Acute effects of physical activity patterns on plasma cortisol and brain-derived neurotrophic factor in relation to corticospinal excitability

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) and cortisol are both capable of modulating synaptic plasticity, but it is unknown how physical activity-induced changes in their plasma levels relate to corticospinal plasticity in humans. Sixteen inactive middle-aged men and women participated in three separate interventions consisting of 3 h prolonged sitting (SIT); 3 h sitting interrupted every 30 min with frequent short physical activity breaks (FPA); and 2.5 h prolonged sitting followed by 25 min of moderate intensity exercise (EXE). These 3 h sessions were each followed by a 30 min period of paired associative stimulation over the primary motor cortex (PAS). Blood samples were taken and corticospinal excitability measured at baseline, pre PAS, 5 min and 30 min post PAS. Here we report levels of plasma BDNF and cortisol over three activity conditions and relate these levels to previously published changes in corticospinal excitability of a non-activated thumb muscle. There was no interaction between time and condition in BDNF, but cortisol levels were significantly higher after EXE compared to after SIT and FPA. Higher cortisol levels at pre PAS predicted larger increases in corticospinal excitability from baseline to all subsequent time points in the FPA condition only, while levels of BDNF at pre PAS did not predict such changes in any of the conditions. Neither BDNF nor cortisol modified changes from pre PAS to the subsequent time points, suggesting that the increased corticospinal excitability was not mediated though an augmented effect of the PAS protocol. The relationship between cortisol and plasticity has been suggested to be inverted U-shaped. This is possibly why the moderately high levels of cortisol seen in the FPA condition were positively associated with changes AURC, while the higher cortisol levels seen after EXE were not. A better understanding of the mechanisms for how feasible physical activity breaks affect neuroplasticity can inform the theoretical framework for how work environments and schedules should be designed.

1. Introduction

Neuroplasticity is the ability of the nervous system to change its function and anatomy in response to experience. Long-term potentiation (LTP) refers to persistent strengthening of synapses in response to recent activity [1]. Together with long-term depression, LTP is considered one of the main cellular mechanisms underpinning learning and memory [2]. LTP-like plasticity has been shown to be higher in young fit compared to older unfit populations [3,4].

In physically active young individuals, an acute bout of high intensity physical activity has been shown to enhance LTP-like motor cortex plasticity induced by paired associative stimulation (PAS) [5,6] and intermittent theta burst stimulation [7]. This increase in plasticity after physical activity seen in young fit individuals sparks the hope that exercising or at least spending less time in sedentary behaviors might benefit LTP-like motor cortex plasticity also in less fit middle-aged and elderly individuals where the risks of neurodegenerative diseases are higher.

In a recent study on middle-aged physically inactive men and women, we used PAS to compare changes in corticospinal excitability and plasticity after 3 h prolonged sitting (SIT) with 2.5 h prolonged sitting followed by 25 min moderate intensity exercise (EXE) or 3 h...
sitting interrupted every 30 min with frequent short physical activity breaks (FPA) [8]. In this middle-aged population, however, LTP-like corticospinal plasticity did not significantly differ between conditions, but exploratory analyses showed that breaking up prolonged sitting was associated with significant increases in corticospinal excitability, while EXE and SIT were not [8]. This finding supports the finding of McDonnell et al. that low intensity rather than moderate intensity exercise promotes neuroplasticity [9]. In their study, they showed that moderate intensity exercise induced increased levels of cortisol as compared to low intensity exercise, while none of the exercise protocols affected peripheral levels of BDNF. Understanding the mechanisms of how physical activity patterns affect neuroplasticity in middle age is important, not least to inform the design of the work environments.

Exercise has been demonstrated to modulate peripheral and central levels of several neuromodulators including BDNF and cortisol. The release of BDNF is linked to exercise intensity in young individuals [10, 11], showing higher concentrations following high-intensity exercise compared to less intense activities. The intensity required for BDNF release is most probably related to relative intensity, i.e., the individual work capacity. Therefore, a given absolute exercise can elicit different responses, depending on the fitness levels of individuals performing them, and thereby explaining the disparities between studies. Peripheral levels of BDNF are typically measured in serum or plasma. The BDNF levels in plasma and serum are typically positively related at baseline, but they measure different pools of BDNF and do not respond to exercise in the same way [12]. While BDNF is one neurotrophic factor that has been suggested to mediate the positive effect of exercise on plasticity, cortisol appears to affect plasticity in a U-shaped manner so that moderate levels are positive for plasticity while higher levels are negative [13].

