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

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[1]*Medical Science Department, University of Ferrara, Ferrara, Italy; and* [2]*Science and Research Centre, University of Primorska, Capodistria, Slovenia*

Soavi C, Marušič U, Sanz JM, Morieri ML, Dalla Nora E, Šimunič B, Pišot R, Zuliani G, Passaro A. Age-related differences in plasma BDNF levels after prolonged bed rest. *J Appl Physiol* 120: 1118–1123, 2016. First published March 3, 2016; doi:10.1152/japplphysiol.01111.2015.—Brain-derived neurotrophic factor (BDNF) is a member of the family of neurotrophins and has been implicated in brain resistance to insults. Murine studies have demonstrated increased hippocampal concentration after acute immobilization and decreased concentration after chronic immobilization. In humans, chronic stress and sedentary lifestyle result in decreased plasma BDNF levels, but there no data exist regarding acute immobilization. The aim of our study was to evaluate age-related responses [comparing 7 younger subjects (age 23 ± 3 yr) and 8 older subjects (age 60 ± 4 yr)] of plasma BDNF before (baseline data collection, BDC) and after 14 days (BR14) of horizontal bed rest (BR). At BDC, BDNF levels were not different between the two groups ($P = 0.101$), although at BR14, BDNF levels were higher in older subjects ($62.02 ± 18.31$) than in younger subjects ($34.36 ± 15.24$ 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 both populations (ALL) ($R = 0.628$, $P = 0.012$), (older, $R = 0.753$, $P = 0.031$; younger, $R = 0.772$, $P = 0.042$), and with total cholesterol in ALL ($R = 0.647$, $P = 0.009$) and older study subjects ($R = 0.805$, $P = 0.016$). At BR14, BDNF correlated with total cholesterol ($R = 0.579$, $P = 0.024$) and age ($R = 0.647$, $P = 0.009$) in ALL. With an increase in 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 in the older study group might reflect a protective overshooting of the brain to counteract the negative effects in such conditions.

brain-derived neurotrophic factor; bed rest; acute stress; aging brain

NEW AND NOTEWORTHY

Blood concentrations of brain-derived neurotrophic factor (BDNF) increase after 14 days of bed rest in older but not younger people, and may indicate that the aging brain is less resistant to acute stresses, and increases in BDNF could represent the protective response to the acute stress of bed rest.

MANY ELDERLY INDIVIDUALS ARE necessarily bedridden at a certain point in their life; for example, an acute medical condition may require hospitalization, with negative effects on metabolism and both motor and cognitive performance. In fact, bed rest was demonstrated to induce insulin resistance, increase total cholesterol and triglyceride plasma levels, impair microvascular regulation, and increase basal arterial tone leading to hypertension (1, 16, 50). Moreover, bed rest has been associated with a rapid decrease in muscle size due to a reduction in muscle protein synthesis and an 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 in brain electrocortical activity (30) and detrimentally effecting 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 tyrosine receptor kinase B (TrkB), a tyrosine kinase receptor, thus mediating neurotrophic signaling (8). During normal development BDNF plays a critical role in cell differentiation, migration, neuronal survival, dendritic arborization, synaptogenesis, and synaptic plasticity. But also following insults, in developing/adult brain, BDNF plays an important role in the repair of damage and resistance to insults (8).

Even if the cellular sources of BDNF found in human plasma are not clearly defined, there is some evidence suggesting a pivotal role in cerebral output. Pan et al. (34) showed that in mice, BDNF can cross the blood-brain barrier bidirectionally, and in studying rat models, Karege et al. (18) demonstrated similar hippocampal and serum levels during maturation and aging processes in which these changes were compatible with the changes in hippocampal BDNF mRNA expression, but not with BDNF mRNA expression in platelets. 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, release of BDNF from the brain has been observed at rest, and a 2- to 3-fold increase occurred during exercise, and Rasmussen et al. (40) estimated that in both conditions brain contribution determined 70-80% of total circulating BDNF.

Alternatively, although BDNF is upregulated in contracting muscle fibers, it has been demonstrated that muscles are 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 behavior 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), whereas Marmigère et al. (29) demonstrated that in a very acute phase of immobilization stress (15–60 min), a transient increase in hippocampal BDNF mRNA expression occurs, followed by a reduction to levels lower than basal at 180 min; similarly, an increase in hippocampal BDNF content was found at 180 min, which rapidly returned at basal levels when the immobilization stimuli lasted up to 300 min.

