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# Apolipoprotein J and Leptin Levels in Patients with Coronary Heart Disease

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**Abstract.** *Background: Preliminary data suggest that apolipoprotein J (ApoJ) may play a role in the development and progression of atherosclerosis. Leptin, an adipose tissue hormone, exerts important cardiovascular effects. The association between serum ApoJ and leptin concentrations was assessed in patients with established or suspected coronary heart disease (CHD). Patients and Methods: Serum ApoJ and leptin concentrations were evaluated in 67 CHD patients undergoing coronary angiography [54 individuals with significant (≥50%) coronary artery stenosis and 13 patients without significant coronary artery stenosis on angiography]. Results: Serum ApoJ concentrations in patients with significant coronary artery stenosis were significantly higher than in those without (303.9±118.6 vs. 121.2±37.5 μg/mL, respectively; p<0.001). The reverse pattern was observed for serum leptin levels (8.6±5.5 vs. 20.6±17.1 ng/ml, respectively; p=0.016). There was a significant negative correlation between ApoJ and leptin levels (r=–0 .353; p=0.003). Conclusion: ApoJ and leptin may be markers for CHD.*

Coronary heart disease (CHD) is a leading cause of morbidity and mortality worldwide (1, 2). The development of noninvasive biomarkers holds implications for the early and more accurate identification of individuals with coronary atherosclerosis, as well as a better understanding of the underlying pathology.

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Apolipoprotein J (ApoJ), also known as clusterin, is a heterodimeric glycoprotein (75-80 kDa) expressed in several tissues and present in all body fluids (3, 4). ApoJ has been implicated in a wide range of physiological and pathophysiological cellular processes, such as reverse lipid transport and redistribution, apoptosis, folding of damaged extracellular proteins (chaperone), cell adhesion and aggregation, membrane recycling, complement regulation, tissue remodeling, tumorigenesis and several age related diseases (*e.g.* atherosclerosis and Alzheimer's disease) (5, 6). In blood, ApoJ is associated with high-density lipoprotein (HDL) particles (7, 8) and forms complexes with apolipoprotein A-I (9) and the human esterase paraoxonase (10). These complexes regulate the transport and local redistribution of lipids. Serum ApoJ concentrations are increased in experimental models of diet-induced atherosclerosis, as well as in patients with diabetes mellitus or CHD (11-14). ApoJ was detected in early atherosclerotic lesions found in the aortic valve region of a mouse model of diet induced atherosclerosis (15) and in human atherosclerotic plaques (16, 17). However, the pathophysiological role of ApoJ in atherosclerosis is still poorly understood.

Leptin is a multifunctional peptide hormone synthesized and released by adipocytes that plays an important role in body weight regulation (18-21). However, leptin has pleiotropic actions including regulation of vascular function, insulin resistance, platelet aggregation and angiogenic effects; this suggests that leptin may play a role in the development of cardiovascular diseases (19-21). The results of two nested case-referent studies suggest that leptin might be a risk factor for myocardial infarction and stroke (22, 23). It was proposed that leptin is an independent predictor of CHD (24-26).

We evaluated the association between serum ApoJ and leptin concentrations in patients with/without angiographically determined coronary artery stenosis.

## Patients and Methods

*Study participants*. A total of 183 Caucasian patients undergoing coronary angiography for the evaluation of established or suspected CHD at the Department of Cardiology, Athens Medical Center, Athens, Greece were recruited over a period of 2 years. After implementing the exclusion criteria, 67 individuals were considered for further analysis. Exclusion criteria were an indication of heart or renal failure, hepatic damage, chronic infection or inflammatory disease (*e.g.* injury or arthritis), malignancy and any autoimmune or hematological disorder. Furthermore, no selected patient had sustained a myocardial infarction, acute coronary syndrome or undertaken any major surgery within 2 months prior to coronary angiography. Those participating in the study also needed to give their approval for the additional fasting blood sample (see below).

The selected patients were subsequently divided into two subgroups: group A included individuals with significant (≥50%; n=54) coronary artery stenosis on angiography, while group B consisted of participants without significant coronary artery stenosis on angiography (n=13). There were no significant differences in terms of gender, age, body mass index (BMI), blood pressure, positive family history for CHD, prevalence of smoking, presence of type 2 diabetes mellitus or cardiovascular medication between the two subgroups. Furthermore, there were no significant differences in total cholesterol, low-density lipoprotein (LDL) cholesterol or blood glucose levels between the two subgroups. Patient classification into the two subgroups was carried out retrospectively after reviewing the angiograms, but with assessors blinded to serum ApoJ and leptin levels.

