Brain, Behavior, and Immunity 25 (2011) 1264–1271

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Brain derived neurotrophic factor, cardiopulmonary fitness and cognition in patients with coronary artery disease

W. Swardfager [a,e], N. Herrmann [a,f], S. Marzolini [g], M. Saleem [a,e], P. Shammi [b], P.I. Oh [c,g], P.R. Albert [h], M. Daigle [h], A. Kiss [d], K.L. Lanctôt [a,e,f,g,*]

[a] Neuropsychopharmacology Research Group, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[b] Neuropsychology, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[c] Division of Clinical Pharmacology, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[d] Clinical Epidemiology, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada
[e] Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada
[f] Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada
[g] Toronto Rehabilitation Institute, Toronto, Ontario, Canada
[h] Ottawa Hospital Research Institute (Neuroscience), University of Ottawa, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 8 February 2011
Received in revised form 4 April 2011
Accepted 25 April 2011
Available online 30 April 2011

Keywords:
Brain derived neurotrophic factor
BDNF
Cognition
Cardiopulmonary fitness
Coronary artery disease
Memory
Executive function
Psychomotor processing speed

ABSTRACT

Objective: To assess serum brain derived neurotrophic factor (BDNF) concentrations as a correlate of cardiopulmonary fitness and as a predictor of cognitive performance in subjects with coronary artery disease (CAD).

Methods: Serum BDNF concentrations were assayed by ELISA and fitness was assessed using a standardized exercise stress test. The Mini Mental Status Examination (MMSE), California Verbal Learning Test 2nd Ed., Stroop, Trail Making Test B and the Digit Symbol-Coding task were administered. The val66met BDNF genotype and serum interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentrations were determined as potential confounders.

Results: In subjects with CAD ($n = 88$; 85.2% male, mean age 62.8 ± 10.5 yr), cardiopulmonary fitness was associated with higher serum BDNF concentrations ($\beta = .305$, $p = .013$). Higher serum BDNF concentrations were associated with higher MMSE scores ($F(1, 87) = 15.406$, $p < .0005$) and better performance on the Digit Symbol-Coding task ($F(1, 87) = 9.620$, $p = .003$). IL-6, TNF-α and the val66met genotype did not influence these results.

Conclusion: Serum BDNF concentrations were associated with cardiopulmonary fitness, psychomotor processing speed and overall cognition in subjects with CAD.

1. Introduction

Cardiopulmonary fitness has been associated with better cognitive performance in older adults (Barnes et al., 2003). Consistent with this observation, physical activity can improve performance on tests of processing speed, attention, executive function and memory (Smith et al., 2010). Physical activity can also increase brain derived neurotrophic factor (BDNF) concentrations in peripheral blood (Ferris et al., 2007; Seifert et al., 2010). Evidence suggests that BDNF may be involved in the beneficial effect of cardiopulmonary fitness on cognitive performance. In the central nervous system, BDNF can support neuronal growth and survival (Berchtold et al., 2010) and attenuate inflammatory damage to axons and midbrain dopaminergic neurons (Fujino et al., 2009; Katsuki et al., 2009; Linker et al., 2010; Wu et al., 2011). In medically and neurologically healthy older adults, higher serum BDNF protein concentrations have been variably associated with better cognitive performance (Gunstad et al., 2008; Komulainen et al., 2008).

In subjects with coronary artery disease (CAD), the association between cardiopulmonary fitness and cognitive performance has been observed independent of other vascular risk factors, demographics, cardiac histories and concomitant medications, suggesting that fitness may be a clinically important factor protecting against cognitive decline (Swardfager et al., 2010). However, peripheral blood BDNF protein concentrations have not been assessed as a predictor of cognitive performance, nor as a correlate of cardiopulmonary fitness in this population.

Previous literature suggests several factors that may influence these relationships. A single nucleotide polymorphism at codon 66 of the BDNF gene, which results in an amino acid substitution

* Corresponding author at: Neuropsychopharmacology Research Group, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Suite FG05, Toronto, Ontario, Canada M4N 3M5. Fax: +1 416 480 6022.
E-mail address: krista.lanctot@sunnybrook.ca (K.L. Lanctôt).

0889-1591/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2011.04.017

(val66met) in the pro-region of the protein, has been associated with poorer cognitive performance (Miyajima et al., 2008). The val66met genotype has also been associated with variation in peripheral blood BDNF protein concentrations (Lang et al., 2009), suggesting the need to assess the val66met genotype as a potential confounder. Moreover, serum BDNF concentrations have been associated with inflammatory biomarkers in subjects with CAD (Lorgis et al., 2010). Exercise-responsive inflammatory biomarkers, notably IL-6 and TNF-α (Goldhammer et al., 2005; Kim et al., 2008), have been associated with the progression of CAD (Jovinge et al., 1998; Luc et al., 2003) and with poorer cognitive function (van Exel et al., 2003; Yaffe et al., 2003) suggesting the need to explore systemic inflammatory activity as a potential confounder.

The present study sought to determine whether peripheral blood BDNF protein concentrations were associated with cardiopulmonary fitness and cognitive function in subjects with CAD independent of two inflammatory markers, a common polymorphism in the BDNF (val66met) genotype, and other clinical characteristics.

2. Methods

The present study assessed cross-sectional associations between (1) serum BDNF concentrations and cardiopulmonary fitness and (2) serum BDNF concentrations and cognitive performance in a cohort of consecutive patients with CAD entering cardiac rehabilitation.

2.1. Participants

Institutional research ethics boards at Sunnybrook Health Sciences Centre and the Toronto Rehabilitation Institute approved the study protocol. Consent to participate was sought from consecutive patients with a documented history of CAD (myocardial infarction, MI; angiographic evidence showing ⩾50% blockage in at least one major coronary artery; percutaneous coronary intervention, PCI; or coronary artery bypass graft surgery, CABG) entering a cardiac rehabilitation (CR) program. Subjects began CR a minimum of 6 weeks post-CABG or MI, or a minimum of 3 weeks post-PCI. Participants were interviewed by a trained study researcher and excluded if they could not complete cognitive testing or if their medical records were otherwise incomplete. Demographics (e.g. age, gender, education), cardiac history (e.g. PCI, CABG, MI), vascular risk factors (body mass index, hyperlipidemia, diabetes, hypertension, waist circumference, smoking), concomitant medication use, medical/psychiatric comorbidities and anthropometrics were ascertained by CR staff or chart review and confirmed by patient interview. Where available, coronary angiography reports were reviewed for indices of CAD severity including the involvement of each major coronary artery (>50% stenosis) and the presence of restenosis in any previously revascularized lesion.

Patients were excluded on the basis of any previously diagnosed neurodegenerative disorder or schizophreniform or bipolar psychiatric illness. As a possible confounder (Sen et al., 2008), major or minor depression were diagnosed by study personnel using the Structured Clinical Interview for Diagnostic and Statistical Manual 4th Edition criteria (SCID, First et al., 1996). The Center for Epidemiological Studies Depression (CES-D) scale (Radloff, 1977) was used to quantify depressive symptoms based on demonstrated utility in patients with CAD (Blumenthal et al., 2003).

