Age-related differences in plasma BDNF levels after prolonged bed rest

Cecilia-Soavi[1], Uroš-Marušič[2], Juana-Maria-Sanz[1], Mario-Luca-Morieri[1], Edoardo-Dalla Nora[1], Bostjan-Šimunič[2], Rado-Pišot[2], Giovanni-Zuliani[1] and Angelina-Passaro[1].

[1] Medical Science Department, University of Ferrara

[2] Science and Research Centre, University of Primorska

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Correspondance to:

Angelina Passaro, MD, PhD,

Medical Science Department, University of Ferrara

Via Ariosto 35, 44121, Ferrara, ITALY

Phone: +39 0532 237017

Mail: psn@unife.it

ORCID code: 0000-0001-8462-7000

Abstract

Brain derived neurotrophic factor (BDNF) is a member of neurotrophin family implied in brain resistance to insults. Murine studies demonstrated increased hippocampal concentration after acute, and decreased after chronic immobilization. In humans, chronic stress and sedentary lifestyle decreased plasmatic BDNF, while there is no data regarding acute immobilization. The aim of our study was to evaluate age-related responses (comparing 7 YOUNG 23 ± 3 years and 8 OLDER subjects 60 ± 4 years) of plasma BDNF before (baseline data collection ,BDC) and after (BR14) 14 days of horizontal bed rest (BR).

At BDC, BDNF levels were not different between the two groups (p=0.101), while at BR14 BDNF levels were higher in OLDER (62.02±18.31 vs YOUNG: 34.36±15.24 pg/ml, p=0.002). A general linear model for repeated measures showed a significant effect of BR on BDNF (p=0.002). The BDC BDNF levels correlated with fat-free mass in the whole population (ALL) (R=0.628, p=0.012), OLDER (R=0.753, p=0.031), and YOUNG (R=0.772, p=0.042), and with total cholesterol in ALL (R=0.647, p=0.009) and OLDER (R=0.805, p=0.016). At BR14 BDNF correlated with total cholesterol (R=0.579, p=0.024) and age (R=0.647, p=0.009) in ALL. With the increase of age, the brain could become naturally less resistant to acute stressors, including the detrimental effects of prolonged bed rest, and thus, the increase in BDNF only in older group might reflect a protective overshooting of the brain to counteract negative effects in such conditions.

Keywords: brain derived neurotrophic factor; bed rest; acute stress; aging brain.

New and Noteworthy

BDNF blood concentration increases after 14 days of bed rest in older but not in young people, maybe indicating that aging brain is less resistant to acute stresses, and the increase of BDNF could represent the protective response to the acute stress of bed rest.

List of abbreviations

BDC: baseline data collection

BDNF: brain-derived neurotrophic factor

BMI: body mass index

BR14: at day 14 of forced bed rest

GLM: general linear model

HDL: high density lipoprotein

HOMA-IR: homeostasis model assessment for insulin resistance

LDL: low density lipoprotein

TG: triglycerides

TNF: tumour necrosis factor

TrkB: tropomiosine receptor kinase B

Introduction

Lots of elderly individuals are necessarily bedridden at a certain point of their life, for example for an acute medical condition needing hospitalization, with negative effects on metabolism and both motor and cognitive performance. In fact bed rest was demonstrated to induce insulin resistance, to increase total cholesterol and triglyceride plasma levels, to impair microvascular regulation, and to increase basal arterial tone leading to hypertension (1; 16; 50); moreover bed rest has been associated to a rapid decrease in muscle size due to both reduction in muscle protein synthesis and increase in protein degradation (57); in addition, prolonged bed rest was shown to act as a stressful agent on the brain, negatively affecting brain structure (23), manifesting changes on the brain electrocortical activity (30) and detrimentally effecting the executive functioning and mood (24; 25).

Brain-derived neurotrophic factor (BDNF) is a growth factor member of the neurotrophin family; it binds specifically to the Tropomiosine receptor kinase B (Trk B), a tyrosine kinase receptor, thus mediating neurotrophic signalling (8). During normal development it plays a critical role in cell differentiation, migration, neuronal survival, dendritic arborisation, synaptogenesis,

and synaptic plasticity, but also following insults in developing/adult brain it play an important role in the repair of damages and resistance to insults (8).

