

## **Evidence for a release of BDNF from the brain during exercise**

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Running title: BDNF release during exercise

Main text: 2.700 words

Abstract: 194 words

2 figures, 1 Table

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## **Abstract**

Brain derived neurotrophic factor (BDNF) has an important role in regulating maintenance, growth and survival of neurons. However, the main source of circulating BDNF in response to exercise is unknown. To identify whether the brain is a source of BDNF during exercise, eight volunteers rowed for 4 hours while simultaneous blood samples were obtained from the radial artery and the internal jugular vein. To further identify putative cerebral region(s) responsible for BDNF release, mouse brains were dissected and analyzed for BDNF mRNA expression following treadmill exercise. In humans, a BDNF release from the brain was observed at rest ( $P < 0.05$ ) and it increased 2-3 fold during exercise ( $P < 0.05$ ). Both at rest and during exercise, the brain contributed 70%-80% of circulating BDNF, while that contribution decreased following 1 hour of recovery. In mice, exercise induced a 3-5 fold increase in BDNF mRNA expression in the hippocampus and cortex, peaking 2 hours after the termination of exercise. These results suggest that the brain is a major but not sole contributor to circulating BDNF. Moreover, the importance of the cortex and hippocampus as a source for plasma BDNF becomes even more prominent during exercise.

Keyword: human, mouse, mRNA, brain derived neurotrophic factor

## Introduction

Brain derived neurotrophic factor (BDNF) is a key protein in regulating maintenance, growth and even survival of neurons (Mattson *et al.*, 2004). BDNF also influences learning and memory (Tyler *et al.*, 2002) and brain tissue from patients with Alzheimer and clinical depression exhibit low expression of BDNF (Karege *et al.*, 2002; Connor *et al.*, 1997). BDNF has also been identified as a key component of the hypothalamic pathway that controls body weight and energy homeostasis (Wisse & Schwartz, 2003). Obese phenotypes are found in BDNF-heterozygous mice and are associated with hyperphagia, hyperleptinemia, hyperinsulinemia and hyperglycemia (Lyons *et al.*, 1999). In addition, BDNF reduces food intake and lowers blood glucose in diabetic mice (Nakagawa *et al.*, 2000). In humans, similar symptoms are associated with the functional loss of one copy of the BDNF gene and with a mutation in the BDNF receptor *Ntrk2* gene (Gray *et al.*, 2006; Yeo *et al.*, 2004).

Physically and socially more complex housing leads to increased neurogenesis, improved learning and less weight gain in rats (Young *et al.*, 1999; Cao *et al.*, 2004) associated with consistent up-regulation in BDNF expression and a direct role for BDNF has recently been reported (Cao *et al.*, 2009). A better understanding of therapeutic actions aimed at increasing BDNF levels, such as exercise (Neeper *et al.*, 1995) is of clinical relevance. It is well-known that BDNF synthesis is centrally-mediated and activity-dependent (Johnson & Mitchell, 2003) and that exercise enhances BDNF transcription in the brain (Oloff *et al.*, 1998). In addition, exercise induces brain uptake of insulin-growth factor 1, which is a prerequisite for the elevation in BDNF mRNA expression (Carro *et al.*, 2000). However, the regions within the brain responsible for the production of BDNF are not known. Physical exercise increases circulating BDNF levels in healthy humans (Ferris *et al.*, 2007; Vega *et al.*, 2006; Gold *et al.*, 2003) although the origin is unclear. We have recently identified BDNF as being a novel contraction-induced muscle cell derived protein that activates and increases

fat oxidation in skeletal muscle in an AMPK dependent fashion (Matthews *et al.*, 2009). Although BDNF was robustly up-regulated in contracting muscle fibres, muscles are not a source of circulating BDNF. Thus, the main source of circulating BDNF at rest and in response to exercise has not been defined.

Tang *et al.* (2008) proposed that platelets can explain that serum BDNF is increased in response to exercise. However, BDNF is also increased in plasma samples, suggesting that BDNF might originate from several other cell sources. We previously demonstrated cerebral output of BDNF in resting healthy humans (Krabbe *et al.*, 2007). Alterations in plasma BDNF levels could therefore reflect variation in the release of BDNF from the brain (Lommatzsch *et al.*, 2005). However, the contribution of the brain to the level of BDNF present in the internal jugular vein in humans during exercise is unknown. While the increase in serum BDNF depends on exercise intensity (Ferris *et al.*, 2007), it is unclear whether exercise duration influences BDNF levels.