2.2. Cognitive testing

A battery of cognitive assessments encompassing processing speed, executive function, memory and global cognition were administered, including the Trail Making Test B, the Victoria version of the Stroop test, and the Digit Symbol-Coding task, a measure of complex attention and psychomotor processing speed from the Wechsler Adult Intelligence Scale 3rd Edition. Verbal memory was assessed as immediate, short and long delayed free recall of the California Verbal Learning Test 2nd Ed. (CVLT-II) word list. Global cognitive status was assessed using the Mini-Mental Status Examination (MMSE). These instruments were chosen based on the National Institute of Neurological Disorders and Stroke and Canadian Stroke Network (NINDS-CSN) harmonized standards (Hachinski et al., 2006). All cognitive testing was carried out at a standardized time (0930 h ± 30 min) and participants refrained from eating or drinking any caffeine-containing beverages for at least 4 h prior and from extensive physical activity. For each cognitive task, a *Z*-score was determined based on published norms. The *Z* distribution maps the mean of the test variable to 0 with 1 *Z*-score unit equal to 1 standard deviation (better performance is more positive and poorer performance is more negative).

2.3. Serum collection and assays

Blood samples were drawn at 0930 h ± 30 min following a 12 h overnight fast and centrifuged at 1000*g* for 10 min at 4 °C. Serum was separated and stored at −80 °C until the time of the assay. IL-6, TNF-α (Alpco Diagnostics, Salem NH, USA) and BDNF (R&D Systems Inc., Minneapolis, MN, USA) were assayed by ultrasensitive enzyme-linked immunosorbant assay according to manufacturers' instructions with sensitivities of 0.16, 0.5 and 20 pg/mL, respectively.

2.4. Genetic sample collection and genotyping

Genomic DNA was extracted from Dacron buccal swabs using the Qiamp DNA mini kit (Qiagen, Missisauga, Ontario, Canada). The Val66Met (rs6265) genotype was determined by polymerase chain reaction (PCR) restriction fragment length polymorphism analysis. The PCR reaction was performed in a final volume of 25 ml containing 1X Phusion HF buffer (Finnzymes, Finland), 2 pmol of each PCR primer, 200 mM dNTP, 50 ng of genomic DNA and 0.625 units of Phusion (Finnzymes, Espoo, Finland). The primers used for amplification were 5′-CCCAAGGCAGGTTCAAGAG-3′ (forward) and 5′-CGTTACCCACTCACTAATACTGTCA-3′ (reverse). The PCR conditions used were: 30 s, 98 °C; 35 cycles of 10 s, 98 °C; 20 s, 68 °C; 10 s, 72 °C; and 5 min, 72 °C. PCR was followed by digestion of amplicons using *NlaIII* (New England Biolabs, Pickering, Ontario, Canada) at 37 °C for 3 h, yielding fragments of 81 and 222 bp (G allele), 81, 54, and 167 bp (A allele) or 81, 54, 167 and 222 bp (heterozygous). Genotypes were confirmed by automated sequencing using the primer: 5′-TGCCCCCATGAAAGAAGC-3′.

2.5. Physical assessments

At the patients' CR intake visit, blood pressure and heart rate were measured by CR staff. Anthropometric measurements were also made; percentage body fat was assessed by bioelectric impedance (Tanita TBF-300A, Tokyo, Japan), waist circumference was measured and body mass index (BMI) was calculated per standard definition.

2.6. Cardiopulmonary fitness assessment

Cardiopulmonary fitness was assessed by a cycle ergometer (Ergoline 800 EL) symptom-limited graded exercise test. Work load was increased by 16.7 W/min and breath-by-breath gas samples were collected and averaged over a 20 s period via calibrated metabolic cart (Vmax) (Hamm and Kavanagh, 2000). The peak volume

of oxygen uptake (VO_{2Peak}) was recorded and normalized for body mass (VO_{2Peak} reported in ml/kg/min). The VO_{2Peak} thus obtained represents a measure of ventilatory capacity at peak effort and it is a highly reliable and reproducible measure of cardiopulmonary fitness (Milani et al., 2006). The respiratory exchange ratio (RER), an indicator of metabolic effort (Naimark et al., 1964), was calculated as the ratio of CO_2/O_2 in gas breath samples at the time of VO_{2Peak} measurement. For each subject, the VO_{2Peak} measured from exercise stress testing was divided by his or her expected VO_{2Peak} to provide a clinically meaningful measure of cardiopulmonary fitness that can be compared across age and gender (Jones and Campbell, 1982). The expected VO_{2Peak} was calculated from established age and gender norms (Jones and Campbell, 1982) as:

Expected $VO_{2Peak} = 60 - (0.55 \times \text{Age})$ ml/kg/min for male subjects and
Expected $VO_{2Peak} = 48 - (0.37 \times \text{Age})$ ml/kg/min for female subjects

2.7. Statistical analyses

All analyses were carried out in SPSS (version 16.0) and considered significant at a two-tailed $\alpha = .05$. Pearson correlations and univariate analyses of variance (ANOVA), as appropriate, were used to identify patient characteristics associated with serum BDNF concentrations. Serum protein concentrations were log-transformed as necessary, and linearity was assessed using P–P plots. Bonferroni correction was applied to comparisons between the val66met genotypes because there are three genotypes (val/val, val/met and met/met), and hence inherent multiple comparisons between them.

A linear regression model was used to determine if cardiopulmonary fitness was a significant predictor of serum BDNF protein concentrations. Possible confounders were chosen *a priori*, including depression (Sen et al., 2008), age (Li et al., 2009), gender (Baker et al., 2010; Komulainen et al., 2008; Li et al., 2009), val66met genotype (Lang et al., 2009) and serum inflammatory markers (Lorgis et al., 2010) for inclusion in the model. Clinical characteristics associated with BDNF concentrations were also included as covariates. As a possible covariate, the number of vascular risk factors (i.e. hypertension, smoking, diabetes, BMI > 30 kg/m^2, waist circumference > 102 for men or >88 cm for women and dyslipidemia) were summed to account for cumulative vascular risk factor burden.

Z-scores from individual cognitive tests were entered into a multiple linear regression model to assess their independent associations with the fraction of the VO_{2Peak} norm. Performance in one cognitive domain can affect performance on tests designed to measure other domains, so a multiple linear regression approach was used. *Z*-scores from individual cognitive tests were also entered into a multiple linear regression model to assess their independent associations with serum BDNF concentrations. In linear regression models, multicollinearity was assessed among predictors using the tolerance statistic (tolerance $\leqslant 0.4$) and if multicollinearity was identified, only one variable identified as part of a multicollinear set was included in the final model.

A multivariate general linear model was used to assess the associations between serum BDNF protein concentration and cognitive test results that were independently associated with BDNF concentrations. Age, gender, angiographic CAD severity measures, cardiac risk factors or other subject characteristics associated with serum BDNF concentrations or cognitive test results were explored as potential confounders.

Subgroup analyses were planned in male subjects due to possible differential effects in males and females (Baker et al., 2010; Komulainen et al., 2008; Li et al., 2009), and in non-depressed subjects who were also free of any antidepressant medication due to an association between depression and lower BDNF concentrations or possible effects of antidepressant medications (Sen et al., 2008).

2.8. Sample size

A sample size of $n = 90$ was considered sufficient (80% power) to detect a clinically meaningful relationship (0.3 standard deviations per one standard deviation change) between serum BDNF concentrations and cardiopulmonary fitness at a two-sided significance of $\alpha = 0.05$.