Even if the cellular sources of BDNF found in human plasma are not clearly defined yet, there is some evidence suggesting a pivotal role in cerebral output. Namely, Pan et al. showed that in mice BDNF can cross blood-brain barrier bi-directionally (34); Karege et al. (18) studied rat models demonstrating similar hyppocampal and serum levels during maturation and aging processes where these changes were compatible with the changes in hyppocampal BDNF mRNA expression, but not with platelets BDNF mRNA expression; on the basis of these results serum concentration of BDNF has already been used as indirect measure of BDNF activity in the central nervous system (9). In humans a BDNF release from brain was observed at rest, and an increase of two- to three-fold occurred during exercise, and Rasmussen et al. estimated that in both conditions brain contribution determined the 70-80% of total circulating BDNF. (40).

On the opposite, although BDNF is up-regulated in contracting muscle fibers, it has been demonstrated that muscles were not a source of circulating BDNF (31, 37).

Low levels of plasma BDNF have been associated with Alzheimer and other forms of dementia (56), diabetes (20; 58), depression (5; 17) and acute coronary syndrome (28); we have recently suggested the existence of a "synergistic" effect of late onset Alzheimer Disease and diabetes on BDNF plasma levels (36).

Interestingly, in murine models, a different behaviour of BDNF was noticed in response to chronic and acute immobilization. Chronic immobilization, as well as other chronic stressors, reduce BDNF concentration in rat hippocampus (41; 42; 49; 54; 55); on the opposite, Marmigere et al. demonstrated that, in the very acute phase of immobilization stress (15-60 minutes), a transient increase in hippocampal BDNF mRNA expression occurs, followed by a reduction at levels lower than basal at 180 minutes; similarly, at 180 minutes was found an increase in hippocampal BDNF content, which rapidly returned at basal levels when the immobilization stimuli lasted up to 300 minutes (29). The same result can be obtained acutely exposing the rat to damaging factors such as hypoxia, ischemia and neurotoxic substances (3), or other kinds of acute stress such as forced swimming in cold water (47). In this context, acute immobilization can be considered as an acute stress factor inducing the activation of short-term protective mechanisms, while chronic stressors as chronic immobilization can lead to excessive stimulation and functional distortion of neuroendocrine systems reducing BDNF expression (33).

Similarly, studies on humans showed a reduction in serum BDNF in sedentary subjects (45) as in people exposed to chronic stress (7; 35), but data are lacking about plasma BDNF response to acute stressors. Thus, we hypothesized that plasmatic levels of BDNF could be associated with long-term bed rest in human subjects and that there will be age-related differences. To test this hypothesis we use data collected during the horizontal 14 days Bed Rest model, where 7 young and 8 older healthy adults were tested before and after 14 days of horizontal bed rest.

Materials and methods

Population

We designed a study composed by two groups of healthy volunteers: the YOUNG group (7 subjects, 19-28 years, mean age 23.3 ± 3.X years) and an OLDER group (8 subjects, mean age 59.5 ± 4.X years). After 3 days of ambulatory period (regulated hospital diet and daily activities) all subjects underwent horizontal bed rest for 14 days in standard air-conditioned hospital rooms of the Orthopedic Hospital of Valdoltra (Slovenia). During the whole bed rest procedure, constant surveillance and 24-hour medical care was provided and all subjects received an individually controlled normo-caloric diet: for each subject resting energy expenditure (estimated by means of bioimpedentiometric- BIA measures) was multiplied by factor 1.2, with 60% caloric content coming from carbohydrates, 25% from fat and 15% from proteins (4). Subjects performed all daily activities in bed, were allowed to freely communicate, watch television and listen to radio, read, use computer and to receive visitors.

Exclusion criteria were: smoking; regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer levels at enrolment greater than 500 µg·L-1; acute or chronic skeletal, neuromuscular, metabolic and cardiovascular disease conditions; pulmonary embolism. All subjects gave their written informed consent. The study was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Biologic samples and measures