The aim of this study was to evaluate the contribution of the human brain to plasma BDNF at rest and during prolonged whole body exercise through the measurement of arterial-to-internal jugular venous difference (a-v diff). To further identify the cerebral region(s) responsible for BDNF release and examine the time pattern of exercise-induced BDNF expression, mice brains were dissected and analyzed for BDNF mRNA expression following treadmill exercise. We hypothesized that the release of BDNF from the human brain would progressively increase throughout the exercise.

## **Methods**

### *Human study*

Eight males aged 22-40 years participated in the study (height  $1.88 \pm 0.08$  m, body mass  $84 \pm 9$  kg and maximal oxygen uptake  $(4.8 \pm 0.5 \text{ l min}^{-1})$ , mean  $\pm$  SD). The subjects provided

informed consent to the study as approved by the Ethics Committee of Copenhagen and Frederiksberg according to the principles established in the Declaration of Helsinki. Each participant visited the laboratory on two occasions. On day 1, the subjects performed incremental exercise to exhaustion on a wind braked rowing ergometer (Concept II, Morrisville, VT, USA) to determine the workload associated with the lactate threshold, defined as the first inflection in the relation between blood lactate and exercise intensity. The subjects were instructed to eat a regular breakfast on the day of the main study. On day two, following 30 min of supine rest, subjects performed a 4-hour bout of ergometer rowing corresponding to a workload 10-15% below the lactate threshold (Hart *et al.*, 2006) to evaluate the influence of exercise duration on BDNF concentration. We did not consider the exercise mode of importance as we did not have any *a priori* indications that exercise modality would impact BDNF response as long as the subjects are familiar with the activity. Subjects were encouraged to row as far as possible while maintaining a steady pace.

On the day of the main study, a catheter was, under local anesthesia (2%, lidocain), placed retrograde with Seldinger technique in the right internal jugular vein (1.6 mm, 14 gauge; ES-04706, Arrow International, PA) guided by an ultrasound image and advanced to the bulb of the vein. Arterial blood was drawn from a catheter in the radial artery (1.1 mm, 20 gauge) of the non-dominant arm. Blood was sampled after 30 min of rest prior to the exercise bout and after 2 and 4 hours of rowing as well as after 1 hour of recovery in the supine position.

Blood was analysed for oxygen, carbon dioxide, glucose and lactate content (ABL 725, Radiometer, Denmark) and heart rate (HR) was recorded on a wrist band monitor (Polar Electro OY, Kempele, Finland). For determination of plasma BDNF, blood samples were drawn into glass tubes containing EDTA, which were immediately spun at 2,600 g for 15 min at 4° C. Plasma was isolated, re-spun at 10,000 g for 10 min at 4° C, re-isolated for complete platelet

removal, and stored at  $-80^{\circ}\text{C}$  until analysed. We used ELISA (R&D Systems, Minneapolis, MN, USA) to measure plasma concentrations of BDNF. The ELISA was specific for BDNF. Samples were analysed in duplicate and mean concentrations were calculated. Two measurements with arterial BDNF values  $>200\text{ pg ml}^{-1}$  at rest was excluded from the analysis as the values lay well outside the mean and 3 SD range compared to the remaining subjects of the group. The cerebral fractional release ( $f_{\text{BDNF}}$ ) of BDNF was calculated as v-a diff/venous concentration.

#### *Animal study*

Forty mice were divided into five groups of 8 mice each. All animals were acclimatized to the treadmill by 10 min of running on three separate days with the last session being held 48 hours before the experiment. One group of mice (pre) did not run acutely and served as controls. The other mice exercised for two hours on a treadmill (18 m/min, 10% slope) until exhaustion and were sacrificed by cervical dislocation either immediately after exercise (0 hour), or after 2 hours, 6 hours or 24 hours of recovery. The brains were dissected immediately and cerebellum, hippocampus and the remaining parts of the brain were separated and quickly frozen in liquid nitrogen for mRNA analysis. One mouse in the 24 hours group had to be taken out of the experiment and accordingly, 7 mice were available for analysis in that group.

All mice were kept at a 12 hours:12 hours light-dark cycle and received standard rodent chow (Altromin nr. 1324, Chr. Pedersen, Ringsted, Denmark). Experiments were approved by the Danish Animal Experimental Inspectorate and complied with the European convention for the protection of vertebrate animals used for experiments and other scientific purposes (council of Europe, No 123, Strasbourg, France, 1985).

