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Original Research

Serum Brain-Derived Neurotrophic Factor is Related to Platelet Reactivity and Metformin Treatment in Adult Patients With Type 2 Diabetes Mellitus

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Key Messages

- Brain-derived neurotrophic factor concentration in serum may be associated with higher platelet reactivity in patients with type 2 diabetes.
- Lipid metabolism serum may be associated with brain-derived neurotrophic factor levels in patients with type 2 diabetes.
- Metformin therapy could have influences on brain-derived neurotrophic factor concentrations in patients with type 2 diabetes.

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ABSTRACT

Objectives: The aim of this study was to investigate the association of serum brain-derived neurotrophic factor (BDNF) levels with platelet reactivity and antidiabetes treatment, as well as serum adipocytokine concentrations.
Methods: This observational, open-label study enrolled 149 patients. Serum BDNF, hematologic, biochemical parameters and platelet reactivity were measured. Blood samples were taken after the last acetylsalicylic acid dose.
Results: Patients with high BDNF levels were younger (65.60±8.956 vs. 68.59±8.516) and smoked cigarettes more frequently (14.6% vs. 4.1%); they were more commonly being treated by metformin (77.3% vs. 54%); had higher platelet counts (245.81±68.85 10^3/mm^3 vs. 206.61±44.48 10^3/mm^3); had shorter collagen-adenosine diphosphate closure time (CADP-CT) values (104.88±69.73 s vs. 140.93±86.63 s); had higher triglyceride concentrations (140.73±67.5 vs. 121.76±60.49) and had higher concentrations of serum thromboxane B2 (0.938±1.59 vs. 0.364±0.76). In univariate linear regression analyses, predictive factors for serum BDNF levels above the median were metformin treatment, current smoking, platelet count, triglyceride concentration, total cholesterol concentration and CADP-CT >74 s. In multivariate backward stepwise analysis CADP-CT >141 s; adiponectin concentration >4.22 μg/mL; total cholesterol and low-density lipoprotein levels were independently associated with serum BDNF levels above the median.
Conclusions: Our results suggest that BDNF may be associated with lipid metabolism and that increased production of BDNF may be related to metformin treatment. Moreover, we showed an association between BDNF levels and platelet reactivity; we found that serum BDNF levels in patients with type 2 diabetes who had high platelet reactivity were higher than in subjects with normal platelet reactivity despite antiplatelet therapy.

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RÉSUMÉ

Objectifs : L'objectif de la présente étude était d'examiner l'association des concentrations sériques du facteur neurotrophique dérivé du cerveau (BDNF, de l'anglais *brain-derived neurotrophic factor*) à la réactivité plaquettaire et au traitement antidiabétique, ainsi qu'aux concentrations sériques d'adipocytokines.
Méthodes : Cette étude observationnelle ouverte regroupait 149 patients. Nous avons mesuré les concentrations sériques du BDNF, les paramètres hématologiques et biochimiques, et la réactivité plaquettaire. Nous avons prélevé les échantillons de sang après la dernière dose d'acide acétylsalicylique.
Résultats : Les patients ayant des concentrations élevées du BDNF étaient plus jeunes (65,60±8,956 vs. 68,59±8,516) et fumaient plus fréquemment la cigarette (14,6% vs. 4,1%); ils étaient plus souvent traités par metformine (77,3% vs. 54%); ils avaient un nombre plus élevé de plaquettes (245,81±68,85 10^3/mm^3 vs. 206,61±44,48 10^3/mm^3); ils montraient des temps d'occlusion plus courts avec collagène et adénosine diphosphate (CADP-CT, de l'anglais *collagen-adenosine diphosphate closure time*) (104,88±69,73 s vs. 140,93±86,63 s); ils avaient des concentrations plus élevées de triglycérides (140,73±67,5 vs. 121,76±60,49); ils avaient des concentrations sériques plus élevées de thromboxane B2 (0,938±1,59 vs. 0,364±0,76). Dans les analyses de régression linéaire univariée, les facteurs prédictifs de concentrations sériques du BDNF au-dessus de la médiane étaient le traitement par metformine, la consommation actuelle de tabac, le nombre de plaquettes, les concentrations de triglycérides, les concentrations de cholestérol total et les CADP–CT>74 s. Dans l'analyse multivariée selon la méthode pas à pas descendante, des CADP–CT>141 s; des concentrations d'adiponectine>4,22 µg/ml; des concentrations de cholestérol total et de lipoprotéines de faible densité étaient indépendamment associés à des concentrations sériques du BDNF au-dessus de la médiane.
Conclusions : Nos résultats montrent que le BDNF peut être associé au métabolisme des lipides et qu'une production accrue du BDNF peut être liée au traitement par metformine. De plus, nous avons démontré une association entre les concentrations du BDNF et la réactivité plaquettaire. Nous avons également observé que les concentrations sériques du BDNF chez les patients atteints du diabète de type 2 qui montraient une réactivité plaquettaire élevée étaient plus élevées que chez les sujets qui montraient une réactivité plaquettaire normale en dépit du traitement antiplaquettaire.

Introduction

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays an important role in the maturation, synaptic connection, neuronal repair and plasticity of the central nervous system; also, it may affect essentially the pathology and the treatment of neurologic diseases (1–3). Apart from the fundamental impact on the nervous system, studies have shown the association between plasma BDNF and systemic or peripheral inflammatory conditions, such as acute coronary syndrome, atherosclerosis and type 2 diabetes mellitus (4–6).

Type 2 diabetes is characterized by hyperglycemia resulting from impaired insulin secretion, increased hepatic glucose production and decreased peripheral glucose utilization (7). Many drugs are available to treat type 2 diabetes, and metformin is 1 of the most commonly used antidiabetes drugs; it reduces glycated hemoglobin (A1C) and fasting glucose concentrations as well as plasma triglyceride and low-density lipoprotein (LDL) levels (8). Some research has shown alterations in BDNF levels due to metformin treatment in vitro, in animal models and in human studies. However, the research was performed in persons with neurodegenerative disease and metabolic syndrome but not in those with type 2 diabetes (9–12).

