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Association between reduced brain-derived neurotrophic factor concentration & coronary heart disease

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***Background & objectives*: Brain-derived neurotrophic factor (BDNF) facilitates neuronal survival, differentiation and synaptic connectivity and affects neurotransmission throughout the brain. However, it has also a modulatory role in energy homeostasis, obesity and cardiovascular function. Obesity, high body mass index (BMI) and dyslipidaemia, among other factors, contribute to coronary heart disease (CHD) development. The exact role of BDNF in development of CHD is not well defined. This study was aimed to evaluate if plasma BDNF concentration was associated with CHD in ethnically homogeneous groups of patients and to correlate plasma BDNF levels with known risk factors for CHD.**

***Methods*: Plasma BDNF concentration, *BDNF* Val66Met polymorphism and other biological and anthropological risk factors for CHD were determined in 208 patients with CHD and 156 healthy controls.**

***Results*: Plasma BDNF concentration was significantly ($P<0.01$) reduced in patients with CHD compared to controls, and it was not influenced by gender, age, smoking or *BDNF* Val66Met polymorphism. It was considerably correlated with cholesterol ($P=0.004$), low-density lipoprotein ($P=0.006$), and diastolic blood pressure ($P=0.018$) in patients with CHD and with platelet number ($P=0.003$) in healthy controls.**

***Interpretation & conclusions*: The results revealed lower plasma BDNF concentration in patients with CHD, suggesting that decreased plasma BDNF concentration might be associated with CHD pathogenesis. Longitudinal studies with a large sample need to be conducted to confirm these findings.**

Key words Body mass index - brain-derived neurotrophic factor - Caucasians - coronary heart disease - lipid profile - risk factors

Obesity, high body mass index (BMI), elevated energy intake and reduced energy consumption contribute to the development of metabolic syndrome, coronary heart disease (CHD), dyslipidaemia, atherosclerosis and diabetes mellitus type II[1-4]. Adiposity measures, altered lipids, high BMI and waist to height ratio (WHtR) are known risk factor for CHD[5]. However, its complicated molecular basis is assumed to be connected to various bioactive compounds and altered biological pathways, including

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brain-derived neurotrophic factor (BDNF). BDNF has diverse important roles in facilitating neuronal survival, connectivity and plasticity[6]. Besides neurotrophic effects, BDNF also modulates energy homeostasis/balance and cardiovascular regulation[6,7] and is involved in the development of obesity[6]. In the periphery, BDNF is mostly stored in platelets, and the changes of peripheral BDNF concentrations may be a consequence of platelet activation[8], which plays an important role in the formulation of platelet thrombus and diseases of the coronary arteries. In various genetic mouse models the cardio-protective roles of BDNF have been demonstrated[9]. BDNF has been reported to be associated with cardio-metabolic diseases[10-12].

The objective of the present study was to evaluate the association of the BDNF concentration with CHD. The secondary aim was to evaluate whether other risk factors such as gender, age, BMI, WHtR, lipid profile or *BDNF* Val66Met polymorphism were associated with the plasma BDNF concentration in Caucasian CHD patients and healthy controls.