Blood samples from each subject were collected after an overnight fasting at enrolment (baseline data collection - BDC) and after 14 days of forced bed rest (BR14) and centrifuged in absence or presence of EDTA to obtain serum and plasma, respectively. Aliquots were stored at -80°C. HDL cholesterol after precipitation of the apo-B containing lipoproteins, (6), total cholesterol and triglycerides (TG) levels were assayed in serum by the Trinder method. The

coefficient of variation was < 2% for Total and HDL cholesterol and < 5% for TG for intra- and inter-batch, respectively. LDL cholesterol plasma levels were calculated by the Friedewald's formula (12).Plasma glucose was measured using standard enzymatic methods (FAR S.R.L., Italy). The coefficient of variation was < 3% for intra-assay. Fasting insulin levels were assayed using an ultrasensitive insulin ELISA kit manufactured by Mercodia AB (Sweden). The coefficient of variation was < 3% for intra-assay. Fasting insulin resistance was evaluated calculating Homeostasis Model Assessment (HOMA-IR) (32). TNF-alpha plasma levels were measured using an ELISA Kit (Invitrogen™ reagents, USA). The coefficient of variation was < 5.2% for intra-assay. C-reactive protein plasma levels were measured using an immunoturbidimetric test (High sensitive C-reactive protein, Roche, Italy). The coefficient of variation was < 2% for intra-assay. BDNF plasma levels were measured by means of ELISA (Promega Italia S.r.l, Italy), following the manufacturer's instructions.

At BDC and BR14 all subjects were tested with BIA for body composition measures using tetra-polar impedance-meter (BIA101, Akern, Florence, Italy) according to manufacturer's instructions (27). All measures were collected by the same trained staff member, after eight hours fasting.

Statistical analysis

Continuous variables were expressed as mean (SD) or, when necessary, as median (range) and categorical variables as the number/percentage. Means were compared by One way ANOVA using the Bonferroni Post Hoc Tests for post-hoc analysis, while medians were compared by non-parametric tests (Kruskal Wallis test). Normality of distribution was tested with Shapiro-Wilk test. Variations between BDC and BR14 in BDNF plasma levels and the other variables of interest were analysed by General Linear Model (GLM) Repeated Measures, Within-Subjects and Between-Subjects test.

Correlations between continuous variables were tested by multivariate linear regression analysis. Variables with non-normal distribution were analyzed after log transformation or with non-parametric test (Spearman's test).

Multivariate linear regression analysis (method stepwise forward) was used to test the association between the plasma BDNF levels and other variables previously selected by univariate analysis. Dichotomous variables were included as dummy variables (0: absent; 1: present).

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) and statistical significance was set a $P < 0.05$.

Results

Baseline characteristics of all subjects are shown in Table 1. In addition to the expected difference in age ($p<0.001$), the OLDER group had significantly higher plasma levels of total ($p<0.001$) and LDL cholesterol ($p<0.001$), triglycerides ($p=0.038$) and C-reactive protein ($p=0.050$) compared with the YOUNG group.

EFFECT OF BED REST ON BDNF

At baseline plasma BDNF levels were not significantly different between the two groups (OLDER: 37.53±18.58 pg/ml, YOUNG: 22.38±13.84 pg/ml, $p=0.101$).

At BR14, BDNF levels were significantly different comparing the two groups (OLDER: 62.02±18.31 pg/ml vs YOUNG: 34.36±15.24 pg/ml, $p=0.002$), the difference was maintained also using BDC BDNF levels as covariate (OLDER vs YOUNG, $p=0.040$) . To evaluate the effect of bed rest on BDNF levels, we performed a GLM for repeated measures which showed a significant bed rest effect ($p=0.002$); however, no interaction effect ($p=0.215$). Post-hoc analysis showed that after bed rest (BR14) there was an increase in plasma BDNF in OLDER subjects ($p=0.009$), while a trend towards an increase was observed in YOUNG group ($p=0.118$) (see Figure 1).

LINEAR REGRESSION ANALISYS BETWEEN BDNF LEVELS AND OTHER VARIABLES

To investigate which anthropometric/metabolic parameters could influence BDNF basal and final levels, we evaluated the correlation between BDNF levels and other variables (age, BMI, fat free mass, fat mass, intracellular water, extracellular water, total body water, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, HOMA-IR, C reactive protein, TNF alpha).

At baseline (BDC), in the whole population, plasma BDNF levels positively correlated with fat-free mass ($R=0.628$, $p=0.012$), total ($R=0.647$, $p=0.009$) and LDL cholesterol levels ($R=0.681$, $p=0.005$); the positive correlation with fat-free mass was maintained also considering only the YOUNG ($R=0.772$, $p=0.042$) and the OLDER group ($R=0.753$, $p=0.031$), and in the OLDER group the correlation with total cholesterol level was confirmed too ($R=0.805$, $p=0.016$).

