

Plasma Adiponectin Levels and Sonographic Phenotypes of Subclinical Carotid Artery Atherosclerosis

Data From the SAPHIR Study

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Background and Purpose—Adipose tissue produces and secretes a number of bioactive molecules, conceptualized as adipocytokines. Adiponectin has been identified as one of the adipocytokines, and hypo adiponectinemia was demonstrated in patients with obesity, diabetes mellitus, and coronary artery disease. Whether decreased adiponectin levels are cause or consequence is an important issue in the discussion on the association between adiponectin and atherosclerosis. In the present study, we investigated the association of plasma adiponectin levels with sonographic phenotypes of subclinical atherosclerosis, which may represent different stages of disease as well as common and distinct determinants.

Methods—A total of 1515 middle-aged healthy white subjects (940 males and 575 females) were included. Common carotid artery intima-media thickness (CIMT) and presence of atherosclerotic plaques were assessed by B-mode ultrasound.

Results—After adjustment for established risk factors, per 1 $\mu\text{g/mL}$ decrease in adiponectin CIMT increased on the average by 3.48 μm in males (95% CI, 1.23 to 5.73 μm) and by 2.39 μm in females (95% CI, 0.50 to 4.27 μm). After dichotomizing adiponectin levels at the median and adjustment for established risk factors, the mean difference of CIMT between subjects with low and high adiponectin levels was 20.42 μm in men (95% CI, 6.80 to 34.04; $P=0.003$) and 20.75 μm in women (95% CI, 1.08 to 40.42; $P=0.039$). No significant relationship was found between adiponectin levels and presence of atherosclerotic plaques.

Conclusion—Our results demonstrate an independent negative association of adiponectin levels and CIMT, whereas no relationship with presence of atherosclerotic plaques was found, thus suggesting hypo adiponectinemia as a risk factor in the development of early atherosclerosis. (*Stroke*. 2005;36:2577-2582.)

Key Words: adiponectin ■ atherosclerosis ■ carotid arteries ■ intima-media thickness ■ risk factors

Recent research has demonstrated that adipose tissue produces and secretes a number of bioactive molecules, conceptualized as adipocytokines, which may contribute to the development of obesity-related diseases.¹ Adiponectin² is an adipose-derived factor and suppresses various mechanisms contributing to atherogenesis, including the expression of adhesion molecules in endothelial cells,^{3,4} proliferation of smooth muscle cells,⁵ and formation of foam cells in vitro.⁶ Physiological concentrations of adiponectin inhibit monocyte adhesion to endothelial cells, transformation of macrophages to foam cells and secretion of tumor necrosis factor- α from macrophages.^{3,4,6,7} Atheroprotective activity of adiponectin was demonstrated in animal models,⁸ and hypo adiponectinemia was found in patients with obesity, type 2 diabetes mellitus, and coronary artery disease (CAD).^{9,10} Weight reduction increases adiponectin plasma concentrations.¹¹ Hy-

po adiponectinemia $<4 \mu\text{g/mL}$ was associated with a 2-fold increase in CAD prevalence in males, independent of established risk factors.⁹ Adipocytes from type 2 diabetics are resistant to insulin,¹² suggesting reduced insulin sensitivity as a reason of diminished adiponectin levels. Moreover, decreased adiponectin expression could reflect the dysfunctional fat cell syndrome.¹³ These results suggest an association between dysregulation of adipocytokines caused by overnutrition and development of atherosclerosis. Whether decreased adiponectin levels are cause or consequence in atherogenesis has not been fully elucidated.¹⁴ In the present study, we investigated the association of plasma adiponectin levels with sonographic phenotypes of subclinical atherosclerosis of the carotid arteries, which may represent different stages of the disease as well as common and distinct determinants.¹⁵

Received August 25, 2005; final revision received September 19, 2005; accepted September 26, 2005.

