Low Adipocyte-Derived Plasma Protein Adiponectin Concentrations Are Associated With the Metabolic Syndrome and Small Dense Low-Density Lipoprotein Particles: Atherosclerosis and Insulin Resistance Study

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Circulating plasma adiponectin, an adipocyte-derived protein, has been shown to be decreased in obese subjects as well as in patients with type 2 diabetes and also in subjects who do not have diabetes, but are insulin resistant. We assessed the relationship between plasma levels of adiponectin, the metabolic syndrome and the occurrence of small dense LDL particles (pattern B) in 101 clinically healthy middle-aged subjects recruited from the general population. Low adiponectin levels were associated with the metabolic syndrome and low-density lipoprotein (LDL) particle size ($r = .55$, $P < .001$). The relationship between adiponectin and LDL particle size remained in a multiple regression model, in which adiponectin and total body fat explained 30% of the variability in LDL particle size. Furthermore, subjects in the lowest tertile of adiponectin had an increased risk of having pattern B (risk odds ratio [ROR] = 5.6). Because this was a cross-sectional study, no conclusions can be drawn about causality. This is the first population-based study in man demonstrating a relationship between small dense LDL particles and adiponectin.

ADIPONECTIN IS exclusively and abundantly produced in white adipose tissue. Despite this close association with body fat, obesity and type 2 diabetes are characterized by low plasma adiponectin concentrations.[1] Adiponectin is more related to whole-body insulin sensitivity than to adiposity[1] and improves insulin signalling in skeletal muscle. A low plasma adiponectin concentration has been found to precede a future decrease in insulin sensitivity.[2] Furthermore, one of the quantitative trait loci associated with the metabolic syndrome has recently been mapped on chromosome 3q27 where the adiponectin gene is located.[3]

The observation that insulin resistance is associated with a clustering of risk factors for cardiovascular disease has lead to the suggestion of a syndrome, which has been given different names, eg, seems to be a central component in the clustering of risk factors constituting the metabolic syndrome or the insulin resistance syndrome. The occurrence of small, dense low-density lipoprotein (LDL) particles (ie, pattern B) is one of the variables suggested to be associated with the metabolic syndrome, as well as with atherosclerosis development.[4,5] The underlying mechanisms seem to be related to changes in lipid metabolism associated with insulin resistance. Recent results from our group have corroborated the hypothesis that the occurrence of small dense LDL particles is associated with insulin resistance, the metabolic syndrome, and subclinical atherosclerosis development, as measured by ultrasound in the carotid artery in clinically healthy, middle-aged men.[5]

The aim of this study was to test the hypothesis that circulating levels of adiponectin are decreased in clinically healthy, middle-aged men with the metabolic syndrome, and also that decreased adiponectin levels are associated with the atherogenic pattern B phenotype.

MATERIALS AND METHODS

The inclusion criteria for study subjects were 58-year-old men and Swedish ancestry. The reasons to include only 58-year-old men were that clinical cardiovascular disease still is scarce at this age, but also to minimize the confounding effect of age and sex. Exclusion criteria were cardiovascular disease, clinical diabetes mellitus or other clinically overt disease, treatment with cardiovascular drugs, which might disturb the measurements performed in the study, or unwillingness to participate. The subjects were randomly selected among men in the County Council register and were invited to a screening examination. Of 818 screened men, 104 men were randomly selected to undergo a euglycemic hyperinsulinemic clamp after having given informed consent. The study was approved by the Ethics Committee at Sahlgrenska University Hospital. In total, 101 subjects had valid measurements of adiponectin and were included in the analysis. An operative definition of the metabolic syndrome, as suggested by a working group associated with the World Health Organization (WHO) 1998, was used.[6] LDL particle size was measured by gradient gel electrophoresis,[5] and plasma levels of adiponectin were determined by a radioimmunoassay kit (LINCO Research, St Charles, MO) that utilizes ^{125}I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum. Human recombinant adiponectin was used as a standard. Inter- and intra-assay coefficient of variation (CV) was 5.2% and 3.6%, respectively. No significant difference was obtained when plasma was compared with serum (n = 20). A euglycemic hyperinsulinemic clamp examination ad modum de Fronzo was performed, slightly modified, as previously published.[7] After the clamp examination, fat-free mass was measured using the dual-energy x-ray absorptiometry body composition model (Lunar DPX-L, Madison, WI; CV = 1.4%). Insulin sensitivity was calculated as the glucose infusion rate (GIR) per minute adjusted for fat-free mass during the final hour of the examination (CV = 15%).[7] High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein (apo) B containing lipoproteins with magnesium chloride and dextran sulphate (CV = 1.25%). LDL was calculated as described by Friedewald et al (CV = 0.81%).[8] Whole blood glucose (b-glucose) was measured with the glucose oxidase technique (CV =

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Submitted March 13, 2003; accepted June 11, 2003.