3. Results

3.1. Recruitment

Study personnel contacted 139 patients at their intake CR visit that met inclusion criteria as determined by clinical personnel. Subsequently, 34 patients declined to be interviewed. Cognitive testing was carried out in all but 18 patients who consented to be interviewed; 11 met further exclusion criteria, 5 were unable to schedule an appointment, 1 screened positive for significant cognitive impairment (MMSE < 24), 1 could not complete cognitive testing because of insufficient English language skills and 1 was unable to complete study tasks due to inadequate visual acuity. There were therefore 88 subjects studied (Table 1).

Table 1
Characteristics of study participants and associations with serum BDNF protein concentrations (n=88).

		Association with serum BDNF concentrations	
		F or r[a]	Sig.[b]
Sociodemographic			
Age, mean ± SD, yr	62.79 ± 10.47	$r = .114$	.292
Employed, n (%)	49 (55.6)	$F(1, 87) = .189$	.665
Partnered, n (%)	68 (77.3)	$F(1, 87) = .004$	.952
Sex, male, n (%)	75 (85.2)	$F(1, 87) = 1.315$	.255
Caucasian, n (%)	79 (89.8)	$F(1, 87) = .066$	.798
Total education, mean ± SD, yr	16.4 ± 3.4	$r = -.038$	.724
Psychometric			
Depressive episode, n (%)	26 (29.5)	$F(1, 87) = .102$	.751
History of depressive episode, n (%)	24 (27.3)	$F(1, 87) = .002$	.964
CES-D score, mean ± SD	11.70 ± 11.34	$r = -.102$	.345
Vascular risk factors			
Hypertension, n (%)	52 (59.1)	$F(1, 87) = .054$	.817
Past smoker, n (%)	53 (60.2)	$F(1, 87) = .080$	.778
Years smoked, mean ± SD	12.92 ± 14.80	$r = .015$	.890
Cigarettes per day, mean ± SD	10.28 ± 11.6	$r = -.021$	.844
Diabetes, n (%)	14 (15.9)	$F(1, 87) = 1.036$	.312
BMI, mean ± SD, kg/m^2	28.73 ± 4.67	$r = -.124$	.257
BMI > 30 kg/m^2 (%)	29 (33.0)	$F(1, 87) = 1.581$	.212
Waist circumference, mean ± SD, cm	101.5 ± 10.8	$r = -.040$	.715
Waist circumference > 88 (female) or > 105 (male) (%)	47 (53.4)	$F(1, 87) = .086$	.770
Dyslipidemia, n (%)	31 (54.4)	$F(1, 87) = 1.544$	.217
Total no. vascular risk factors, mean ± SD	2.59 ± 1.35	$r = -.043$	.692
Cardiac history			
PCI, n (%)	53 (60.2)	$F(1, 87) = .361$	.549
Myocardial infarction, n (%)	43 (48.9)	$F(1, 87) = .082$	.775
CABG, n (%)	28 (31.8)	$F(1, 87) = 0.922$	.340
Angina, n (%)	25 (28.4)	$F(1, 87) = .011$	.918
Peripheral vascular disease, n (%)	4 (4.5)	$F(1, 87) = .347$	.557
No. vessels involved, mean ± SD	2.03 ± 0.96	$r = -.071$	.533

Table 1 (*continued*)

		Association with serum BDNF concentrations	
		F or *r*[a]	Sig.[b]
Time since event, mean ± SD, weeks	14.2 ± 8.1	*r* = −.031	.785
Concomitant medications			
Statins, *n* (%)	86 (97.7)	$F(1, 87) = 2.342$	.130
ASA, *n* (%)	84 (95.5)	$F(1, 87) = .059$	.808
ß-blockers, *n* (%)	66 (75.0)	$F(1, 87) = 2.256$	.137
Antihypertensive, *n* (%)	55 (62.5)	$F(1, 87) = 4.725$	.032
Nitroglycerin, *n* (%)	51 (58.0)	$F(1, 87) = .248$	.620
Diuretics, *n* (%)	14 (15.9)	$F(1, 87) = .359$	.551
Ca^{2+}-channel antagonists, *n* (%)	13 (14.8)	$F(1, 87) = .167$	.684
Anxiolytics, *n* (%)	9 (10.2)	$F(1, 87) = .088$	.768
Antidiabetic agents, *n* (%)	9 (10.2)	$F(1, 87) = 1.565$	.214
Antidepressants, *n* (%)	6 (6.8)	$F(1, 87) = 1.211$	.274
Resting physiology			
Resting heart rate, mean ± SD, BPM	67.66 ± 110.20	*r* = .079	.465
Resting systolic BP, mean ± SD, mm Hg	127.91 ± 17.04	*r* = −.138	.202
Resting diastolic BP, mean ± SD, mm Hg	74.93 ± 11.17	*r* = −.103	.342
Fitness parameters			
Fraction VO_{2Peak} norm, mean ± SD	.858 ± .263	*r* = .252	.019
Maximum heart rate, mean ± SD, BPM	122.61 ± 20.56	*r* = .009	.933
Maximum systolic BP, mean ± SD, mm Hg	181.08 ± 26.49	*r* = −.035	.752
Maximum diastolic BP, mean ± SD, mm Hg	79.93 ± 10.39	*r* = −.083	.450

[a] *F* represents the *F* statistic in one-way analyses of variance (ANOVA) comparing serum BDNF protein concentrations between categorical subject characteristics and *r* represents the Pearson correlation between serum BDNF protein concentrations and continuous subject characteristics, as appropriate.

[b] *P*-value represents the two-sided significance in one-way ANOVA or Pearson correlations ASA = acetylsalicylic acid; BP = blood pressure; BPM = beats per minute; CABG = coronary artery bypass graft; CES-D = Center for Epidemiological Studies-Depression Scale; PCI = percutaneous coronary intervention; VO_{2Peak} = peak volume of oxygen uptake.

3.2. Serum assays and genotyping

Mean serum concentrations of BDNF, IL-6 and TNF-α are presented in Table 2. For parametric analyses serum IL-6 concentrations were log-transformed due to skew and kurtosis. One third of TNF-α concentrations were below the limit of detectability; therefore, for analysis, patients were stratified into tertiles, with non-detectable samples comprising the lowest tertile. The coefficients of variation for BDNF, IL-6 and TNF-α were 3.5%, 11% and 24%, respectively. Higher serum concentrations of IL-6 were associated with higher BDNF concentrations (Table 2). The time since most recent acute coronary syndrome was not associated with serum IL-6 ($p = .559$) or TNF-alpha ($p = .890$) concentrations suggesting that the population may have been relatively "stable" with respect to inflammatory markers after acute events.

Genotype frequencies for the val66met polymorphism are presented in Table 2. Serum BDNF concentrations were numerically higher in subjects carrying at least one copy of the met allele (17,193 ± 7931 pg/ml) than in subjects of the val/val genotype (14,798 ± 7931 pg/ml) in agreement with previous findings (Lang et al., 2009), but this difference was not significant ($F(1, 83) = 1.724$, $p = .193$). The two subjects of the met/met genotype had serum BDNF concentrations of 13,334 and 22,580 pg/ml.

Table 2
Results of serum assays and genotyping.