#### *RNA isolation. RT and PCR*

RNA isolation was performed on cerebellum, hippocampus and remaining brain (mainly cortex) using a guanidinium thiocyanate-phenol-choloroform method modified from

Chomczynski & Sacchi (Chomczynski & Sacchi, 2006) as described previously (Pilegaard *et al.*, 2000). Reverse transcription (RT) was performed using the superscript II RNase H-system (Invitrogen, Carlsbad, CA, USA) as previously described (Pilegaard *et al.*, 2000). The amount of single-stranded DNA (ssDNA) was determined in the RT samples using the OliGreen reagent (Molecular Probes, The Netherlands) as previously described (Lundby *et al.*, 2005). The BDNF mRNA content was determined by fluorescence-based real-time PCR (ABI PRISM 7900 Sequence Detection System, Applied Biosystems, CA, USA). Forward (FP) and reverse (RP) primers and TaqMan probe were designed from mouse specific sequence data (Ensembl, Sanger Institute) using computer software (Primer Express, Applied Biosystems). The oligo sequences used to amplify a fragment of the BDNF mRNA were forward primer: 5' GGACAGCAAAGCCACAATGTTC 3' ; reverse primer: 5' TCCGTGGACGTTTACTTCTTTCAT 3' and TaqMan probe: 5' CGGTTGCATGAAGGCGGCG 3'. The probe was 5' 6-carboxyfluorescein (FAM) and 3' 6-carboxy-N,N,N',N'-tetramethylrhodamine (TAMRA) labelled. Prior optimization was conducted determining optimal primer concentrations, probe concentration and verifying the efficiency of the amplification. PCR amplification was performed (in triplicates) in a total reaction volume of 10  $\mu$ l and the Ct values were converted to a relative amount using the standard curve (Lundby *et al.*, 2005). The BDNF mRNA content was normalised to the total single-stranded DNA (ssDNA) content in each sample and this BDNF mRNA /ssDNA ratio is presented.

### **Statistical analysis**

The effect of time was evaluated by using a one-way ANOVA with repeated measures in the human experiment and a one-way ANOVA in the mouse study (*proc mixed*, SAS 9.1, SAS Institute Inc., Cary, NC). Following a significant F-test, Student Neuman Keuls *post hoc* test was

used for multiple comparisons and statistical significance was accepted at  $P < 0.05$ . Values are expressed as mean  $\pm$  standard deviation, except in the figures where error bars indicate SEM.

## Results

### *Human experiment*

All volunteers completed 4 hours ergometer rowing, however, one subject had to reduce pace after 3 hours and 15 min of rowing to be able to complete the bout. Mean work rate and HR were  $160 \pm 38 \text{ W min}^{-1}$  and  $143 \pm 8 \text{ beats min}^{-1}$ , respectively, and the subjects rowed  $51.1 \pm 8.3$  km. No changes were observed in arterial hemoglobin oxygen saturation ( $S_aO_2$ ; **Table 1**). Rowing exercise caused a marked impact on cerebral hemodynamics. Internal jugular venous hemoglobin oxygen saturation ( $S_{jv}O_2$ ) decreased after 4 hours of exercise compared with rest and the decrease became statistically significant ( $P < 0.05$ ). Similarly, arterial carbon dioxide tension ( $P_aCO_2$ ) decreased throughout rowing to  $4.5 \pm 0.5 \text{ kPa}$  after 4 hours but the resting  $P_aCO_2$  ( $5.0 \pm 0.3 \text{ kPa}$ ) was restored after 1 hour of recovery. During the experiment arterial glucose was relatively stable ranging from  $6.3 \pm 0.9 \text{ mmol l}^{-1}$  at rest to  $5.3 \pm 0.9 \text{ mmol l}^{-1}$  after 1 hour of recovery. No changes in arterial lactate concentration were observed.