The same result can be obtained acutely by exposing rats 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 that induces activation of short-term protective mechanisms, whereas chronic stressors as chronic immobilization can lead to excessive stimulation and functional distortion of neuroendocrine systems by reducing BDNF expression (33).

Similarly, studies with humans have showed a reduction in serum BDNF levels in sedentary subjects (45), such as people exposed to chronic stress (7, 35), but data are lacking about plasma BDNF responses to acute stressors. Thus we hypothesized that plasma 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 used data collected during 14 days of horizontal bed rest in which seven younger and eight older healthy adults were tested before and after 14 days of horizontal bed rest.

MATERIALS AND METHODS

Population. We designed a study that consisted of two groups of healthy volunteers: younger subjects [$n = 7$, age 19–28 yr (mean 23.3 ± 3.X)] and older subjects ($n = 8$, mean age 59.5 ± 4.X yr). After 3 days of an ambulatory period (regulated hospital diet and daily activities), all subjects underwent horizontal bed rest for 14 days in standard air conditioned hospital rooms at the Orthopedic Hospital of Valdoltra (Slovenia). Continuous surveillance and 24-h medical care was provided during the entire bed rest period, and all subjects received an individually controlled, normal caloric diet: for each subject, resting energy expenditure (estimated by means of bioimpedentiometric measures) was multiplied by a factor of 1.2, with caloric content consisting of 60% carbohydrates, 25% fats, and 15% proteins (4). Subjects performed all daily activities in bed, and were allowed to freely communicate, watch television, listen to radio, read, use a computer, and receive visitors.

Exclusion criteria included smoking; regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer levels at enrollment >500 μg/l; acute or chronic skeletal, neuromuscular, metabolic, or cardiovascular disease; and 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.

Biological samples and measurements. Blood samples were collected from each subject after an overnight fasting at enrollment [i.e., baseline data collection (BDC)] and after 14 days of forced bed rest (BR14) and centrifuged in the absence or presence of EDTA to obtain serum and plasma, respectively. Aliquots were stored at −80°C. Levels of HDL cholesterol after precipitation of lipoproteins containing apo-B (6), total cholesterol, and triglycerides (TG) were assayed in serum using the Trinder method. The coefficient of variation was <2% for total and HDL cholesterol and <5% for TG for intrabatch and interbatch, respectively. LDL cholesterol plasma levels were calculated using the Friedewald formula (12). Plasma glucose was measured using standard enzymatic methods (FAR, Italy). The intra-assay coefficient of variation was <3%. Fasting insulin levels were assayed using an ultrasensitive insulin ELISA kit manufactured by Mercodia (Sweden), and the intra-assay coefficient of variation was <3%. Fasting insulin resistance was evaluated by calculating the homeostasis model assessment (HOMA-IR) (32). TNF-α plasma levels were measured using an ELISA Kit (Invitrogen, USA). The intra-assay coefficient of variation was <5.2%. C-reactive protein plasma levels were measured using an immunoturbidimetric test (High sensitive C-reactive protein, Roche, Italy). The intra-assay

coefficient of variation was <2%. BDNF plasma levels were measured by means of ELISA (Promega, Italy), following the manufacturer's instructions.

At BDC and BR14 all subjects were tested with bioimpedentiometric measures for body composition using a tetrapolar impedancemeter (BIA101; Akern, Florence, Italy) according to the manufacturer's instructions (27). All measurements were collected by the same trained staff member after subjects had fasted for 8 h.

Statistical analysis. Continuous variables were expressed as means (SD) or, when necessary, as median (range), and categorical variables as the number/percentage. Means were compared by one-way ANOVA using the Bonferroni test for post hoc analysis, whereas medians were compared using the nonparametric Kruskal-Wallis test. Normality of distribution was tested with the Shapiro-Wilk test. Variations in BDNF plasma levels and the other variables of interest between BDC and BR14 were analyzed by general linear model repeated measures, within-subjects and between-subjects test.

Correlations between continuous variables were tested by multivariate linear regression analysis. Variables with nonnormal distribution were analyzed after log transformation or with a nonparametric test (Spearman's test).

Multivariate linear regression analysis (stepwise forward method) 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, Chicago, IL) and statistical significance was set $P < 0.05$.

