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Research article

High plasma leptin levels are associated with impaired diastolic function in patients with coronary artery disease☆

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ABSTRACT

Background and aims: Obese subjects have elevated leptin levels, which have been associated with increased risk of cardiovascular events. Because leptin has direct cellular effects on various tissues, we tested the hypothesis that leptin levels are associated with cardiac structure or function in patients with coronary artery disease (CAD).

Methods and results: The study population consisted of 1 601 CAD patients, of whom 42% had type 2 diabetes mellitus. Plasma leptin was measured in fasted state and an echocardiography performed. Leptin levels were not related to LV dimensions or LV ejection fraction (NS for all), but higher leptin levels were associated with elevated E/E' (9.43 vs. 11.94 in the lowest and the highest leptin quartile, respectively; p = 0.018 for trend). Correspondingly, a decreasing trend was observed in E/A (1.15 vs. 1.06; p = 0.037). These associations were independent of obesity and other relevant confounding variables.

Conclusion: We conclude that elevated plasma leptin levels are associated with impaired left ventricular diastolic function in patients with CAD independently of obesity and other confounding variables. Leptin may be one of the mechanistic links explaining the development of congestive heart failure in obese subjects.

1. Introduction

Leptin is a 16-kDa peptide hormone encoded by the *ob* gene. It was discovered in the 1990s and first identified as expressed and secreted by adipose tissue [1]. Leptin is an important regulator of human energy homeostasis. Circulating levels of leptin are usually increased in obesity [2], which has led to the hypothesis that leptin works as a signal of adiposity to the brain in order to regulate body weight [3]. In addition to adipose tissue, leptin and its receptor isoforms are expressed in various other tissues. These include cardiovascular tissues, such as endothelial cells [4], smooth muscle cells [5], and cardiomyocytes [6]. These findings suggest that leptin has specific effects on the myocardium [7].

Considering cardiac structure and function, leptin levels have been suggested to be associated with left ventricular hypertrophy [8], increased myocardial wall thickness [9], and impaired cardiac contractile function [10]. In addition, *ob/ob* mice have displayed diastolic dysfunction coincided with an increase in myocardial collagen upon leptin treatment, which indicates that leptin has profibrotic properties [11]. Indeed, elevated leptin levels have been associated with heart failure in patients with both reduced and preserved ejection fraction [12–14]. Recently, Fontes-Carvalho et al. (2015) showed that high leptin levels are independently associated with diastolic dysfunction in the general population, especially in women [15].

Thus, leptin exhibits a wide range of cardiovascular effects and is associated with cardiovascular outcome. The associations between leptin and cardiovascular diseases could emerge from direct cellular effects in both cardiac and vascular tissues. To our knowledge, the association between leptin levels and cardiac structure and function has not been studied in large coronary artery disease patient cohorts. Therefore, we tested the hypothesis that leptin levels are associated with cardiac structure or function in patients with coronary artery disease (CAD).

☆ Study registered at ClinicalTrials.gov, Record 1539/31/06, Identifier NCT01426685.

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2. Material and methods

2.1. Study population

The present study is a part of the ARTEMIS study (Cardiovascular Complications in Type II Diabetes Study; registered at ClinicalTrials.gov, Record 1539/31/06, Identifier NCT01426685). The study population consisted of 1 601 coronary artery disease (CAD) patients, of whom 42% had type 2 diabetes mellitus, 22% had pre-diabetes (impaired fasting glucose or impaired glucose tolerance), and 36% were non-diabetic. The patients were recruited from the consecutive series of patients undergoing coronary angiography in the Division of Cardiology of the Oulu University Hospital. Patients with angiographically diagnosed coronary artery disease (at least 50% stenosis in a coronary artery) were included in the study. The diabetes and non-diabetes groups were matched in terms of the following variables: sex, age, history of myocardial infarction, and type of coronary intervention after angiography. A flow chart of the matching procedure can be found in a previous article from the same study group [16].

Exclusion criteria were New York Heart Association (NYHA) Functional Classification class IV despite appropriate treatment of heart failure, pacemakers or planned pacemaker implantation, participation in a competing clinical trial that was not accepted by the Steering Committee, physical or psychological unfitness for participation in the study according to the opinion of the investigator, doubtful compliance, geographical or other inaccessibility for follow-up, pregnancy, life expectancy less than one year, end-stage renal failure requiring dialysis or plasma creatinine > 250 μmol/l, age less than 18 years, or a malignancy (except for breast or prostate cancer in remission). The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the Regional Ethics Committee of the Northern Ostrobothnia Hospital District. Informed consent was obtained from the subjects.