At BR14, in the whole population BDNF plasma levels were found to correlate with total cholesterol ($R=0.579$, $p=0.024$), but also with age ($R=0.647$, $p=0.009$); no variables were found to significantly correlate with plasma BDNF dividing the subject into OLDER and YOUNG group.

The variation of BDNF plasma levels from BDC to BR14 (ΔBDNF) did not correlate with any of the mentioned variables. Figure 2 shows the correlation between total cholesterol and plasma BDNF both in OLDER and YOUNG group at BDC and BR14.

We finally produced two Multiple Linear Regression Models: the first using as dependent variable BR14 plasma BDNF levels, and as independent variables the membership in one or another of the groups (YOUNG/OLDER), BDC plasma levels of BDNF, BR14 plasma levels of total cholesterol and Fat free mass. We found that only the membership in one or the other of the two groups predicted final BDNF plasma levels (R^2=0.658, p=0.008, unstandardized beta coefficient 27.656).

We also performed a second Multiple Linear Regression Model using as dependent variable plasma BDNF (both at BDC and BR14), and as independent variables the membership in one or another of the two groups, total cholesterol (both at BDC and BR14), fat-free mass (both at BDC and BR14), and another variable that we named "time", obtained giving number "0" to all the detections collected at BDC from each subject, and "1" to all the detections collected at BR14. So doing we evaluated the influence of the factor "time" on BDNF levels, which means the effect of undergoing bed rest. The results are shown in Table 2.

Discussion

The present study investigated the age-related differences in plasma BDNF levels after prolonged physical inactivity (bed rest) acting as an acute stressor on human body. To our knowledge, this was the first study aiming to investigate plasma BDNF fluctuations in younger and older healthy subjects undergoing 14-day of strict bed rest, therefore straight forward comparison with other studies is not possible. However, in the animal studies BDNF hippocampal levels were demonstrated to increase in murine models during acute stresses such as immobilization stress, explicating a protective role (13; 29; 39). On the other hand, chronic immobilization (repeated immobilization stress) as well as other chronic stressors, reduced BDNF concentration in rat hippocampus (41; 42; 49; 54; 55). Here we show an increase in plasma BDNF after bed rest in older subjects, but not in young people.

Our aim was not to assimilate human model of immobilization with murine; clearly, the two situations cannot be compared, neither for what regards time intervals, nor about the intensity of the stress: it is clear that mice experience a very strong psychological stress during forced immobilization, rather than a physical stress; on the opposite, human volunteers experience

scarce or no psychological stress, but a substantial physical stress, as shown in a recent work of our group (38). Nevertheless, the finding that acute and chronic stresses differently modulate BDNF expression in mice led us to hypothesize that also in humans such a modulation could take place.

As previously said, even if there is no agreement about the cellular source of human plasma BDNF, there is some evidence, both in human and in animal, of a central role of brain in determining circulating BDNF levels (18; 40), and its contribution to total circulating BDNF has been considered of about 70-80% (40). A massive presence of BDNF in platelets and in skeletal muscle was described too, but while platelet release upon activation does not seem to explain the variations of circulating BDNF occurring with aging or with exercise (26; 40), skeletal muscle was demonstrated not to be a source of circulating BDNF (31, 37). On the basis of all these results blood concentration of BDNF has already been used as indirect measure of BDNF activity in the central nervous system (9).

We hypothesize that, with increasing age, the brain might become less resistant to acute stresses such as bed rest, so that a condition like bed rest could become more stressful for the brain, and therefore a greater effort in order to counteract its negative effects could be required.

Our correlation analyses showed a positive correlation between BDNF and fat-free mass, both in OLDER and YOUNG, as well as in the whole population at BDC. Several studies which examined changes in BDNF levels in humans revealed an augmentation of BDNF after acute bout of exercise (43; 14) or, for instance, after five weeks (59) and three months of endurance training (46), a well-known condition which influences also muscle mass in younger and older individuals (15; 48; 53). On the opposite, studies showed lower BDNF values in sedentary individuals (45). Therefore our result might reflect the fact that people with higher levels of fat-free mass were likely more physically active. The loss of such a correlation at the end of bed rest (BR14) might reflect the different behaviour of the two variables during bed rest: in fact, while an increase is observed in BDNF, on the opposite a decrease in fat-free mass is observed at the end of bed rest period in the whole population (60.3 $\rightarrow$56.5 kg, $P<0.001$) as well as in OLDER group (59.8 $\rightarrow$56.7, $P<0.001$). The decrease in muscle mass was confirmed in other bed rest studies involving younger (11) and older individuals (19).