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Stroke is available at <http://www.strokeaha.org>

DOI: 10.1161/01.STR.0000190834.00284.fd

Subjects and Methods

Subjects

The cohort of 1515 white subjects consisted of 940 males (40 to 55 years of ages) and 575 females (50 to 65 years of age) who are participants of the Salzburg Atherosclerosis Prevention program in subjects at High Individual Risk (SAPHIR). The study objectives, recruitment procedures, and population characteristics have been detailed.^{16,17} Subjects with clinical manifestations of atherosclerosis, congestive heart failure, valvular heart disease, chronic alcohol (>3 drinks per day) or drug abuse, morbid obesity (body mass index [BMI] >40 kg/m²) were excluded to reduce possible confounding from therapeutic interventions. Diabetes mellitus was defined by a fasting glucose level of ≥ 7 mmol/L or antidiabetic treatment. Twenty-four-hour ambulatory blood pressure measurement was performed in all participants (TM 2430 PC system; Bosch+Soehn). Means of daytime (7 AM to 10 PM) measurements of systolic blood pressure (SBP-d) were used in the analyses. Abdominal adipose tissue areas were assessed by computed tomography (MX TWIN Picker CT; Marconi Medical Systems) as described.¹⁸ Informed consent was obtained from all participants, and the study was approved by the local ethics committee.

Laboratory Data

Venous blood was collected after an overnight fast. Adiponectin was measured using the human adiponectin ELISA kit (BioCat GmbH). Total serum cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), high-sensitive C-reactive protein (hs-CRP), fasting glucose, LDL size, insulin sensitivity (k-ITT), and homeostasis model assessment (HOMA) were determined as described.¹⁷

Ultrasonography

Carotid artery B-mode ultrasound measurements (ATL HDI 3000 CV; Philips Medical Systems) were performed and read by a single experienced ultrasound operator blinded to clinical and laboratory

characteristics. The protocol included multiple longitudinal and transverse imaging planes of the carotid arteries. Common carotid artery intima-media thickness (CIMT) was measured end-diastolic according to the leading edge method for the near and far walls,¹⁹ and mean values of left and right arteries were used in the statistical analyses. The intraobserver variability of this method is low (<3% or <25 μm).²⁰ Atherosclerotic plaque was defined as a focal structure encroaching into the arterial lumen of ≥ 0.5 mm or 50% of the surrounding CIMT value or by a thickness of ≥ 1.5 mm as measured from the media-adventitia interface to the intima-lumen interface.²¹

Statistics

All analyses were performed using SPSS 12.0 package (SPSS Inc). Characteristics of subjects are described as means and SDs (unless otherwise indicated) and compared using *t* tests. Associations between adiponectin levels and various risk factors were measured by Pearson correlation. The dependence of CIMT on the explanatory variables was analyzed using general linear model (GLM), the dependence of the presence or absence of atherosclerotic plaques on the explanatory variables by logistic regression. The effect size Eta^2 was used to analyze individual associations of the variables with CIMT. GLM analysis was performed with adiponectin as a continuous covariate as well as dichotomized (high/low) at the median. All linear models were adjusted for age, blood pressure, LDL, BMI, and smoking. For additional risk factors (HDL, LDL size, triglycerides, diabetes mellitus, blood glucose, fasting insulin, HOMA index, k-ITT, and hs-CRP), a forward stepwise procedure (*P* in ≤ 0.05 ; *P* out ≥ 0.10) was used to choose those covariates that, in addition to the aforementioned risk factors, had significant effects on dependent variables.