Type 2 diabetes is a hypercoagulable state and is associated with platelet hyper-reactivity (13). The cause of high platelet reactivity is complex and is related to metabolic disturbances, hyperglycemia and impaired lipid metabolism (14,15). It has been reported that BDNF is produced partially by vascular endothelial cells or smooth muscle cells, and large amounts of BDNF are stored mainly in the platelets; later, they are released into plasma through activation or clotting processes (16,17). However, the regulation of BDNF in peripheral blood remains poorly understood. Several studies suggest that age, sex and smoking history play important roles in BDNF activity (18,19). Stoll et al investigated the impact of various antiplatelet drugs on BDNF concentrations in serum and plasma and on the release of BDNF from platelets in healthy volunteers (20).

The primary aim of this study was to investigate the correlation between platelet reactivity and BDNF serum levels in patients with type 2 diabetes who are in long-term acetylsalicylic acid (ASA) therapy. The secondary aim of the project was to investigate the influence of metformin treatment on BDNF serum levels in patients with type 2 diabetes as well as to study biochemical parameters, such as those of adiponectin.

Methods

This was a preliminary, exploratory analysis of the results from the Aspirin Vs/Or Clopidogrel in Aspirin-resistant Diabetics inflammation Outcomes (AVOCADO) study, a multicentre, prospective, randomized, open-label study. The ethics committee of the Medical University of Warsaw approved both the study protocol and the informed-consent form. The study was conducted in accordance with the current version of the Declaration of Helsinki at the time when the study was designed, and informed written consent was obtained. In the present analysis, we included only fully characterized subjects (i.e. those with all biochemical results, including plasma BDNF concentrations and adipocytokines) participating in the AVOCADO study as it was previously published (21,22).

Blood sample and assay procedures

All blood samples were taken in fasting conditions at 9 AM at least 2 h after the last dose of antiplatelet drugs. Standard laboratory techniques were used for regular laboratory testing. Serum BDNF concentrations were measured using BDNF Quantikine Immunoassay (R&D Systems, Minneapolis, Minnesota, United States) as previously described and as used in our laboratory (23). ELISA kits were also used to determine concentrations of the following parameters: serum thromboxane B2 (sTxB2) (EIA kits, Cayman Chemicals, Ann Arbor, Michigan, United States); von Willebrand factor molecule; tumour necrosis factor-alpha (Quantikine HS ELISA Human TNF-α Immunoassay), interleukin-6 (Quantikine HS ELISA Human IL-6 Immunoassay; R&D Systems); soluble CD40 ligand (Human soluble CD40 Ligand Immunoassay; R&D Systems) and soluble

P-selectin (human P-selectin/CD62P ELISA kit; R&D Systems). High-sensitivity C-reactive protein concentrations were assessed using a Cobas Integra 800 device (Roche, Basel, Switzerland), as previously described (22,24). The compliance with ASA treatment was defined according to previously described criteria (21). Serum concentrations of high-molecular-weight adiponectin, leptin and resistin were measured using Human High Molecular Weight Adiponectin ELISA (Millipore, Billerica, Massachusetts, United States), Human Leptin Quantikine ELISA Kit (R&D Systems) and Human Resistin Quantikine ELISA Kit (R&D Systems), respectively (25).

Platelet function analysis

Platelet function analysis was performed using both the VerifyNow Aspirin Assay (ASA) (Accumetrics, San Diego, California, United States) and the platelet function analysis (PFA)-100 assay (Dade-Behring International, Newark, Delaware, United States). For VerifyNow ASA, reaction units <550 were used to determine platelet dysfunction as the result of ASA (21,22). Using PFA-100, collagen-epinephrine bitartrate (CEPI) and collagen-adenosine diphosphate (CADP) closure times (CTs) were determined (21,26). To determine high platelet reactivity, we applied a cut-off value for CEPI-CT ≥193 s and for CADP-CT above the lower quartile value (i.e. >74 s) (21,22).

Statistical analysis

Statistical analysis was performed using STATA/SE v. 13.1 statistical software (Stata, College Station, Texas, United States). Normally distributed continuous variables are presented as mean ± SD, whereas variables with non-normally distributed continuous variables are presented as medians with corresponding range. Categorical variables are presented as frequencies (percentages). Normality of distribution was assessed using the Shapiro-Wilk test. All the parametric data were compared by independent *t* tests and the nonparametric data by the Mann-Whitney U test. Selected biochemical parameters, such as CADP, sTxB2, insulin, leptin and adiponectin levels, were shown as concentrations below and above 75 percentiles.

Univariate and multivariate linear regression analyses were performed to determine the predictive factors of serum BDNF concentration. Moreover, univariate and multivariate logistic regression analyses were performed to determine the predictive factors of serum BDNF concentrations above the median. A backward conditional stepwise method was used to select the parameters, which were included in the final model. Multivariate regression analysis was performed to identify the predictors of high serum BDNF concentration after adjustment for demographic, biochemical factors and medications. A value of p≤0.05 was considered significant for all tests.

Results

We included 149 patients in the analysis. Mean ± SD demographic data, concurrent medications, biochemical and hematologic parameters for the study population are presented in Table 1.

The subjects were divided into 2 groups based on the observed median value of serum BDNF concentrations (28.28 ng/mL): BDNF ranges (11.59 to 28.09 ng/mL) in the low BDNF group (74 patients, 49.7% of the population) and BDNF ranges (28.47 to 57.50 ng/mL) in the high BDNF group (75 patients, 50.3% of the population).

Patients with high BDNF were younger (65.60±8.956 vs. 68.59±8.516; p=0.038) and smoked cigarettes more frequently than patients with low BDNF (14.6% vs. 4.1%; p=0.025); they were taking metformin treatment more commonly than patients with low BDNF (77.3% vs. 54%; p=0.002); also, they were more likely to be taking calcium channel blockers (50.7% vs. 35.1%; p=0.04); had higher concentrations of platelets (245.81±68.85 10^3/mm^3 vs. 206.61±44.48 10^3/mm^3; p=0.005); had shorter CADP-CT values (104.88±69.73 s vs. 140.93±86.63 s; p<0.0001); had higher triglyceride concentrations (140.73±67.5 vs. 121.76±60.49; p=0.049) and had higher concentrations of serum TxB_2 (0.938±1.59 vs. 0.364±0.76; p=0.02) (Table 2).

