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ORIGINAL ARTICLE

Brain-derived neurotrophic factor concentrations in tetraplegic athletes

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Study design: A prospective cohort with acute tetraplegia.
Objective: The purpose of this study was to investigate acute changes in serum brain-derived neurotrophic factor (BDNF) concentrations in tetraplegic spinal cord-injured (SCI) athletes during a typical training session of wheelchair rugby.
Settings: German Sport University Cologne, Cologne, Germany.
Methods: Eleven male SCI (AIS A and B) athletes completed a 90-min training session: The warm-up period included continuous pushing, submaximal increasing sprints and agility drills. The main training section comprised ball handling, passing drills, scrimmage activity and tactical practice. At the end of the training session, the athletes did moderate continuous pushing as a short cool-down. Venous blood samples were taken at rest before exercise, after the warm-up period and immediately following the first part of the main training section. Serum was pipetted after 30 min of blood sample resting and a subsequent centrifugation. BDNF concentrations were measured using an enzyme immunoassay ELISA kit.
Results: Heart rate ($P<0.01$) and lactate ($P=0.04$ and $P<0.01$) concentration differed significantly in warm-up and main training part in comparison with basal values at rest. At rest, BDNF concentrations were $33.2 \pm 21.6\,ng\,ml^{-1}$, after warm up $31.9 \pm 18.9\,ng\,ml^{-1}$ and after the training session $29.9 \pm 11\,ng\,ml^{-1}$, without significant differences ($P>0.05$).
Conclusions: A typical wheelchair rugby training session does not affect basal serum BDNF concentration in elite SCI athletes. In comparison with concentrations previously reported in healthy subjects, the current values at rest were slightly higher or rather at the upper limit.
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INTRODUCTION

Regular or single bouts of physical activity, such as aerobic endurance exercise or strength training, are associated with manifold positive effects for able-bodied people.[1] Beside an improved preventive cardiorespiratory fitness and muscular capacity, associated with a reduced risk of cardiovascular diseases, mental health and memory performance are increased as well.[2] In this context, neurotrophic factors such as the brain-derived neurotrophic factor (BDNF) have a vital role in terms of neurogenesis, neuroplasticity and nerve protection.[3] Regular physical exercise maintains higher basal BDNF levels and the reaction to exercise is stronger than with other neurotrophins like NGF (neurotrophic growth factor) or NT (neurotrophin) 3-5.[2] The effect of endurance and weight training on BDNF concentration in blood plasma differs.[2,4] Intensity, duration and type of endurance exercise lead to different BDNF concentrations in blood serum[5,6] and even a singular sodium lactate infusion raised serum BDNF levels.[7]

In animals with a SCI, exercise leads to higher concentrations of neurotrophic factors like BDNF.[8,9] These modulations might have an impact on the survival of motoneurons, sprouting and synaptic remodelling of injured axons. Local injections of adeno-associated virus constructs or fibroblasts implying BDNF allows rats with complete paraplegia spinal cord transection injuries to reorganize locomotor skills.[10,11] In addition, BDNF concentration is immediately increased after experimentally induced injury in the rat spinal cord.[11–13]

There is strong evidence that in depression, Alzheimer's and multiple sclerosis, basal concentrations of BDNF are decreased. To raise circulating BDNF levels, exercise has been applied in several neuronal disorders like depression,[14,15] schizophrenia[16] and neurodegenerative diseases like Alzheimer's[17] as well as multiple sclerosis.[18] Depending on lesion levels in tetra- and paraplegia SCI, there are major influences on central nerve system and peripheral nerve system. Impairments in terms of muscle functions or organ and hormonal regulation may also trigger different reactions of neurotrophic factors like BDNF.[19]

To the best of our knowledge, there is only one study that examines BDNF levels in spinal cord injury (SCI) subjects.[20] Rojas and colleagues[20] demonstrate higher basal BDNF values ($37.2 \pm 19.8\,ng\,l^{-1}$) for paraplegic SCI athletes than previously reported in able-bodied subjects. BDNF in paraplegic athletes increases after short (10 min) moderate hand cycling activity but not after a following marathon race simulation. Wheelchair rugby, an attractive sport for athletes with a tetraplegical SCI, is well analyzed in respect of performance, testing and analytics of energy expenditure during games and training.[21–23] Moreover, the beneficial impact of regular participation in wheelchair rugby training on functional abilities is well documented.[24] Although according to general guidelines about the

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energy expenditure to maintain cardiovascular health and fitness, SCI subjects can ensure the amount of energy expenditure through activities like wheelchair rugby[25] and can improve pulmonary function after 1 year of wheelchair rugby training,[26] it is not known whether these positive effects also come along with neurophysiological alterations like an increase in BDNF serum concentration.

Thus, the main objectives of this study were (i) to evaluate the influence of a wheelchair rugby training session on serum BDNF concentrations in tetraplegic athletes and (ii) to compare these results with previously reported BDNF concentrations in paraplegic athletes as well as able-bodied subjects at rest and after exercise.