Results

Tables 1, 2, and 3 summarize characteristics of the subjects studied, correlations between plasma adiponectin levels and

TABLE 1. Clinical and Metabolic Characteristics of Subjects Studied

	All (n=1515)		Males (n=940)		Females (n=575)		<i>P</i> Value (males vs females)
	Mean	SD	Mean	SD	Mean	SD	
CIMT, μm	759.5	125.1	757.0	124.2	763.7	126.5	0.317
Adiponectin, $\mu\text{g/mL}$	8.46	4.50	6.85	3.01	11.10	5.23	<0.0005
Age, y	51.9	6.0	49.3	5.4	56.1	4.4	<0.0005
BMI, kg/m ²	26.7	3.9	27.0	3.5	26.3	4.5	0.004
Visceral fat, mm ²	7750	4450	8200	4450	7010	4360	<0.0005
Subcutaneous fat, mm ²	19 980	9980	17 240	8230	24 530	1930	<0.0005
SBP-d, mm Hg	133.6	12.9	135.1	12.5	131.2	13.3	<0.0005
DBP-d, mm Hg	82.0	7.9	82.5	8.0	81.1	7.6	0.001
LDL, mmol/L	3.77	0.94	3.78	0.93	3.72	0.96	0.185
HDL, mmol/L	1.54	0.40	1.41	0.34	1.75	0.42	<0.0005
Total cholesterol, mmol/L	5.92	1.04	5.86	1.03	6.01	1.04	0.007
Triglycerides, mmol/L	1.43	0.99	1.58	1.15	1.18	0.58	<0.0005
Fasting glucose, mmol/L	5.21	1.02	5.27	1.04	5.1	0.97	0.004
Fasting Insulin, $\mu\text{U/mL}$	7.48	5.11	7.74	5.65	7.06	4.05	0.007
k-ITT	4.15	1.31	4.10	1.38	4.24	1.20	0.038
HOMA	1.81	1.54	1.88	1.60	1.70	1.43	0.021
hs-CRP, mg/L	2.90	6.57	2.63	7.44	3.35	4.79	0.037
LDL size, nm	266.5	11.2	264.1	11.8	270.3	9.1	<0.0005
Smoking, yes (%)	503 (33.2%)		365 (38.8%)		138 (24.0%)		<0.0005
Diabetes mellitus, yes (%)	61 (4.0%)		42 (4.5%)		19 (3.3%)		0.116

K-ITT indicates the short insulin tolerance test.

TABLE 2. Correlations Between Adiponectin Plasma Concentrations and Metabolic Parameters

Correlation of Adiponectin With		Males			Females		
		Crude	Adjusted for Age	Adjusted for Age and BMI	Crude	Adjusted for Age	Adjusted for Age and BMI
Fasting insulin	<i>r</i>	-0.316	-0.323	-0.210	-0.278	-0.291	-0.179
k-ITT	<i>r</i>	0.237	0.243	0.161	0.222	0.240	0.153
HOMA	<i>r</i>	-0.257	-0.262	-0.149	-0.240	-0.251	-0.141
LDL size	<i>r</i>	0.284	0.286	0.243	0.275	0.276	0.243
HDL	<i>r</i>	0.344	0.344	0.281	0.425	0.424	0.360
TG	<i>r</i>	-0.250	-0.251	-0.185	-0.227	-0.235	-0.182

All correlations were significant, with $P < 0.0005$ with the exception for HOMA adjusted for age and BMI in females with $P < 0.001$.

r indicates correlation coefficient (Pearson); TG, triglycerides; k-ITT, the short insulin tolerance test.

metabolic parameters, and correlations of CIMT with traditional risk factors. A significant sex difference was observed for adiponectin levels $< 4 \mu\text{g/mL}$, which were found in 135 (14.4%) men but only in 20 (3.5%) women ($P < 0.0005$). GLM analysis was performed with age, blood pressure (SBP-d), LDL, BMI, and smoking as main effects, demonstrating a significant negative association between adiponectin levels and CIMT ($P = 0.004$). Because significant ($P < 0.05$) interaction effects with sex were found for LDL, smoking, and age, males and females were subsequently analyzed separately, with age, SBP, LDL, BMI, and smoking in the linear model alongside adiponectin. For males, all variables showed significant impact on CIMT, including adiponectin with $P = 0.032$. For females only, age, SBP-d, and adiponectin with $P = 0.047$ were significant (Table 4). Because of the strong correlation between body fat and adiponectin, supplementary analysis was performed without adjustment for BMI, yielding the expected stronger impact of adiponectin (Table 5). Extending GLM analysis by the stepwise procedure described above yielded the following results. In males, hs-CRP had significant impact on CIMT, whereas diabetes mellitus and LDL size proved significant for females. After including these variables in the model, the adiponectin effect remained significant in males ($P = 0.040$) but not in females ($P = 0.114$).