In univariate logistic regression analysis, predictive factors for serum BDNF levels above the median were: age (odds ratio [OR] 0.961, 95% confidence interval [CI], 95% CI 0.926 to 0.998; p=0.04); metformin treatment (OR 1.703, 95% CI 1.195 to 2.426; p=0.003); current smoking (OR 2.017, 95% CI 1.042 to 3.903; p=0.037); CADP-CT above the 75th percentile (OR 0.531, 95% CI 0.341 to 0.828; p=0.005); sTxB2 levels above the 75th percentile (OR 2.016, 95% CI 1.300 to 3.125; p=0.002) and adiponectin levels above the 75th percentile (OR 0.538, 95% CI 0.329 to 0.881; p=0.014) (Table 3). In multivariate backward stepwise analysis CADP-CT >141 s (p=0.014), adiponectin concentration >4.22 μg/mL (p=0.016), total cholesterol (p=0.007) and LDL (p=0.032) were risk factors for serum BDNF above the median (Table 3). In univariate linear regression analysis, predictive factors for high serum BDNF levels were: metformin

Table 1
Demographic and clinical characteristics of the study's patients

Demographic data	N (%) or mean/median ± SD
Male gender	82 (55%)
Age (y)	67.1±8.8
SBP (mmHg)	139.6±19.4
DBP (mmHg)	78.9±10.8
BMI (kg/m^2)	30.8±5.9
WHR	1.0±0.1
Hypertension	141 (94.6%)
Ischemic heart disease	75 (50.3%)
Previous MI	44 (29.5%)
Dyslipidemia	123 (82.6%)
Chronic heart failure	50 (33.5%)
Previous stroke	15 (10%)
Past smokers	86 (57.7%)
Current smokers	14 (9.5%)
Drug therapy	
Metformin	98 (65.8%)
Insulin	46 (30.9%)
Beta blocker	109 (73.2%)
ACE-I	95 (63.8%)
ARB	32 (21.5%)
Statin	107 (71.8%)
Proton pump inhibitor	17 (11.7%)
Clopidogrel	32 (21.5%)
Laboratory parameters	
Leukocyte count (×10^3/μL)	7.1±2.1
Hemoglobin (g/dL)	14.0±1.4
Hematocrit (%)	41.9±3.9
Platelet count (×10^3/μL)	226.3±61.1
MPV (fl)	9.9±1.2
eGFR (MDRD formula) (mL/min/1.73 m^2)	71.6±22.1
A1C (%)	7.0±1.4
TC (mg/dL)	162.5±38.8
HDL-C (mg/dL)	49.5±13.6
LDL-C (mg/dL)	86.6±33.7
Triglycerides (mg/dL)	131.3±64.6
vWF (%)	143.1±54.2
Adiponectin	3.5±2.3
hsCRP (mg/dL)	3.1±3.5
TxB2 (pg/mL)	68.1±70.7

Note: Data are presented as mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; *A1C,* glycated hemoglobin; *ARB,* angiotensin receptor blocker; *BMI,* body mass index; *DBP,* diastolic blood pressure; *eGFR,* estimated glomerular filtration rate; *HDL-C,* high density lipoprotein cholesterol; *hsCRP,* high-sensitivity C-reactive protein; *LDL-C,* low density lipoprotein cholesterol; *MDRD,* modification of diet in renal disease; *MI,* myocardial infarction; *MPV,* mean platelet volume; *SBP,* systolic blood pressure; *TC,* total cholesterol; *TxB_2,* thromboxane B2; *vWF,* von Willebrand factor; *WHR,* waist-to-hip ratio.

Table 2
Patients' characteristics grouped by serum BDNF concentration: demographic data

Characteristics	BDNF low (n=74) 11.59 to 28.09 ng/mL	BDNF high (n=75) 28.47 to 57.50 ng/mL	p
Demographic data			
Age (years)	68.59±8.52	65.60±8.96	0.038
Female	29 (39.2%)	38 (50.6%)	0.107
BMI (kg/m^2)	30.43±6.19	31.26±5.67	0.392
WHR	0.97±0.09	0.96±0.08	0.903
Waist circumference (cm)	104.16±15.47	105.08±15.21	0.712
Hip circumference (cm)	107.63±12.62	110.01±13.30	0.263
SBP (mmHg)	140.24±21.26	138.87±17.47	0.666
DBP (mmHg)	77.86±10.87	79.99±10.64	0.231
History of smoking	39 (52.7%)	47 (62.7%)	0.143
Current smoking	3 (4.05%)	11 (14.66%)	0.025
Dyslipidemia	64 (86.5%)	59 (77.6%)	0.149
Hypertension	71 (95.9%)	70 (93.3%)	0.367
CAD	35 (47.3%)	40 (53.3%)	0.283
Prior MI	23 (31.1%)	21 (28%)	0.408
CHF	30 (40.1%)	20 (26.7%)	0.052
Diabetes mellitus treatment and concomitant medication			
Beta blockers	52 (70.2%)	57 (76%)	0.273
ACE-I	47 (63.5%)	48 (64%)	0.543
Angiotensin receptor blockers	19 (25.7%)	13 (17.3%)	0.149
Loop diuretics	12 (16.2%)	10 (13.3%)	0.396
Statins	56 (75.7%)	51 (68%)	0.195
Fibrates	9 (12.2%)	15 (20%)	0.140
Calcium channel blockers	26 (35.1%)	38 (50.7%)	0.040
Metformin	40 (54%)	58 (77.3%)	0.002
Sulphonylurea derivatives	38 (51.4%)	34 (45.3%)	0.284
Insulin	22 (29.7%)	24 (32%)	0.451
Additional antiplatelet therapy (clopidogrel)	16 (21.6%)	16 (21.3%)	0.562
Biochemical parameters			
WBC (10^3/mm^3)	6.79±2.02	7.36±2.15	0.100
HGB (g/dL)	13.84±1.39	14.14±1.35	0.182
HCT (%)	41.46±3.87	42.26±3.89	0.211
PLT (10^3/mm^3)	206.61±44.48	245.81±68.85	0.000
MPV (fL)	9.84±1.30	9.87±1.19	0.862
FG (mg/dL)	134.86±41.14	150.81±63.77	0.135
eGFR (mL/min/1.73 m^2)	70.42±23.23	72.75±21.01	0.523
UA (mg/dL)	5.83±1.70	5.50±1.40	0.149
TCh (mg/dL)	157.00±34.49	168.04±42.22	0.084
TG (mg/dL)	121.76±60.50	140.73±67.50	0.049
LDL (mg/dL)	84.97±30.68	88.22±36.61	0.942
HDL (mg/dL)	47.89±12.78	51.18±14.29	0.143
A1C (%)	6.91±1.15	7.15±1.59	0.836
CEPI-CT (s)	226.39±76.88	218.88±81.98	0.681
CADP-CT (s)	140.93±86.63	104.88±69.73	0.000
VerifyNow ARU	451.08±109.28	448.27±97.46	0.545
sTXB2 (ng/mL)	0.36±0.80	0.94±1.59	0.020
hsCRP (mg/L)	3.05±3.24	3.24±3.72	0.782
sP-selectin	87.00±31.96	91.76±38.04	0.942
sCD40L (ng/mL)	0.62±0.59	0.99±2.21	0.615
TNF-α (pg/mL)	2.51±1.45	2.36±1.26	0.400
IL-6 (pg/mL)	2.31±1.37	2.93±2.20	0.188
vWF (%)	147.06±60.80	139.14±46.86	0.690
Hyperuricemia	17 (23%)	14 (18.7%)	0.328
CADP-CT above 75th percentile (s)	22 (29.7%)	8 (10.7%)	0.003
sTxB2 above 75th percentile (ng/mL)	8 (11%)	25 (33.3%)	0.001
Insulin above 75th percentile	12 (24%)	14 (28%)	0.410
Leptin above 75th percentile	15 (20.8%)	15 (28.8%)	0.181
Adiponectin above 75th percentile	18 (36%)	7 (14%)	0.010
Adiponectin	4.01±2.78	2.90±1.59	0.048