		Association with serum BDNF protein concentration	
		F or *r*[a]	Sig.[b]
Serum biomarkers (n = 88)			
BDNF, mean ± SD, pg/ml	15,347 ± 7989	–	–
IL-6, mean ± SD, pg/ml	2.97 ± 3.61	*r*=.322	.002
TNF-α, mean ± SD, pg/ml	1.73 ± 3.49	–	–
TNF-α, tertile	–	$F(2, 87) = 2.578$	.082
BDNF val66met Polymorphism (*n* = 84)	–	$F(2, 83) = .861$	.651
val/val, *n* (%)	55 (65.5)	–	–
val/met, *n* (%)	27 (32.1)	–	–
met/met, *n* (%)	2 (2.4)	–	–

[a] F represents the *F* statistic in one-way analyses of variance (ANOVA) comparing serum BDNF protein concentrations between categorical subject characteristics and r represents the Pearson correlation; IL-6 concentrations were log-transformed; Bonferroni correction was applied to the val66met genotype comparison.

[b] *P*-value represents the two-sided significance in one-way ANOVA or Pearson correlations.

3.3. Cardiopulmonary fitness and serum BDNF protein concentrations

The mean VO_{2Peak} of the present cohort was 14.2% below expected age and gender norms (range 45% below to 67% above their predicted VO_{2Peak}) and 38.4% of subjects presented with VO_{2Peak} below average (<73% of their predicted VO_{2Peak}). Of all subject characteristics, poorer cardiopulmonary fitness, the use of an antihypertensive medication, and lower serum concentrations of IL-6 were associated with lower BDNF concentrations (Tables 1 and 2) in bivariate comparisons.

In a linear regression model ($F(7, 85) = 3.173$, $p = .005$, adjusted $R^2 = .152$) controlling for age, sex, antihypertensive use, depression, antidepressant use, and serum IL-6 concentrations, better cardiopulmonary fitness was a significant independent predictor of serum BDNF concentrations ($\beta = .305$, $p = .013$). Of all covariates, only serum IL-6 concentrations also independently predicted higher serum BDNF concentrations ($\beta = .304$, $p = .006$). Including the val66met genotype, angiographic CAD severity measures, or the number of vascular risk factors in this model did not significantly influence the strength of this association between fitness and serum BDNF concentrations ($p < .02$). In planned subgroup analyses, fitness and serum BDNF concentrations remained associated when excluding depressed subjects or subjects using an antidepressant medication ($\beta = .276$, $p = .041$, $n = 59$) and among male subjects ($\beta = .329$, $p = .011$, $n = 75$).

3.4. Cardiopulmonary fitness and cognitive performance

Cognitive test results are summarized in Table 3. Significant associations were identified between cardiopulmonary fitness and all cognitive *Z*-scores except CVLT-II short and long delayed free recall *Z*-scores in Pearson correlations (Table 3). MMSE scores were significantly associated with cardiopulmonary fitness when controlling for age and gender ($\beta = 0.240$, $p = .028$); however, in a multiple linear regression model (overall model $F(5, 85) = 3.414$, $p = .008$, adjusted $R^2 = .124$), only Digit Symbol-Coding *Z*-scores were associated with cardiopulmonary fitness independently of performance on the other cognitive tests ($\beta = 0.285$, $p = .023$; Table 3).

3.5. Serum BDNF protein concentrations and cognitive performance

In bivariate comparisons, serum BDNF concentrations were associated with all cognitive measures except CVLT-II short and long delayed free recall and Stroop *Z*-scores (Table 4). To identify cognitive domains independently associated with serum BDNF

Table 3
Cognitive testing results and associations with cardiopulmonary fitness (n=88).

Cognitive test	Raw score	Correlations with fraction of VO_{2Peak} norm[a]			Coefficients of a multiple linear regression model predicting fraction of VO_{2Peak} norm[b]			
		Z-Score	r	Sig.	B	SE	β	Sig.
Stroop	28.6 ± 9.3 s	0.36 ± 1.02	.281	.009	.032	.033	.118	.118
Trail making B	88.2 ± 42.0 s	−0.05 ± 0.90	.248	.021	.022	.037	.074	.554
Digit symbol-coding	61.3 ± 16.7 symbols	0.18 ± 1.07	.380	<.0005	.073	.031	.285	.023
CVLT-II encoding	43.2 ± 12.2 words	0.17 ± 1.2	.243	.024	.022	.026	.096	.407
CVLT-II short delayed free recall	9.3 ± 3.4 words	.25 ± 1.1	.100	.360	–	–	–	–
CVLT-II long delayed free recall	9.3 ± 3.5 words	0.14 ± 1.1	.162	.136	–	–	–	–
MMSE score	28.9 ± 1.7 points	–	.240	.028	−.018	.026	−.078	.489

CVLT-II = California Verbal Learning Test 2nd Ed.; MMSE = Mini Mental Status Exam; SD = standard deviation.
[a] Pearson correlations (r) between the fraction of age and gender predicted VO_{2Peak} (see Methods; Cardiopulmonary fitness assessment) and Z-score; for MMSE scores, correlation represents the association (β) between VO_{2Peak} and MMSE score in multiple linear regression controlling for age and gender.
[b] Coefficients of a multiple linear regression model predicting the fraction of expected age and gender VO_{2Peak} norm with Z-scores on cognitive tests and MMSE scores entered as independents; CVLT-II measures exceeded tolerance for multicolinearity so only the encoding score was entered in the final linear regression model.

Table 4
Associations between cognitive testing and serum BDNF protein concentrations (n=88).

Cognitive test	Correlation with serum BDNF protein concentration[a]		Coefficients of a multiple linear regression model predicting serum BDNF protein concentrations[b]			
	r	Sig.	B	SE	β	Sig.
Stroop	−.059	.586	−3176	909	−.388	.001
Trail making test B	.212	.047	1291	1007	.145	.204
Digit symbol-coding	.412	<.0005	3150	847	.411	<.0005
CVLT-II encoding	.253	.017	534	705	.077	.451
CVLT-II short delayed free recall	.096	.376	–	–	–	–
CVLT-II long delayed free recall	.135	.210	–	–	–	–
MMSE	.342	.001	1710	691	.244	.015

CVLT-II = California Verbal Learning Test 2nd Ed.; MMSE = Mini Mental Status Exam; SD = standard deviation.
[a] Pearson correlations (r) between serum BDNF concentrations and cognitive test Z-scores; for MMSE scores, the association (β) between serum BDNF concentration and MMSE score in multiple linear regression systematically controlling for age and gender is presented.
[b] Coefficients of a multiple linear regression model predicting serum BDNF concentrations with Z-scores from cognitive tests entered as independents; CVLT-II measures exceeded tolerance for multicolinearity so only the encoding score was entered in the final linear regression model.

concentrations, all cognitive test scores were entered as independent variables into a multiple linear regression model (overall model $F(5, 87) = 7.937$, $p < .0005$, adjusted $R^2 = 0.285$). In this model, BDNF concentrations were associated with higher Digit Symbol-Coding Z-scores, higher MMSE scores, and lower Stroop Z-scores (Table 4).