TABLE 3. Correlation Between CIMT and Established Risk Factors (unadjusted)

	Pearson	All	Males	Females
Adiponectin	<i>r</i>	-0.065	-0.092	-0.086
	<i>P</i> value	0.012	0.005	0.039
Age	<i>r</i>	0.360	0.478	0.290
	<i>P</i> value	< 0.0005	< 0.0005	< 0.0005
BMI	<i>r</i>	0.205	0.253	0.152
	<i>P</i> value	< 0.0005	< 0.0005	< 0.0005
LDL	<i>r</i>	0.128	0.186	0.039
	<i>P</i> value	< 0.0005	< 0.0005	0.352
SBP-d	<i>r</i>	0.218	0.233	0.211
	<i>P</i> value	< 0.0005	< 0.0005	< 0.0005
Smoking	<i>r</i>	0.017	0.077	-0.081
	<i>P</i> value	0.516	0.019	0.051

For males, per 1 $\mu\text{g/mL}$ decrease of adiponectin, CIMT increased on the average by 3.48 μm (95% CI, 1.23 to 5.73 μm). For females, a decrease of 1 $\mu\text{g/mL}$ adiponectin increased CIMT on average by 2.39 μm (95% CI, 0.50 to 4.27 μm). Using the dichotomized adiponectin level (above/below the median [750 μm]) as explanatory variable and adjustment for age, SBP, LDL cholesterol, BMI, and smoking, the mean difference of CIMT between subjects with low and such with high adiponectin levels was 15.61 μm in men (SE 7.05; $P = 0.027$; 95% CI, 1.77 to 29.45) and 17.02 μm in women (SE 10.21; $P = 0.096$; 95% CI, -3.03 to 37.07). Removing BMI from the model resulted in a pronounced adiponectin effect: Mean CIMT difference increased to 20.42 μm in men (SE 6.94; $P = 0.003$; 95% CI, 6.80 to 34.04) and to 20.75 μm in women (SE 10.01; $P = 0.039$; 95% CI, 1.08 to 40.42). The Figure shows 95% CIs for mean adjusted CIMT (at 53 years of age; SBP 133 mm Hg and LDL 3.8 mmol/L) by adiponectin dichotomized at the median.

Atherosclerotic plaques were observed in 355 (23.4%) subjects (209 men [22.2%], 146 women [25.4%], NS). For investigation of the association of adiponectin with atherosclerotic plaques, logistic regression analysis with the above-mentioned explanatory variables was performed. Age, blood pressure (SBP-d), LDL, and triglycerides were significant predictors in males, whereas age, LDL, smoking, and diabetes proved significant in females. Adiponectin was far from significance in males and females.

Discussion

To our best knowledge, this is the first study demonstrating an association of adiponectin levels and CIMT in a large population, thus confirming similar observations in a limited cohort.²² An important issue in the discussion on the association between adiponectin and atherosclerosis is whether the decreased adiponectin levels are cause or consequence. Putative mechanisms include reduced production in adipocytes as a marker of the dysfunctional fat cell syndrome,¹³ increased consumption in the blood stream, or a combination of both.¹⁴ This is of particular interest considering the growing evidence suggesting that different ultrasound determinations of carotid atherosclerosis may represent different stages of disease as well as distinct and common determinants.¹⁵ Among carotid ultrasound parameters, CIMT probably re-