A short questionnaire regarding conventional cardiovascular risk factors was obtained at baseline from all participants. Smoking was defined as any history of regular smoking. Blood pressure was measured under resting conditions at least 6 h after hospital admission. Hypertension was considered if the patient was taking anti hypertensive treatment or if a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg measurement were recorded at least twice. Diabetes mellitus was diagnosed according to the World Health Organization criteria (27) or if the patient was on antidiabetic medications. A positive family history of CHD was defined as any first-degree relative with confirmed CHD (<55 years in males; <65 years in females). Height and weight were recorded and the body mass index (BMI) was calculated [BMI=body weight (kg)/height $(m)^2$]. All medications at the time of hospital admission were recorded.

Coronary angiography was performed by the standard Judkins technique using a semiquantitative coronary angiographic system. This method provides a quantification of the atherosclerotic burden. The angiograms were assessed by two observers blinded to the serum ApoJ, leptin levels or other risk factors. The level of agreement between the two observers was >90%. Disagreements were resolved by discussion between the two reviewers.

The Ethics Committee of the Medical School of the University of Athens approved the present study and written informed consent was obtained from all participants.

*Collection of blood samples*. Venous blood, obtained after overnight fasting and 2 h before coronary angiography, was collected in the absence of anticoagulant into Wasserman® tubes and allowed to clot for 30 min at 4˚C. Serum was isolated from blood by centrifugation at 3000g for 15 min at 4˚C. The serum was stored at –80˚C and analyzed within 2 months from collection.

*Measurement of biochemical variables*. Total serum cholesterol was determined by the cholesterol oxidase peroxidase-amidopyrine (CHOD-PAP) method (Biosis, Athens, Greece). Following precipitation of other lipoproteins with heparin and $MnCl_2$, serum HDL cholesterol was measured using a commercially available kit (Roche, Manheim, Germany). Serum triglycerides were measured by the enzymatic glycerol-3-phosphate-oxidase peroxidase-amidopyrine (GPO-PAP) method (Biosis). Fasting blood glucose levels were determined enzymatically by the hexokinase method (Biosis). All the aforementioned biochemical measurements were performed using a Technicon RA-XT automated analyzer (Technicon Instruments, Tanytown, NT, USA). LDL cholesterol was estimated by the Friedewald formula: LDL cholesterol=total serum cholesterol – (HDL cholesterol + triglycerides/5) mg/dL.

C-reactive protein (CRP) was determined by a sensitive sandwich enzyme immunoassay (CRP ELISA kit; Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The detection limit of this assay was 0.124 mg/l and there was no cross-reactivity with other acute phase proteins. The intra-assay and interassay precision variations were 5.8% and 12.7%, respectively. Each sample was assayed in duplicate.

*Determination of serum ApoJ concentrations*. Serum ApoJ concentrations were determined using an in-house sandwich ELISA with monoclonal and polyclonal antibodies to human clusterin (both purchased from Santa Cruz Biotechnologies, USA) and pure ApoJ used as standards, as previously described (11, 12, 28). A calibration curve was obtained, using purified ApoJ at concentrations ranging from 80 to 1,000 μg/ml. Serum samples were assayed in duplicate.

*Determination of serum leptin concentrations*. Serum leptin concentrations were measured using a commercially available sandwich ELISA (Human Leptin "Dual Range" ELISA, Linco Research Inc., Missouri, USA). The detection limit was 0.5ng/ml and there was no cross-reactivity with other cytokines. Each sample was assayed in duplicate. The intra-assay and interassay variation coefficients were 2.6-4.6% and 2.6-6.2%, respectively.

*Statistical analysis*. Data are expressed as mean±1 standard deviation (SD) for continuous variables and as percentages for categorical data. The Kolmogorov-Smirnov test was used to assess whether the distribution of variables followed a Gaussian pattern. Comparisons of continuous variables were analyzed using the Mann-Whitney *U*-test. Differences in categorical variables were tested for significance with the chi-squared test. Spearman rank correlation coefficients were calculated as a measure of correlation between continuous variables. Independent associations with the presence of significant coronary artery stenosis were evaluated by multivariate logistic regression analysis. Significance was defined as two-tailed $p$-value <0.05. Statistical analyses were performed with the software package SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

## Results

The demographic characteristics of the study population are listed in Table I.