Older individuals typically have lower baseline levels of BDNF [14] and higher baseline levels of cortisol [15], possibly making them more sensitive to exercise induced increases in cortisol. It appears possible therefore that the lack of effect of moderate intensity exercise on corticospinal excitability in Bojsen-Møller et al. [8] could be related to the exercise causing too high increases in cortisol.

The primary aim of this study was to investigate the acute effects of 3 h SIT, EXE, and FPA on plasma BDNF and cortisol in middle-aged inactive individuals. These conditions mimic feasible activity behaviors for office workers. The secondary aim was to, within each condition, investigate the possible impact of plasma cortisol and BDNF levels after each 3 h activity on changes in corticospinal excitability and on the LTP-like corticospinal neuroplasticity induced by PAS. We hypothesized that plasma BDNF would increase more after EXE, than FPA or SIT (hypothesis 1), that plasma cortisol would decrease from baseline to later in the day (hypothesis 2), but that immediately after the 3 h activities, cortisol would be higher in the EXE than the SIT and FPA conditions (hypothesis 3), and higher in the FPA than the SIT condition (hypothesis 4). We also hypothesized that the changes in corticospinal excitability and the LTP-like neuroplasticity induced by PAS would be related differently to plasma BDNF and cortisol immediately after EXE, SIT, and FPA (hypothesis 5).

For EXE and FPA, but not SIT, we hypothesized (hypothesis 6) higher plasma levels of BDNF before the PAS to predict larger increases in corticospinal excitability from baseline to 5 and 30 min after PAS as well as larger increases from pre PAS to subsequent time-points (as an indication of LTP-like neuroplasticity). For EXE, we hypothesized the plasma levels of cortisol before the PAS to be too high and therefore (hypothesis 7) relate negatively to LTP-like neuroplasticity induced by PAS and changes in corticospinal excitability from baseline to 5 and 30 min after PAS.

For EXE and SIT we hypothesized higher plasma levels of cortisol before the PAS to predict larger increases in corticospinal excitability from baseline to 5 and 30 min after PAS as well as larger increases from pre PAS to subsequent time-points (as an indication of LTP-like neuroplasticity) (hypothesis 8).

### Table 1

<table>
<thead>
<tr>
<th>Characteristics of the participants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall n – 16</td>
</tr>
<tr>
<td>Age mean (SD) years 52.6 (8.1)</td>
</tr>
<tr>
<td>VO2 mean (SD) mL/kg/minute 31.3 (6.2)</td>
</tr>
<tr>
<td>Gender (male%/female%) 50%/50%</td>
</tr>
<tr>
<td>Body mass (kg) 82.8 (12.8)</td>
</tr>
</tbody>
</table>

### 2. Methods

Data was derived from a recent study investigating the effects of breaking up sedentary behavior on corticospinal excitability. The main aim of that study was to compare the effects of three different physical activity conditions on PAS-induced plasticity. A more extensive account of the experimental protocol and the primary findings were recently published [8].

#### 2.1. Participants

Physically inactive middle-aged participants were recruited through advertisement primarily among sedentary office workers. Sixteen middle-aged adults who self-reported spending less than 150 min per week in moderate to vigorous physical activity participated in the study (Table. 1). All participants reported no use of medication and were screened with the transcranial magnetic stimulation adult safety screening tool [16] for any contraindications. The study was approved by the Stockholm regional ethical review board (2016/2096–31 and 2017/198).

#### 2.2. Study design

Participants attended the lab for one preparatory session and three experimental sessions, each separated by at least seven days and preceded by a four-day long run-in period, during which physical activity was measured. Furthermore, diet was standardized and participants were instructed to abstain from exercise and alcohol 24 h prior to each experiment. On the day preceding each experimental condition participants were asked to record their food intake and to consume a standardized dinner no later than 8:00 pm. Participants fasted and were only allowed to drink water from after the dinner until a standardized breakfast was served in the lab at approximately 8:30 am. Each participant was given the same amount of breakfast on all experimental days, but standardization of calories or macronutrients from body mass was not attempted.