Arterial BDNF increased during rowing ( $P < 0.05$ ; **Fig. 1**). The internal jugular venous BDNF concentration increased from  $442 \pm 272 \text{ pg ml}^{-1}$  at rest to  $1172 \pm 968 \text{ pg ml}^{-1}$  after 4 hours of exercise ( $P < 0.05$ ) and returned to the resting level after 1 hour of recovery ( $P < 0.05$ ). At rest, there was BDNF release from the brain with the a-v diff being  $-347 \pm 316 \text{ pg ml}^{-1}$  and this release increased with exercise ( $-902 \pm 876 \text{ pg ml}^{-1}$ ,  $P < 0.05$ ). After 1 hour of recovery, the release of BDNF from the brain returned to resting levels. The  $f_{BDNF}$  was  $72 \pm 32\%$  at rest and  $84 \pm 8\%$  during exercise, without reaching statistical significance. In the recovery period, the  $f_{BDNF}$  decreased to  $35 \pm 44\%$  ( $P < 0.05$  vs. rest).

### *Mouse experiment*

At rest, the level of BDNF mRNA was 2 fold higher ( $P < 0.05$ ) in cortex than in the hippocampus and approximately 3 fold higher ( $P < 0.05$ ) in hippocampus than in cerebellum. In response to 2 hours of treadmill exercise, the expression of BDNF mRNA was increased in hippocampus and cortex, but not the cerebellum (**Fig. 2**). The BDNF mRNA expression peaked both in hippocampus and cortex at 2 hours of recovery from exercise with levels 3-5 fold higher ( $P < 0.05$ ) than in control mice.

### **Discussion**

The present results suggest that the brain has a significant BDNF production both at rest and during prolonged exercise, and that it may be a major source for increased plasma BDNF during exercise in healthy subjects. These observations are supported by BDNF mRNA present in all three examined brain parts in the mouse and an exercise-induced increase in BDNF mRNA expression in mouse hippocampus and cortex in response to a single exercise bout. The peak in mRNA expression extended into recovery suggesting that exercise-induced BDNF gene regulation within the brain occurs into the recovery phase.

At rest, BDNF is released into the internal jugular vein suggesting that the brain delivers BDNF to the circulation (Krabbe *et al.*, 2007). The present findings confirm the presence of a cerebral output of BDNF at rest in trained subjects. Because BDNF can cross the blood-brain barrier in both directions (Pan *et al.*, 1998; Poduslo & Curran, 1996), it is of interest to discern the contribution of the brain to the internal jugular venous concentration of BDNF at rest and during exercise. The finding that almost 3/4 of the BDNF present in the venous circulation originated from brain structures suggests that brain tissue is the main contributor to the circulating BDNF. However,

other possible explanations for the release exist. BDNF is released from the cerebral vascular endothelium following hypoxic stress (Guo *et al.*, 2008; Wang *et al.*, 2006). While such stress may not be present at rest, exercise could result in cerebral hypoxic stress since cerebral oxygen tension decreases during strenuous exercise (Nybo & Rasmussen, 2007). It remains to be determined from which cells the mRNA induction originates. Cerebral blood volume is reported to be less than 5% (Ito *et al.*, 2005). Thus, the bulk of the cells in the central nervous system are not vascular endothelium cells. It is therefore most likely that the mRNA increase occurs in tissues outside the vascular endothelium. BDNF may also be released from activated platelets in the cerebral circulation as suggested in conditions such as sleep apnea treatment (Staats *et al.*, 2005). BDNF platelet content represents the vast majority of BDNF in circulating blood and the increase in a-v diff across the brain for BDNF during exercise could originate from activation of platelets transitioning through the cerebral vasculature. Conversely, the increase in brain BDNF mRNA expression is in accordance with findings by Neeper *et al.* (1995) and that BDNF found in the internal jugular vein originates from brain structures such as the hippocampus or the cortex. Contribution from other sources, however, cannot be excluded.

There was a dramatic reduction of BDNF concentration from the jugular vein to the radial artery. The fate, however, of the BDNF in the periphery remains unclear. BDNF enhances lipid oxidation in the muscles (Matthews *et al.*, 2009) and it could thus be speculated that the muscles take BDNF up. However, clearance of BDNF by the liver as well as simple dilution cannot be ruled out.