TABLE 4. GLM Analysis for CIMT on Age, SBP, LDL, BMI, Smoking, and Adiponectin

	Trait	Coefficient B	SE(B)	P Value	95% CI		Eta ²	
					Low	High		
Males	Age	9.84	0.64	<0.0005	8.58	11.10	0.201	
	SBP	1.35	0.29	<0.0005	0.78	1.92	0.023	
	<i>R</i> ² =0.295	LDL	0.432	0.096	<0.0005	0.244	0.620	0.021
	<i>R</i> ² adjusted=0.291	BMI	3.44	1.08	0.001	1.32	5.55	0.011
	Smoking	12.81*	7.07	0.070	-1.07	26.68	0.004	
	Adiponectin	-2.53	1.18	0.032	-4.85	-0.22	0.005	
Females	Age	7.60	1.18	<0.0005	5.291	9.913	0.068	
	SBP	1.33	0.39	0.001	0.56	2.10	0.020	
	<i>R</i> ² =0.127	LDL	0.072	0.134	0.592	-0.191	0.335	0.001
	<i>R</i> ² adjusted=0.118	BMI	1.92	1.21	0.111	-0.446	4.293	0.004
	Smoking	-10.52*	11.79	0.373	-12.636	33.667	0.001	
	Adiponectin	-1.98	0.99	0.047	-3.922	-0.029	0.007	

*The mean difference in CIMT between smokers and exsmokers and nonsmokers/pipe smokers, adjusted for the other covariates in the model.

*R*² indicates coefficient of determination of the whole model; *R*² adjusted, coefficient of determination adjusted for the No. of parameters in the whole model.

flects earlier stages of atherogenesis, notably a hypertrophic response of intimal and medial cells to lipid infiltration and hypertension.¹⁵ Furthermore, modest to moderate associations of CIMT with various cerebrovascular and cardiovascular end points have established CIMT as a surrogate parameter of clinical end points.^{23–25} Adiponectin suppresses various mechanisms contributing to atherogenesis,^{5,6,26,27} and our results are consistent with this background. In contrast, our data do not support the mechanism of hypoadiponectinemia caused by accumulation in the injured artery wall⁷ because we found no significant association between decreased adiponectin levels and atherosclerotic plaque.

Previously described associations of adiponectin levels and various markers for progression of atherosclerosis (insulin-resistant state^{28,29} and atherogenic lipoprotein profile^{29,30}) are confirmed by our findings (Table 2).

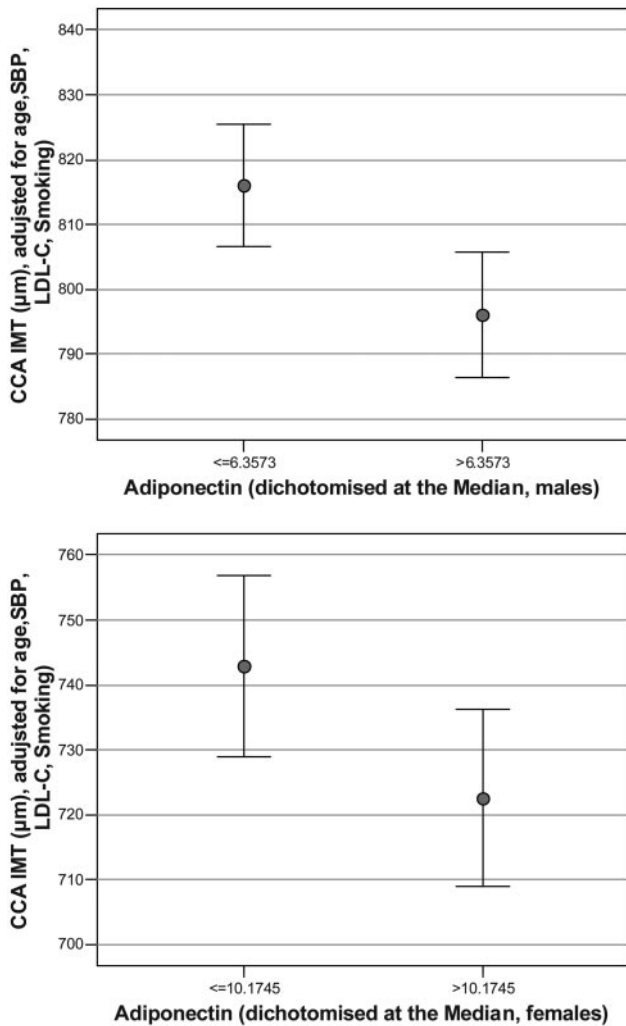
The sex differences of plasma adiponectin concentrations in our population remained significant after adjustment for parameters of body fat (BMI and visceral and subcutaneous adipose tissue) and may be explained by the higher amount of visceral fat in men. A sex-based difference of adiponectin levels is supported by some^{30,31} but not all other studies.²⁸ Animal and in vitro models suggest androgen-induced hypoadiponectinemia as connecting link between visceral fat, insulin resistance, and atherosclerosis in men,^{30,31} whereas other studies reported similar adiponectin concentrations for men and women with comparable amounts of liver fat, which were clearly associated with visceral fat mass.³² The stronger impact of adiponectin on CIMT in men is consistent with data reporting an association of low adiponectin, CAD, and atherosclerosis almost exclusively in male populations.^{9,33,34} This finding may be explained by the significantly higher

