Adiponectin Predicts High-Density Lipoprotein Cholesterol Efflux Capacity in Adults Irrespective of Body Mass Index and Fat Distribution

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Context: Obesity is associated with hypoadiponectemia, dyslipidemia, and increased risk of cardiovascular disease (CVD). Mechanisms linking these conditions remain to be fully understood. Cholesterol efflux capacity (CEC) is a crucial functional property of high-density lipoprotein (HDL) that strongly predicts CVD incidence.

Objective: We investigated whether age, fat distribution, and other obesity-related factors affect CEC in juvenile and adult overweight/obese participants of the STYJOBS/EDECTA cohort (NCT00482924).

Design: We performed an observational study.

Main Outcome Measures: CEC and its association with body measures and related metabolic parameters was assessed in 683 participants (281 juveniles, of whom 227 were overweight/obese; 402 adults, of whom 197 were overweight/obese).

Results: Pearson correlation analysis showed that, after Bonferroni correction, CEC was significantly inversely correlated with body mass index (BMI), carotid diameter, waist circumference, waist-to-hip, waist-to-height ratio, oxidized low-density lipoprotein, and uric acid and with the liver markers alanine-aminotransferase and choline esterase. CEC was positively correlated with HDL cholesterol, total cholesterol, apolipoprotein A1, and adiponectin in adults, whereas in juveniles only apolipoprotein A1 showed a significant positive correlation with CEC. Age-stratified linear regression analyses with CEC as the outcome variable identified adiponectin as the most significant predictor of CEC in adults. The results did not change when either BMI or waist-to-hip ratio as a factor of fat distribution was included in the models.

Conclusions: Hypoadiponectemia is a robust predictor of reduced cholesterol efflux capacity in adults irrespective of BMI and fat distribution. Further investigations are needed to assess whether adiponectin is a causal determinant of CEC. (***J Clin Endocrinol Metab*** **102: 4117–4123, 2017)**

Obesity is a growing burden. For the first time in human history, the number of obese people worldwide exceeds those who are underweight (1). It is alarming that childhood obesity has reached epidemic proportions within this millennium (2, 3). Hence, obesity affects all age groups and races. Pathological consequences include type 2 diabetes, nonalcoholic fatty liver disease, certain cancers, and cardiovascular disease (CVD) (4). Lifelong obesity can

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Abbreviations: ALT, alanine transaminase; apo, apolipoprotein; BMI, body mass index; CEC, cholesterol efflux capacity; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; IL, interleukin; LDL, low-density lipoprotein.

lead to more fatal clinical endpoints, such as myocardial infarction or stroke. This will cause for the first time offspring with lower life expectancy than their parents, which is mainly due to the burden of CVD caused by atherosclerotic vascular disease. In particular, visceral obesity is associated with disturbed adipokine dysbal ance (*i.e.*, decreased adiponectin, increased leptin), chronic low-grade inflammation, and dyslipidemia, characterized by decreased high-density lipoprotein (HDL) blood levels.

Adipose tissue is a highly active endocrine organ (5). Adiponectin, a cytokine that is exclusively expressed in adipose tissue (6), is decreased in obesity (7). Hypoadiponectinemia is associated with insulin resistance (8), type 2 diabetes (9), metabolic syndrome (10), dyslipidemia (11), and hypertension (12). Adiponectin exerts protective effects against atherosclerosis due to its anti-inflammatory and antiatherogenic properties (13, 14) and suppresses foam cell formation of macrophages isolated from patients with type 2 diabetes mellitus (15). Thus, decreased adiponectin levels indicate CVD (16) and endothelial dysfunction (17, 18).