Serum ApoJ concentrations were significantly higher in men than in women (298.1±131.7 *vs.* 198.8±96.3 μg/ml, respectively; $p$=0.005). There was a trend for an increase in

Table I. *Characteristics of the study population (n=67).*

| | |
|---|---|
| Male gender (%) | 70.1 |
| Age (years) | 64.4±8.4 |
| Body mass index (kg/m$^2$) | 28.8±6.2 |
| Hypertension (%) | 50.7 |
| Smoking (%) | 40.3 |
| Type 2 diabetes mellitus (%) | 25.4 |
| Positive family history (%) | 23.9 |
| Systolic blood pressure (mmHg) | 135±21 |
| Diastolic blood pressure (mmHg) | 77±8 |
| Total cholesterol (mg/dl) | 169±42 |
| Triglycerides (mg/dl) | 136±81 |
| HDL-cholesterol (mg/dl) | 35±10 |
| LDL-cholesterol (mg/dl) | 107±37 |
| Cholesterol/HDL ratio | 5.1±1.5 |
| Fasting glucose (mg/dl) | 100±14 |
| ApoJ (μg/ml) | 268.4±129.8 |
| Leptin (ng/ml) | 10.9±10.0 |
| CRP (mg/l) | 4.8±4.7 |
| ASA (%) | 65.7 |
| Beta-blockers (%) | 50.7 |
| ACE inhibitors (%) | 28.4 |
| Statins (%) | 52.2 |

All values are reported as mean±standard deviation (SD), absolute number (n) or as perecentage (%); LDL: low-density lipoprotein, HDL: high-density lipoprotein, ApoJ: apolipoprotein J, CRP: C-reactive protein, ASA: acetylsalicylic acid, ACE inhibitors: angiotensin-converting enzyme inhibitors, CHD: coronary heart disease.

Table II. *Spearman correlation coefficients for circulating ApoJ and leptin concentrations in patients with coronary heart disease.*

| Variables | Leptin | | ApoJ | |
|---|---|---|---|---|
| | rho | *p*-value | rho | *p*-value |
| Leptin | 1 | | **–0.353** | **0.003** |
| Age | –0.142 | 0.253 | 0.159 | 0.200 |
| Body mass index | **0.457** | **<0.001** | –0.133 | 0.282 |
| Systolic blood pressure | 0.125 | 0.315 | –0.031 | 0.804 |
| Diastolic blood pressure | 0.063 | 0.611 | –0.050 | 0.687 |
| Total cholesterol | 0.021 | 0.869 | –0.057 | 0.649 |
| Triglycerides | **0.278** | **0.023** | 0.122 | 0.325 |
| HDL-cholesterol | 0.123 | 0.323 | **–0.320** | **0.008** |
| LDL-cholesterol | –0.099 | 0.427 | 0.056 | 0.651 |
| Cholesterol/HDL ratio | –0.134 | 0.278 | **0.325** | **0.007** |
| Fasting glucose | **0.290** | **0.017** | –0.169 | 0.173 |
| CRP | –0.116 | 0.348 | **0.557** | **<0.001** |

All values are reported as mean±standard deviation (SD), absolute number (n) or as perecentage (%); LDL: low-density lipoprotein, HDL: high-density lipoprotein, ApoJ: apolipoprotein J, CRP: C-reactive protein, ASA: acetylsalicylic acid, ACE inhibitors: angiotensin-converting enzyme inhibitors, CHD: coronary heart disease.

serum ApoJ concentrations in smokers compared with non-smokers (298.9±139.4 *vs*. 247.9±120.4 μg/ml, respectively; $p$=0.079), but this difference was not significant. Serum ApoJ concentrations were similar in diabetic and non-diabetic patients (274.7±156.8 *vs*. 266.3±121.1 μg/ml, respectively; $p$=0.954), as well as in normotensive and hypertensive patients (277.1±117.6 *vs*. 260.0±142.0 μg/ml, respectively; $p$=0.272).

Serum leptin concentrations were significantly lower in men than in women (8.3±6.6 *vs*. 17.2±13.6 ng/ml, respectively; $p$=0.001). Smokers had lower serum leptin levels than non-smokers (8.3±6.0 *vs*. 12.7±11.8 ng/ml, respectively; $p$=0.109) but this difference was not significant. Serum leptin levels were similar in diabetic and non-diabetic patients (13.4±14.9 *vs*. 10.1±7.8 ng/ml, respectively; $p$=0.589) and in normotensive and hypertensive patients (10.6±8.5 *vs*. 11.3±11.4 ng/ml, respectively; $p$=0.985).