Material & Methods

The study included a consecutive sample of 364 Caucasian individuals: 208 patients with CHD and 156 healthy controls, of Croatian origin. Diagnosis, screening (physical examination, assessment of blood pressure and electrocardiogram) and sampling of patients with CHD was done during four months (from March 18, 2014 to July 18, 2014), in the University Hospital Thalassotherapia Opatija, Clinics for Treatment, Rehabilitation and Prevention of Cardiovascular Diseases; Opatija, Croatia. CHD was diagnosed using the International Classification of Diseases-tenth revision criteria, categories I20-I25 (angina pectoris, myocardial infarction, current complications following myocardial infarction and chronic ischaemic heart disease)[13]. The criteria for diagnosing CHD included >50 per cent stenotic lesions in at least one major coronary vessel determined by coronary angiography or multi-slice computed tomography (MSCT), myocardial infarction, coronary stent implantation and coronary artery bypass surgery. Patients with CHD had stable (n=177) or unstable (n=31) angina. The unstable angina (considered as a type of acute coronary syndrome) group consisted of patients with chest pain occurring at rest or minimal exertion. These patients had significant coronary artery disease, diagnosed with MSCT, coronary angiography or invasive coronary angiography (with stenotic lesions >70<100%), and fulfilled only coronary stent implantation criterion. The stabile angina group consisted of patients with mild chest discomfort after coronary revascularization. Most of the patients had heart failure with preserved left ventricle ejection function (LEVF ≥50%; n=159) ejection fraction, but there were also patients with moderately (40%≤ LVEF ≤49%; n=40) and severely (LVEF <40%; n=9) reduced ejection fraction. Exclusion criteria for CHD individuals included neurodegenerative and neuropsychiatric disorders, the use of antidepressants and anxiolytic medication, acute infections and inflammations. Control individuals (N=156 healthy controls) were screened during routine physical check-ups in the same hospital. Exclusion criteria were CHD, neurodegenerative and neuropsychiatric disorders, the current use of antidepressants, anxiolytic medication, acute infections and inflammations.

The study protocol was approved by the Ethics Committee of the School of Medicine, University of Zagreb, Zagreb, Croatia, and all participants signed written informed consent before participation.

Anthropological measures: Measurement of height was done without shoes to the nearest 0.5 cm, and body weight with a digital scale; calculation of BMI was done as weight (kg) over height (m^2); waist circumference was determined with inelastic measuring tape, to the nearest 0.1 cm, at the end of normal expiration, in a standing position at the level of the umbilicus (midway between the lowest rib and the superior border of the iliac crest). Calculation of WHtR was done by waist circumference (cm) divided by height (cm). Hypertension was defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or having used antihypertensive medication for the previous nine years.

Biological measures: Blood (8 ml) was drawn between 0730 h and 0800 h after overnight fasting. Determination of total cholesterol (normal values <5 mmol/l) was done with cholesterol oxidase-phenol+aminophenazone and readings on a Dimension Xpand Plus Integrated Chemistry System (Siemens Healthcare GmbH, Germany). Determination of triglycerides (normal values <1.7 mmol/l), low-density lipoprotein (LDL), cholesterol (normal values <3 mmol/l) and high-density lipoprotein (HDL) cholesterol (normal values >1.2 mmol/l) was done using a Dimension Xpand Plus Integrated Chemistry System. Glucose (referral values 4.4-6.4 mmol/l) was determined using spectrophotometric determination using hexokinase (Glucose System Reagent 800,

Olympus, Japan) and was read on a Dimension Xpand Plus Integrated Chemistry System. Insulin (referral values 18-173 pmol/l) was determined with a chemiluminescent microparticle immunoassay (ARCHITECT® insulin assay, Abbott Core Laboratory, USA) and was read on an ARCHITECT i-1000SR immunoassay analyzer (Abbott Core Laboratory, USA). The homeostasis model assessment (HOMA) index[14] (referral values 0.40-1.8) was calculated. The high sensitivity C-reactive protein (hsCRP) (referral values 0.0-5.0 mg/l) was determined using a particle-enhanced turbidimetric immunoassay[15] and was read on a Siemens Dimension Xpand Plus Integrated Chemistry System.

BDNF plasma concentration: Assay of plasma BDNF concentration was measured with enzyme-linked immunosorbent assay (ELISA) based on the manufacturer's instructions (Quantikine ELISA, R&D Systems, Minneapolis, Minnesota, USA), in duplicates. Coefficients of variations (CV), both intra- and inter-assay CV, were <10 per cent.

Genotyping of the BDNF Val66Met polymorphism: The *BDNF* Val66Met polymorphism (rs6265) was genotyped as described previously by using Real-Time PCR System 7300 (Applied Biosystems, USA)[16], from DNA isolated using the salting out method, with TaqMan® SNP Genotyping Assay (Applied Biosystems, USA), according to instructions from manufacturer.

