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

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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 yet 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 ± 3.8 yr)] and older subjects (n = 8, mean age 59.5 ± 4.2 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 bioimpendiometric measures) was multiplied by a factor of 1.2, with caloric intake (calculated per gram of protein) being 1.5, 1.7, and 2.2 for younger and eight older healthy adults, respectively.

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. younger, 37.53 ± 18.58 pg/ml; P = 0.001).

Table 1. Baseline characteristics of the two groups

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

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

RESULTS

Table 1. Baseline characteristics of the two groups

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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 levels, and total cholesterol and fat-free mass as dependent and independent variables. To evaluate 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-

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

<table>
<thead>
<tr>
<th>Model</th>
<th>( R^2 )</th>
<th>( P ) for model</th>
<th>Predictors</th>
<th>Nonstandard ( \beta ) coefficient</th>
<th>( P ) for variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.229</td>
<td>&lt;0.001</td>
<td>Group</td>
<td>20.506</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.341</td>
<td>&lt;0.001</td>
<td>Group</td>
<td>20.506</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.497</td>
<td>&lt;0.001</td>
<td>Group</td>
<td>10.492</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time</td>
<td>18.054</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total cholesterol</td>
<td>0.232</td>
<td>0.001</td>
</tr>
</tbody>
</table>
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, 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.

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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