RESULTS

Baseline characteristics of all subjects are shown in Table 1. In addition to the expected difference in age ($P < 0.001$), the group of older individuals had significantly higher plasma levels of total ($P < 0.001$) and LDL cholesterol ($P < 0.001$), TG ($P = 0.038$), and C-reactive protein ($P = 0.050$) compared with the younger 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; younger, 22.38 ± 13.84 pg/ml; $P = 0.101$).

At BR14, BDNF levels were significantly different between the two groups (older, 62.02 ± 18.31 pg/ml vs.

Table 1. *Baseline characteristics of the two groups*

	Younger, $n = 7$	Older, $n = 8$	P
Demographic and anthropometric characteristics			
Age, yr	23.3 ± 3	59.5 ± 4	<0.001
Body mass index, kg/m²	24.0 ± 2.3	26.9 ± 4.2	0.128
Fat-free mass, kg	60.9 ± 3.9	59.8 ± 6.9	0.722
Fat mass, kg	14.0 ± 6.2	19.9 ± 4.9	0.062
Metabolic profile			
Total cholesterol, mg/dl	151.1 ± 15.2	212.4 ± 24.4	<0.001
LDL cholesterol, mg/dl	89.0 ± 11.6	143.0 ± 18.6	<0.001
HDL cholesterol, mg/dl	43.1 ± 7.4	42.1 ± 7.7	0.804
Triglycerides, mg/dl	95.0 ± 35.5	136.5 ± 33.9	0.038
Insulin, mU/l	5.42 ± 1.58	5.69 ± 1.11	0.697
Glucose, mg/dl	77.2 ± 6.1	79.6 ± 9.5	0.565
HOMA-IR	1.02 ± 0.25	1.12 ± 0.27	0.440
Inflammation/stress markers			
C-reactive protein, mg/dl	0.05 ± 0.02	0.11 ± 0.06	0.050
TNFα	0.98 ± 1.32	1.45 ± 2.65	0.680
BDNF, pg/ml	22.38 ± 13.84	37.53 ± 18.58	0.101

BDNF, blood-derived neurotrophic factor; HOMA-IR, homeostasis model assessment.

younger, 34.36 ± 15.24 pg/ml, $P = 0.002$), and the difference was also maintained when BDC BDNF levels were used as a covariate (older vs. younger $P = 0.040$). To evaluate the effect of bed rest on BDNF levels, we performed a general linear model for repeated measures, which showed a significant bed rest effect ($P = 0.002$) but 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 ($P = 0.009$), whereas a trend toward an increase was observed in the younger group ($P = 0.118$) (see Fig. 1).

Linear regression analysis 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, body mass index, fat-free mass, fat mass, intracellular water, extracellular water, total body water, total cholesterol, HDL cholesterol, LDL cholesterol, TG, glucose, insulin, HOMA-IR, C-reactive protein, and TNFα).

At baseline (BDC) in the study population, plasma BDNF levels were positively correlated with fat-free mass ($R = 0.628$, $P = 0.012$), and 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 in both groups (younger, $R = 0.772$, $P = 0.042$; older, $R = 0.753$, $P = 0.031$), and in the older group the correlation with total cholesterol level was also confirmed ($R = 0.805$, $P = 0.016$).

At BR14, BDNF plasma levels were found to correlate with total cholesterol ($R = 0.579$, $P = 0.024$) and with age ($R = 0.647$, $P = 0.009$); no variables were significantly correlated with plasma BDNF in either group.

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

Finally, we produced two multiple linear regression models using BDC plasma levels of BDNF, BR14 plasma BDNF

Fig. 2. Correlation between plasma BDNF and total cholesterol levels in the older and younger study groups both at BDC and BR14.