In response to prolonged exercise, the contribution from the human brain to BDNF in the circulation was larger than at rest. The transient increase in BDNF plasma concentration in response to prolonged exercise confirms that a single exercise bout changes plasma BDNF concentrations (Vega *et al.*, 2006; Gold *et al.*, 2003). The internal jugular venous BDNF

concentration increased by 2-3 folds after 4 hours of exercise. The brain did not increase its BDNF release after 2 hours but only after 4 hours of exercise, suggesting that, at this specific exercise intensity, more than 2 hours is needed to increase the cerebral BDNF release. This observation emphasizes the importance of the prolonged nature of this specific exercise stimulus. Accordingly, exercise duration seems to influence circulating BDNF in addition to exercise intensity (Ferris *et al.*, 2007). We speculate that the volume of exercise could be the trigger to increase BDNF release or that BDNF is released as exercise becomes strenuous and several of the subjects reported high perceived exertion towards the end of the rowing bout. The reduction in  $f_{\text{BDNF}}$  during recovery might reflect that while the brain transiently increases the release of BDNF in response to exercise, plasma BDNF levels return to resting concentration during recovery through a reduction of BDNF release by the brain. While hyperglycemia attenuates BDNF release (Krabbe *et al.*, 2007), the release of BDNF by the brain in the present study was not an effect of exercise-induced hypoglycemia but exercise *per se* as the release of BDNF returned to baseline after 1 hour of recovery where blood glucose was the lowest.

The levels of BDNF mRNA in the various brain parts and the transient up-regulation of BDNF mRNA expression in mouse hippocampus and cortex, but not cerebellum, in response to exercise emphasises the likely importance of specific parts of the brain as a source of BDNF at rest as well as during and after exercise. Surprisingly, as the cerebellum is involved in motor tasks, BDNF mRNA was not up-regulated in the cerebellum following exercise. Complex motor learning and moderate exercise produce different effects on the expression of BDNF (Klintsova *et al.*, 2004). It may be that the synthesis of BDNF is part of the adaptation to a new stimulus, such as exercising to exhaustion or coping with a new or stressful environment e.g. the exercise laboratory. Since both running and rowing are relatively simple motor tasks once they have been learned, the cerebellum may not have been heavily activated. The cerebellum may thus not have been activated to the same

level as the hippocampus and cortex and that a threshold needs to be passed before exhibiting an increase in BDNF mRNA. Given the involvement of the hippocampus and cortex in memory and cognition, sensation of exertion may be part of the signal for BDNF mRNA increase rather than the motor task itself. We may hypothesize a link between the metabolic challenges imposed by strenuous exercise on the brain (Nybo & Rasmussen, 2007; Dalsgaard, 2006) and BDNF production. Unfortunately, we did not systematically evaluate perceived exertion. Further studies should evaluate if a relation exists between perceived exertion and BDNF release from the brain. Moreover, the observation that the mRNA expression in the mouse brain peaked 2 hours after the end of exercise, whereas the release of BDNF from the human brain peaked during the exercise period may indicate that exercise elicits multiple roles of BDNF exerted at different times, although species differences may exist. We suggest that the increase in circulating BDNF in humans engaged in prolonged, strenuous exercise originates from the brain. Our results from exercising mice to exhaustion support cerebral contribution to circulating BDNF. We do, however, acknowledge that comparison of the exercise intensity and duration may be troublesome between humans and mice and this may explain the different time courses by the two responses. We can, though, state that both protocols introduced a significant BDNF response. The findings in mouse brain, however, suggest that a likely increased protective role of BDNF within the brain in response to exercise mainly occurs in the recovery phase. Rather, repeated bouts of exercise may well be needed for detectable BDNF protein changes to be evident. Thus, training-induced elevated BDNF mRNA levels are suggested to derive from cumulative effects of transient increases after each exercise bout eventually leading to elevated protein levels. Still, it is likely that the increased BDNF levels detected in the internal jugular vein from the human subjects after exercise comes from a release of BDNF by the brain and not by other blood-borne sources. In this context, up-regulation of BDNF

mRNA in the recovery may serve as a super-compensatory adaptation to the increased demand for BDNF release by the brain during and after exercise.