Recent evidence has shed light on the complex nature of HDL metabolism and function. The mechanisms by which HDLs might confer atheroprotection have been extensively investigated. These include reverse cholesterol transport, improved endothelial function through stimulating production of nitric oxide, anti-inflammatory actions on endothelial cells and leukocytes, prevention of apoptosis of endothelial cells, and antithrombotic effects (19). A key function of HDL is transportation of cholesterol from peripheral tissues, such as the arterial wall, to the liver for excretion. Strategies to measure the cholesterol efflux capacity (CEC) of apolipoprotein (apo) B-depleted serum, an integrated measure of HDL quantity and quality, have successfully been applied in clinical cohort studies (5, 6, 20). There are compelling data that the ability of HDL to promote cholesterol efflux from macrophages, the first step in the "reverse cholesterol transport" pathway, is inversely associated with risk for atherosclerotic vascular disease even after controlling for HDL cholesterol. CEC inversely associates with early, asymptomatic atherosclerotic vascular disease in the general population (20), and with incident cardiovascular events among apparently healthy individuals (5, 6).

These advanced measures of lipoprotein function may be useful for early detection of cardiometabolic risk. We hypothesized that obesity would reduce CEC after adjusting for known risk factors as early as in young persons afflicted with obesity. Referring to the antiatherogenic and antidiabetogenic profile of adiponectin, we hypothesize that decreased adiponectin associates with reduced CEC.

Subjects and Methods

Participants

Study participants were from the STYJOBS/EDECTA study (STYrian Juvenile Obesity Study, Early DEteCTion of atherosclerosis), which is designed to investigate early stages of atherosclerosis and metabolic disorders in obese juveniles and middle-aged adults. STYJOBS is registered at ClinicalTrials.gov (Identifier NCT00482924), where detailed information of the study is available. For participants ≤18 years of age, overweight was defined as a body mass index (BMI) >90th but ≤97th percentile, and obesity was defined as a BMI >97th percentile. For adults ≥18 years of age, overweight was defined as a BMI >25 but ≤30 kg/m^2, and obesity was defined as a BMI >30 kg/m^2. Participants were 402 overweight/obese adults ≥18 years (female, 240; male, 162; age range, 18.0 to 68.0; mean, 35.8) and 281 juveniles aged <18 years (female, 145; male, 136; age range, 4.0 to 17.9; mean, 12.7) from the STYJOBS cohort. Standard anthropometric data (height, weight, and waist and hip circumference) were obtained from each subject. Subjects wore light clothing (*e.g.*, shorts and a light top) and no shoes during the measurements. Standing height was measured to the nearest 0.1 cm using a portable calibrated stadiometer (SECA-220; Hamburg, Germany). Body mass was measured to the nearest 0.01 kg using calibrated electronic scales (Soehnle 7700; Murrhardt, Germany). The BMI was calculated as the weight in kilograms divided by the square of height in meters. Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. All subjects enrolled were apparently healthy and free of chronic diseases. Exclusion criteria for overweight/obese participants were endocrine diseases (*e.g.* hypothyreosis, manifest type 2 diabetes), infectious diseases, inflammatory or any other chronic diseases. The study was approved by the ethical committee of the Medical University of Graz. Blood collection and ultrasonography were performed after written informed consent was given by the participants.

Statistical methods

Categorical parameters are summarized as absolute and relative frequencies, and continuous parameters are presented as mean (standard deviation) or as median (interquartile range) as appropriate. Continuous parameters were checked for normality, and the two age cohorts were analyzed separately. Pearson correlations between CEC and further continuous parameters were computed to quantify the degree of association. Using a Bonferroni correction for multiple outcomes, only P values <0.002 are considered significant. Furthermore, linear regression analyses were used to identify parameters most predictive for CEC levels. Parameters identified in a univariable regression analysis were then entered into a stepwise procedure, excluding certain parameters due to collinearities and missing values. Statistical analyses were performed using R version 3.3.3 and SPSS version 24.

Clinical and biochemical analysis

Resting blood pressure was measured in the right arm, at the end of the physical examination with the participant sitting. Fasting blood samples were collected from 8:00 to 10:30 AM. Leptin and adiponectin were determined from human plasma by