levels, and total cholesterol and fat-free mass as dependent and independent variables in both groups separately (younger/older). We found only that one or the other group 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 plasma BDNF, total cholesterol, and fat-free mass (both at BDC and BR14) as a dependent variable and as independent variables for one or the other of the two groups, and another variable, time, obtained by assigning "0" to all the detections collected at BDC from each subject, and "1" to all the detections collected at BR14. In doing so we evaluated the influence of the 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 the human body. To our knowledge, this was the first study aiming to investigate plasma BDNF fluctuations in younger and older healthy subjects undergoing 14 days of strict bed rest; therefore, it is not possible to carry out a straight-forward comparison with other studies. However, in animal studies, BDNF hippocampal levels were demonstrated to increase in murine models during acute stresses such as immobilization, explicating a protective role (13, 29, 39). On the other hand, chronic immobilization (re-
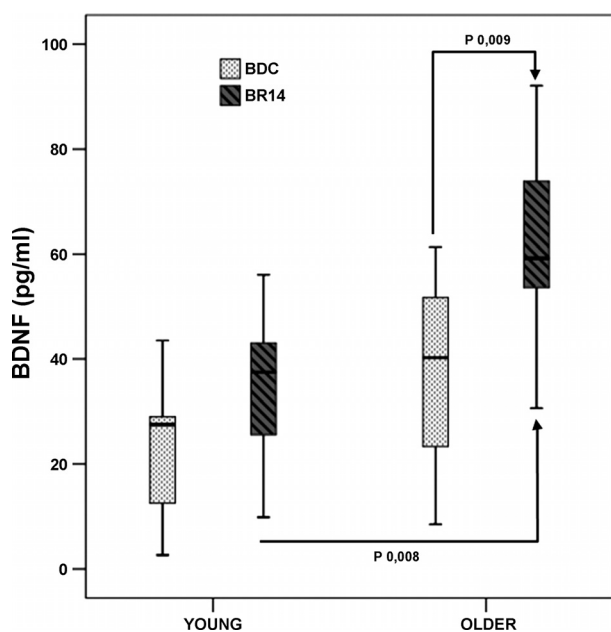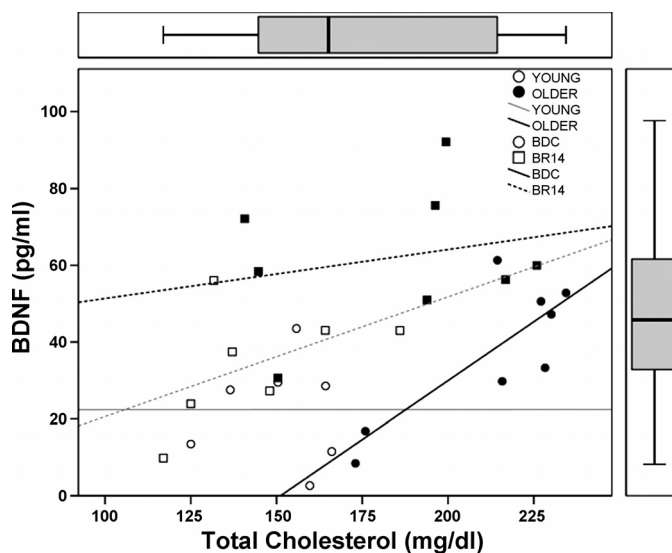

Fig. 1. Variation in plasma levels of brain-derived neurotrophic factor (BDNF) from baseline data collection (BDC) to day 14 of bed rest (BR14) in the study population.

Table 2. *Multiple linear regression model indicating predictors of BDNF plasma levels.*

Model	R^2	P for model	Predictors	Nonstandard β coefficient	P for variable
1	0.229	0.001	Group	20.506	0.001
2	0.341	<0.001	Group	20.506	<0.001
			Time	13.109	0.010
3	0.497	<0.001	Group	10.492	0.061
			Time	18.054	<0.001
			Total cholesterol	0.232	0.001

peated immobilization stress) as well as other chronic stressors, has led to reduced BDNF concentrations 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 younger subjects.

Our aim was not to replicate a murine model of immobilization with humans; clearly, the two situations cannot be compared, either with time intervals nor intensity of stress. It is clear that mice experience a very strong psychological stress rather than a physical stress during forced immobilization; on the contrary, human volunteers experience little or no psychological stress but substantial physical stress, as shown in recent research by our group (38). Nevertheless, the finding that acute and chronic stresses modulate BDNF expression differently in mice led us to hypothesize that such a modulation could also take place in humans.

Even if there is no agreement about the cellular source of human plasma BDNF, there is some evidence, both in humans and animals, that the brain plays a central role in determining circulating BDNF levels (18, 40), and that its contribution to total circulating BDNF has been considered to be about 70–80% (40). A massive presence of BDNF in platelets and in skeletal muscle was also described, but whereas platelet release upon activation does not seem to explain the variations in circulating BDNF occurring with aging or 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 concentrations of BDNF have already been used as an 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 such as bed rest could become more stressful for the brain, and therefore a greater effort to counteract its negative effects could be required.