In a multivariate general linear model predicting Digit Symbol-Coding Z-scores (corrected model $F(5, 87) = 4.345$, $p = .001$, adjusted $R^2 = .187$), Stroop Z-scores (corrected model $F(6, 87) = 1.220$, $p = .304$, adjusted $R^2 = .015$) and MMSE scores (corrected model $F(6, 87) = 3.617$, $p = .003$, adjusted $R^2 = .153$), and controlling for age, gender, serum IL-6 concentrations, depression and antidepressant use, higher serum BDNF concentrations were associated with higher Digit Symbol-Coding Z-scores ($F(1, 87) = 9.620$, $p = .003$) and higher MMSE scores ($F(1, 87) = 15.406$, $p < .0005$). In this model, higher serum IL-6 concentrations were associated with lower MMSE scores ($F(1, 87) = 4.035$, $p = .048$). The associations between serum BDNF concentrations and Digit Symbol-Coding Z-scores ($p = .002$) and MMSE scores ($p < .001$) were unchanged when repeating the model with the val66met genotype included as a covariate ($n = 84$). Similarly, angiographic CAD severity measures, the use of an antihypertensive medication and cardiac risk factors (cumulatively or individually) did not affect the strength of the observed associations when explored as covariates in the multivariate model ($p < .005$ for all associations between serum BDNF concentrations and MMSE scores or Digit Symbol-Coding Z-scores). In the planned subgroup analysis of non-depressed and antidepressant medication free subjects ($n = 59$), serum BDNF concentrations remained associated with MMSE scores ($F(1, 58) = 13.028$, $p = .001$) and Digit Symbol-Coding Z-scores ($F(1, 58) = 9.136$, $p = .004$). In the planned subgroup of male subjects ($n = 75$), serum BDNF concentrations remained associated with MMSE scores and ($F(1, 74) = 14.281$, $p < .0005$) and Digit Symbol-Coding Z-scores ($F(1, 74) = 8.342$, $p = .005$).

4. Discussion

An association between cardiopulmonary fitness and cognitive function was observed in this cohort of subjects with CAD, consistent with previous reports from CAD and medically healthy older subject populations (Barnes et al., 2003; Swardfager et al., 2010). Of the cognitive tests examined, cardiopulmonary fitness was independently associated with performance in the Digit Symbol-Coding task, which is predominantly a test of psychomotor processing speed and complex attention. This result is consistent with previous studies showing that physical activity reliably improved performance on cognitive tests involving these domains (Angevaren et al., 2008). Poorer performance on the Digit Symbol-Coding task is clinically important in older adults because it has been prospectively associated with incident disability and increased risks of coronary syndromes, stroke and mortality (Elkins et al., 2005; Fried et al., 1998; Rosano et al., 2008).

In a model explaining 15.2% of the variance in serum BDNF protein concentrations, a significant association with cardiopulmonary fitness was observed. This is consistent with reports that physical activity can increase peripheral blood BDNF concentrations in medically healthy subjects (Seifert et al., 2010; Tang et al., 2008). However, since this effect has not been consistent in

younger medically healthy and physically active subjects, the present study focused on an older and largely sedentary patient population (Goekint et al., 2010). The VO_{2Peak} of the present cohort was 14.2% below expected age and gender norms, consistent with low self-reported habitual physical activity in patients entering this cardiac rehabilitation program (Marzolini et al., 2010). Due to this and the decline in serum BDNF concentrations associated with age (Ziegenhorn et al., 2007), cardiopulmonary fitness may have been a more relevant predictor of serum BDNF concentrations in this population.

In the present cohort, bivariate comparisons suggested associations between serum BDNF concentrations and better performance across multiple cognitive domains. However, like cardiopulmonary fitness, serum BDNF concentrations were most strongly associated with better performance on the Digit Symbol-Coding task in multiple linear regression analysis and in a multivariate model. In older subjects, a decline in performance on this test has been associated with vascular risk factors such as diabetes and hypertension (Knopman et al., 2001). In the present study, higher peripheral BDNF concentrations also predicted higher scores on the MMSE, the instrument most commonly used to screen for cognitive status in clinical practice. These findings are in agreement with a correlation between MMSE scores and serum BDNF concentrations observed in subjects with vascular dementia (Yasutake et al., 2006), suggesting the involvement of BDNF along a continuum of vascular cognitive impairment. These findings may be important because vascular risk factors can be managed clinically, and their effects on cognitive outcomes might be partially mitigated by physical activity.

Associations between circulating BDNF concentrations and vascular risk factors have been identified previously, but it remains unclear whether these associations reflect a role in pathogenesis or in an adaptive physiological response (Golden et al., 2010). In diabetic mouse models, BDNF modulates central control of energy balance and chronic administration of BDNF can improve glucose metabolism (Nakagawa et al., 2000). Spontaneously hypertensive rats show deficits in hippocampal neurogenesis that can be restored by treatment with oestradiol, which normalizes BDNF expression in the dentate gyrus (Pietranera et al., 2010). Clinically, vascular risk factors lead to dysfunction of the vascular endothelium (Puddu et al., 2000), which synthesizes and releases BDNF (Kim et al., 2008; Nakahashi et al., 2000). Vascular endothelial function can be improved by physical activity (Walsh et al., 2003), which might therefore attenuate the effects of vascular risk factors on neuroprotection and neurogenesis. Increased brain volumes observed in older subjects undertaking regular physical activity (Colcombe et al., 2006) would be consistent with the associations between cardiopulmonary fitness, BDNF concentrations and cognitive function observed in the present study.

As reviewed by Zhang and colleagues, physical activity can improve cerebral ischemic tolerance by enhancing blood brain barrier function and increasing cerebral small vessel number (Zhang et al., 2011). Patients with CAD are susceptible to subtle cerebral ischemic damage that appears on magnetic resonance images as white matter hyperintensities. These are an important mediator of the effects of vascular risk factors on cognitive performance, and Digit Symbol-Coding scores in particular (Hajjar et al., 2011). The response to cerebral ischemia involves angiogenesis and neurogenesis, which interact to salvage and remodel affected tissue (Chopp et al., 2007; Louissaint et al., 2002). This "neurovascular niche" contributes to functional recovery from stroke (Chopp et al., 2007; Ohab et al., 2006) and possibly to mitigating subtle cognitive deficits resulting from disease of the smaller vessels. Roles of BDNF in the dynamic interactions between neurogenesis (Guo et al., 2008; Louissaint et al., 2002) and angiogenesis (Qin et al., 2011) have been described, but remain to be fully characterized (Kim et al., 2004; Li et al., 2006). The clinical impact of BDNF signaling on cerebrovascular disease has been suggested by greater white matter hyperintensity volumes in carriers of the met BDNF allele 60 years of age or older (Taylor et al., 2008). While the significance of peripheral BDNF concentrations in this process is not known, damaged or early angiogenic vessels are known to be highly permeable to intravascular components that may affect neurovascular remodelling (Zhang et al., 2000).

The present study did not identify any significant independent predictors of serum BDNF concentrations in addition to cardiopulmonary fitness, with the exception of serum IL-6 concentrations. This may reflect a previously observed correlation between plasma BDNF concentrations and activated mononuclear cell number in medically healthy adults (Lommatzsch et al., 2005) and the stimulatory effect of IL-6 on BDNF secretion from peripheral mononuclear cells (Schulte-Herbruggen et al., 2005). In the present study, BDNF was assayed from serum, which largely reflects activation-dependent release of BDNF from platelets (Fujimura et al., 2002; Lommatzsch et al., 2005). An association between serum BDNF and soluble P-selectin, a platelet pro-coagulant inflammatory marker, has been observed previously in subjects with CAD (Lorgis et al., 2010). In the present study, serum BDNF and IL-6 concentrations were correlated, but higher BDNF concentrations were associated with higher MMSE scores while higher serum IL-6 was associated with lower MMSE scores. These clinical data would be consistent with pleiotrophic inflammatory effects, which might both exacerbate neural insult and promote compensatory BDNF signaling, a possible role of "neuroprotective immunity" previously described (Bayas et al., 2002; Kerschensteiner et al., 2003; Linker et al., 2010).

