30]. Patients and controls were matched for age, gender, and body mass index (BMI).

Diagnosis of FCH was based on plasma levels of total cholesterol, triglycerides, and apoB using the nomogram recently published by Veerkamp et al. [29]. CVD was defined as presence of peripheral artery disease or history of myocardial infarction, angina pectoris, coronary artery bypass or angioplasty, transient ischemic attack or stroke.

After withdrawal of lipid-lowering medication for four weeks and an overnight fast, blood was drawn by venipuncture. BMI was calculated as body weight (in kilograms) divided by the square of height (in meters). The maximum hip circumference and waist circumference (at the umbilical level) were measured in the late exhalation phase while standing. These two measurements were used to calculate waist–hip ratio (WHR).

The study protocol is approved by the ethical committee of the Radboud University Nijmegen Medical Centre and the procedures followed were in accordance with institutional guidelines. All subjects gave written informed consent.

Biochemical analyses. Plasma total cholesterol and total triglycerides were determined by enzymatic, commercially available reagents (Catalog No. 237574 (Boehringer-Mannheim) and Catalog No. 6639 (Sera Pak), respectively). HDLc was determined by the polyethylene glycol 6000 method. In the FCH population VLDL was isolated and cholesterol of this fraction was determined as above. LDLc was calculated by subtraction of VLDLc and HDLc from plasma total cholesterol. In the control population, LDLc was calculated according to the method of Friedewald. Total plasma apoB concentrations were determined by immunonephelometry. Glucose concentrations were measured in duplicate using the oxidation method (Beckman®, Glucose Analyser2, Beckman Instruments Inc., Fullerton, CA 92634, USA).

Intima media thickness (IMT) measurement. Carotid IMT was determined using an AU5 Ultrasound machine (Esaote Biomedica, Genova, Italy) with a 7.5 MHz linear-array transducer. Longitudinal images of the distal-most 10 mm of both the far wall and the near wall of both common carotid arteries were obtained in the optimal projection (anterolateral, lateral, or posterolateral). The sonographer performed the actual measurement of the IMT off-line at the time of the examination, using semi-automatic edge-detection software (M'Ath®Sdt version 2.0, Metris, Argenteuil, France). All measurements were carried out in end-diastole, using the R-wave of a simultaneously recorded ECG as a reference frame. From each frame the mean IMT was calculated over at least 7.5 mm of the above mentioned 10 mm segment (yielding a quality index of at least 75%). The outcome variable was defined as the mean IMT of the near and far wall of both common carotid arteries [31].

Plasma adiponectin multimer assay. Plasma levels of total, LMW, MMW, and HMW adiponectin were determined in duplicate using a commercially available enzyme-linked immunosorbent assay (ELISA) from ALPCO Diagnostics (Catalog No. 47-ADPH-9755, NH, USA). This assay is able to quantify total adiponectin, HMW + MMW and HMW directly. The concentrations of LMW and MMW are obtained by subtracting HMW + MMW from total adiponectin and HMW from HMW + MMW, respectively. The adiponectin sensitivity index (ASI) was calculated as the percentage of HMW from total adiponectin [28]. The LMW/HMW ratio was calculated as the ratio of LMW to HMW adiponectin. Inter-assay and intra-assay coefficients of variance were 2.7% and 1.3% for total adiponectin, 1.5% and 2.1% for HMW adiponectin, and 11.1% and 2.4% for HMW + MMW, respectively.

Statistical analysis. Continuous variables are expressed as means ± SD unless otherwise indicated. Variables showing skewed distribution were logarithmically transformed before the analyses. Student's unpaired *t* test was used to assess statistical significance of differences observed between patients with FCH and controls. Two-tailed *P*-values less than 0.05 were considered significant. All statistical analyses were performed with the SPSS 14.0 software package.

Results

Characteristics

The anthropometric and metabolic characteristics of the patients with FCH and controls are presented in Table 1. Compared to control subjects, patients with FCH showed significantly higher levels of TC, TG, and apoB. HDLc level was significantly decreased in the FCH population.