Note: Data are presented as mean ± SD.
ACE-I, angiotensin-converting enzyme inhibitor; *A1C*, glycated hemoglobin; *ARU*, aspirin reaction unit; *BDNF*, brain-derived neurotrophic factor; *BMI*, body mass index; *CAD*, coronary artery disease; *CADP-CT*, collagen/adenosine diphosphate closure time; *CEPI-CT*, collagen/epinephrine bitartrate closure time; *CHF*, congestive heart failure; *DBP*, diastolic blood pressure; *eGFR*, estimated glomerular filtration rate; *FG*, fasting glucose; *HCT*, hematocrit; *HDL*, high-density lipoprotein; *HGB*, hemoglobin; *hsCRP*, high sensitivity C-reactive protein; *IL-6*, interleukin 6; *LDL*, low-density lipoprotein; *MI*, myocardial infarction; *MPV*, mean platelet volume; *PLT*, platelet count; *SBP*, systolic blood pressure; *sCD40L*, soluble CD40 ligand; *sP-selectin*, soluble platelet selectin; *sTXB2*, serum thromboxane B2; *TCh*, total cholesterol; *TG*, triglycerides; *TNF-α*, tumor necrosis factor alpha; *UA*, uric acid; *vWF*, von Willebrand factor; *WBC*, white blood cells; *WHR*, waist-to-hip ratio.

treatment (p<0.001); current smoking (p=0.044); platelet count (p<0.001); triglyceride concentration (p=0.006); total cholesterol (p=0.022); CADP-CT >74 s (p<0.001) (Table 4). In multivariate linear regression analysis, predictive factors for high serum BDNF levels were: CADP-CT (p=0.001), metformin treatment (p=0.0282) and triglyceride concentration (p=0.0449).

Patients taking metformin (n=98) had significantly higher serum BDNF levels than patients without metformin therapy (n=51) (p=0.0002) (Figure 1A). Patients with normal platelet reactivity based on CADP-CT (i.e. >141 s; n=30) had lower serum BDNF concentrations than patients with high platelet reactivity (i.e. <141 s; n=119) (p=0.0013) (Figure 1B).

Table 3
Univariate logistic regression analysis of factors that correlate with higher BDNF levels

	OR (95% CI)	p
Gender	1.262 (0.912–1.747)	0.160
Age	0.961 (0.926–0.998)	0.040
BMI (kg/m^2)	1.024 (0.970–1.082)	0.390
WHR	0.163 (0.003–8.336)	0.366
Sulfonylurea treatment	0.886 (0.642–1.223)	0.463
Metformin treatment	1.703 (1.195–2.426)	0.003
Diet treatment	0.822 (0.587–1.153)	0.257
Insulin treatment	1.055 (0.745–1.493)	0.764
Hypertension	0.769 (0.369–1.603)	0.484
CAD	1.128 (0.818–1.557)	0.462
Dyslipidemia	0.759 (0.492–1.170)	0.212
MI	0.929 (0.653–1.321)	0.680
CHF	0.730 (0.517–1.032)	0.075
Hiperurycemia	0.877 (0.590–1.305)	0.518
Statins	0.826 (0.577–1.184)	0.299
Fibrates	1.344 (0.858–2.105)	0.197
Current smoking	2.017 (1.042–3.903)	0.037
Total cholesterol	1.008 (0.999–1.017)	0.090
Tryglycerides	1.005 (0.999–1.011)	0.081
HDL (mg/dL)	1.018 (0.994–1.044)	0.145
A1C (%)	1.134 (0.893–1.440)	0.303
LDL (mg/dL)	1.003 (0.993–1.013)	0.558
CADP-CT above 75th percentile (s)	0.531 (0.341–0.828)	0.005
sTXB2 above 75th percentile (ng/mL)	2.016 (1.300–3.125)	0.002
Level of insulin above 75th percentile	1.003 (0.993–1.013)	0.558
Level of leptin above 75th percentile	1.239 (0.846–1.813)	0.271
Level of adiponectin above 75th percentile	0.538 (0.329–0.881)	0.014
Predictors of high serum BDNF concentration in multivariate logistic regression analysis		
	OR (95% CI)	p
Adiponectin above 75th percentile	0.504 (0.288–0.880)	0.016
CADP >141 above 75th percentile	0.398 (0.191–0.828)	0.014
TCh (mg/dL)	1.049 (1.013–1.087)	0.007
LDL (mg/dL)	0.956 (0.918–0.996)	0.032

A1C, glycated hemoglobin; *BDNF,* brain-derived neurotrophic factor; *BMI,* body mass index; *CAD,* coronary artery disease; *CADP-CT,* collagen/adenosine diphosphate closure time; *CHF,* congestive heart failure; *HDL,* high-density lipoprotein; *LDL,* low-density lipoproteins; *MI,* myocardial infarction; *sTXB2,* serum thromboxane B2; *TCh,* total cholesterol; *WHR,* waist-to-hip ratio.