TABLE 5. GLM Analysis for CIMT on Age, SBP, LDL, Smoking and Adiponectin

	Trait	Coefficient B	SE(B)	P Value	95% CI		Eta ²	
					Low	High		
Males	Age	10.17	0.64	<0.0005	8.91	11.42	0.214	
	SBP	1.64	0.28	<0.0005	1.10	2.19	0.036	
	<i>R</i> ² adjusted=0.284	LDL	0.449	0.096	<0.0005	0.260	0.638	0.023
	<i>R</i> ² =0.288	Smoking	14.41*	7.09	0.042	0.51	28.32	0.004
	Adiponectin	-3.48	1.15	0.002	-5.73	-1.23	0.010	
Females	Age	7.69	1.18	<0.0005	5.38	10.00	0.070	
	SBP	1.50	0.38	<0.0005	0.75	2.24	0.027	
	<i>R</i> ² =0.123	LDL	0.084	0.134	0.530	-0.179	0.347	0.001
	<i>R</i> ² adjusted=0.115	Smoking	-11.27*	11.79	0.340	-34.44	11.89	0.002
	Adiponectin	-2.39	0.96	0.013	-4.27	-0.50	0.011	

*The mean difference in CIMT between smokers and exsmokers and nonsmokers/pipe smokers, adjusted for the other covariates in the model.

*R*² indicates coefficient of determination of the whole model; *R*² adjusted, coefficient of determination adjusted for the No. of parameters in the whole model.



95% CIs for mean adjusted CIMT (age 53 years; SBP 133 mm Hg; LDL 3.8 mmol/L) by adiponectin dichotomized at the median.

number of men with an adiponectin level $<4 \mu\text{g/mL}$, which was demonstrated to be associated with an increased risk for CAD prevalence,⁹ thus suggesting a threshold value for the atheroprotective effect of adiponectin. The main determinants of CIMT are age and SBP,¹⁵ and the impact of all other risk factors on CIMT is comparatively weak. The effect size of low adiponectin levels on CIMT is stronger than that of smoking in both sexes and stronger than the effect of LDL cholesterol in females. Thus, the results of our analyses strongly suggest low adiponectin as a risk factor for early atherosclerosis as reflected by CIMT. This effect is independent from the abovementioned relationships to obesity, glucose, and lipid metabolism. However, the cross-sectional design of our study warrants cautious interpretation of results, and further investigations are necessary to substantiate these findings.

Acknowledgments

All statistical analyses were performed by Mynda Schreuer, PhD. The Salzburg Atherosclerosis Prevention Program in subjects at High Individual Risk (SAPHIR) is supported by grants from the

Medizinische Forschungsgesellschaft Salzburg and the Kamillo Eisner Foundation.

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