Mean WHR tended to be somewhat higher in FCH subjects than in controls, but this difference was not significant. However, WHR was higher and HDLc and LDLc lower in males compared to females, both in patients with FCH and in controls. Plasma glucose was not different between patients with FCH and controls. Mean IMT value was 0.86 mm in both groups. The incidence of CVD was higher in patients with FCH than in control subjects (43% vs 18%, $P < 0.05$).

Levels of total adiponectin and the different multimers in FCH

In both FCH and control group, we observed higher levels of adiponectin in females compared to males (Table 2). Because of this sexual dimorphism, we analyzed the data stratified by gender. Mean total adiponectin levels were lower in FCH patients compared to control subjects, but the difference reached statistical significance for females only. This decrease in total adiponectin in FCH was associated with reduced levels of HMW and LMW adiponectin, again reaching statistical significance in females only. The level of MMW adiponectin did not differ between patients with FCH and controls (Table 2).

Significant correlation was observed with WHR for total adiponectin (controls $r = -0.49$, FCH $r = -0.54$), HMW (controls $r = -0.47$, FCH $r = -0.54$), MMW (controls $r = -0.38$, FCH $r = -0.46$), and LMW (controls $r = -0.39$, FCH $r = -0.30$). Total plasma adiponectin, HMW- and MMW adiponectin also significantly correlated with HDLc within FCH patients (total adiponectin $r = -0.30$, HMW $r = 0.28$, MMW $r = 0.35$). Similar correlations of total adiponectin and its multimers with HDLc were found within control subjects. Significant correlation of adiponectin levels with plasma glucose was seen only in the patients with FCH and only for total adiponectin ($r = -0.38$), HMW ($r = -0.36$), and MMW ($r = -0.36$). Total adiponectin and its multimers did not correlate

Table 1
Characteristics of patients with familial combined hyperlipidemia and controls

Characteristics	FCH patients			Controls		
	All	Men	Women	All	Men	Women
N	51	22 (43%)	29 (57%)	51	22 (43%)	29 (57%)
Age (years)	63.9 (8.0)	62.5 (7.7)	65 (8.2)	62.8 (6.6)	61.6 (6.9)	63.7 (6.3)
BMI (kg/m^2)	28.5 (3.4)	27.9 (3.0)	28.9 (3.6)	28.4 (3.3)	27.8 (2.9)	28.9 (3.6)
WHR	0.93 (0.06)	0.98 (0.04)‡	0.89 (0.05)	0.90 (0.08)	0.96 (0.07)‡	0.85 (0.06)
TC (mmol/L)	7.4 (1.2)*	7.2 (1.2)	7.5 (1.1)	6.3 (1.3)	5.9 (0.9)‡	6.6 (1.4)
TG (mmol/L)	3.7 (2.0)*	4.4 (2.5)	3.3 (1.5)	1.7 (0.8)	1.8 (1.0)	1.6 (0.7)
HDLc (mmol/L)	1.1 (0.3)*	1.1 (0.23)‡	1.2 (0.24)	1.4 (0.4)	1.3 (0.3)	1.4 (0.4)
LDLc (mmol/L)	4.4 (1.2)	3.9 (1.1)‡	4.8 (1.2)	4.3 (1.1)	3.9 (0.8)‡	4.5 (1.3)
ApoB (mg/L)	1481 (245)*	1421 (176)	1528 (281)	1127 (268)	1105 (240)	1151 (301)
Glucose (mmol/L)	5.5 (0.9)	5.7 (1.0)	5.3 (0.8)	5.6 (1.1)	5.7 (1.3)	5.6 (0.9)
IMT	0.86 (0.13)	0.90 (0.14)	0.83 (0.11)	0.86 (0.13)	0.87 (0.14)	0.86 (013)
CVD	22 (43%)*	11	11	9 (18%)	5	4

Data are presented as means ± SD; CVD data are presented as number (%). TG, glucose, and IMT data are skewed distributed. **P*-value < 0.05, compared to controls; ‡*P*-value < 0.05, compared to females within the same subgroup. BMI, body mass index; WHR, waist-to-hip ratio; TC, total cholesterol; TG, triglyceride; ApoB, apolipoprotein B; IMT, intima media thickness; CVD, cardiovascular disease.