enzyme-linked immunosorbent assays (ELISAs) from Biovendor Laboratory Medicine, Inc. (Brno, Czech Republic) according to manufacturer's instructions. Intra- and interassay coefficients of variation for all ELISAs in our study were below 10%. Cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were measured by means of electrochemiluminescence assay on an Elecsys2010 analyzer (Roche Diagnostics, Mannheim, Germany). Oxidized LDL and apo-AI were measured by a commercially available ELISA (oxidized LDL Competitive ELISA, SE-754 50; Mercodia, Uppsala, Sweden; Human Apolipoprotein AI ELISA Kit APOA1 ab108803; Abcam). Plasma insulin was measured by ELISA (Mercodia), and plasma glucose was measured by the glucose hexokinase method on a Cobas 6000 chemical analyzer (Roche Diagnostics). Homeostatic model assessment–insulin resistance was calculated as the product of the fasting plasma insulin value (in microunits per milliliter) and the fasting plasma glucose value (in millimoles per liter) divided by 22.5 (21). High sensitive-CRP interleukin (IL)-6; liver transaminases aspartate transaminase/glutamic oxaloacetic transaminase, alanine transaminase (ALT)/glutamic-pyruvic transaminase, and gamma glutamyl transferase; creatinine; and uric acid were measured by routine laboratory methods on a Cobas 6000 chemical analyzer.

Deep-frozen (−80°C) aliquots of platelet-free plasma derived from citrated blood samples were used for endogenous thrombin potential analysis, which was performed by the new CE *in vitro* diagnostic–labeled Innovance endogenous thrombin potential test kit on a BCS-XP analyzer (Siemens Healthcare Diagnostics GmbH, Wien, Austria). Standard clotting parameters, including prothrombin time, activated thromboplastin time, fibrinogen, antithrombin, and D-dimer, were measured with LIATEST reagents using a STAGO STA-R Evolution coagulation analyzer (Roche Diagnostics).

Preparation of apoB-depleted serum

To prepare apoB-depleted serum, 40 μL polyethylene glycol (20% in 200 mmol/L glycine buffer) was added to 100-μL serum samples and incubated for 20 minutes at room temperature. Samples were centrifuged (10,000 rpm, 20 minutes, 4°C), and the supernatants were recovered (20).

CEC

We used a previously validated protocol (20, 22) to quantify the CEC of apoB-depleted serum samples of study subjects. A mouse macrophage cell line (J774 cells) was plated in 48-well plates followed by addition of 1 μCi/mL ^{3}H-cholesterol (Perkin Elmer, Boston, MA) for 24 hours. During the labeling period, J774 cells were stimulated with 0.3 mmol/L 8-(4-chlorophenylthio)-cyclic AMP (Sigma, Darmstadt, Germany) to upregulate ATP-binding cassette transporter A1. Cells were rinsed several times to remove ^{3}H-cholesterol from cell supernatants. In some wells, the ^{3}H-cholesterol content of J774 cells was measured to determine ^{3}H-cholesterol taken up by J774 cells during the labeling period. To determine the ^{3}H-CEC of individual serum samples of study subjects, apoB-depleted serum (2.8%) was added to cells for 4 hours at 37°C. All steps were performed in the presence of 2 μg/mL of the acyl coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma, Darmstadt, Germany). All assays were performed in two independent experiments, and samples were assessed in duplicate. We included a serum control on each plate to correct for interassay variation across plates. Values obtained for serum samples from study subjects were normalized to this serum control. Duplicate measures of efflux capacity in two independent experiments were highly correlated across the entire cohort (r = 0.91).

Results

Baseline characteristics for the juveniles and adults are displayed in Table 1. Pearson correlation analysis (Table 2) showed that, after Bonferroni correction, in adults CEC was significantly inversely correlated with BMI, carotid diameter, waist circumference, waist-to-hip and waist-to-height ratio, oxidized LDL, uric acid, and the liver markers alanine-aminotransferase and choline esterase. CEC was positively correlated with HDL cholesterol, total cholesterol, apoA1, and serum adiponectin in adults, whereas in juveniles only apoA1 showed a significant positive correlation with CEC.