The three experimental sessions consisted of three separate experimental conditions; a) 3 h of sitting interrupted every 30 min with short social breaks (SIT), b) 3 h of sitting interrupted with short frequent physical activity breaks (FPA) every 30 min, and c) 2.5 hs of sitting followed by 25 min of biking at a perceived exertion of 13 on the Borg scale (EXE). Participants were allowed to read a book, but were not allowed to watch television, use a smartphone or tablet during the sitting time. Participants were also not allowed to sleep. Each FPA break consisted of three rounds of three exercises performed for 20 s each resulting in a 3-minute break. The exercises were body weight box squats, calf-raises, and gluteus maximus contractions combined with knee-raises. Participants watched a video of the exercises to keep the pace. The video was adopted from a previous study [17]. In the SIT and EXE conditions, the experimenter had a social break with the participants every 30 min to control for the social interaction of the FPA condition. The social breaks consisted of a 3 min casual conversation with the research leader. All experimental sessions involved assessment of blood markers and corticospinal excitability. The order of physical activity conditions was block randomized.

An outline of the experimental sessions is visualized in Fig. 1. In brief, corticospinal excitability of the primary motor cortex was assessed.
and venous blood samples were drawn from the antecubital vein at baseline (at approximately 8:20 am), immediately after the 3 h activity condition, within 5 min after a 30 min long paired associative stimulation protocol (PAS), and at 30 min after PAS.

To ensure that the blood sampling caused a minimal disturbance, a catheter was inserted into the participant’s antecubital vein at the start of the experiment and was kept throughout the experiment. To avoid contamination and coagulation of the catheter between samples, the catheter was flushed with saline solution after each blood sampling.

2.3. Familiarization session

At the familiarization session, participants filled out a health declaration and the informed consent form, were given verbal and written information, and tried out the transcranial magnetic stimulation and electrical stimulation to be used in the subsequent sessions. They were then instructed on how to carry activity monitors and attach the glucose measurement device and were provided with a standardized meal to eat on the night prior to each experimental session (i.e. the same amount and composition of meal was consumed at the three time points). They then participated in a submaximal cycle ergometer test used for the estimation of cardiorespiratory fitness. Changes in heart rate (ΔHR) and power output (ΔPO) between a standardized lower work-load and an individualized higher workload were calculated. These data together with age and sex were then entered into a validated algorithm [18] to derive an estimation of cardiorespiratory fitness. Based on this test and the heart rate recordings during the test, the workload to be used in the EXE session was determined.

2.4. Measures of corticospinal excitability and plasticity

The procedures for measuring corticospinal excitability are...
illustrated in Fig. 2 and described in more detail in a previous publication [8]. In brief, transcranial magnetic stimulation was applied over the primary motor cortex controlling the Abductor Pollicis Brevis (APB) of the dominant hand, and motor evoked potentials were collected via AgCl electrodes positioned over the muscle belly of the APB (Fig. 2a). Stimulations of different intensities were applied in a random order at a 3 s interval. The peak-to-peak amplitudes of the motor evoked potentials from the transcranial magnetic stimulation (Fig. 2b) were normalized to the peak-to-peak amplitude of the motor evoked potentials from supramaximal stimulation of the medial nerve (Mmax). The Mmax normalized magnetic evoked potentials were used to create a recruitment curve (Fig. 2c). The area under this magnetic evoked potential/Mmax recruitment curve (AURC) was used as a measure of corticospinal excitability. Changes in corticospinal excitability over the PAS protocol are interpreted as LTP-like neuroplasticity, while changes from baseline to subsequent time points are more carefully described just as changes in corticospinal excitability as they may be ascribed to other mechanisms than LTP-like neuroplasticity.

2.5. Paired associative stimulation

The PAS protocol used consisted of paired peripheral electrical stimulation and primary motor cortex transcranial magnetic stimulation applied at a frequency of 0.1 Hz over 30 min. Within each pair, the peripheral stimulation of the medial nerve (stimulus intensity at 300% of the sensory threshold) was delivered 25 ms prior to the transcranial magnetic stimulation (stimulus intensity at 120% of the resting motor threshold). The PAS protocol used in the current study is described in more detail in Bojsen-Møller et al. [8].