Table 2
Levels of total plasma adiponectin and its multimers in patients with FCH and controls

	FCH patients			Controls		
	All	Men	Women	All	Men	Women
Total adiponectin (μg/ml)	4.8 (2.3)*	3.3 (1.2)†	5.9 (2.3)‡	6.2 (3.1)	4.1 (1.7)†	7.7 (3.0)
HMW (μg/ml)	2.0 (1.2)*	1.2 (0.6)†	2.6 (1.3)‡	2.8 (1.9)	1.5 (0.9)†	3.7 (2.0)
MMW (μg/ml)	1.1 (0.7)	0.7 (0.3)†	1.4 (0.7)	1.2 (0.7)	0.8 (0.4)†	1.5 (0.8)
LMW (μg/ml)	1.7 (0.8)*	1.4 (0.7)	1.9 (0.9)‡	2.2 (1.0)	1.7 (0.7)†	2.6 (1.0)

Data are presented as means ± SD; Total adiponectin, HMW, MMW, and LMW data are skewed distributed. **P*-value < 0.05, compared to controls; †*P*-value < 0.05, compared to females within the same subgroup; ‡*P*-value < 0.05, compared to female controls. HMW, high molecular weight adiponectin; MMW, middle molecular weight adiponectin; LMW, low molecular weight adiponectin.

with IMT, neither in patients with FCH nor in controls (data not shown).

Besides the gender specificity in total plasma adiponectin level and its multimers, adiponectin multimer distribution was also different between males and females (Fig. 1). Male subjects showed lower ASI and higher LMW/HMW adiponectin ratio compared to females in both patients with FCH and control subjects, reaching statistical significance in control subjects only (ASI: 36.7 ± 11.3% and 42.1 ± 9.8% for FCH males and females, respectively, and 36.4 ± 11.2% and 45.6 ± 11.0% for control males and females, respectively; LMW/HMW adiponectin ratio: 1.3 ± 0.8 and 0.9 ± 0.7 for FCH males and females, respectively, and 1.4 ± 0.7 and 0.9 ± 0.7 for control males and females, respectively). However, no differences were observed in the adiponectin multimer distribution between the patients with FCH and the control subjects (Fig. 1).

Adiponectin multimer distribution and CVD

No difference was observed between mean total plasma adiponectin of patients with FCH with or without CVD stratified by gender (Fig. 2A). We did not compare adiponectin levels of controls

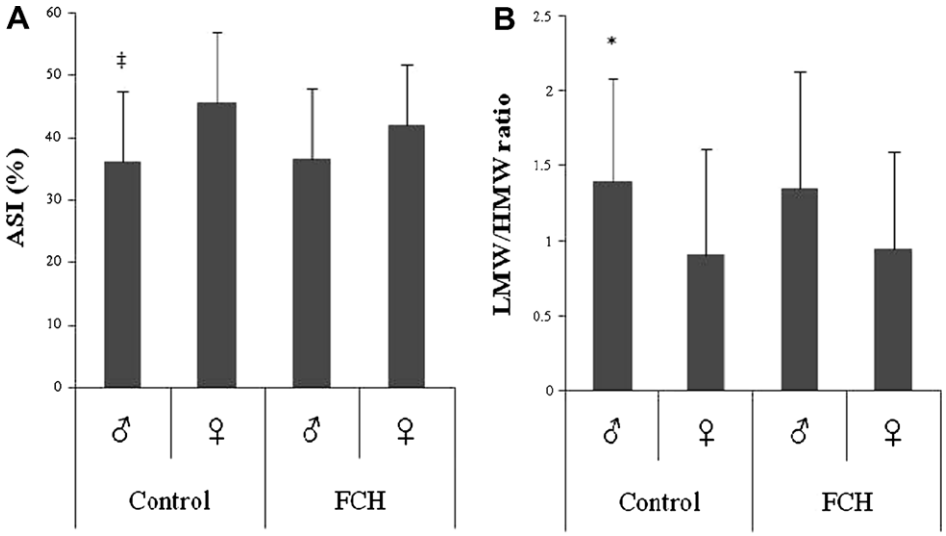

Fig. 1. Adiponectin sensitivity index (ASI), reflected by percentage HMW adiponectin to total adiponectin (A) and ratio of LMW and HMW adiponectin in patients with FCH and controls, stratified by gender (B). **P*-value < 0.01, ‡*P*-value < 0.05, compared to females of the control group.