Table 4
Predictors of high serum BDNF concentration regression analysis

Predictors of high serum BDNF concentration in univariate linear regression analysis			
Variable	Coefficient	95% CI	p
Age	–0.164	–0.331 to 0.002	0.052
PLT (10^3/mm^3)	0.053	0.030 to 0.075	<0.000
A1C (%)	0.917	–0.145 to 1.978	0.090
TCh (mg/dL)	0.044	0.007 to 0.082	0.022
TG (mg/dL)	0.028	0.006 to 0.051	0.006
CADP-CT >74 (s)	–0.030	–0.047 to –0.012	0.001
sTxB2 (ng/mL)	0.920	–0.227 to 2.067	0.115
Current smoking	5.167	0.151 to 10.182	0.044
Calcium channel blockers	2.618	–0.349 to 5.585	0.083
Metformin	5.849	2.870 to 8.827	0.000
Leptin above 75th percentile	–0.995	–2.737 to 0.748	0.2612
Adiponectin above 75th percentile	2.120	0.032 to 4.208	0.0467
Insulin above 75th percentile	–0.296	–2.398 to 1.807	0.7809
A1C	0.917	–0.145 to 1.978	0.0900
Predictors of high serum BDNF concentration in multivariate linear regression analysis			
	Coefficient	95% CI	p
CADP-CT	–0.039	–0.062 to –0.016	0.0010
TG	0.032	0.001 to 0.062	0.0449
TCh	0.015	–0.036 to 0.066	0.5581
PLT (10^3/mm^3)	0.026	–0.005 to 0.056	0.1011
Current smoking	3.638	–2.034 to 9.310	0.2059
Metformin	4.327	0.473 to 8.182	0.0282
Adiponectin above 75th percentile	0.864	–1.189 to 2.916	0.4056

A1C, glycated hemoglobin; *BDNF,* brain-derived neurotrophic factor; *CADP-CT,* collagen/adenosine diphosphate closure time; *CI,* confidence interval; *OR,* odds ratio; *PLT,* platelet count; *sTXB2,* serum thromboxane B2; *TCh,* total cholesterol; *TG,* triglycerides.

Discussion

In the present study, we evaluated the association between serum BDNF concentrations and both platelet reactivity and antidiabetes therapy. For the first time, we showed the association between BDNF and metformin treatment in patients with type 2 diabetes. Additionally, we confirmed our previous results in a larger number of patients with diabetes: that serum BDNF concentrations could, at least in part, be related to platelet reactivity to antiplatelet therapy in type 2 diabetes.

A limited amount of research has shown the alteration of BDNF levels to be due to antidiabetes drug treatment in vitro, in animal models and in studies in humans (9–12). In our study, we found increased BDNF levels in patients who were taking metformin treatment. Yoo et al investigated the effects of a high-fat diet and the subsequent treatment by metformin on BDNF levels in mice. They observed significantly decreased BDNF levels with the high-fat diet + vehicle-treated group in the dentate gyrus. However, the administration of metformin prevented reduction of the BDNF levels. Additionally, injection of K252a, a potent BDNF receptor (tropomyosin receptor kinase B) inhibitor, significantly reversed the amelioration of the reduction in cell proliferation induced by high-fat diet in a metformin-treated group (10). Another study showed significantly increased BDNF levels in the metformin-treated group than in both the control group and the group with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinsonism in mice (11). In a human study, nonsignificantly higher plasma BDNF levels were observed in patients with metabolic syndrome after treatment with metformin (9). It was also shown that metformin treatment ameliorated the decreased mRNA of BDNF levels in Schwann cells under conditions of hypoxia. This beneficial effect of metformin was significantly inhibited by compound C, which is an inhibitor of adenosine monophosphate (AMP)-activated protein kinase (AMPK) and an important cellular regulator of lipid and glucose metabolism (12,27). It has been shown in many studies that the lipid-lowering effect of metformin is attributed largely to the activation of the energy sensor AMPK in hepatocytes and can suppress de novo lipogenesis in hepatocytes by activating AMPK (28–30). Thus, the correlation between BDNF and metformin may be the reason for metformin-induced insulin action by insulin receptor binding, metformin-induced high BDNF levels due to increasing AMPK and enhanced tyrosine kinase receptor activity, which may amplify BDNF signalling (31).

Various methods are available to assess platelet function under antiplatelet treatment. Platelet function assays should be easy to use, conducted in whole blood, reproducible, available at point-of-care and have clear cut-offs associated with thrombotic and bleeding complications. For ASA, certain assays that monitor the conversion of arachidonic acid into thromboxane A2 and ensuing platelet aggregation are often preferred over more global tests of platelet function (32). In our study, we used both PFA-100, CEPI-CT and CADP-CT tests in order to measure global platelet reactivity because they are quite insensitive to both ASA and clopidogrel therapy (32). As in our previous research, we found association only with PFA-100 CADP-CT, which reflects the combined effects of platelet activation by collagen, ADP and high shear (33). It is well known that ADP is 1 of the most important pro-aggregating substances released and that it interacts with purinergic receptors (P2X, P2Y1 and P2Y12) on the platelets' surfaces (34). It has previously been described that increased residual platelet reactivity, measured by PFA-100 CADP-CT, consistently predicts the occurrence of cardiovascular events (33,35). Because of the general insensitivity of CADP to platelet inhibition induced by ASA, it was hypothesized that the