Our analyses showed a positive correlation between BDNF and fat-free mass, both in the older and younger groups, and in both groups at BDC. Several studies that have examined changes in BDNF levels in humans revealed an augmentation of BDNF after an acute bout of exercise (43, 14) or, for instance, after 5 wk (59) and 3 mo (46) of endurance training, a condition that is well known to influence muscle mass in younger and older individuals (15, 48, 53). Alternatively, studies have shown lower BDNF values in sedentary individuals (45). Therefore, our results might reflect that people with higher levels of fat-free mass likely were more physically active. The loss of such a correlation at the end of bed rest (BR14) might reflect the different behaviors of the two variables during bed rest: in fact, whereas an increase is observed in BDNF, a decrease in fat-free mass was observed at the end of bed rest in both study populations (60.3 → 56.5 kg, $P < 0.001$) and in the older group (59.8 → 56.7 kg, $P < 0.001$). A similar decrease in muscle mass has been 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 study population and in our older study group, but not in the younger study group; this might reflect a different significance in total cholesterol plasma levels at different ages. It is well known from the literature that although a higher level of cholesterol is a risk factor for all causes and cardiovascular mortality in younger subjects (10), in older people the same levels seem to be associated with lower mortality (a concept known as "reverse epidemiology"). This probably derives from the fact that in older people, lower levels of total cholesterol are the result of chronic inflammatory conditions rather than a healthier lifestyle, so that higher levels of total cholesterol actually reflect better overall health (2).

Interestingly, at the end of bed rest (BR14), this positive correlation between BDNF and total cholesterol is no longer present: this could reflect the behavior of cholesterol as an acute-phase molecule. In acute stress conditions, cholesterol plasma levels decrease (44), in this context, considering bed rest to be a stressful condition, although BDNF increases to counteract its negative effect on the brain, total and LDL cholesterol levels decrease, thereby losing the positive correlation.

On the other hand, the role of bed rest as an acute stress condition is confirmed by the behavior of several variables: first, total cholesterol, which decreases from BDC to BR14 both in the general population (183.79 → 165.14 mg/dl, $P < 0.001$) and in our group of older study subjects (212.36 → 183.51 mg/dl, $P < 0.024$), but not in our group of younger subjects (151.11 → 144.14, $P < 0.23$), but in addition, TNFα, a well-known inflammatory marker, increases after bed rest both in the general population (1.23 → 1.46 mg/dl, $P < 0.001$), and it also did so in our older study subjects (1.45 → 1.59 mg/dl, $P < 0.001$) and our younger study subjects (0.98 → 1.30 mg/dl, $P < 0.001$). Even C-reactive protein shows an increase in plasma concentration after bed rest in the general population (0.080 → 0.236 mg/dl, $P < 0.033$).

Age emerged as a variable at BR 14 that correlated with plasma BDNF and reflects the significant increase in BDNF observed in the older group but not the younger group. On the other hand, the multiple linear regression models demonstrate that age (i.e., one or the other group) is an independent predictor of final plasma BDNF levels, which is influenced, as expected, by bed rest (i.e., "time" factor), but also by total cholesterol. Despite what already has been said regarding cholesterol and its behavior as an acute-phase molecule, only a few studies exist in literature that link BDNF with cholesterol, and most of them refer to brain content of cholesterol and not to plasma 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 for the general population, so we aim to study a larger population to confirm this result.

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

Study limitations. We acknowledge some limitation of this study. First, the small number of subjects makes it difficult to derive any conclusions. However, it is necessary to keep in mind that bed rest studies that include older individuals are rare, and bed rest studies are very costly, labor-intensive, and

limited by hospital capacity. The total costs are estimated at >$20,000 per participant. However, despite having such a small population sample size, we were able to detect variables that were correlated with moderate significance. We hypothesize that an even stronger statistical significance could be reached with a larger study population.

Finally, because we did not perform functional neuroimaging and did not obtain samples of cerebrospinal fluid from subjects, we have only surrogate evaluations of brain stress, such as TNFα, and C-reactive protein.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.S., U.M., M.L.M., E.D.N., B.S., R.P., and A.P. conception and design of research; U.M., M.L.M., B.S., R.P., and A.P. performed experiments; C.S., J.M.S., E.D.N., and A.P. analyzed data; C.S., U.M., J.M.S., E.D.N., R.P., G.Z., and A.P. interpreted results of experiments; C.S., U.M., and A.P. prepared figures; C.S. and A.P. drafted manuscript; C.S., U.M., J.M.S., M.L.M., E.D.N., B.S., R.P., G.Z., and A.P. edited and revised manuscript; C.S., U.M., J.M.S., M.L.M., E.D.N., B.S., R.P., G.Z., and A.P. approved final version of manuscript.