Moreover, total cholesterol was found to positively correlate with BDC plasma BDNF levels in the whole population and in the OLDER group, but not in the YOUNG subjects: this might reflect a

different significance of total cholesterol plasma levels at different ages. It is well known from literature that while in the young subjects a higher level of cholesterol is a risk factor for all-causes and cardiovascular mortality (10), in older people the same levels seem to be associated with a lower mortality (concept known as “Reverse epidemiology”). This probably derives from the fact that in older population lower levels of total cholesterol, rather than reflecting a healthier lifestyle, are the result of chronic inflammatory conditions, so that higher levels of total cholesterol reflect actually a better global health (2)

Interestingly, at the end of bed rest (BR14), this positive correlation between BDNF and total cholesterol is not found any more: this could reflect the behaviour of cholesterol as an acute phase molecule: in acute stress conditions cholesterol plasma levels decrease (44), in this context, considering bed rest a stressful condition, while BDNF increases in order to counteract its negative effect on brain, total and LDL cholesterol levels decrease, losing the positive correlation.

On the other hand, the role of bed rest as an acute stress condition is confirmed by the behaviour of several variables: first of all total cholesterol, which decreases from BDC to BR14 both in the whole population (183.79 →165.14 mg/dl, P <0.001) and in OLDER group (212,36 →183.51 mg/dl, P 0.024), but not in YOUNG (151.11 →144.14, P 0.23), but also TNFα, a well-known inflammatory marker, increases after bed rest both in the whole population (1.23 →1.46 mg/dl, P<0.001), in OLDER (1.45 →1.59 mg/dl, P<0.001) and YOUNG group (0.98 →1.30 mg/dl, P<0.001), and even CRP shows an increase in plasma concentration after bed rest in the whole population (0.080 →0.236 mg/dl, P0.033).

The fact at BR14 age emerges as a variable correlating with plasma BDNF reflects the significant increase in BDNF seen in OLDER, but not in YOUNG group; on the other hand, the Multiple Linear regression models demonstrate that age (i.e. the membership to one or the other group) is an independent predictor of the final plasma BDNF levels, which is influenced, as expected, by the bed rest (i.e. “time” factor), but also by total cholesterol. Despite what already said regarding cholesterol and its behaviour as acute phase molecule, there are only a few studies in literature linking BDNF with cholesterol, and most of them refer to brain content of cholesterol and not to plasmatic levels (21; 51; 52). Our result seems in line with a recent study conducted in a cohort of patients suffering from bipolar disorder (22), but data are lacking regarding the general population, so we aim to study a larger population in order to confirm this result.

The potential mechanisms involved in plasma BDNF expression during prolonged bed rest are further discussed. During acute stress, as during an intense and prolonged physical activity inducing hypoxia, the response of central nervous system (in particular cortex and hippocampus, as suggested by Rasmussen et al.) (40) is an increased release of BDNF. If confirmed, our data seem to suggest that, also in case of prolonged bed rest, central nervous system increase BDNF production as a stereotyped response in order to repair the damage induced by acute stress, and its release in circulation to counteract the negative effects of acute stress on metabolism.

Limitations of the study

We must finally acknowledge some limitation of this study, first of all the small number of subjects, that makes it difficult to drive any conclusions. However, it is necessary to keep in mind that bed rest studies which included older individuals are rare and that bed rest studies are very costly, labour intensive, and limited by hospital capacity. The total costs are estimated at the level over $20,000 per participant. However, in spite of having such a small population sample size we were able to detect variables, which were correlated with moderate significance. We hypothesize that in a larger population even stronger statistical significance could be reached.

Finally, since we did not perform functional neuroimaging and we did not obtain samples of cerebro-spinal fluid from subjects, we have only surrogate evaluations of brain stress, such as TNFα, and CRP.

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Figure captions:

Figure 1. BDNF plasma levels variation from BDC to BR14 in our population

Figure 2. Correlation between plasma BDNF and total cholesterol levels in OLDER and YOUNG group both at BDC and BR14.