2.6. Biomarker analyses

Before each blood sampling, the catheter was flushed with saline solution. The blood samples were collected into a heparinized and centrifuged immediately at 6000 rpm at 4 °C for 3 min to separate the plasma. Thereafter, separated plasma samples were transferred to Eppendorf tubes and stored at −80 °C until analysis. For the quantification of plasma BDNF (pBDNF) levels, samples were analyzed in duplicate using the Quantikine Human Free BDNF ELISA kit (#DBD00, R&D Systems, Inc., Minneapolis, MN, United States) according to manufacturer’s instructions. For the analysis of plasma levels of cortisol, samples were analyzed in duplicate by an ELISA kit (#CO368S, Calbiotech Inc., El Cajon, CA, United States) according to the manufacturer’s instructions. Plasma levels of BDNF and cortisol are expressed in pg × mL⁻¹ and ng × mL⁻¹, respectively.

2.7. Statistics

The normal distribution of data was first evaluated using Shapiro-Wilk test. Since the pBDNF data was not normally distributed, the values were log-transformed prior to further analyses (denoted as Ln-pBDNF). Linear mixed effects models were then used to address hypotheses 1–4 by investigating the association of plasma BDNF and cortisol with conditions, time, and their interactions while taking into account the random intercept and random slope. In addition, when the time was treated as a continuous variable in the model, we reported the p-value of interactions between conditions and time to represent the linear trend of intervention effect over time.

Using linear mixed effects models while controlling for the random effects, the hypotheses 5–8 were addressed by relating exposures (Ln-pBDNF or cortisol at pre PAS), time (baseline, pre PAS, 5 min post PAS, and 30 min post PAS), conditions (SIT, FPA, and EXE), and their
interactions to repeated AURC. Specifically, three steps of modeling were introduced when testing our hypotheses 5–8. In the first step, we estimated the effect of pre PAS Ln-pBDNF on changes of AURC from baseline to pre PAS, 5 min post PAS, and 30 min post PAS in each condition. We then introduced pre PAS cortisol in the same model to check its confounding effect. In the second step, we estimated the effect of pre PAS cortisol on the changes in AURC from the baseline in each condition, while the confounding effect of Pre PAS Ln-pBDNF was adjusted after. In the third step, similar to the previous two steps, we estimated the effect of Ln-pBDNF and cortisol at pre PAS on the changes in AURC from the pre PAS in each condition, respectively.

Stata/SE 16.0 was used in the analysis. The level of statistical significance was set to \( p < 0.05 \). Sensitivity analyses were performed using only the subset of participants who were without missing value in plasma BDNF, cortisol, and AURC.

3. Results

3.1. BDNF and cortisol

Fig. 3 displays the levels of Ln-pBDNF at baseline, pre PAS, 5 min post PAS and 30 min post PAS for each condition. The results of the linear mixed effects model showed no main effect of condition on level of Ln-pBDNF (Fig. 3), but overall Ln-pBDNF was higher at 30 min post PAS compared to baseline (Coefficient 0.54, CI 0.25–0.83) (indicated as * in Fig. 2). Furthermore, there was an interaction between condition and time at 30 min post PAS where Ln-pBDNF had increased less from baseline to 30 min post in the FPA compared to in the SIT condition (Coefficient –0.43, CI –0.86 to –0.00).

Fig. 4 displays the levels of cortisol at baseline, pre PAS, 5 min post PAS and 30 min post PAS. For cortisol, the linear mixed effects model showed that plasma levels did not differ between conditions. There was a significant decrease from baseline to pre PAS (Coefficient –32, CI –46, –18), to 5 min after PAS (Coefficient –26, CI –41, –12) and to 30 min post PAS (Coefficient –25, CI –40, –10). There was also an interaction between time and condition, such that cortisol levels were higher pre PAS in the EXE compared to the SIT condition (Fig. 4).

Table 2
Mean area under the recruitment curve (SD) at different time points for the three different conditions.

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>Baseline</th>
<th>Pre PAS</th>
<th>5 min Post PAS</th>
<th>30 min post PAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIT</td>
<td>15.29 (10.7)</td>
<td>15.36 (8.7)</td>
<td>16.94 (11.1)</td>
<td>17.42 (11.5)</td>
</tr>
<tr>
<td>FPA</td>
<td>14.64 (8.6)</td>
<td>18.31 (10.9)</td>
<td>19.75 (10.1)</td>
<td>20.51 (10.6)</td>
</tr>
<tr>
<td>EXE</td>
<td>15.56 (8.5)</td>
<td>15.94 (9.3)</td>
<td>16.47 (8.2)</td>
<td>17.95 (9.1)</td>
</tr>
</tbody>
</table>

Exercise (EXE), sitting (SIT), and sitting with frequent physical activity breaks (FPA). Paired associative stimulation (PAS). Area under the transcranial magnetic stimulation induced recruitment curve (AURC).