Statistical analysis: Evaluation of results was done with Sigma Stat 3.5 (Jandel Scientific Corp., CA, USA). A multiple linear regression analysis was done to check the possible influence of age, gender, smoking and *BDNF* Val66Met polymorphism on BDNF concentration. A two-tailed Pearson coefficient of correlation was used to assess the possible correlation between different measures for obesity and CHD with BDNF concentration. Chi-square goodness-of-fit test was used to determine if the frequency of the BDNF Val66Met genotypes was in the Hardy-Weinberg equilibrium[17]. Chi-square-test was used to evaluate the frequency of male and female individuals, smoking, the presence of high blood pressure and the distribution of the *BDNF* Val66Met genotypes (Met/Met, Met/Val and Val/Val). Standardized residuals and *R* value[18] were calculated to evaluate a main contributor to significant differences. The Kolmogorov-Smirnov test assessed the normality of distribution. Since the normality of the data failed, age, height, weight, BMI, WHtR, glucose, insulin, HOMA index, plasma lipids and hsCRP values were evaluated with the Mann-Whitney U test. All used tests were two-tailed. G*Power 3 Software[19] was used to calculate in advance statistical power and sample size (with α=0.05; power=0.800 and corresponding small effect sizes). The total desired sample size was 244 (for the multiple linear regression analysis), 197 (for the Chi-square-test) and 352 (for the *t* test of independent samples), respectively.

Results

Table I shows the characteristics of the included individuals, while the frequency of gender, smoking, high blood pressure and *BDNF* Val66Met genotypes are shown in Table II. There were significant (P<0.001) differences between patients with CHD and healthy controls in age, BMI, WHtR, glucose, insulin, total cholesterol, HDL, LDL, triglycerides, hsCRP and HOMA index (Table I). Patients with CHD were significantly older and had significantly higher concentrations of glucose, insulin, triglycerides and significantly higher values of BMI, WHtR, hsCRP and HOMA index than healthy controls. Controls had significantly higher cholesterol, HDL and LDL concentrations than patients with CHD (Table I).

The frequency of male and female individuals (Table II) differed significantly (P<0.001), due to significantly more (R=3.65) women (67%) in control group compared to men (33%). No significant differences in the frequency of smoking, presence of high blood pressure and the distribution of *BDNF* Val66Met genotype was found between patients with CHD and controls (Table II).

Frequency of the *BDNF* Val66Met genotypes was in the Hardy-Weinberg equilibrium in patients with CHD (P=0.358) or controls (P=0.139). Plasma BDNF concentration did not differ between patients with stable [4.27 (2.34-9.60); 6.58±5.78 ng/ml] and unstable [5.31 (2.63-9.42); 6.49±4.87 ng/ml] angina. Therefore, all further analyses included total CHD patients. Plasma BDNF concentration was significantly (U=13642.5; z=-2.598; P<0.01) decreased (38%) in CHD patients [4.36 (2.35-9.51)] compared to controls [6.36 (3.81-10.13)].

To examine a relation of plasma BDNF concentration with the angiographic severity of CHD, partial correlation of plasma BDNF concentration and left ventricle ejection function (LVEF), corrected for the effect of age, gender and *BDNF* Val66Met

Table I. Demographic and clinical data of patients with coronary heart disease (CHD) and healthy controls