REFERENCES

1. **Aciero PJ, Smith DL, Calles-Escandon J.** Effects of short-term inactivity on glucose tolerance, energy expenditure, and blood flow in trained subjects. *J Appl Physiol* 84: 1365–1373, 1998.
2. **Ahmadi SF, Streja E, Zahmatkesh G, Streja D, Kashyap M, Moradi H, Molnar MZ, Reddy U, Amin AN, Kovesdy CP, Kalantar-Zadeh K.** Reverse epidemiology of traditional cardiovascular risk factors in the geriatric population. *J Am Med Dir Assoc* 16: 933–999, 2015.
3. **Binder DK, Scharfman HE.** Brain-derived neurotrophic factor. *Growth Factors* 22: 123–131, 2004.
4. **Biolo G, Agostini F, Simunic B, Sturma M, Torelli L, Preiser JC, Deby-Dupont G, Magni P, Strollo F, di Prampero P, Guarnieri G, Mekjavic IB, Pisot R, Narici MV.** Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest. *Am J Clin Nutr* 88: 950–958, 2008.
5. **Brunoni AR, Lopes M, Fregni F.** A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol* 11: 1169–1180, 2008.
6. **Burstein M, Scholnick HR, Morfin R.** Rapid method for the isolation of lipoproteins from human serum by precipitations with polyanions. *J Lipid Res* 11: 583–595, 1970.
7. **Chui DH, Marcellino M, Marotta F, Sweed H, Solimene U, Vignali AI, Xiao W, Ayala A, Cagnuolo U, Zerbinati N.** A double-blind RCT testing beneficial modulation of BDNF in middle-aged, life style-stressed subjects: a clue to brain protection? *J Clin Diagn Res* 8: MC01–MC06, 2014.
8. **Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S.** Brain-derived neurotrophic factor and the development of structural neuronal connectivity. *Dev Neurobiol* 70: 271–288, 2010.
9. **Cui H, Jin Y, Wang J, Weng X, Li C.** Serum brain-derived neurotrophic factor (BDNF) levels in schizophrenia: a systematic review. *Shanghai Arch Psychiatry* 24: 250–261, 2012.
10. **Eckel RH, Cornier MA.** Update on the NCEP ATP-III emerging cardio-metabolic risk factors. *BMC Med* 12: 115, 2014.
11. **Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR.** Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol Endocrinol Metab* 270: E627–E633, 1996.
12. **Friedewald WT, Levy RI, Fredrickson DS.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin Chem* 18: 499–502, 1972.
13. **Givalois L, Marmigère F, Rage F, Ixart G, Arancibia S, Tapia-Arancibia L.** Immobilization stress rapidly and differentially modulates BDNF and TrkB mRNA expression in the pituitary gland of adult male rats. *Neuroendocrinology* 74: 148–153, 2001.
14. **Gold SM, Schulz KH, Hartmann S, Mladek M, Lang UE, Hellweg R, Reer R, Braumann KM, Heesen C.** Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *J Neuroimmunol* 138: 99–105, 2003.
15. **Häkkinen K, Alen M, Kraemer WJ, Gorostiaga E, Izquierdo M, Rusko H, Mikkola J, Häkkinen A, Valkeinen H, Kaarakainen E, Romu S, Erola V, Ahtiainen J, Paavolainen L.** Neuromuscular adaptations during concurrent strength and endurance training versus strength training. *Eur J Appl Physiol* 89: 42–52, 2002.
16. **Hamburg NM, McMackin CJ, Huanf AL, Shenouda SM, Widlansky ME, Schulz E, Gocke N, Ruderman NB, Keaney JF Jr, Vita JA.** Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. *Arterioscler Thromb Vasc Biol* 27: 2650–2656, 2007.
17. **Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM.** Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* 109: 143–148, 2002.
18. **Karege F, Schawald M, Cisse M.** Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* 328: 261–264, 2002.
19. **Kortebein P, Symons TB, Ferrando A, Paddon-Jones D, Ronsen O, Protas E, Conger S, Lombeida J, Wolfe R, Evans WJ.** Functional impact of 10 days of bed rest in healthy older adults. *J Gerontol A Biol Sci Med Sci* 36: 1076–1081, 2008.
20. **Krabbe KS, Nielsen AR, Krogh-Madsen R, Plomgaard P, Rasmussen P, Eriksturp C, Fischer CP, Lindegaard B, Petersen AM, Taudorf S, Secher NH, Pilegaard H, Bruunsgaard H, Pedersen BK.** Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia* 50: 431–438, 2007.
21. **Lee BH, Kim YK.** Potential peripheral biological predictors of suicidal behaviour in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 35: 842–847, 2011.
22. **Lee SY, Chen SL, Chang YH, Chen PS, Huang SY, Tzeng NS, Wang CL, Lee IH, Wang TI, Chen KC, Yang YK, Hong JS, Lu RB.** Correlation of plasma brain derived neurotrophic factor and metabolic profiles in drug-naïve patients with bipolar II disorder after a twelve-week pharmacological intervention. *Acta Physiatr Scand* 131: 120–128, 2015.
23. **Li K, Guo X, Jin Z, Ouyang X, Zeng Y, Feng J, Ma L.** Effect of simulated microgravity on human brain gray matter and white matter - evidence from MRI. *PLoS One* 10: e0135835, 2015.
24. **Lipnicki DM, Gunga HC, Belavy DL, Felsenberg D.** Bed rest and cognition: effects on executive functioning and reaction time. *Aviat Space Environ Med* 80: 1018–1024, 2009.
25. **Liu Q, Zhou R, Chen S, Tan C.** Effects of head-down bed rest on the executive functions and emotional response. *PLoS One* 7: e52160, 2012.
26. **Lommatzsch M, Zingler D, Schuhbaeck K, Schloetcke K, Zingler C, Schuff-Werner P, Virchow JC.** The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol Aging* 26: 115–123, 2005.
27. **Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI.** Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr* 41: 810–817, 1985.
28. **Manni L, Nikolova V, Vyagova D, Chaldakov VN, Aloe L.** Reduced plasma levels of NGF and BDNF in patients with acute coronary syndromes. *Int J Cardiol* 102: 169–171, 2005.
29. **Marmigère F, Givalois L, Rage F, Arancibia S, Tapia-Arancibia L.** Rapid induction of BDNF expression in the hippocampus during immobilization stress challenge in adult rats. *Hippocampus* 13: 646–655, 2003.