2.2. Laboratory measurements and echocardiography

After fulfilling the inclusion and exclusion criteria, the patients underwent extensive risk profiling at the baseline. Before inclusion in the study, the patients without known diabetes underwent a 2-h oral glucose tolerance test (OGTT) to exclude diabetes and abnormal glucose tolerance. Diabetes was defined as fasting capillary plasma glucose levels $\geq$ 7.0 mmol/l or a 2-h post-load value in the OGTT $\geq$ 12.2 mmol/l or both, according to the WHO definition and diagnostic criteria for diabetes mellitus and intermediate hyperglycaemia. Impaired glucose tolerance was defined as fasting capillary plasma glucose < 7.0 mmol/l and 2-h glucose $\geq$ 8.9 mmol/l but < 12.1 mmol/l. Impaired fasting glucose was defined as fasting capillary plasma glucose $\geq$ 6.1 mmol/l but < 6.9 mmol/l and 2-h glucose < 8.9 mmol/l.

Body weight was measured with a digital scale. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference, rounded to the nearest 0.5 cm, was measured with a tape measure midway between the lower rib margin and the iliac crest in light expirium. Arterial blood pressure was measured in a sitting position from the right arm with an oscillometric device (Dinamap® Model 18465X, Criticon Ltd, Ascot, UK) after a 12-h overnight fast and a rest of 10–15 min. Three blood pressure measurements were performed at 1-min intervals and the mean of the last two was used in the analyses.

Venous blood samples were drawn into EDTA tubes after a 12-h overnight fast using standardized methods. Plasma leptin levels were determined by a commercial enzyme-linked immunosorbent assay kit (BioVendor; Cat No: RD 191001100, USA). Capillary glucose was determined by glucose oxidase method with the OneTouch® UltraEasy® test strips with FastDraw™ (LifeScan, Switzerland). High-sensitivity C-reactive protein was determined by immunonephelometry (BN ProSpec® and CardioPhase®, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Plasma lipids and creatinine were analysed by the laboratory of Oulu University Hospital.

Two-dimensional, M-mode, and Doppler echocardiographies were performed according to the American Society of Echocardiography (ASE) guidelines by three cardiologists utilising a General Electric Vivid 7 ultrasound machine. The echocardiographic parameters chosen for further study were left ventricular mass index (LVMI), left ventricular ejection fraction (EF), tissue Doppler-derived peak early diastolic septal mitral annular velocity (E'), ratio of peak early diastolic mitral velocity to tissue Doppler-derived peak early diastolic mitral annular velocity (E/E'), ratio of peak early mitral velocity to peak atrial velocity (E/A), ratio of E-wave velocity-time integral to A-wave velocity-time integral (Ei/Ai), left atrial diameter (LA), isovolumic relaxation time (IVRT), velocity-time integral of systolic (Sint) and diastolic (Dint) flow of the pulmonary vein, left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), and septal wall thickness. Left ventricular mass was calculated using the formula recommended by the American Society of Echocardiography (LV mass = $0.8 \times (1.04 \quad ((\mathrm{LVIDd} + \mathrm{PWTd} + \mathrm{SWTd})^3 - (\mathrm{LVIDd})^3)) + 0{,}6$ g). Left ventricular mass index (LVMI) was calculated by dividing LV mass by both body surface area (BSA) and squared height.

2.3. Statistical analyses

Statistical analyses were performed using IBM® SPSS® Statistics for Windows, Version 19 software (IBM Corp., Armonk, NY). Correlations between leptin and the chosen variables were analysed with univariate analysis of covariance (ANCOVA) or chi-square test. Fasting plasma levels of leptin were divided into quartiles. Characteristics and general cardiovascular risk factors were adjusted for age, sex, and BMI except for BMI, which was adjusted for age and sex. Echocardiographic parameters were adjusted for age, sex, body mass index (except for left ventricular mass index derived from the BSA formula), waist-hip ratio, heart rate during echocardiography, systolic blood pressure, high-sensitivity C-reactive protein, stage of the glucose metabolism disorder, history of myocardial infarction, and history of revascularisation (coronary artery bypass graft surgery or percutaneous coronary intervention). In the case of left ventricular mass index derived from the height formula, ANCOVA analyses were adjusted for age, sex, weight, waist-hip ratio, heart rate during echocardiography, systolic blood pressure, high-sensitivity C-reactive protein, stage of the glucose metabolism disorder, history of myocardial infarction, and history of revascularisation (CABG or PCI). Significant interaction terms of the nominal variables were included in the adjustments. P values of less than 0.05 were considered significant.

3. Results

Characteristics and general cardiovascular risk factors of the subjects are shown in Table 1. The proportion of diabetic subjects, age, sex, BMI, waist circumference, hip circumference, waist-hip ratio, plasma total and HDL cholesterol, plasma triglycerides, plasma high-sensitivity C-reactive protein ($p < 0.001$ for all) as well as LDL cholesterol ($p = 0.008$), plasma creatinine ($p = 0.009$), mean heart rate obtained from Holter monitoring ($p < 0.001$), and systolic blood pressure ($p = 0.006$) differed significantly between leptin quartiles before adjustments. The proportion of patients in different Canadian Cardiovascular Society grades of angina pectoris varied significantly between leptin quartiles. As expected, female subjects were overrepresented in the highest leptin quartile.