Risk factors	Patients with CHD (n=208)	Healthy controls (n=156)
Age (yr)	57.00 (52.00-61.00)***	44.50 (35.00-54.00)
	55.67±7.66	44.11±11.63
BMI (kg/m^2)	27.75 (26.03-31.05)***	25.30 (23.13-30.10)
	28.61±4.14	26.39±5.19
WHtR	0.35 (0.51-0.61)***	0.33 (0.44-0.55)
	0.56±0.07	0.49±0.08
Glucose concentration (mmol/l)	5.50 (5.12-6.30)***	5.10 (4.82-5.40)
	6.02±1.51	5.16±0.50
Insulin concentration (pmol/l)	64.85 (45.70-90.53)***	48.15 (36.95-70.88)
	74.73±47.88	60.51±41.74
HOMA index	1.23 (0.88-1.77)***	0.900 (0.70-1.31)
	1.44±0.95	1.13±0.77
Total cholesterol concentration	4.45 (3.60-5.70)***	5.55 (4.80-6.40)
(mmol/l)	4.71±1.42	5.63±1.24
HDL concentration (mmol/l)	1.25 (0.992-1.500)***	1.72 (1.380-2.108)
	1.30±0.40	1.76±0.53
LDL concentration (mmol/l)	2.63 (1.87-3.57)***	3.25 (2.66-4.01)
	2.78±1.11	3.37±0.97
Triglyceride concentration	1.29 (0.87-1.77)***	0.94 (0.56-1.34)
(mmol/l)	1.44±0.79	1.10±0.72
hsCRP (mg/l)	2.65 (1.60-6.48)***	1.75 (1.30-3.00)
	7.59±14.19	2.70±2.52
Platelet count (×10^3/µl)	261.00 (225.00-312.50)	259.50 (230.00-297.00)
	279.50±90.59	263.92±52.78

***P<0.001 compared to controls. The values are shown as median (25-75% percentiles) and mean±SD. BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; HOMA, homeostasis model assessment; n, number of individuals; SD, standard deviation; WHtR, waist to height ratio

genotypes, was performed. Most of the patients had heart failure with preserved (LVEF ≥50%; n=159) ejection fraction, but there were also patients with moderate (40% ≤LVEF ≤49%; n=40) and severe (LVEF <40%; n=9) ejection function. Plasma BDNF concentration was in weak positive correlation (r=0.214; P=0.002) with the LVEF.

In the multiple linear regression analysis, dependent variable was plasma BDNF concentration, while age, gender, smoking and *BDNF* Val66Met were independent variables. This analysis showed a lack of significant model (P=0.234) and no significant effects of age (P=0.094), gender (P=0.407), smoking (P=0.481) or *BDNF* Val66Met (P=0.180) on BDNF values, revealing that these independent variables were not associated with BDNF concentration in plasma.

Partial correlation was used to assess the influence of BMI, height, weight, waist circumference, WHtR, glucose, insulin, HOMA index, hsCRP, total cholesterol, HDL, LDL, triglycerides, systolic or diastolic blood pressure, platelet number or platelet volume on plasma BDNF concentration in patients with CHD and in controls, corrected for the possible effect of age, gender and *BDNF* Val66Met genotypes (Table III). In patients with CHD, a significant positive correlation between plasma BDNF concentration and cholesterol (P=0.004), LDL (P=0.006), and diastolic blood pressure (P=0.018) was found. In controls, there was a significant positive correlation of plasma BDNF concentration with platelet number (P=0.003) or hsCRP (P=0.038) and significant negative correlation with HDL cholesterol (P=0.041). Other risk factors for CHD were not significantly correlated to plasma BDNF concentration (Table III).

Table II. Frequency of gender, smoking, high blood pressure and brain-derived neurotrophic factor (*BDNF*) genotypes in patients with coronary heart disease (CHD) and healthy controls

Risk factors	Patients with CHD (n=208)	Healthy controls (n=156)
Gender		
Men	142 (68.3)***	52 (33.3)
Women	66 (31.7)	104 (66.67)
Smoking†		
Yes	46 (22.3)	42 (27.3)
No	160 (77.8)	112 (72.7)
High blood pressure (mmHg)		
Yes	98 (47.1)	89 (57.1)
No	110 (52.9)	67 (42.9)
BDNF Val66Met$		
Met/Met	8 (3.9)	8 (5.2)
Met/Val	55 (26.7)	41 (26.4)
Val/Val	143 (69.4)	106 (68.4)

***P<0.001 compared to healthy controls. Values are shown as n (%). †2 healthy controls and 2 CHD patients could not be genotyped due to low DNA concentration; $1 healthy control and 2 CHD patients could not be genotyped due to low DNA concentration.