There were no significant differences in serum ApoJ (289.3±149.3 *vs*. 245.6±102.1 μg/ml, respectively; $p$=0.359) or leptin concentrations (10.9±11.4 *vs*. 10.9±8.5 ng/ml, respectively; $p$=0.841) between patients on statins compared with those not taking statins.

Serum ApoJ positively correlated with the total cholesterol/HDL cholesterol ratio and CRP, and inversely with serum HDL cholesterol and leptin values. Correlations of serum ApoJ are shown in Table II. Serum leptin levels correlated positively with BMI, triglycerides and fasting blood glucose and inversely with serum ApoJ levels.

The independent association between serum ApoJ concentrations and other variables was analysed by using stepwise multiple regression analysis with serum ApoJ as the dependent variable and age, gender, BMI, HDL, LDL, triglycerides, fasting serum glucose, CRP and serum leptin as the independent variables. CRP (B=1.68±0.24, Beta=0.61, $p$<0.001) and serum leptin levels (B= –3.67±1.14, Beta= –0.28, $p$=0.002) (R2=51.1% , $p$<0.001) were independent predictors of increased serum ApoJ levels.

In patients with significant coronary artery stenosis, serum ApoJ concentrations were significantly higher compared with patients without stenosis (303.9±118.6 *vs*. 121.2±37.5 μg/ml, respectively; $p$<0.001). Table III summarizes the characteristics of our study cohort with respect to the presence or absence of significant coronary artery stenosis.

By multivariate logistic regression analysis, serum ApoJ levels remained significantly and positively associated with the presence of significant coronary artery stenosis after adjustment for age and gender ($p$<0.001). Further adjustment for diabetes, hypertension, smoking, BMI, total serum cholesterol, HDL cholesterol and LDL cholesterol did not affect the independent positive association between serum ApoJ concentrations and the presence of significant coronary artery stenosis ($p$<0.001). Moreover, the positive association between serum ApoJ values and coronary artery stenosis remained significant after adjustment for cardiovascular medications [acetylsalicylic acid (aspirin), beta-blockers, angiotensin-converting enzyme (ACE) inhibitors and statins; $p$=0.001].

Table III. *Analysis of the cohort with respect to the presence (CAD+) or absence (CAD–) of significant (≥50%) coronary artery stenosis.*

| | CAD+ | CAD– | *p*-value |
|---|---|---|---|
| n | 54 | 13 | |
| Male gender (%) | 74.1 | 53.8 | 0.185 |
| Age (years) | 64.6±8.1 | 63.3±9.6 | 0.617 |
| Body mass index (kg/m$^2$) | 28.6±5.2 | 29.7±9.5 | 0.623 |
| Hypertension (%) | 51.9 | 46.2 | 0.952 |
| Smoking (%) | 40.7 | 38.5 | 0.880 |
| Type 2 diabetes mellitus (%) | 25.9 | 23.1 | 0.832 |
| Positive family history (%) | 24.1 | 23.1 | 0.940 |
| Systolic blood pressure (mmHg) | 135±21 | 135±20 | 0.898 |
| Diastolic blood pressure (mmHg) | 78±7 | 76±9 | 0.485 |
| Total cholesterol (mg/dl) | 165±43 | 188±37 | 0.068 |
| Triglycerides (mg/dl) | 145±87 | 99±32 | 0.014 |
| HDL-cholesterol (mg/dl) | 31±6 | 52±8 | 0.0001 |
| LDL-cholesterol (mg/dl) | 104±36 | 116±40 | 0.326 |
| Cholesterol/HDL ratio | 5.4±1.5 | 3.7±0.9 | 0.0001 |
| Fasting glucose (mg/dl) | 99±14 | 102±16 | 0.733 |
| ApoJ (μg/ml) | 303.9±118.6 | 121.2±37.5 | 0.0001 |
| Leptin (ng/ml) | 8.6±5.5 | 20.6±17.1 | 0.016 |
| CRP (mg/l) | 5.8±4.7 | 0.6±0.5 | 0.0001 |
| ASA (%) | 68.5 | 53.8 | 0.344 |
| Beta-blockers (%) | 51.9 | 46.2 | 0.952 |
| ACE inhibitors (%) | 31.5 | 15.4 | 0.321 |
| Statins (%) | 55.6 | 38.5 | 0.425 |

All values are reported as mean±standard deviation (SD), absolute number (n) or as perecentage (%); CAD+: significant (≥50%) coronary artery stenosis, CAD–: no significant coronary artery stenosis; other abbreviations as in Table I.