## **Limitations**

Healthy trained males were considered and the generalisation of the present results might be limited to this population. However, the aim of this study was to identify whether the brain is a source of BDNF during exercise in humans. Estrogen is known to regulate the expression of BDNF (Sohrabji & Lewis, 2006) but plasma BDNF levels has been shown to be similar between sexes in healthy adults (Lommatzsch *et al.*, 2005). However, women displayed significantly lower platelet BDNF levels than men in that study. Accordingly, a difference in BDNF release from the brain between sexes cannot be ruled out and limit the generalization of this study. Thus, to avoid potential sex hormonal effects on BDNF expression and/or release, we chose only to include male subjects in the present study. There was also some inter-individual variance in BDNF a-v diff. Two subgroups may be differentiated maybe because the superior sagittal sinus drains into the right internal jugular vein in only approximately 50% of individuals (Ferrier *et al.*, 1993; Lambert *et al.*, 2000; Lambert *et al.*, 1991) and whether BDNF overflow is markedly different between cortical and subcortical brain regions is not known. Given that BDNF mRNA expression increased in cortex, it may be that for some subjects the a-v diff. for BDNF could have been even larger than observed had the contra-lateral jugular vein been catheterized. Also, no significant elevation in arterial BDNF was present after 4 h of exercise. However, when the two subjects with outlying resting BDNF levels were excluded from analysis, the increase in arterial BDNF became significant. As the exercise-induced increase in systemic BDNF depends of its resting levels and that BDNF concentration is influenced by acute exercise (Ferris *et al.*, 2007), it may be speculated that some of the variability in the resting BDNF levels may be due to subjects having performed some physical

activity before reporting to the laboratory, e.g. commuting by cycling. We did not evaluate the general activity level performed by the subjects. We included active rowers and they must be assumed to be above average physically active subjects. Accordingly, fitness level may have influenced our results but the main goal was to verify whether the brain is a source of BDNF during exercise, rather than evaluating the impact of fitness level on BDNF response to exercise. We did not measure cerebral blood flow and it could be speculated that the changes in the BDNF a-v diff was related to changes in cerebral blood flow. However, the changes in CBF were moderate when gauged from  $S_{jv}O_2$  or  $P_aCO_2$  (**Table 1**) and are unlikely to account for the 2-5 folds increase in the BDNF a-v diff. Furthermore, when measuring a-v diff, it is not possible to discriminate whether the neurons, glia cells or the cerebrovascular endothelium produce BDNF. However, exercise is associated with an elevation of BDNF mRNA in the hippocampus and cerebral cortex in mice. Therefore, the brain is the likely main source of systemic BDNF during prolonged exercise. Since we did not measure the contribution of platelets or other peripheral sources of BDNF, the importance of the brain as a source of BDNF might be overestimated relative to peripheral sources in the blood circulation draining the brain. The platelets were spun down and thus removed from the surfactant and thereby the analysis. The  $f_{BDNF}$  does not take recirculation of BDNF into account and the brain could thus contribute to a larger proportion of BDNF than illustrated by  $f_{BDNF}$ .

In conclusion, the human and mice data suggest that BDNF mRNA expression peaks in recovery after exercise, while an increased release occurs during exercise. We suggest that BDNF gene regulation during recovery from exercise serves as the basis for cumulative effects of repeated exercise bouts eventually leading to detectable increases in BDNF protein content and concomitantly increased potential for BDNF release and neuroprotection in specific brain parts.

## **Funding**

This work was supported by Centre of Inflammation and Metabolism and the Copenhagen Muscle Research Centre. This study was further supported by the Danish Medical Research Council, the Commission of the European Communities (contract no. LSHM-CT-2004-005272 EXGENESIS) and the Lundbeck Foundation. Patrice Brassard is the recipient of a postdoctoral fellowship from the Fonds de la recherche en santé du Québec (FRSQ).

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**Table 1:** Arterial ( $S_aO_2$ ) and jugular venous ( $S_{jv}O_2$ ) haemoglobin oxygen saturation and arterial carbon dioxide tension ( $P_aCO_2$ )