As a potential limitation of the present study, physical activity was not quantified; however cardiopulmonary fitness has been more reliably associated with cognitive performance than self-reported metrics of physical activity (Barnes et al., 2003). In the present cohort, depression and depressive symptom severity were not associated with lower serum BDNF concentrations, contrasting findings in younger adult populations (Sen et al., 2008); however, the present findings are consistent with other studies of older patients (Ziegenhorn et al., 2007) and of those with cardiovascular disease (Jimenez et al., 2009; Yang et al., 2011). As a potential limitation, depressive symptom severity was measured by a self-zreport instrument rather than by clinician rating. This study was also not powered to detect associations between BDNF serum concentrations and the val66met genotype or other clinical characteristics; however, these covariates did not mitigate the associations between serum BDNF concentrations and study outcomes. While concentrations of IL-6 and BDNF in serum were correlated, the clinical significance of this association is unclear, other inflammatory markers were not explored and many TNF-α assay results were below the limit of detectability. Since BDNF serum assays reflect the release of BDNF from platelets, it is unclear whether plasma concentrations, thought to reflect more immediate systemic BDNF availability, would have been similarly associated with cardiopulmonary fitness or cognitive function. As a further limitation, the antibody used to detect BDNF by ELISA was not necessarily specific for the mature BDNF peptide and concentrations of pro-BDNF were not determined (Woo et al., 2005). The observational study design precludes causal inferences. Finally, this cohort of subjects entering cardiac rehabilitation may have been more motivated or perceived to be more amenable to rehabilitation by prescribing physicians, potentially introducing some selection bias and limiting the generalizability of results.

In conclusion, serum BDNF protein concentrations were associated with better cardiopulmonary fitness, better psychomotor processing speed/complex attention and better overall cognition in subjects with CAD. These relationships were independent of IL-6

concentrations and the val66met polymorphism despite the observed association between IL-6 and BDNF concentrations in serum. These findings suggest that serum BDNF concentrations are clinically relevant in subjects with CAD. Further studies are needed to understand the nature of their involvement in neurobiological adaptations related to cardiopulmonary fitness.

5. Conflict of Interest Statement

WS is supported by an Alzheimer Society of Canada doctoral award.

NH receives research funding from Sonexa Therapeutics Inc. and Lundbeck Canada Inc., and holds grants from the National Institute of Health, the Ontario Mental Health Foundation, the Canadian Institute of Health Research, Alzheimer Society of Canada, Heart and Stroke Foundation, and Physicians' Services Incorporated Foundation. NH received speaker's honoraria from Lundbeck, Pfizer, Janssen Ortho and Novartis

SM reports no disclosures.

MS reports no disclosures.

PS reports no disclosures.

PIO sits on the steering committee for clinical trial sponsored by Pfizer.

PRA reports no disclosures.

MD reports no disclosures.

AK reports no disclosures.

KLL is the executive director of the Medical Outcomes and Research in Economics (MORE) Research group, received research funding from Abbott Laboratories, Sonexa Therapeutics Inc., Workers Safety and Insurance Board (WSIB) and Lundbeck Canada, is funded by grants from the National Institute of Health, Canadian Institute of Health Research, the Alzheimer's Society of Canada, the Ontario Mental Health Foundation, the Heart and Stroke Foundation and Physician's Services Incorporated Foundation.

6. Sources of financial support

The study was supported by research grants from The Drummond Foundation (Montréal, Québec, Canada), the Physicians' Services Incorporated Foundation (Toronto, Ontario, Canada), the Heart and Stroke Foundation (Toronto, Ontario, Canada) and the Alzheimer Society of Canada (Toronto, Ontario, Canada). The above institutions financially supported the study, but they were not involved in study management, the collection analysis and interpretation of data, and the preparation of the manuscript and manuscript review or approval.

Acknowledgments

The authors would like to gratefully acknowledge Yekta Dowlati, MSc, and Marilyn Sherman, BScN, Reg. N, COHN, for their contributions to recruitment and data collection.

References

Angevaren, M., Aufdemkampe, G., Verhaar, H.J., Aleman, A., Vanhees, L., 2008. Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. Cochrane Database Syst Rev (3), CD005381.

Baker, L.D., Frank, L.L., Foster-Schubert, K., Green, P.S., Wilkinson, C.W., McTiernan, A., Plymate, S.R., Fishel, M.A., Watson, G.S., Cholerton, B.A., Duncan, G.E., Mehta, P.D., Craft, S., 2010. Effects of aerobic exercise on mild cognitive impairment: a controlled trial. Arch. Neurol. 67 (1), 71–99.

Barnes, D.E., Yaffe, K., Satariano, W.A., Tager, I.B., 2003. A longitudinal study of cardiorespiratory fitness and cognitive function in healthy older adults. J. Am. Geriatr. Soc. 51 (4), 459–465.

Bayas, A., Hummel, V., Kallmann, B.A., Karch, C., Toyka, K.V., Rieckmann, P., 2002. Human cerebral endothelial cells are a potential source for bioactive BDNF. Cytokine 19 (2), 55–58.

Berchtold, N.C., Castello, N., Cotman, C.W., 2010. Exercise and time-dependent benefits to learning and memory. Neuroscience 167 (3), 588–597.

Blumenthal, J.A., Lett, H.A., Babyak, M.A., White, W., Smith, P.K., Mark, D.B., Jones, R., Mathew, J.P., Newman, M.F.NORG Investigators, 2003. Depression as a risk factor for mortality after coronary artery bypass surgery. Lancet 362 (9384), 604–609.

Chopp, M., Zhang, Z.G., Jiang, Q., 2007. Neurogenesis, angiogenesis, and MRI indices of functional recovery from stroke. Stroke 38 (2 Suppl.), 827–831.

Colcombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L., Kramer, A.F., 2006. Aerobic exercise training increases brain volume in aging humans. J. Gerontol. A: Biol. Sci. Med. Sci. 61 (11), 1166–1170.

Elkins, J.S., Knopman, D.S., Yaffe, K., Johnston, S.C., 2005. Cognitive function predicts first-time stroke and heart disease. Neurology 64 (10), 1750–1755.

Ferris, L.T., Williams, J.S., Shen, C.L., 2007. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. Med. Sci. Sports Exerc. 39 (4), 728–734.

First, M.B., Spitzer, R.L., Gibbon, M. et al., 1996. Structured Clinical Interview For DSM-IV Diagnoses (SCID): Clinician and Research Versions. Biometrics Research Department, Columbia University, New York, NY.

Fried, L.P., Kronmal, R.A., Newman, A.B., Bild, D.E., Mittelmark, M.B., Polak, J.F., Robbins, J.A., Gardin, J.M., 1998. Risk factors for 5-year mortality in older adults: the Cardiovascular Health Study. JAMA 279 (8), 585–592.

Fujimura, H., Altar, C.A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J., Sun, B., Tandon, N.N., 2002. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb. Haemost. 87 (4), 728–734.

Fujino, H., Kitaoka, Y., Hayashi, Y., Munemasa, Y., Takeda, H., Kumai, T., Kobayashi, S., Ueno, S., 2009. Axonal protection by brain-derived neurotrophic factor associated with CREB phosphorylation in tumor necrosis factor-alpha-induced optic nerve degeneration. Acta Neuropathol. 117 (1), 75–84.