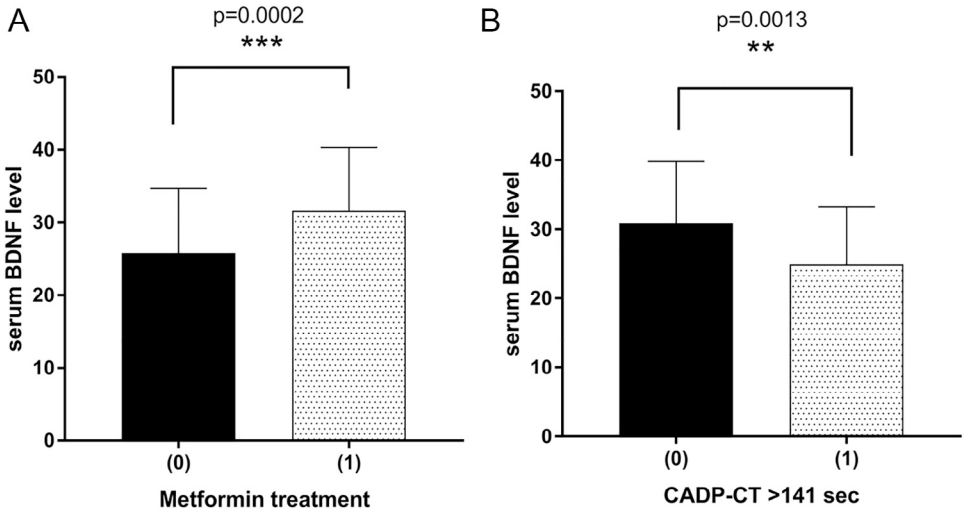

Figure 1. (A) Distribution of serum BDNF concentration across patients with metformin treatment (group 1, n=98, 31.66±0.87) and no metformin treatment (group 0, n=51, 25.81±1.24) (p<0.001). (B) Distribution of serum BDNF concentration across patients with normal platelet reactivity based on CADP-CT (i.e. >141 s, group 1, n=30, 24.93±1.52) and high platelet reactivity (i.e. <141 s, group 0, n=119, 30.85±0.823) in the study group (p=0.001). Data are mean ± SE. BDNF, brain-derived neurotrophic factor; CADP-CT, collagen/adenosine diphosphate closure time (s).

correlation of CADP-CT with the composite events would have been observed in these patients, whether or not they were taking ASA (36). If so, this suggests that CADP-CT may allow detection of an underlying ADP-dependent platelet activity that could be clinically significant. It should be noted that in our study, 21.5% of patients were also taking clopidogrel; however, we did not observe a correlation between BDNF levels and ASA and clopidogrel treatment. Similarly, Stoll et al also showed that ASA and clopidogrel had no significant effects on the plasma BDNF levels; however, a single loading dose (600 mg) of clopidogrel but not ASA (500 mg) significantly reduced the release of BDNF from platelets in 24 healthy volunteers. Clopidogrel inhibits irreversibly the membrane receptors of platelets, which are activated by ADP and impact on platelet alpha-granule degranulation (20). That study was consistent with the decrease in the platelet alpha-granule marker, transforming growth factor beta 1 after clopidogrel treatment, and the correlation between the effects of clopidogrel on BDNF and transforming growth factor beta 1 concentrations but not those after ASA treatment. In our previous studies, in a cohort of patients with type 2 diabetes, we found that platelet reactivity could be related to a number of clinical factors, biochemical variables and genetic polymorphisms (21,22,24,37–41). Moreover, it was found that genetic polymorphisms within genes related to platelet reactivity could also be useful prognostic tools (42). Also, noncoding RNAs (for example microRNA) are emerging biomarkers for cardiovascular diseases and platelet function and activity. Upon activation, platelets secrete microvesicles abundant in growth factors, as well as various proteins that might exert extracellular effects. Recent studies have indicated that platelet microvesicles may deliver platelet miRNAs at the action site in the cardiovascular system (43). Further analyses are needed to understand the mechanism of platelet reactivation and BDNF due to differing antiplatelet therapies and potential associations with new platelet-function biomarkers.

Moreover, we found significantly higher serum BDNF levels in current smokers than in nonsmokers, but there was no significant difference between former smokers and nonsmokers. An important study examined the levels of serum BDNF in nonsmokers, former smokers and current smokers with and without nicotine dependence in 2,088 participants. Smokers with and without nicotine dependence had higher levels of serum BDNF than former and never smokers, which indicates that the association between upregulation of BDNF and nicotine use is related to the duration of smoking (18). It was also observed that BDNF mRNA and protein expression in the hippocampus is increased after nicotine administration in animal models (44). Acute nicotine administration decreased the hippocampal BDNF gene expression by indirectly activating 5-HT_{2A} receptors. Alternatively, it may decrease due to the inhibitory effect of acute nicotine on BDNF mRNA. However, after chronic administration, tolerance may develop to the inhibitory effect of nicotine on BDNF mRNA expression, which may lead to a gradual increase of BDNF levels in chronic smokers (45).

Aging is a relevant factor affecting BDNF's ability to protect neuronal activity. In humans, BDNF levels in plasma have been found to decrease with increasing age (19). In our study, we also noted that patients with greater age had lower serum BDNF levels.

The increased risk for type 2 diabetes with obesity can be described partially by altered functions of adipose tissue, which is a major endocrine organ that secretes a number of active adipocytokines, such as leptin, resistin and adiponectin (25,46). We found that adiponectin levels were significantly associated with serum BDNF levels. It has been reported that the BDNF expression in hypothalamus is regulated by leptin and adiponectin (47). BDNF administration ameliorates hyperphagia and hyperglycemia in leptin receptor-deficient db/db animal models, which leads to the speculation that BDNF may play a role in leptin-resistance obesity and type 2 diabetes (48). Maekawa et al have demonstrated that low BDNF expression in the ventromedial hypothalamus is associated with blood glucose levels, increased leptin secretion and visceral fat mass in a rat model of type 2 diabetes. In their study, administration of BDNF significantly decreased plasma leptin levels in a long-lasting manner concurrently with feeding suppression in rats with type 2 diabetes and hyperleptinemia (49). Human studies have also showed an inverse correlation between BDNF and adiponectin and a positive correlation between BDNF and leptin (50,51). Altogether, our study and previous findings may indicate that BDNF and leptin play important roles in the central regulation of energy metabolism and the dysregulation of the neurotrophin signalling result in obesity.