Next, age-stratified linear regression analyses with CEC as the outcome variable were performed. After univariable analysis and consideration of collinearities between the parameters as well as exclusion of parameters with missing values, the following parameters were entered into a stepwise procedure: age, sex, glucose, systolic blood pressure, and parameters of body fat (BMI or waist-to-hip ratio), inflammation (IL-6), liver function (ALT), metabolism (adiponectin, uric acid). HDL cholesterol and apolipoprotein A1 were not considered. In adults CEC was most significantly associated with adiponectin, followed by ALT and uric acid, whereas in juveniles the only significant predictor was IL-6 (Table 3). Forward and backward selection yielded the same results. The same results were seen with either BMI or waist-to-hip ratio as a factor of fat distribution included in the models.

Discussion

Recent evidence has shed light on the complex nature of HDL metabolism and function. There are compelling data that CEC, an integrated measure of HDL quantity and quality, is most likely the most relevant component of atheroprotection (5, 6, 20). Our study shows that plasma adiponectin levels are associated with reduced CEC in adults older than 18 years, independently of sex, BMI, fat distribution, blood pressure, kidney function, and liver function. Interestingly, this association was not seen in juveniles younger than 18 years, suggesting that some long-lasting behaviors (*e.g.*, healthy or unhealthy diets, consumption of soft drinks) may affect CEC. It is important to establish the extent of this risk early and to identify potential targets for future intervention and treatment. Especially, visceral obesity is associated with disturbed adipokine dysbalance (*i.e.*, decreased adiponectin,

Table 1. Characteristics of the Study Cohort

	Age <18 y (n = 281) [Mean ± SD/Median (IQR 25th–75th)]	Age ≥18 y (n = 402) [Mean ± SD/Median (IQR 25th–75th)]
HDL-CEC, %	11.3 ± 2.6[a]	12.0 ± 2.4
Sex (male), n	136 (48%)	162 (40%)
Smoking, yes	10 (3.5%)	82 (23%)
Physical activity	187 (75%)	355 (90%)
Age, y	12.7 ± 2.9	35.8 ± 11.9
BMI, kg/m^2	27.1 ± 6.8	27.0 ± 7.1
IMT, cm	0.1 ± 0.0	0.1 ± 0.0
Carotis diameter, cm	0.5 ± 0.1	0.5 ± 0.1
Waist circumference, cm	87.7 ± 16.2	89.5 ± 18.6
Waist-to-hip ratio	0.9 ± 0.1	0.9 ± 0.1
Waist-to-height ratio	0.6 ± 0.1	0.5 ± 0.1
Systolic BP, mm Hg	121.3 ± 14.7	124.1 ± 16.2
Diastolic BP, mm Hg	68.3 ± 9.2	77.8 ± 10.5
Cholesterol, mmol/L	161.3 ± 26.6	196.6 ± 50.2
Triglyceride, mmol/L	95.0 (68.0–140.5)	90.0 (61.0–134.2)
HDL cholesterol, mmol/L	45.1 ± 13.1	62.5 ± 18.9
LDL cholesterol, mmol/L	101.3 ± 24.7	117.2 ± 41.2
Oxidized LDL, mmol/L	40.4 ± 17.4	54.9 ± 23.7
Glucose, mmol/L	90.5 ± 12.9	86.7 ± 12.8
HbA1c, mmol/mol	4.8 ± 0.4	6.0 ± 5.0
HOMA-IR	2.9 (1.6–6.0)	1.7 (1.0–2.9)
Uric acid, ng/dL	5.3 ± 1.4	5.2 ± 1.4
Fibrinogen, g/L	347.3 ± 72.8	309.4 ± 73.2
hsCRP, mg/L	1.5 (0.5–3.9)	1.6 (0.6–3.3)
IL-6, ng/L	2.9 (1.8–4.6)	1.7 (1.5–2.8)
AST, U/L	28.0 (23.0–33.0)	26.0 (22.0–32.0)
ALT, U/L	21.0 (16.0–28.0)	22.0 (16.0–33.0)
GGT, U/L	16.0 (12.0–20.0)	21.0 (15.0–31.0)
CHE, U/L	9389.9 ± 1772.6	8165.9 ± 1882.6
Alcaline phosphatase, U/L	213.0 ± 94.8	62.9 ± 18.7
PON activity, nmol/min/mL	0.4 (0.2–0.6)	0.3 (0.2–0.7)
ApoA-I, mg/dL	193.3 ± 36.9	200.2 ± 36.0
Leptin, ng/mL	29.7 (14.7–44.7)	10.9 (4.4–27.1)
Adiponectin, μg/mL	11.1 ± 5.4	10.7 ± 5.3

Abbreviations: AST, aspartate transaminase; BP, blood pressure; CHE, cholinesterase; eGFR, estimated glomerular filtration rate; GGT, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin; hsCRP, high-sensitive C-reactive protein; IMT, intima-media thickness; PON, paraoxonase.