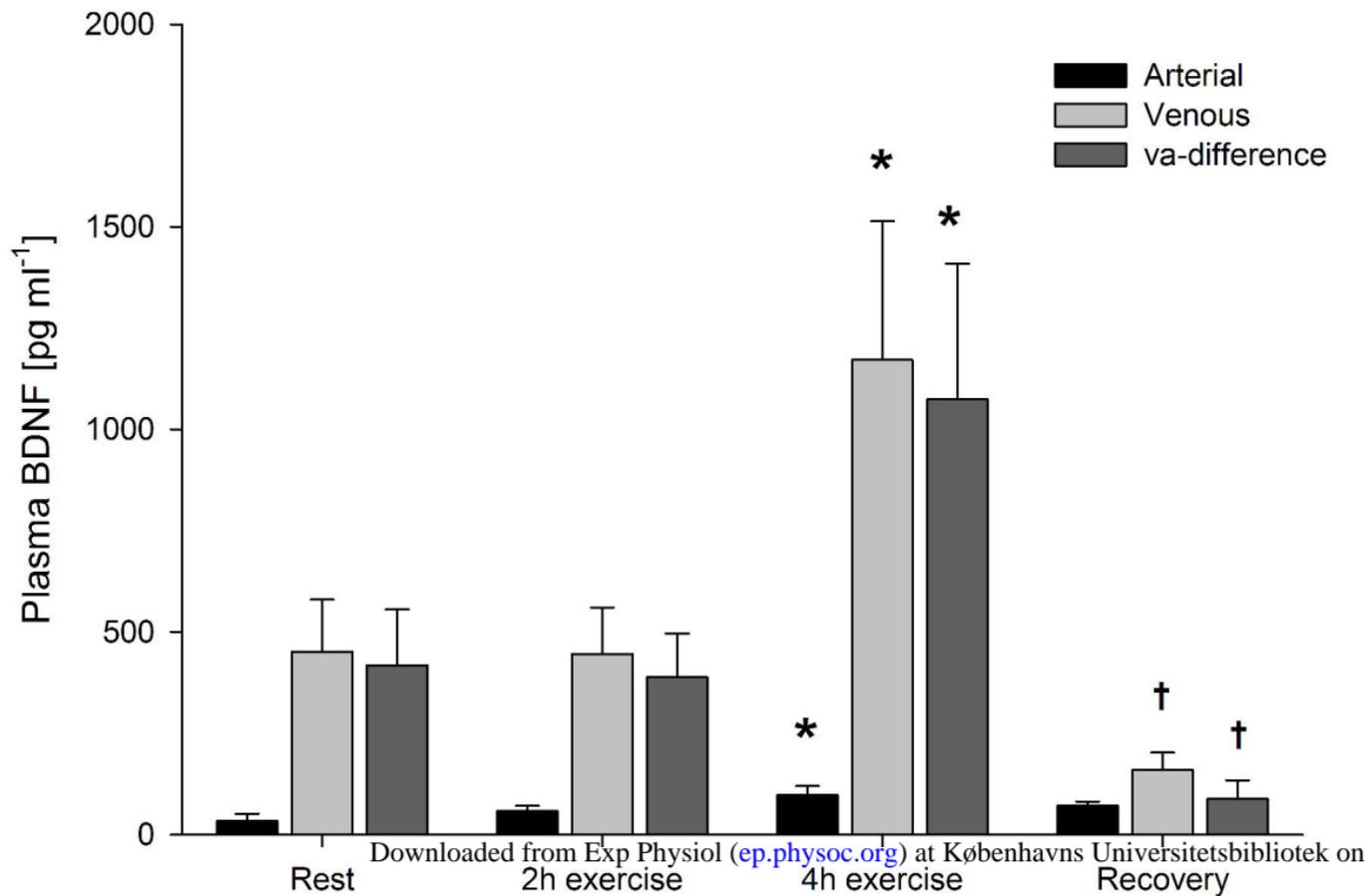
		Rest	Rowing		Recovery
			2 h	4 h	1 h
$S_aO_2$	[%]	97.3±0.6	96.5±1.0	95.3±4.0	96.1±1.4
$S_{jv}O_2$	[%]	60.9 ± 9.8	59.0 ± 7.2	62.2 ± 5.7	64.5 ± 8.7
$P_aCO_2$	[kPa]	5.0 ± 0.3	4.6 ± 0.2	4.5 ± 0.5	5.0 ± 0.28

Values are mean ± SD for 8 subjects.

### Figure legends

**Figure 1:** Arterial, jugular venous and venous-arterial difference across the brain for brain-derived neurotrophic factor (BDNF) before, during and 1h after a four hour rowing bout. Mean ± SEM for N = 8. \*, different from rest,  $P < 0.05$ , † different from rest and exercise,  $P < 0.05$ .

**Figure 2:** Brain-derived neurotrophic factor (BDNF) in mouse brain cerebellum (white bars), hippocampus (hatched bars) and cortex (crossed bars) before (Pre), immediately after (0') and 2h, 6h and 24h after a 2h treadmill exercise bout. The BDNF mRNA content is normalized to the single-stranded DNA content in the samples. Each bar represents means of eight animals (except the group at 24h in which seven animals were available for analysis) and SEM. \*, significantly different from 'Pre'; †, significantly different from cerebellum,  $P < 0.05$ .



BDNF mRNA