Recent studies have shown that BDNF treatment in animals that are obese and have diabetes has a positive effect on glucose and lipid metabolism. Nakagawa et al demonstrated that subcutaneous injection of BDNF reduces food intake and ameliorates impaired glucose

tolerance in mice with diet-induced obesity (52). BDNF also reduces serum insulin and glucose levels when injected into rats with diabetes (48). Along with glucose metabolism, studies have also shown that BDNF is associated with lipid metabolism. In our study, we found that serum BDNF levels were significantly positively correlated with total cholesterol, triglyceride and LDL levels, which is a confirmation of other recent studies (5,51,53,54). These results may suggest that BDNF plays a role in the treatment of diabetes and dyslipidemia.

There are 2 main limitations of our study. First, it was observational, and it is impossible to account for all possible confounding influences. Second, this study was limited by its small sample size and lack of controls who did not have type 2 diabetes. Further study of a large population with type 2 diabetes and known platelet reactivity status would add further information about the role of platelet activation in BDNF.

Conclusions

In conclusion, the findings of this study suggest that BDNF may contribute to lipid metabolism, and increased production of BDNF may be related to metformin treatment. Importantly, we showed a strong association between BDNF concentration and platelet reactivity; serum BDNF levels in patients with type 2 diabetes and high platelet reactivity were higher than in subjects with normal platelet reactivity. Further studies are required to investigate this correlation.

Author Disclosures

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References

1. Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S. Brain-derived neurotrophic factor and the development of structural neuronal connectivity. Dev Neurobiol 2010;70:271–88.
2. Mirowska-Guzel D. The role of neurotrophic factors in the pathology and treatment of multiple sclerosis. Immunopharmacol Immunotoxicol 2009;31:32–8.
3. Gezen-Ak D, Dursun E, Hanağası H, et al. BDNF, TNFα, HSP90, CFH, and IL-10 serum levels in patients with early or late onset Alzheimer's disease or mild cognitive impairment. J Alzheimers Dis 2013;37:185–95.
4. Manni L, Nikolova V, Vyagova D, et al. Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. Int J Cardiol 2005;102:169–71.
5. Suwa M, Kishimoto H, Nofuji Y, et al. Serum brain-derived neurotrophic factor level is increased and associated with obesity in newly diagnosed female patients with type 2 diabetes mellitus. Metabolism 2006;55:852–7.
6. Lorgis L, Amoureux S, de Maistre E, et al. 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 2010;24:525–30.
7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009;32(Suppl. 1):S62–7.
8. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: Pharmacokinetics and pharmacodynamics. Pharmacogenet Genom 2012;22:820–7.
9. Hristova MG. Metabolic syndrome and neurotrophins: Effects of metformin and non-steroidal antiinflammatory drug treatment. Eurasian J Med 2011;43:141–5.
10. Yoo DY, Kim W, Nam SM, et al. Reduced cell proliferation and neuroblast differentiation in the dentate gyrus of high fat diet-fed mice are ameliorated by metformin and glimepiride treatment. Neurochem Res 2011;36:2401–8.
11. Patil SP, Jain PD, Ghumatkar PJ, Tambe R, Sathaye S. Neuroprotective effect of metformin in MPTP-induced Parkinson's disease in mice. Neuroscience 2014;277:747–54.
12. Ma J, Liu J, Yu H, Chen Y, Wang Q, Xiang L. Effect of metformin on Schwann cells under hypoxia condition. Int J Clin Exp Pathol 2015;8:6748–55.
13. Yazbek N, Bapat A, Kleiman N. Platelet abnormalities in diabetes mellitus. Coron Artery Dis 2003;14:365–71.
14. Ferreiro JL, Gomez-Hospital JA, Angiolillo DJ. Platelet abnormalities in diabetes mellitus. Diab Vasc Dis Res 2010;7:251–9.
15. Coban E, Yazicioglu G, Ozdogan M. Platelet activation in subjects with subclinical hypothyroidism. Med Sci Monit 2007;13:CR211–14.
16. Prakash YS, Martin RJ. Brain-derived neurotrophic factor in the airways. Pharmacol Ther 2014;143:74–86.
17. Fujimura H, Altar CA, Chen R, et al. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb Haemost 2002;87:728–34.
18. Jamal M, Van der Does W, Elzinga BM, Molendijk ML, Penninx BW. Association between smoking, nicotine dependence, and BDNF Val66Met polymorphism with BDNF concentrations in serum. Nicotine Tob Res 2015;17:323–9.
19. Lommatzsch M, Zingler D, Schuhbaeck K, et al. The impact of age, weight and gender on BDNF levels in human platelets and plasma. Neurobiol Aging 2005;26:115–23.
20. Stoll P, Plessow A, Bratke K, et al. Differential effect of clopidogrel and aspirin on the release of BDNF from platelets. J Neuroimmunol 2011;238:104–6.
21. Postula M, Kapłon-Cieślicka A, Rosiak M, et al. Genetic determinants of platelet reactivity during acetylsalicylic acid therapy in diabetic patients: Evaluation of 27 polymorphisms within candidate genes. J Thromb Haemost 2011;9:2291–301.
22. Rosiak M, Postula M, Kapłon-Cieślicka A, et al. Effect of ASA dose doubling versus switching to clopidogrel on plasma inflammatory markers concentration in patients with type 2 diabetes and high platelet reactivity: The AVOCADO study. Cardiol J 2013;20:545–51.
23. Mirowska-Guzel D, Gromadzka G, Seniow J, et al. Association between BDNF-196 G>A and BDNF-270 C>T polymorphisms, BDNF concentration, and rTMS-supported long-term rehabilitation outcome after ischemic stroke. Neuro Rehab 2013;32:573–82.
24. Postula M, Janicki PK, Rosiak M, et al. Effect of common single-nucleotide polymorphisms in acetylsalicylic acid metabolic pathway genes on platelet reactivity in patients with diabetes. Med Sci Monit 2013;19:394–408.
25. Kapłon-Cieślicka A, Postuła M, Rosiak M, et al. Association of adipokines and inflammatory markers with lipid control in type 2 diabetes. Pol Arch Med Wewn 2015;125:414–23.
26. Chakroun T, Addad F, Abderazek F, et al. Screening for aspirin resistance in stable coronary artery patients by three different tests. Thromb Res 2007;121:413–18.
27. Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: Possible roles in type 2 diabetes. Am J Physiol 1999;277:1–10.
28. Li Y, Wong K, Giles A, et al. Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. Gastroenterology 2014;146:539–49.
29. Wang QA, Scherer PE, Gupta RK. Improved methodologies for the study of adipose biology: insights gained and opportunities ahead. J Lip Res 2014;55:605–24.
30. Zang MW, Xu SQ, Maitland-Toolan KA, et al. Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes 2006;55:2180–91.
31. Stith BJ, Goalstone ML, Espinoza R, Mossel C, Roberts D, Wiernsperger N. The antidiabetic drug metformin elevates receptor tyrosine kinase activity and inositol 1,4,5-trisphosphate mass in Xenopus oocytes. Endocrinology 1996;137:2990–9.
32. Lordkipanidzé M. Advances in monitoring of aspirin therapy. Platelets 2012;23:526–36.
33. Gianetti J, Parri MS, Sbrana S, et al. Platelet activation predicts recurrent ischemic events after percutaneous coronary angioplasty: A 6 months prospective study. Thromb Res 2006;118:487–93.
34. Hechler B, Cattaneo M, Gachet C. The P2 receptors in platelet function. Semin Thromb Hemost 2005;31:150–61.
35. Chiu FC, Wang TD, Lee JK, et al. Residual platelet reactivity after aspirin and clopidogrel treatment predicts 2-year major cardiovascular events in patients undergoing percutaneous coronary intervention. Eur J Intern Med 2011;22:471–7.
36. Jilma B. Platelet function analyzer (PFA-100): A tool to quantify congenital or acquired platelet dysfunction. J Lab Clin Med 2001;138:152–63.
37. Postula M, Janicki PK, Rosiak M, et al. New single nucleotide polymorphisms associated with differences in platelets reactivity in patients with type 2 diabetes treated with acetylsalicylic acid: Genome-wide association approach and pooled DNA strategy. J Thromb Thrombol 2013;36:65–73.
38. Rosiak M, Postula M, Kaplon-Cieslicka A, et al. The effect of doubling the dose of acetylsalicylic acid (ASA) on platelet function parameters in patients with type 2 diabetes and platelet hyperreactivity during treatment with 75 mg of ASA: A subanalysis of the AVOCADO study. Kardiol Pol 2013;71:552–7.
39. Milanowski L, Pordzik J, Janicki PK, Postula M. Common genetic variants in platelet surface receptors and its association with ischemic stroke. Pharmacogenomics 2016;17:953–71.
40. Kaplon-Cieslicka A, Rosiak M, Postula M, et al. Predictors of high platelet reactivity during aspirin treatment in patients with type 2 diabetes. Kardiol Pol 2013;71:893–902.