30. **Marusic U, Meeusen R, Pisot R, Kavcic V.** The brain in micro- and hypergravity: the effects of changing gravity on the brain electrocortical activity. *Eur J Sport Sci* 14: 813–822, 2014.

31. **Matthews VB, Aström MB, Chan MH, Bruce CR, Krabbe KS, Prelovsek O, Akerström T, Yfanti C, Broholm C, Mortensen OH, Penkowa M, Hojman P, Zankari A, Watt MJ, Bruunsgaard H, Pedersen BK, Febbraio MA.** Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein-kinase. *Diabetologia* 52: 1409–1418, 2009.

32. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412–419, 1985.

33. **Nowacka M, Obuchowicz E.** BDNF and VEGF in the pathogenesis of stress-induced affective diseases: an insight from experimental studies. *Pharmacol Rep* 65: 535–546, 2013.

34. **Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ.** Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 37: 1553–1561, 1998.

35. **Paska AV, Zupanc T, Pregelj P.** The role of brain-derived neurotrophic factor in the pathophysiology of suicidal behaviour. *Psychiatr Danub* 25: Suppl *2*, 341–344, 2013.

36. **Passaro A, Dalla Nora E, Morieri ML, Soavi C, Sanz JM, Zurlo A, Fellin R, Zuliani G.** Brain-derived neurotrophic factor plasma levels: relationship with dementia and diabetes in the elderly population. *J Gerontol A Biol Sci Med Sci* 70: 294–302, 2015.

37. **Pedersen BK, Pedersen M, Krabbe KS, Bruunsgaard H, Matthews VB, Febbraio MA.** Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals. *Exp Physiol* 94: 1153–1160, 2009.

38. **Pisot R, Marusic U, Biolo G, Mazzucco S, Lazzer S, Grassi B, Reggiani C, Toniolo L, di Prampero PE, Passaro A, Narici MV, Mohammed S, Rittweger J, Gasparini M, Gabrijelčič M, Simunic B.** Greater loss in muscle mass and function but smaller metabolic alterations in older compared to younger men following two weeks of bed rest and recovery. *J Appl Physiol*. First published January 28, 2016; doi: 10.1152/japplphysiol.00858.2015.