MATERIALS AND METHODS

Subjects

Eleven male elite wheelchair rugby athletes (age: 31.7 ± 5.9 years; height: 185 ± 0.1 cm; weight: 74 ± 7 kg) with a tetraplegical SCI (spinal lesion level C5-C7; AIS A and B according to the American Spinal Injury Association) were recruited for the study. They were active in wheelchair rugby for 5.7 ± 4.1 years and all members of the German or Polish national team. All subjects were medically checked before the investigation and were informed about the aim, risks and procedures of the tests. All procedures received institutional ethics approval (according to the Helsinki Declaration) and each participant provided written informed consent.

Training session

The training session included a warm-up period, a main section and finally a cool-down. During the warm-up period, the athletes did continuous pushing for about 10 min, followed by eight 20-m submaximal increasing sprints and agility drills. Stretching of the upper extremities completed the warm-up.

The main training section was separated in two segments and contained ball handling, passing drills, scrimmage activity and tactical practice, which lasted 45 min. Parts of a full game were simulated afterwards (second segment). At the end of the training session, the athletes did moderate continuous pushing for cool-down. Overall, the training session lasted 90 min.

At rest, immediately after the warm-up and the first main-training segment (at the same time as the BDNF measurement), heart rate (HR) was measured (FT4, Polar Electro GmbH, Büttelborn, Germany). In addition, the maximum HR during the warm-up and during the first main-training segment was recorded. Capillary blood samples for lactate determination were taken from the earlobe at the same time as HR was measured. For the analysis of lactate concentration, the enzymatic-amperometric measuring method was applied (Biosen C line, EKF, Barleben, Germany).

BDNF measurement

Venous blood samples were taken at rest (after 10 min of sitting in the wheelchair without pushing), immediately (within 1 min) after the warm-up period and after the first half of the main training segment (before the simulated game). These time ranges are within the literature, whereas it has been shown that BDNF values return to baseline after 10–60 min.[2] Serum was pipetted after 30 min of sample resting and a following centrifugation at 4000 r.p.m. for 10 min at 4 °C (EBA 21, Hettich, Tuttlingen, Germany). Samples were stored in Eppendorf tubes (Eppendorf, Hamburg, Germany) first for 4 h at − 18 °C in a mobile freezer for transport and after that at − 40 °C. Enzyme immunoassay ELISA kits (R&D Systems, Minneapolis, MN, USA) were used with an assay range of 62.5–4.000 pg ml^{-1} and cross-reactivity with one or more available related molecules. Measuring sensitivity was 20 pg ml^{-1} and intra- and inter-assay variations were 5% and 11%, respectively. The samples were passively defrosted 2 h before analysis and vortexed to ensure homogeneous solutions. The analytical procedure was arranged accurately according to instructions of the kit. The photometric analysis was conducted using a Multiskan FC photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

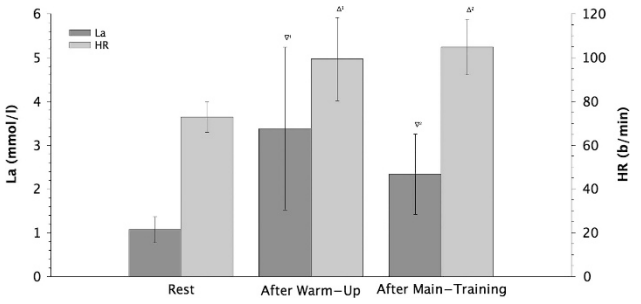

Figure 1 HR and lactate (La) values during the training session—∇1: La warm-up significantly ($P<0.01$) higher than Rest; ∇2: La main-training significantly ($P=0.04$) higher than rest; Δ1: HR warm-up significantly ($P<0.01$) higher than rest; Δ2: HR warm-up significantly ($P<0.01$) higher than rest.

Statistics

All data are shown as mean ± s.d. Analysis of variance for repeated measurement was conducted by using the software Statistica (STATSOFT, Tulsa, OK, USA). The rectangular distribution is conducted automatically initially with the analysis of variance by this program. *Post hoc* comparison with baseline of the significant results were evaluated with the Bonferroni *post hoc* test. The level of significance for all analyses was set at $P \leqslant 0.05$.

RESULTS

Heart rate and lactate levels

Lactate concentration and HR at rest before exercise differed significantly from values after the warm-up and the main training section (Figure 1).

After the warm-up period, the lactate concentration increased significantly in mean about 316% ($P<0.01$) and after the first main-training part about 219% ($P=0.04$) in comparison with rest.

HR increased by 136% ($P<0.01$) and 144% ($P<0.01$) in contrast to the rested state. Here, the maximal HR was found during the warm-up (129 ± 16.3 b.p.m.).

BDNF concentration

Before exercise, BDNF concentrations were 33.23 ± 21.58 ng ml^{-1}, after warm up 31.89 ± 18.93 ng ml^{-1} and during the training session 29.9 ± 10.98 ng ml^{-1}. Analysis of variance revealed no significant ($P>0.05$) difference between the different measurements.

DISCUSSION

The primary purposes of this study were (i) to investigate the influence of a wheelchair rugby training session on serum BDNF concentrations in tetraplegic athletes and (ii) to compare the results with previously reported values in paraplegics and healthy subjects at rest and after exercise.