41. Postula M, Janicki PK, Eyileten C, et al. Next-generation re-sequencing of genes involved in increased platelet reactivity in diabetic patients on acetylsalicylic acid. Platelets 2016;27:357–64.
42. Iaconetti C, Sorrentino S, De Rosa S, Indolfi C. Exosomal miRNAs in heart disease. Physiology (Bethesda) 2016;31:16–24.
43. Willeit P, Zampetaki A, Dudek K, et al. Circulating microRNAs as novel biomarkers for platelet activation. Circul Res 2013;112:595–600.
44. Andresen JH, Løberg EM, Wright M, Goverud IL, Stray-Pedersen B, Saugstad OD. Nicotine affects the expression of brain-derived neurotrophic factor mRNA and protein in the hippocampus of hypoxic newborn piglets. J Perinat Med 2009;37:553–60.
45. Kenny PJ, File SE, Rattray M. Acute nicotine decreases, and chronic nicotine increases the expression of brain-derived neurotrophic factor mRNA in rat hippocampus. Brain Res Mol Brain Res 2000;85:234–8.
46. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 2008;93:64–73.
47. Noble EE, Billington CJ, Kotz CM, Wang C. The lighter side of BDNF. Am J Physiol Regul Integr Comp Physiol 2011;300:1053–69.
48. Tonra JR, Ono M, Liu X, et al. Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. Diabetes 1999;48:588–94.
49. Maekawa F, Fujiwara K, Toriya M, et al. Brain-derived neurotrophic factor in VMH as the causal factor for and therapeutic tool to treat visceral adiposity and hyperleptinemia in type 2 diabetic Goto-Kakizaki rats. Front Synap Neurosci 2013;5:7.
50. Fujinami A, Ohta K, Obayashi H, et al. Serum brain-derived neurotrophic factor in patients with type 2 diabetes mellitus: Relationship to glucose metabolism and biomarkers of insulin resistance. Clin Biochem 2008;41:812–17.
51. Golden E, Emiliano A, Maudsley S, et al. Circulating brain-derived neurotrophic factor and indices of metabolic and cardiovascular health: Data from the Baltimore Longitudinal Study of Aging. PLoS ONE 2010;5:e10099.
52. Nakagawa T, Ogawa Y, Ebihara K, et al. Anti-obesity and anti-diabetic effects of brain-derived neurotrophic factor in rodent models of leptin resistance. Int J Obes Relat Metab Disord 2003;27:557–65.
53. Boyuk B, Degirmencioglu S, Atalay H, et al. Relationship between levels of brain-derived neurotrophic factor and metabolic parameters in patients with type 2 diabetes mellitus. J Diabetes Res 2014;2014:978143.
54. Eyileten C, Zaremba M, Janicki PK, et al. Serum brain-derived neurotrophic factor is related to platelet reactivity but not to genetic polymorphisms within BDNF encoding gene in patients with type 2 diabetes. Med Sci Monit 2016;22:69–76.