3.2. Corticospinal excitability

Corticospinal excitability was measured as AURC. Table 2 describes the AURC at the different time points for each condition.

3.3. Association between biomarkers and changes in corticospinal excitability

To test hypotheses 5–8 we used the linear mixed effects model to investigate whether plasma levels of cortisol and Ln-pBDNF at pre-PAS moderated changes in AURC. These analyses showed that BDNF levels at pre PAS did not predict changes in AURC in any of the conditions. Higher levels of cortisol at pre PAS were however associated with larger changes in AURC from baseline to all subsequent timepoints in the FPA.
condition only (see Table 3). Neither Ln-pBDNF nor cortisol at pre PAS moderated changes in AURC from pre PAS to any of the subsequent time points. Multiple regression analyses were performed as sensitivity analyses including only participants with complete data. When we calculated the %change in AURC and related the %change in AURC to Ln-pBDNF and cortisol at pre PAS in each condition, similar results were shown as mixed effects model.

Results in Table 4 are revealing that levels of cortisol after the 3 h intervention (pre PAS) were associated with a higher %change in AURC in the FPA condition, but not in the SIT or the EXE conditions. Adjusted R² was 0.457 for immediately post FPA and 0.255 for 30 min post PAS. There were no significant associations between Ln-pBDNF at any time point and %change in AURC. Neither cortisol nor Ln-pBDNF significantly predicted the %change in AURC from pre PAS to any of the time points after PAS in any of the conditions.

4. Discussion

This study aimed to investigate the effects of 3 h of SIT, FPA, and EXE on cortisol and plasma BDNF among middle-aged participants with a sedentary lifestyle. A further purpose was to relate the levels of plasma BDNF and cortisol after these 3 h of SIT, FPA, or EXE to changes in corticospinal excitability and PAS-induced plasticity within each condition.

The three experimental conditions were chosen because they are considered feasible for implementation in an office-setting. From a neurophysiological perspective, the conditions are chosen because an acute bout of exercise has previously been shown to increase the response to PAS in young adults [5,6], and because frequent short breaks in sitting have been shown to improve blood glucose regulation [19] which is hypothesized to be essential for brain function [20].

Cortisol levels decreased from baseline to 5 min post PAS and 30 min post PAS in all three conditions. Since cortisol levels have a circadian rhythm, with a strong peak just after awakening and a progressive lowering throughout the day, this lowering of cortisol plasma levels for all conditions was expected since the 5 min post PAS and 30 min post PAS were taken 4 h and 4.5 h after the baseline measure (see Fig. 1). Cortisol levels decreased from baseline to pre PAS in the SIT and FPA conditions, but remained high in the EXE condition. Plasma BDNF increased slightly from baseline to 30 min post PAS, irrespective of condition. Corticospinal plasticity, as measured as increases in AURC between pre PAS and 5 or 30 min after the PAS intervention were not related to levels of BDNF or cortisol. Levels of cortisol prior to the PAS protocol were, however, related to changes in AURC from baseline to after the intervention and from baseline to 30 min post PAS. This was true only for the FPA condition. While higher cortisol after the FPA

Table 3

 Associations of AURC with Ln-pBDNF and Cortisol at pre PAS over time and conditions.

<table>
<thead>
<tr>
<th>Time × Conditions × Pre Pas Ln-pBDNF</th>
<th>β-coefficients</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Upper bounds</td>
</tr>
<tr>
<td>Baseline × SIT Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre Pas × FPA</td>
<td>-0.021</td>
<td>-0.252</td>
</tr>
<tr>
<td>Pre Pas × EXE</td>
<td>-0.038</td>
<td>-0.313</td>
</tr>
<tr>
<td>5 min post PAS × FPA</td>
<td>-0.086</td>
<td>-0.322</td>
</tr>
<tr>
<td>5 min post PAS × EXE</td>
<td>-0.087</td>
<td>-0.361</td>
</tr>
<tr>
<td>30 min post PAS × FPA</td>
<td>0.009</td>
<td>-0.226</td>
</tr>
<tr>
<td>30 min post PAS × EXE</td>
<td>0.029</td>
<td>-0.246</td>
</tr>
</tbody>
</table>