[a]Data are presented as n (%) for categorical parameters and mean ±standard deviation (SD) or median [interquartile range (IQR)] for continuous parameters.

increased leptin) (23), chronic low-grade inflammation (24), and dyslipidemia, characterized by decreased HDL blood levels.

Our results showed a robust and independent correlation of adiponectin with CEC. This is of particular interest because recent studies have provided strong evidence that CEC, the first step in the process of reverse cholesterol transport, is inversely associated with incident coronary heart events, independent of established cardiovascular risk factors (5, 6, 20, 25). Uric acid has also been described as an excellent predictor of increased cardiovascular risk (26) and cardiovascular events, including all causes of death (27). Indeed, although ranked below adiponectin, uric acid remained significant in the multivariable modeling of our study. It will be interesting to clarify in future studies whether algorithms including adiponectin, uric acid, and CEC are useful for prognostic profiling.

We observed that IL-6 levels showed an independent and inverse association with CEC in juveniles but not in adults. IL-6 plays a critical role in the induction of proinflammatory serum amyloid A (28). Serum amyloid A rapidly interacts with HDL particles and negatively affects CEC (19) and might therefore affect CEC in juveniles who are *per se* affected with higher rates of infections, leading to activation of acute phase proteins.

Our results raise the possibility that adiponectin directly affects CEC in adults. However, further studies are needed to draw firm conclusions and to demonstrate causality and the underlying mechanisms. Previous studies reported a correlation between plasma adiponectin levels and lipoprotein concentrations. A positive association of adiponectin with levels of HDL cholesterol was reported in individuals with diabetes (11, 29) and in individuals without diabetes (16, 30).

A significant inverse association between adiponectin and plasma hepatic lipase activity was previously reported,

Table 2. Pearson Correlation Analysis With CEC

	<18 y		≥18 y	
	r	*P* value	*r*	*P* value
Age, y	0.01	0.921	−0.11	0.031
BMI, kg/m^2	−0.08	0.181	−0.20	**<0.001**[a]
IMT, cm	0.06	0.399	−0.10	0.146
Carotis diameter, cm	−0.13	0.054	−0.26	**<0.001**
Waist circumference, cm	−0.13	0.057	−0.27	**<0.001**
Waist-to-hip ratio	−0.08	0.224	−0.27	**<0.001**
Waist-to-height ratio	−0.10	0.122	−0.22	**<0.001**
Systolic BP, mm Hg	0.03	0.659	−0.15	0.003
Diastolic BP, mm Hg	−0.02	0.753	−0.08	0.093
Cholesterol, mmol/L	0.10	0.109	0.18	**<0.001**
Triglyceride, mmol/L	0.09	0.124	0.06	0.202
HDL cholesterol, mmol/L	0.19	0.002	0.46	**<0.001**
LDL cholesterol, mmol/L	−0.06	0.359	−0.04	0.487
Oxidized LDL, mmol/L	−0.09	0.152	−0.17	**0.001**
Glucose, mmol/L	0.08	0.209	−0.10	0.049
HbA1c, mmol/mol	0.04	0.531	−0.06	0.264
HOMA-IR	0.06	0.363	−0.11	0.036
Uric acid, ng/dL	−0.11	0.060	−0.26	**<0.001**
Fibrinogen, g/L	−0.04	0.678	−0.14	0.026
hsCRP, mg/L	−0.16	0.006	0.08	0.111
IL-6, ng/L	−0.17	0.007	0.02	0.631
AST, U/L	−0.05	0.380	−0.13	0.008
ALT, U/L	−0.05	0.411	−0.26	**<0.001**
GGT, U/L	−0.08	0.160	−0.05	0.312
CHE, U/L	−0.02	0.766	−0.22	**<0.001**
Alcaline phosphatase, U/L	0.04	0.466	−0.05	0.360
PON activity, nmol/min/mL	0.06	0.295	0.08	0.101
ApoA-I, mg/dL	0.39	**<0.001**	0.41	**<0.001**
Leptin, ng/mL	−0.12	0.054	−0.04	0.446
Adiponectin, μg/mL	0.06	0.362	0.32	**<0.001**