Goekint, M., Roelands, B., De Pauw, K., Knaepen, K., Bos, I., Meeusen, R., 2010. Does a period of detraining cause a decrease in serum brain-derived neurotrophic factor? Neurosci. Lett. 486 (3), 146–149.

Golden, E., Emiliano, A., Maudsley, S., Windham, B.G., Carlson, O.D., Egan, J.M., Driscoll, I., Ferrucci, L., Martin, B., Mattson, M.P., 2010. Circulating brain-derived neurotrophic factor and indices of metabolic and cardiovascular health: data from the Baltimore Longitudinal Study of Aging. PLoS One 5 (4), e10099.

Goldhammer, E., Tanchilevitch, A., Maor, I., Beniamini, Y., Rosenschein, U., Sagiv, M., 2005. Exercise training modulates cytokines activity in coronary heart disease patients. Int. J. Cardiol. 100 (1), 93–99.

Gunstad, J., Benitez, A., Smith, J., Glickman, E., Spitznagel, M.B., Alexander, T., Juvancic-Heltzel, J., Murray, L., 2008. Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. J. Geriatr. Psychiatry Neurol. 21 (3), 166–170.

Guo, S., Kim, W.J., Lok, J., Lee, S.R., Besancon, E., Luo, B.H., Stins, M.F., Wang, X., Dedhar, S., Lo, E.H., 2008. Neuroprotection via matrix-trophic coupling between cerebral endothelial cells and neurons. Proc. Natl. Acad. Sci. USA 105 (21), 7582–7587.

Hachinski, V., Iadecola, C., Petersen, R.C., Breteler, M.M., Nyenhuis, D.L., Black, S.E., Powers, W.J., DeCarli, C., Merino, J.G., Kalaria, R.N., Vinters, H.V., Holtzman, D.M., Rosenberg, G.A., Dichgans, M., Marler, J.R., Leblanc, G.G., 2006. National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards. Stroke 37 (9), 2220–2241.

Hajjar, I., Quach, L., Yang, F., Chaves, P.H., Newman, A.B., Mukamal, K., Longstreth Jr., W., Inzitari, M., Lipsitz, L.A., 2011. Hypertension, white matter hyperintensities, and concurrent impairments in mobility, cognition, and mood: the cardiovascular health study. Circulation 123 (8), 858–865.

Hamm, L.F., Kavanagh, T., 2000. The Toronto Cardiac Rehabilitation and Secondary Prevention Program: 1968 into the new millennium. J. Cardiopulm. Rehabil. 20 (1), 16–22.

Jimenez, I., Sobrino, T., Rodriguez-Yanez, M., Pouso, M., Cristobo, I., Sabucedo, M., Blanco, M., Castellanos, M., Leira, R., Castillo, J., 2009. High serum levels of leptin are associated with post-stroke depression. Psychol. Med. 39 (7), 1201–1209.

Jones, N.L., Campbell, E.J.M., 1982. Clinical Exercise Testing, second ed. W.B. Saunders, Philadelphia.

Jovinge, S., Hamsten, A., Tornvall, P., Proudler, A., Bavenholm, P., Ericsson, C.G., Godsland, I., de Faire, U., Nilsson, J., 1998. Evidence for a role of tumor necrosis factor alpha in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. Metabolism 47 (1), 113–118.

Katsuki, H., Kurimoto, E., Takemori, S., Kurauchi, Y., Hisatsune, A., Isohama, Y., Izumi, Y., Kume, T., Shudo, K., Akaike, A., 2009. Retinoic acid receptor stimulation protects midbrain dopaminergic neurons from inflammatory degeneration via BDNF-mediated signaling. J. Neurochem. 110 (2), 707–718.

Kerschensteiner, M., Stadelmann, C., Dechant, G., Wekerle, H., Hohlfeld, R., 2003. Neurotrophic cross-talk between the nervous and immune systems: implications for neurological diseases. Ann. Neurol. 53 (3), 292–304.

Kim, H., Li, Q., Hempstead, B.L., Madri, J.A., 2004. Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells. J. Biol. Chem. 279 (32), 33538–33546.

Kim, Y.J., Shin, Y.O., Bae, J.S., Lee, J.B., Ham, J.H., Son, Y.J., Kim, J.K., Kim, C., Lee, B.K., Oh, J.K., Othman, T., Min, Y.K., Yang, H.M., 2008. Beneficial effects of cardiac rehabilitation and exercise after percutaneous coronary intervention on hsCRP and inflammatory cytokines in CAD patients. Pflugers Arch. 455 (6), 1081–1088.

Knopman, D., Boland, L.L., Mosley, T., Howard, G., Liao, D., Szklo, M., McGovern, P., Folsom, A.R., 2001. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 56 (1), 42–48.

Komulainen, P., Pedersen, M., Hanninen, T., Bruunsgaard, H., Lakka, T.A., Kivipelto, M., Hassinen, M., Rauramaa, T.H., Pedersen, B.K., Rauramaa, R., 2008. BDNF is a novel marker of cognitive function in ageing women: the DR's EXTRA study. Neurobiol. Learn. Mem. 90 (4), 596–603.

Lang, U.E., Hellweg, R., Sander, T., Gallinat, J., 2009. The Met allele of the BDNF Val66Met polymorphism is associated with increased BDNF serum concentrations. Mol. Psychiatry 14 (2), 120–122.

Li, G., Peskind, E.R., Millard, S.P., Chi, P., Sokal, I., Yu, C.E., Bekris, L.M., Raskind, M.A., Galasko, D.R., Montine, T.J., 2009. Cerebrospinal fluid concentration of brain-derived neurotrophic factor and cognitive function in non-demented subjects. PLoS One 4 (5), e5424.

Li, Q., Ford, M.C., Lavik, E.B., Madri, J.A., 2006. Modeling the neurovascular niche: VEGF- and BDNF-mediated cross-talk between neural stem cells and endothelial cells: an in vitro study. J. Neurosci. Res. 84 (8), 1656–1668.

Linker, R.A., Lee, D.H., Demir, S., Wiese, S., Kruse, N., Siglienti, I., Gerhardt, E., Neumann, H., Sendtner, M., Luhder, F., Gold, R., 2010. Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. Brain 133 (Pt 8), 2248–2263.

Lommatzsch, M., Zingler, D., Schuhbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., Virchow, J.C., 2005. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol. Aging 26 (1), 115–123.

Lorgis, L., Amoureux, S., de Maistre, E., Sicard, P., Bejot, Y., Zeller, M., Vergely, C., Sequeira-Le Grand, A., Lagrost, A.C., Berchoud, J., Cottin, Y., Rochette, L., 2010. Serum brain-derived neurotrophic factor and platelet activation evaluated by soluble P-selectin and soluble CD-40-ligand in patients with acute myocardial infarction. Fundam. Clin. Pharmacol. 24 (4), 525–530.

Louissaint Jr., A., Rao, S., Leventhal, C., Goldman, S.A., 2002. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 34 (6), 945–960.

Luc, G., Bard, J.M., Juhan-Vague, I., Ferrieres, J., Evans, A., Amouyel, P., Arveiler, D., Fruchart, J.C., Ducimetiere, P., 2003. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. Arterioscler. Thromb. Vasc. Biol. 23 (7), 1255–1261.