Supported by grants from the Swedish Heart-Lung Foundation , the Swedish Medical Research Council (12270 and 10880), AstraZeneca Mölndal, Sweden. J.H. is Medical Adviser at AstraZeneca R&D, Mölndal, Sweden.

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0026-0495/03/5212-0057$30.00/0

doi:10.1016/S0026-0495(03)00313-5

Table 1. Descriptives of Study Group When Divided in Tertiles on Basis of Circulating Adiponectin Levels

	Tertiles of Adiponectin			
	Lowest (<25th, n = 33)	Middle (25th-75th, n = 34)	Highest (>75th, n = 34)	*P* for Trend
Adiponectin* (μg/mL)	7.76 (3.53-10.84)	12.92 (11.14-16.66)	21.68 (16.85-32.79)	†
LDL cholesterol (mmol/L)	4.14 ± 0.87	4.12 ± 1.29	3.98 ± 0.93	>.30
HDL cholesterol (mmol/L)	1.05 ± 0.26	1.23 ± 0.29	1.50 ± 0.37	<.001
Triglycerides (mmol/L)*	1.65 (0.59-9.89)	1.42 (0.62-4.31)	1.08 (0.47-2.96)	.001
LDL particle size (nm)	25.89 ± 0.75	26.25 ± 0.66	26.71 ± 0.61	<.001
Blood glucose mmol/L	4.66 ± 0.54	4.75 ± 0.66	4.78 ± 0.54	>.30
Plasma insulin μU/mL	10.5 (3.9-32.9)	8.2 (2.9-24.4)	6.9 (3.2-29.9)	<.001
Body weight (kg)	89.7 ± 13.2	83.3 ± 16.6	78.1 ± 11.1	<.001
Body height (cm)	178 ± 7	177 ± 6	179 ± 5	>.30
BMI (kg/m^2)	28.3 ± 4.1	26.4 ± 4.9	24.3 ± 3.3	<.001
Total body fat (kg)	25.0 ± 7.9	21.3 ± 8.8	18.9 ± 7.5	.003

*Values are geometric mean and range.

†Not tested for statistical significance because of selection criteria.

3.0%). Total plasma insulin was determined in all subjects with radioimmunoassay (RIA) (Pharmacia Insulin, Uppsala, Sweden; CV = 22%). Body weight was measured on a balance scale with the subject dressed in underwear. Body-mass index (BMI) was calculated according to the formula body weight/height^2 (CV = 0.6%). All statistics were analyzed using SPSS for Windows 9.0 (SPSS, Chicago, IL). Subjects were arbitrarily divided in tertiles to have a reasonable number of subjects in each group (n = 30). Trend analysis was performed by using the Mantel's test. Logistic regression was used to explore the association between circulating adiponectin levels and small LDL particle size. Simple Spearman's rank correlation coefficients were used to calculate univariate associations, and adjustment for body fat and insulin sensitivity (calculated as the GIR per minute adjusted for fat-free mass during the final hour of the examination of glucose infusion) regarding the relationship between LDL particle size and adiponectin was made in a multiple regression model.

RESULTS

When divided in tertiles, circulating adiponectin levels were positively associated with HDL cholesterol and LDL particle size and negatively associated with triglycerides, plasma insulin, body weight, BMI, and total body fat (Table 1). No significant association was seen between adiponectin, LDL cholesterol, and body height, respectively (Table 1). In total 11 subjects (11%) fulfilled the criteria of the metabolic syndrome, 70 subjects (69%) had at least 1 risk factor, but not the full syndrome, and 20 subjects (20%) had no risk factors of the metabolic syndrome. There was a significant association towards lower adiponectin levels for subjects with the metabolic syndrome as compared with the other groups (Fig 1). LDL particle size was not included in this definition of the metabolic syndrome. However, circulating adiponectin levels were also highly related to LDL particle size ($r = .55$, $P < .001$). In a multiple regression model including adiponectin, GIR, and total body fat, adiponectin (F value = 35.9, $P < .001$) and total body fat (F-value = 21.9, $P < .001$) were independent predictors of LDL particle size and explained 30% of the variability. Furthermore, the risk odds ratio for having the atherogenic pattern B phenotype in the lowest versus the highest tertile of circulating adiponectin levels was risks odds ratio (ROR) = 5.64 (95% confidence interval [CI] 1.24 to 25.9), $P = .02$.