Abbreviations: AST, aspartate transaminase; BP, blood pressure; CHE, cholinesterase; eGFR, estimated glomerular filtration rate; GGT, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin; hsCRP, high-sensitive C-reactive protein; IMT, intima-media thickness; PON, paraoxonase; *r*, Pearson correlation coefficient.

[a]Bold *P* values are considered significant after Bonferroni correction.

suggesting that adiponectin may represent an important factor contributing to the regulation of hepatic lipase activity (31). Hepatic lipase functions as a lipolytic enzyme that hydrolyzes triglycerides and phospholipids in lipoproteins of intermediate and high density. It is thereby involved in the formation of small, dense LDL and represents a major determinant of the plasma HDL cholesterol concentration (32). In line with this study, a more recent report showed that adiponectin was strongly associated with HDL particle size and negatively associated with small HDL particles, which is in good agreement with the reported inverse association of hepatic lipase activity and adiponectin (25). The inhibitory effect of adiponectin on hepatic lipase activity might therefore explain the HDL cholesterol–elevating activity of adiponectin. It is well known that CEC from individuals with similar levels of HDL cholesterol or apolipoprotein A-I may have very different capacities to remove cholesterol from macrophages (5, 6, 20). Most importantly, the HDL phospholipid content is a major factor determining CEC (33). Hence, adiponectin-induced inhibition of hepatic lipase (and HDL–phospholipid hydrolysis) is expected to directly affect CEC of HDL.

Table 3. Regression Analysis of HDL CEC With Baseline Characteristics of the STYJOBS Cohort

Parameter	β (95% CI)	*P* value
≥18 y of age		
Adiponectin, μg/ml	0.11 (0.05 to 0.15)	<0.001
ALT, U/L	−0.02 (−0.03 to −0.004)	0.012
Uric acid, ng/dL	−0.20 (−0.39 to −0.02)	0.030
<18 y of age		
IL-6, ng/L	−0.17 (−0.30 to −0.05)	0.007

Resulting linear regression models after forward selection with the following parameters: age; sex; glucose; systolic blood pressure; and parameters of body fat (BMI or waist-to-hip ratio), inflammation (IL-6), liver function (ALT), and metabolism (adiponectin, uric acid).

In addition to the capacity of serum to accept cholesterol, cholesterol exporters expressed on macrophages protect from foam cell formation. Interesting in this regard, a previous study showed that adiponectin treatment significantly increased macrophage expression of the cholesterol exporter ATP-binding cassette transporter G1 without affecting protein expression of scavenger receptors (34). *In vitro* studies demonstrated that adiponectin markedly suppressed foam cell formation in macrophages, which was mainly attributed to an increase in ABCG1-mediated cholesterol efflux (34). Pharmacological or genetic inhibition of liver X receptor α blocked the adiponectin-mediated ABCG1 expression, suggesting that liver X receptor α activation inhibited lipid accumulation of macrophages by adiponectin.

The current study has some limitations. Our population was European, limiting the generalizability of our results outside of this population. Further, the correlations may explain only about 5% of the variation, and determining causality remains an objective. Nevertheless, any cross-sectional study can only show association and not causation, so these data support the need for further longitudinal studies.

In summary, our study demonstrates that hypoadiponectemia is an effective predictor for decreased CEC in adult normal-weight and overweight/obese participants. Thus, variation in adiponectin production may be a crucial modulator of the functionality of HDL, representing a mechanism for increased cardiovascular risk. Our results add to the current concept that anti-inflammatory factors such as adiponectin may play a central role in modulating obesity-related comorbidities.

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