In our study, there was no influence of wheelchair rugby training on serum BDNF concentrations. An influence of the increased autonomic dysfunction in tetraplegics on BDNF in comparison with paraplegic subjects could not been shown.

Under the present test conditions, BDNF values of tetraplegic athletes at rest were slightly higher or rather at the upper limit in comparison with previously reported values in able-bodied subjects.[2,3,27] Knaepen *et al.*[2] summarized a high variability in basal values of 1.5–30.9 ng ml^{-1} in able-bodied subjects. The data by Rojas Vega *et al.*[20] in paraplegic athletes were somewhat higher than those in our study (Table 1). Increased BDNF concentrations after moderate

Table 1 Basal BDNF values (present study and literature) in SCI, AB and MS subjects

Study (year)	*Population*	n	*Gender*	*Age (years)*	*BDNF at rest (ng ml^{-1})*
Present study	SCI athletes (tetra)	11	Male	31.7 ± 5.9	33.3 ± 21.6
Rojas *et al.* (2008)	SCI athletes (para)	11	Male	40.6 ± 6.3	37.2 ± 19.8
Zoladz *et al.* (2008)	AB healthy humans	13	Male	22.7 ± 0.5	10.3 ± 1.4
Goda *et al.* (2013)	AB Healthy humans	40	Male	24.1 ± 2.9	14.9 ± 5.0
Casetllano and White (2008)	AB - MS	11	Male	40.0 ± 10.0	10.1 ± 20.2

Abbreviations: AB, able-bodied; BDNF, brain-derived neurotrophic factor; MS, multiple sclerosis; SCI, spinal cord injury.

exercise described in paraplegic athletes[20] were not found in the present study with tetraplegical athletes.

In healthy able-bodied persons, lactate concentration seems to be a major mediator for BDNF accumulation. High lactate concentrations elicited by high intensity exercise were linked to increased BDNF values. A potential indicator for the effect of lactate was given by Schiffer *et al.*[7] BDNF concentrations increased immediately after sodium lactate infusion (resulting in blood lactate concentration up to 15 mmol l^{-1}), but not 24 or 60 min after injection. These results were supported by Rojas Vega *et al.*[28] who compared the effects of short low and high intensity exercise with an additional sodium bicarbonate infusion. After high intensity exercise, the BDNF concentrations were elevated until 3 min after exercise. Lactate concentrations remained at the same level in both conditions with and without infusion, while the pH, HCO_3^- and base excess were influenced because of the bicarbonate infusion.

To date, there is only one study that examined the influence of exercise intensity on BDNF in SCI (paraplegic) athletes. Rojas Vega *et al.*[20] compared BDNF values at rest, during warm-up and after a marathon simulation on a treadmill. The warm-up intensity was about 54% of HR max and a metabolic situation represented by lactate concentrations of 2.1 ± 1.0 mmol l^{-1}. During a following marathon race simulation, the intensity was about 89% of HR max and an accompanying lactate concentration of 7.5 ± 3.7 mmol l^{-1}. Post-marathon exercise BDNF values were not significantly different compared with resting values before exercise. According to Leicht *et al.*[29] the current metabolic conditions of our subjects already during the warm-up (3.38 ± 1.86 mmol l^{-1}) period but as well at the following main part (2.34 ± 0.92 mmol l^{-1}) of the training session can be estimated as very intensive, whereas BDNF values were not elevated. The influence of high intensity exercise on BDNF levels in able-bodied subjects could not be proved in paraplegic athletes[20] nor in the current study in tetraplegic wheelchair rugby athletes. Moreover, there is strong evidence that low-to-moderate intensity exercise leads to raised BDNF levels in SCI athletes and during neurodegenerative diseases.[18]

The complete and incomplete lesion (AIS A and B) of the current subjects influence physiological factors and can be a parameter of altered neurophysiological reactions in comparison with able-bodied healthy subjects.[28] Interestingly, studies[30] of BDNF in SCI and multiple sclerosis subjects showed greater standard deviations. Types of lesions, process of diseases like multiple sclerosis may result in greater differences in BDNF concentrations in the rested state.

In healthy able-bodied persons, elevated BDNF levels characterize chronic adaptations through regular physical exercise on a neurophysiological level.[2] These results are not validated[2] to date and cannot be compared with SCI athletes, while there are no studies comparing trained and untrained subjects.

CONCLUSION

A typical wheelchair rugby training session has no influence on serum BDNF concentration in tetraplegical SCI athletes. In comparison with previously reported BDNF concentrations in healthy able-bodied subjects at rest, current values were slightly higher or rather at the upper limit in tetraplegic SCI athletes. These findings are in line with BDNF concentrations of paraplegic SCI athletes although the metabolic, respiratory and neurologic situation of these populations differs. In addition, it appears as if the effect of lactate in paralyzed athletes is not comparable with that of able-bodied persons. Intensity and duration of exercise in the present study correspond to a typical training intervention in wheelchair rugby. Further studies should focus on low intensity activities and their impact on neurotrophic factors in SCI.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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