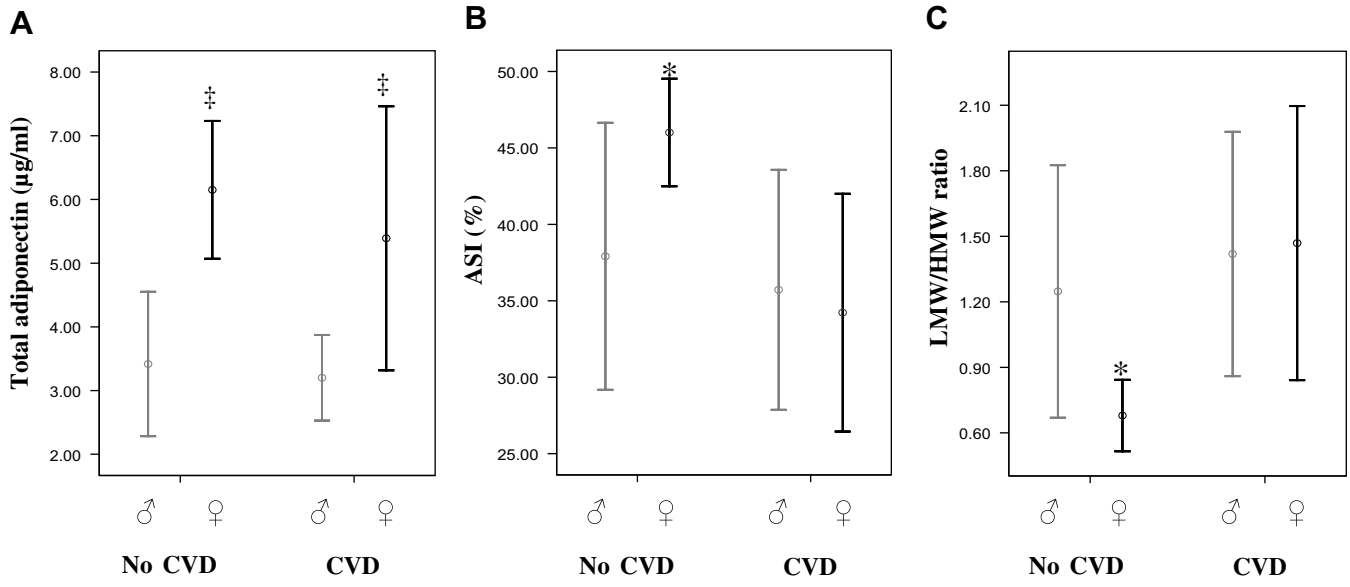

Fig. 2. Plasma levels of total adiponectin (A), adiponectin sensitivity index (ASI), reflected by percentage HMW adiponectin to total adiponectin (B) and ratio of LMW and HMW adiponectin in FCH patients with and without CVD, stratified by gender (C). *P-value < 0.05, compared to FCH females with CVD, ‡P-value < 0.05, compared to FCH males with or without CVD.

with and without CVD, because the number of control subjects with CVD was too low (five males and four females). However, within the FCH population, women with CVD exhibited significantly lower ASI (34.2 ± 10.1% vs 46.0 ± 7.1%) and higher LMW/HMW ratio (1.5 ± 0.8 vs 0.7 ± 0.3) compared to women without CVD (Fig. 2B and C). Both these ratios were not different in male patients with FCH with and without CVD.

Discussion

In this study, we show that patients with FCH have a reduced level of total adiponectin, but their adiponectin multimer distribution does not differ from age-, gender-, and BMI-matched controls. However, FCH females with CVD have a less favorable adiponectin multimer distribution with significant lower ASI and higher LMW/HMW adiponectin ratio than FCH females without CVD. Consequently, the profile of adiponectin multimers in FCH females with CVD reflects a more atherogenic multimer distribution.