Ln-pBDNF: log-transformed plasma brain-derived neurotrophic factor. SIT: prolonged sitting. FPA: sitting with frequent short physical activity breaks. EXE: sitting followed by exercise. The β-values represent the association of repeated measurements in area under the recruitment curve (AURC) with three way interactions between Time, exposures (Ln-pBDNF or cortisol at pre PAS), and conditions. The * signifies a significant association between pre PAS cortisol and change in AURC from baseline to pre PAS (p = 0.042), to 5 min post PAS (p = 0.023) in the FPA condition only.

Table 4

Associations between changes in area under the recruitment curve and biomarkers.

<table>
<thead>
<tr>
<th>%change in AURC from baseline to pre PAS</th>
<th>%change in AURC from baseline to 5 min post PAS</th>
<th>%change in AURC from baseline to 30 min post PAS</th>
<th>%change in AURC from pre PAS to 5 min post PAS</th>
<th>%change in AURC from pre PAS to 30 min post PAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIT Ln-pBDNF pre PAS</td>
<td>β = -0.342</td>
<td>β = -0.278</td>
<td>β = -0.022</td>
<td>β = -0.641</td>
</tr>
<tr>
<td>SIT Cortisol pre PAS</td>
<td>β = -0.229</td>
<td>β = -0.401</td>
<td>β = -0.344</td>
<td>β = -0.627</td>
</tr>
<tr>
<td>FPA Ln-pBDNF pre PAS</td>
<td>β = -0.166</td>
<td>β = -0.191</td>
<td>β = 0.426</td>
<td>β = -0.267</td>
</tr>
<tr>
<td>FPA Cortisol pre PAS</td>
<td>β = -0.791 *</td>
<td>β = -0.264</td>
<td>β = -0.662 *</td>
<td>β = -0.378</td>
</tr>
<tr>
<td>EXE Ln-pBDNF pre PAS</td>
<td>β = -0.281</td>
<td>β = -0.390</td>
<td>β = 0.120</td>
<td>β = -0.209</td>
</tr>
<tr>
<td>EXE Cortisol pre PAS</td>
<td>β = -0.035</td>
<td>β = -0.129</td>
<td>β = -0.027</td>
<td>β = -0.339</td>
</tr>
</tbody>
</table>

Paired associative stimulation. Ln-pBDNF: log-transformed plasma brain-derived neurotrophic factor. SIT: prolonged sitting. FPA: sitting with frequent short physical activity breaks. EXE: sitting followed by exercise. The β-values represent the standardized independent regression coefficients for the pre PAS Ln-pBDNF, and cortisol on %change in area under the recruitment curve (AURC). The * signifies a significant association between pre PAS cortisol and %change in AURC from baseline to pre PAS (p = 0.007) and from baseline to 30 min post PAS (p = 0.04) in the FPA condition only.
condition was associated with increased corticospinal excitability at 30 min post PAS, there was an even stronger association with increased AURC immediately after the FPA condition. In our study, this means that the cortisol levels were associated with changes in AURC even before the plasticity protocol was applied. The association between cortisol and changes in AURC was not completely unexpected since in rodent models cortisol levels have been shown to relate to plasticity in an inverted U-shape manner, so that intermediate levels of cortisol promote and higher levels prevent plasticity [13].

BDNF induces pro-neuroprotection signaling cascades by binding to tropomyosin receptor kinase B, however, some of these mechanisms are indirectly affected by cortisol actions. It has been demonstrated that chronic exposure to dexamethasone, a synthetic glucocorticoid, suppresses BDNF-induced glutamate release [21], which has an impact on the processes of long-term memory and the development of mental illnesses [22,23]. While in support of our interpretation, artificially elevated cortisol levels have been demonstrated to reduce PAS-induced plasticity [24], there are other mechanisms that may also have contributed to the changes in corticospinal excitability, such as increased levels of noradrenaline [25].