DISCUSSION

Previous studies have shown that obesity, insulin resistance, and type 2 diabetes are accompanied by low circulating adiponectin concentrations.[1] Adiponectin has also been shown to be closely related to different features of the metabolic syndrome, such as high trigylycerides,[9,10] low HDL,[9-11] and decreased insulin sensitivity.[10] The results from the present study showing a relationship between HDL, triglycerides, and adiponectin corraborate these previous studies. However, the present study extends the observed relationship between dyslipidemia and low adiponectin levels to also encompass small LDL particles. It has previously been shown that small LDL particle size is related to insulin resistance,[12] components of the

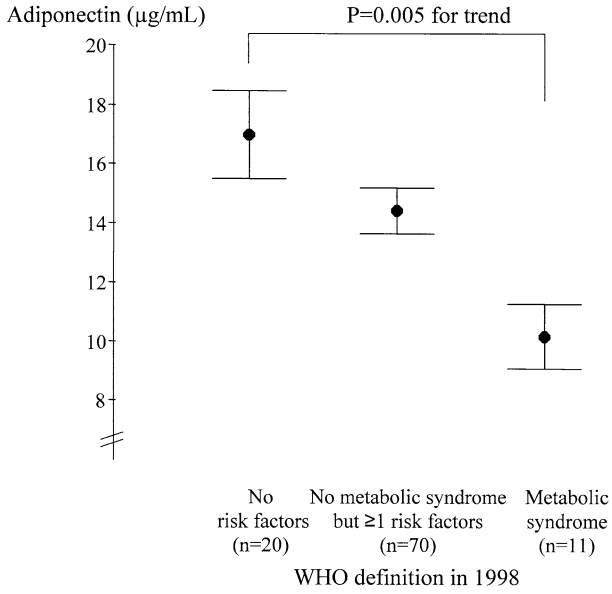

Fig 1. Means (SE) of circulating adiponectin concentrations in relationship to the risk factors of the metabolic syndrome, as defined by a working group associated with the WHO.

metabolic syndrome, as well as to the full metabolic syndrome,[5] atherosclerosis,[5] and to prospective risk of coronary artery disease.[13] The mechanisms underlying the development of small LDL particles are not fully known. Insulin resistance is associated with an influx of fatty acids from the splanchnic circulation to the liver, causing an increased production of very–low-density lipoprotein (VLDL) particles.[14,15] The concomitant reduction in lipoprotein lipase action in the peripheral tissues and the liver is believed to lead to the production of small LDL particles. However, insulin resistance, as measured by clamp, was not independently associated with LDL size in the present study. In a multiple regression model, adiponectin and total body fat turned out to be independent predictors of LDL size, explaining 30% of the variability. Furthermore, subjects in the lowest tertile of adiponectin levels had an increased risk (ROR = 5.6) of having pattern B (that is, a small LDL particle phenotype) as compared with subjects in the highest tertile of adiponectin levels. Taken together, low adiponectin levels seem to be closely related to several of the risk factors in the metabolic syndrome, including dyslipidemia, small LDL particle size, and decreased insulin sensitivity. These results are also in line with recent studies suggesting a close genetic link between the metabolic syndrome and adiponectin.[3]

These results were obtained in a population-representative sample of 58-year-old, white untreated men who were selected to minimize the effect of confounding factors, such as race, sex, age, and treatment with different drugs for cardiovascular disease. To summarize, the present study showed an independent relationship between LDL particle size and adiponectin. Furthermore, subjects in the lowest tertile of adiponectin had an increased risk of having pattern B (ROR = 5.6) as compared with subjects in the highest tertile. Tentatively, the strong independent association between small LDL particle size and decreased adiponectin levels may relate to the suggested antiatherosclerotic effects of adiponectin. However, the mechanistic relationship between adiponectin and LDL particle size remains to be elucidated. Because this was a cross-sectional study, no conclusions can be drawn about causality.

ACKNOWLEDGMENT

We thank Dr Lena Bokemark for screening of subjects and laboratory technologist, Carita Fagerlund, for excellent research assistance.

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