Marzolini, S., Candelaria, H., Oh, P., 2010. Prevalence and impact of musculoskeletal comorbidities in cardiac rehabilitation. J. Cardiopulm. Rehabil. Prev. 30 (6), 391–400.

Milani, R.V., Lavie, C.J., Mehra, M.R., Ventura, H.O., 2006. Understanding the basics of cardiopulmonary exercise testing. Mayo Clin. Proc. 81 (12), 1603–1611.

Miyajima, F., Ollier, W., Mayes, A., Jackson, A., Thacker, N., Rabbitt, P., Pendleton, N., Horan, M., Payton, A., 2008. Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. Genes Brain Behav. 7 (4), 411–417.

Naimark, A., Wasserman, K., Mcllroy, M.B., 1964. Continuous measurement of ventilatory exchange ratio during exercise. J. Appl. Physiol. 19, 644–652.

Nakagawa, T., Tsuchida, A., Itakura, Y., Nonomura, T., Ono, M., Hirota, F., Inoue, T., Nakayama, C., Taiji, M., Noguchi, H., 2000. Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. Diabetes 49 (3), 436–444.

Nakahashi, T., Fujimura, H., Altar, C.A., Li, J., Kambayashi, J., Tandon, N.N., Sun, B., 2000. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. FEBS Lett. 470 (2), 113–117.

Ohab, J.J., Fleming, S., Blesch, A., Carmichael, S.T., 2006. A neurovascular niche for neurogenesis after stroke. J. Neurosci. 26 (50), 13007–13016.

Pietranera, L., Lima, A., Roig, P., De Nicola, A.F., 2010. Involvement of brain-derived neurotrophic factor and neurogenesis in oestradiol neuroprotection of the hippocampus of hypertensive rats. J. Neuroendocrinol. 22 (10), 1082–1092.

Puddu, P., Puddu, G.M., Zaca, F., Muscari, A., 2000. Endothelial dysfunction in hypertension. Acta Cardiol. 55 (4), 221–232.

Qin, L., Kim, E., Ratan, R., Lee, F.S., Cho, S., 2011. Genetic variant of BDNF (Val66Met) polymorphism attenuates stroke-induced angiogenic responses by enhancing anti-angiogenic mediator CD36 expression. J. Neurosci. 31 (2), 775–783.

Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl. Psychol. Measure. 1, 385–401.

Rosano, C., Newman, A.B., Katz, R., Hirsch, C.H., Kuller, L.H., 2008. Association between lower digit symbol substitution test score and slower gait and greater risk of mortality and of developing incident disability in well-functioning older adults. J. Am. Geriatr. Soc. 56 (9), 1618–1625.

Schulte-Herbruggen, O., Nassenstein, C., Lommatzsch, M., Quarcoo, D., Renz, H., Braun, A., 2005. Tumor necrosis factor-alpha and interleukin-6 regulate secretion of brain-derived neurotrophic factor in human monocytes. J. Neuroimmunol. 160 (1–2), 204–209.

Seifert, T., Brassard, P., Wissenberg, M., Rasmussen, P., Nordby, P., Stallknecht, B., Adser, H., Jakobsen, A.H., Pilegaard, H., Nielsen, H.B., Secher, N.H., 2010. Endurance training enhances BDNF release from the human brain. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298 (2), R372–R377.

Sen, S., Duman, R., Sanacora, G., 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol. Psychiatry 64 (6), 527–532.

Smith, P.J., Blumenthal, J.A., Hoffman, B.M., Cooper, H., Strauman, T.A., Welsh-Bohmer, K., Browndyke, J.N., Sherwood, A., 2010. Aerobic exercise and neurocognitive performance. a meta-analytic review of randomized controlled trials. Psychosom. Med. 72 (3), 239–252.

Swardfager, W., Herrmann, N., Marzolini, S., Saleem, M., Kiss, A., Shammi, P., Oh, P.I., Lanctot, K.L., 2010. Cardiopulmonary fitness is associated with cognitive performance in patients with coronary artery disease. J. Am. Geriatr. Soc. 58 (8), 1519–1525.

Tang, S.W., Chu, E., Hui, T., Helmeste, D., Law, C., 2008. Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. Neurosci. Lett. 431 (1), 62–65.

Taylor, W.D., Zuchner, S., McQuoid, D.R., Payne, M.E., MacFall, J.R., Steffens, D.C., Speer, M.C., Krishnan, K.R., 2008. The brain-derived neurotrophic factor VAL66MET polymorphism and cerebral white matter hyperintensities in late-life depression. Am. J. Geriatr. Psychiatry 16 (4), 263–271.

van Exel, E., de Craen, A.J., Remarque, E.J., Gussekloo, J., Houx, P., Bootsma-van der Wiel, A., Frolich, M., Macfarlane, P.W., Blauw, G.J., Westendorp, R.G., 2003. Interaction of atherosclerosis and inflammation in elderly subjects with poor cognitive function. Neurology 61 (12), 1695–1701.

Walsh, J.H., Bilsborough, W., Maiorana, A., Best, M., O'Driscoll, G.J., Taylor, R.R., Green, D.J., 2003. Exercise training improves conduit vessel function in patients with coronary artery disease. J. Appl. Physiol. 95 (1), 20–25.

Woo, N.H., Teng, H.K., Siao, C.J., Chiaruttini, C., Pang, P.T., Milner, T.A., Hempstead, B.L., Lu, B., 2005. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat. Neurosci. 8 (8), 1069–1077.

Wu, S.Y., Wang, T.F., Yu, L., Jen, C.J., Chuang, J.I., Wu, F.S., Wu, C.W., Kuo, Y.M., 2011. Running exercise protects the substantia nigra dopaminergic neurons against inflammation-induced degeneration via the activation of BDNF signaling pathway. Brain Behav. Immun. 25 (1), 135–146.

Yaffe, K., Lindquist, K., Penninx, B.W., Simonsick, E.M., Pahor, M., Kritchevsky, S., Launer, L., Kuller, L., Rubin, S., Harris, T., 2003. Inflammatory markers and cognition in well-functioning African–American and white elders. Neurology 61 (1), 76–80.

Yang, L., Zhang, Z., Sun, D., Xu, Z., Yuan, Y., Zhang, X., Li, L., 2011. Low serum BDNF may indicate the development of PSD in patients with acute ischemic stroke. Int. J. Geriatr. Psychiatry 26 (5), 495–502.

Yasutake, C., Kuroda, K., Yanagawa, T., Okamura, T., Yoneda, H., 2006. Serum BDNF, TNF-alpha and IL-1beta levels in dementia patients: comparison between Alzheimer's disease and vascular dementia. Eur. Arch. Psychiatry Clin. Neurosci. 256 (7), 402–406.

Zhang, F., Wu, Y., Jia, J., 2011. Exercise preconditioning and brain ischemic tolerance. Neuroscience 177, 170–176.

Zhang, Z.G., Zhang, L., Jiang, Q., Zhang, R., Davies, K., Powers, C., Bruggen, N., Chopp, M., 2000. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J. Clin. Invest. 106 (7), 829–838.

Ziegenhorn, A.A., Schulte-Herbruggen, O., Danker-Hopfe, H., Malbranc, M., Hartung, H.D., Anders, D., Lang, U.E., Steinhagen-Thiessen, E., Schaub, R.T., Hellweg, R., 2007. Serum neurotrophins – a study on the time course and influencing factors in a large old age sample. Neurobiol. Aging 28 (9), 1436–1445.