It was unexpected that plasma BDNF did not increase more after the EXE intervention compared to the SIT or FPA. In a meta-regression of studies investigating acute effects of exercise on BDNF increases Dinoff et al. showed a trend towards larger increases after exercise at higher intensities [26]. At the same time, however, they showed that cardiorespiratory fitness was positively associated with an increase in BDNF [26]. The low levels of cardiorespiratory fitness in our sample (31.3 ± 6.2 mL kg⁻¹ min⁻¹) may therefore partly explain the low increase in BDNF. To our knowledge, only one previous study has investigated the effects of breaking up prolonged sitting in combination with exercise on BDNF [27]. Wheeler et al. found an increase in serum BDNF after morning exercise of moderate intensity combined with breaking up sedentary behavior during the day with FPA bouts equivalent to those used in the current study [27]. Wheeler et al. did however, not compare the effects of FPA with the effects of exercise, but rather compared a morning exercise with 8 h of subsequent sitting to a morning bout with 8 h of subsequent sitting with FPA bouts. Due to these rather substantial differences in study design, the different findings are not surprising. Furthermore, increased serum BDNF after exercise likely reflects the spleen release of BDNF rich platelets into the blood stream and does not necessarily imply that the bioavailable levels are increased in the central nervous system [28]. Ideally, measuring both serum and plasma levels of BDNF would give a more complete understanding of the neurotrophic response to exercise. We showed in a trial on elderly individuals that serum and plasma levels of BDNF do not always change in parallel after a single session or 12 weeks of cognitive or physical exercise [12], but that when physical exercise was followed by cognitive exercise, the levels of increase in plasma BDNF were related to improvements in working memory over the 12 weeks of exercise [29]. Along the same lines, plasma levels of cortisol may not be reflective of bioavailable levels. The effects of corticosteroids on corticospinal excitability and synaptic plasticity are rather complicated, since glucocorticoid and mineralocorticoid receptors have separate distributions and opposing effects [30]. Recent animal experiments indicate that the context of a stressor may influence the effects of corticosteroids on learning [30]. While the current findings are commensurable with the notion that among middle-aged sedentary individuals, breaking up sedentary behavior with frequent short physical activity breaks would keep the cortisol levels on a level beneficial for corticospinal excitability, the findings need to be confirmed in more studies. These findings reinforce the notion that investigations comparing brain plasticity and biochemical markers of neuroplasticity between different populations need to standardize physical activity patterns, at least in the hours preceding the investigation [31].

4.1. Methodological considerations

It is important to the interpretation of the current results to highlight that in this study we measured BDNF, in plasma. Plasma concentrations of BDNF were rather high and we cannot exclude the possibility that samples may have been contaminated by platelet release of BDNF during sampling into the vacutainers. Cortisol levels are known to have a circadian rhythm with a large awakening response followed by a gradual lowering over the day. All experiments started in the morning, well after the end of the awakening response, but when cortisol levels are still high. It is possible, therefore, that the results both for cortisol and corticospinal plasticity may have been different if the activities were performed later in the afternoon. We choose to perform the experiments in the morning so that the participants could come to the lab with minimal physical activity after an overnight fast. In this way, we minimized the risk of variations in physical activity and food intake prior to the experiment.

5. Conclusion

While plasma BDNF did not vary between different physical activity conditions, cortisol levels were significantly higher after EXE compared to after the SIT and FPA conditions. Levels of cortisol after the activity condition were positively associated with changes in corticospinal excitability in the FPA condition only, but levels of plasma BDNF were not associated with changes in corticospinal excitability in any of the conditions. While higher cortisol levels predicted greater increases in corticospinal excitability in the FPA condition, our findings support previous findings suggesting that corticospinal plasticity is reduced with too high levels of cortisol, as seen in the EXE condition. Future investigations trying to evaluate neuroplasticity responses to different physical activity protocols should therefore include cortisol along with different pools of peripheral BDNF.

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Author contributions

ME, OE and VB outlined the study to acquire funding, ME, OE, and EBM designed the final study, OE wrote the ethics application and coordinated the data collection, EBM collected the data with assistance from OT, OE, ME and MP. MM and MP performed biomarker analyses, RW and ME performed statistical analyses and prepared tables, ME drafted the manuscript, and all authors contributed in editing the manuscript and approving the final manuscript. RW was added to the list of authors in the revision stage since her involvement in the analyses and interpretation of the data was substantially expanded. This has been discussed and agreed upon by all co-authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data are available from the corresponding author upon reasonable request.
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