Patients with significant coronary artery stenosis had lower serum leptin concentrations compared with those individuals without coronary stenosis (8.6±5.5 *vs.* 20.6±17.1 ng/ml, respectively; $p$=0.016). After adjustment for age and gender, serum leptin levels remained significantly and inversely associated with the presence of significant coronary artery stenosis ($p$<0.001). In the fully adjusted model, the independent inverse association between serum leptin levels with the presence of significant coronary artery stenosis remained significant ($p$=0.022).

Finally, CRP levels were elevated in patients with significant coronary artery stenosis compared with those without (5.8±4.7 *vs.* 0.6±0.5 mg/l, respectively; $p$<0.001).

## Discussion

In the present study, an association between ApoJ, leptin and coronary artery stenosis was demonstrated in a cohort of patients undergoing coronary angiography. Our findings show that serum ApoJ levels are positively associated with coronary artery stenosis. In contrast, serum leptin levels are inversely associated with coronary artery stenosis.

Our results are consistent with previous studies supporting an association between serum ApoJ concentrations and CHD (11, 12). Several studies hold that ApoJ is implicated in various cardiovascular diseases (*e.g.* atherosclerosis, CHD and myocardial infarction) (12-17). ApoJ accumulation in the coronary artery wall during the progression of atherosclerosis is accompanied by a significant increase in serum ApoJ levels (12-14). Even though the exact mechanism by which ApoJ is associated with CHD remains incompletely understood, our findings strengthen the hypothesis that serum ApoJ levels may play a role in coronary atherosclerosis.

A positive association was observed between serum ApoJ and CRP levels. Several studies showed that CRP is an independent risk factor for CHD (29-33). CRP is a marker of atherosclerosis; its production increases during chronic vascular inflammatory that occurs in response to vascular injury induced by various stimuli (*e.g.* oxidative stress, shear stress or infection) (29-33). ApoJ exhibits antiinflammatory properties (34). Thus, the presence of vascular inflammation may enhance ApoJ expression. This suggests that ApoJ may be involved in the vascular defence against vascular inflammation and injury (5, 6).

Serum leptin concentrations were reduced in patients with significant coronary stenosis compared with patients without. Our findings are in agreement with a study involving patients with angiographically determined CHD (26), where low serum leptin levels were associated with increased susceptibility to coronary atherosclerosis. In our study, the similar BMI and blood glucose values in the two subgroups, excludes the possibility that the lower serum leptin levels in patients with significant coronary artery stenosis can be attributed to these variables (35, 36).

Previous studies reported that statins lower serum leptin levels in hypercholesterolemic rabbits and in patients with accelerated atherosclerosis (37, 38). In our study there were no significant differences between the two subgroups based on the use of statins. Therefore, the reduction in serum leptin levels in patients with significant coronary artery stenosis is unlikely to be attributed to statins.

The mechanisms underlying the observed association between increased ApoJ and decreased serum leptin levels in patients with significant coronary artery stenosis are unknown. Leptin stimulates vascular smooth muscle cell proliferation (39), enhances vascular calcification (40), induces oxidative stress in vascular endothelial cells (41) and enhances platelet aggregation (42); these mechanisms contribute to atherogenesis. In contrast, ApoJ is associated with inhibition of vascular smooth muscle cell proliferation, migration and adhesion (43). ApoJ can also respond to inflammatory or oxidative stress stimuli and protect vascular tissue from damage via its antiinflammatory and antiapoptotic properties (6, 44, 45). The presence of established CHD may thus be characterized by an enhanced

expression and secretion of ApoJ and the pro-atherosclerotic effects of leptin. A direct interaction of leptin with ApoJ has already been demonstrated (46). The effects of this association on the pathogenesis of CHD remain to be determined.

In summary, our study provides evidence that raised serum ApoJ and decreased leptin concentrations are associated with significant coronary artery stenosis and may therefore serve as markers of CHD. Verification of these preliminary findings holds implications for a better understanding of the pathogenesis of CHD.

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