39. **Rage F, Givalois L, Marmigère F, Tapia-Arancibia L, Arancibia S.** Immobilization stress rapidly modulates BDNF mRNA expression in the hypothalamus of adult male rats. *Neuroscience* 112: 309–318, 2002.

40. **Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H.** Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* 94: 1062–1069, 2009.

41. **Rasmusson AM, Shi L, Duman R.** Downregulation of BDNF mRNA in the hippocampal dentate gyrus after re-exposure to cues previously associated with foot-shock. *Neuropsychopharmacology* 27: 133–142, 2002.

42. **Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA.** Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in the rat hippocampus. *Mol Psychiatry* 7: 609–616, 2002.

43. **Rojas Vega S, Strüder HK, Vera Wahrmann B, Schmidt A, Bloch W, Hollmann W.** Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Res* 1121: 59–65, 2006.

44. **Rott D, Klempfner R, Goldenberg I, Leibowitz D.** Cholesterol levels decrease soon after myocardial infarction. *Isr Med Assoc J* 17: 370–373, 2015.

45. **Schmolesky MT, Webb DL, Hansen RA.** The effects of aerobic exercise intensity and duration on levels of brain-derived neurotrophic factor in healthy men. *J Sports Sci Med* 12: 502–511, 2013.

46. **Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknecht B, Adser H, Jakobsen AH, Pilegaard H, Nielsen HB, Secher NH.** Endurance training enhances BDNF release from the human brain. *Am J Physiol Regul Integr Comp Physiol* 298: R372–R377, 2010.

47. **Shi SS, Shao SH, Yuan BP, Pan F, Li ZL.** Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus. *Yonsei Med J* 51: 661–671, 2010.

48. **Sipila S, Suomeinen H.** Effects of strength and endurance training on thigh and leg muscle mass and composition in elderly women. *J Appl Physiol* 78: 334–340, 1995.

49. **Smith MA, Makino S, Kvetnansky R, Post RM.** Stress and glucocorticoids affect the expression of brain derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci* 15: 1768–1777, 1995.

50. **Stuart CA, Shoiraw RE, Prince MJ, Peters EJ, Wolfe RR.** Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism* 37: 802–806, 1998.

51. **Suzuki S, Kiyosue K, Hazama S, Ogura A, Kashihara M, Hara T, Koshimizu H, Kojima M.** Brain-derived neurotrophic factor regulates cholesterol metabolism for synapse development. *J Neurosci* 27: 6417–6427, 2007.

52. **Suzuki S, Numakawa T, Shimazu K, Koshimizu H, Hara T, Hatanaka H, Mei L, Lu B, Kojima M.** BDNF-induced recruitment of TrkB receptor into neuronal lipid rafts: roles in synaptic modulation. *J Cell Biol* 167: 1205–1215, 2004.

53. **Tarpenning KM, Hamilton-Wessler M, Wiswell RA, Hawkins SA.** Endurance training delays age of decline in leg strength and muscle morphology. *Med Sci Sports Exerc* 36: 74–78, 2004.

54. **Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Tone S, Senba E.** Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci Res* 28: 103–110, 1997.

55. **Vaidya VA, Marek GJ, Aghajanian GK, Duman RS.** 5HT2A receptor-mediated regulation of brain derived neurotrophic factor mRNA in the hippocampus and the neocortex. *J Neurosci* 17: 2785–2795, 1997.

56. **Ventriglia M, Zanardini R, Bonomini C, Zanetti O, Volpe D, Pasqualetti P, Gennarelli M, Bocchio-Chiavetto L.** Serum brain derived neurotrophic factor levels in different neurological diseases. *Biomed Res Int* 2013: 901082, 2013.

57. **Wiggs MP.** Can endurance exercise preconditioning prevention disuse muscle atrophy? *Front Physiol* 6: 63, 2015.

58. **Zhen YF, Zhang J, Liu XY, Fang H, Tian LB, Zhou DH, Kosten TR, Zhang XY.** Low BDNF is associated with cognitive deficits in patients with type 2 diabetes. *Physiopharmacology* 227: 93–100, 2013.

59. **Zoladz JA, Pilc A, Majerczak J, Grandys M, Zapart-Bukowska J, Duda K.** Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. *J Physiol Pharmacol* 59, *Suppl* 7: 119–132, 2008.