Like earlier studies, our study shows a clear sexual dimorphism of adiponectin levels in the control population [6]. Females have higher total plasma adiponectin than males, and this difference is due to elevated levels of all multimers, but HMW multimer in particular [28,32]. As a result of these differences, ASI is higher and LMW/HMW ratio is lower in females compared to males. Gonadal steroids are presumed to be involved in the gender-related difference in adiponectin levels. Testosterone was previously shown to selectively inhibit the secretion of HMW adiponectin [32], and plasma estradiol concentration was shown to negatively correlate with plasma total adiponectin concentration in postmenopausal women [6,33]. This effect of testosterone on HMW adiponectin production was hypothesized to partly explain the higher risk of CVD in males. Lara-Castro et al. demonstrated that the HMW adiponectin multimer exhibits close associations with insulin sensitivity, high concentrations of less atherogenic LDL and more cardioprotective HDL [34]. In the present study, we observed significant correlations of HMW and MMW adiponectin with HDLc and with plasma glucose. These features might contribute to the increased CVD risk in subjects having decreased relative amounts of the HMW adiponectin multimer. Consistent with this, cross-sectional studies have also demonstrated the selective reduction of the HMW adiponectin multimer in type 2 diabetes [25], in CVD [27] and in the metabolic syndrome [35].

Concurrent with the reduction of the relative amount of the HMW multimer of adiponectin we show that in a subset of FCH females, the protective effect of HMW adiponectin against CVD diminishes and CVD risk may rise. Previously, in cross-sectional studies HMW adiponectin has consistently been shown to be a better marker than total adiponectin in the prediction of insulin resistance and the metabolic syndrome [34], endothelial dysfunction [36], and type 2 diabetes [37]. In addition, plasma HMW adiponectin was reported to serve as a marker for severity of CAD [38]. With respect to prediction of future cardiovascular events, reports, however, are rather contradictory. Inoue et al. showed that plasma HMW adiponectin levels may also predict future CVD events in patients with CAD [38]. In contrast, others [39,40], who measured only total adiponectin, reported that high adiponectin level was associated with increased total mortality in patients with chronic heart failure and with recurrence of cardiovascular events in patients who had a recent clinical manifestation of vascular disease. Poor physical condition may have confounded the outcome of the latter two studies. Patients with chronic heart failure experience weight loss (wasting) due to increased resting energy expenditure and low-grade chronic inflammation precedes recurrence of vascular events. The FCH patients included in the present study were in good physical condition, suggesting that physical condition may not have confounded the analyses.

It has often been shown that visceral adiposity is an independent negative predictor of adiponectin. In addition, Lara-Castro et al. showed that this close association of plasma total adiponectin with reduced abdominal fat, is attributed primarily to the HMW adiponectin multimer [34]. Therefore, the reduced levels of HMW adiponectin found in the present study in a subset of FCH females may be related to increased visceral adiposity. An increase in visceral adiposity may be associated with adipocyte hypertrophy and lead to less functional adipocytes and altered adipokine production. Consistent with this, we did find a significant inverse correlation of HMW adiponectin with WHR, which is surrogate marker of visceral adiposity.

A limitation of this study is that the cross-sectional design limits inferences about causality. Furthermore, the age of the subjects

ranged from 50 to 70 years. As a consequence, most females included in the study were postmenopausal and may have relatively high adiponectin levels due to reduced estradiol concentration. Moreover, due to an impairment in renal function, age is positively associated with plasma adiponectin levels. Unfortunately, no data on renal function of the present population are available. For these reasons our results cannot be generalized to younger subjects.

In conclusion, despite reduced total adiponectin, FCH males show normal adiponectin multimer distribution. However, due to a more pronounced reduction of the HMW multimer in FCH females with CVD, these females have a more atherogenic adiponectin multimer distribution with decreased ASI and increased LMW/HMW adiponectin ratio.

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