Discussion

The present study showed a significantly lower plasma BDNF concentration in CHD patients compared to healthy control group. The present results supported the data that reduced serum BDNF levels were related to increased risk for cardiovascular disease in large cohorts of individuals followed longitudinally (n=3687)[20]. Reduced plasma BDNF levels in CHD patients were similar to the previous results from Caucasian patients with acute coronary syndromes[21] or CHD patients with metabolic syndrome[1] or Japanese patients with angina pectoris[10]. Plasma BDNF concentration did not differ between patients with stable and unstable angina; however, there was weak positive correlation of plasma BDNF levels with LVEF, suggesting the association of BDNF with severity of CHD clinical presentation.

In our study, BDNF concentration was not affected by smoking status, age and gender or by the *BDNF* Val66Met genotypes[20,22]. No relationship between peripheral BDNF concentration and *BDNF* Val66Met polymorphism was found in the present, and other studies[23,24]. These differences might be due to different diagnoses, methods of BDNF determination, serum and plasma BDNF concentration or *BDNF* genotyping[8,25].

Since peripheral BDNF might be affected by the risk factors associated with CHD[5], although this was not confirmed recently[20], possible confounders were chosen in advance, such as age[26,27], gender[26,28], smoking, systolic blood pressure, hypertension treatment, plasma lipid levels and BMI[27]. A significant correlation was observed between plasma BDNF concentration and total cholesterol, LDL and diastolic blood pressure in patients with CHD. These results corresponded to the findings showing that risk factors for cardiovascular diseases correlated with BDNF in plasma in individuals of Chinese origin with angina pectoris[10]. In agreement with an ability of platelets to release BDNF on activation[8], plasma BDNF concentration in healthy controls was correlated with platelet number. However, in CHD patients, such correlation was not observed. As these two groups did not differ in platelet number, lower plasma BDNF concentration in CHD patients might be due to the lower platelet activation and consequently less BDNF excretion, presumably associated with anti-platelet medication[8]. The present and previous results[20] suggest a protective role of BDNF in the CHD pathogenesis.

BDNF is reported to be associated with energy expenditure, BMI and obesity[6,29,30]. In the present study, plasma BDNF was not associated with body weight, BMI and obesity. No correlation between BDNF and obesity/BMI might be due to the relatively high BMI values and relatively low BMI range, in both healthy controls and CHD group, with almost all individuals in the overweight category. These results corroborated with studies demonstrating lack of association of BDNF with obesity[31-33].

The limitation of the study was its cross-sectional design and the strength was in a sufficiently large sample size and statistical power, individuals from the same ethnicity, collected from the same centre, with a narrowly diagnosed patients.

In conclusion, the present study revealed a significant association of lower plasma BDNF concentration with CHD. Longitudinal multicentric studies with a large sample size should be done to confirm the present findings.

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Table III. Partial correlation analysis (two-tailed) between brain-derived neurotrophic factor (BDNF) concentration and risk factors for coronary heart disease (CHD), corrected for the possible effect of age, gender and *BDNF* Val66Met polymorphism

Risk factors	Patients with CHD (n=208)		Healthy individuals (n=156)	
	Partial correlation		Partial correlation	
	r	*P*	*r*	*P*
BMI	0.133	0.059	0.133	0.104
Height	0.034	0.633	-0.036	0.662
Weight	0.099	0.162	0.131	0.109
Waist circumference	0.013	0.855	0.153	0.059
WHtR	0.011	0.878	0.156	0.055
Total cholesterol	0.201	0.004	-0.019	0.813
HDL cholesterol	0.048	0.498	-0.166	0.041
LDL cholesterol	0.192	0.006	0.016	0.848
Triglycerides	0.139	0.048	0.098	0.231
hsCRP	-0.043	0.542	0.168	0.038
Insulin	0.015	0.829	-0.015	0.855
Glucose	0.131	0.063	-0.048	0.554
HOMA index	0.032	0.648	-0.013	0.876
Systolic blood pressure	0.115	0.103	-0.101	0.214
Diastolic blood pressure	0.165	0.018	-0.078	0.339
Platelet number	0.040	0.575	0.244	0.003
Platelet volume	0.015	0.834	-0.140	0.085

BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; HOMA, homeostasis model assessment; n, number of individuals; WHtR, waist to height ratio

***Conflicts of Interest*:** None.

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