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Lactate infusion at rest increases BDNF blood concentration in humans

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ABSTRACT

Studies in humans use blood lactate to determine the degree of the exercise intensity, suggesting that exercise with elevated blood lactate concentrations results in increased BDNF plasma concentrations. However, it is not clear if lactate per se or rather other mechanisms are responsible for changes in blood BDNF concentrations. The lactate clamp method at rest is an appropriate method to examine physiological responses of lactate on the human organism without the effects of exercise. Eight male sport students placed in a sitting position received intravenous infusions with a 4 molar sodium-lactate solution in an incremental design starting with an infusion rate of 0.01 ml/kgBW/min for the first three minutes, which was increased every three minutes by 0.01 ml/kgBW/min up to 0.08 ml/kg/min in the 24th minute. All together each subject received 4.2 mmol of infusion. Venous blood samples were taken before and immediately after the infusion as well as in the 24th and the 60th min after the infusion period and analysed for BDNF. Blood gases and capillary blood lactate (La) were analysed before the test, every three minutes directly before increasing the infusion rate, at the end of the infusion and in the post infusions period until the 12th min and after 24 and 60 min. BDNF and La increased significantly after the infusion and reached baseline values at the end of the experiment ($p < 0.05$, $p < 0.01$, respectively). pH and hydrogen ions increased from the beginning until the end of the infusion period ($p < 0.01$). This data suggest that blood lactate is involved in the regulation of BDNF blood concentrations.

Animal studies provide evidence that physical activity (PA) results in neurocyte neogenesis, long-term potentiation and long-term plasticity regulated by neurotrophins such as BDNF [4,5,32]. Increases of brain and blood BDNF are considered to be of major importance for mediating the benefits of PA in the brain by the induction of central and peripheral growth factor cascades [reviews 6,9].

The methods, executed in animal studies, e.g. microdialysis and histological brain slices cannot be transferred onto humans due to ethical reasons. However, increased BDNF blood concentrations after PA seem to reflect increased BDNF concentrations in the human brain, since BDNF crosses the blood–brain barrier [10,21]. Independent from regulatory mechanisms, PA has proven to impact the nervous system and the cognitive performance in humans [33]. In this context vocabulary learning was 20% faster after intensified physical exercise compared to lower intensities [33]. Moreover, BDNF is suggested to be a neuromodulator in small diameter nociceptive nerves within the spinal dorsal horn and is released by acid stimuli [14,23].

Data from previous studies suggest an exercise intensity dependent effect on BDNF blood concentrations. There is still no substantial physiologic evidence on how acute exercise regulates BDNF plasma concentrations. However, most studies in humans used blood lactate to determine the degree of the exercise intensity, suggesting that exercise with higher blood lactate concentrations results in elevated BDNF plasma concentrations [7,8,28]. However, it is not clear if lactate per se or other mechanisms are responsible for the described changes in blood BDNF concentrations.

The use of the lactate clamp method at rest is an established method to examine physiological and neurological responses of lactate in the human organism without the previously described effects of exercise. The infusion of sodium-lactate provides an increase of blood lactate without a metabolic acidosis, which is accompanied during high intensity exercise. It is the purpose of this study to determine the effects of acute blood lactate elevations in healthy men on plasma BDNF concentrations at rest, since it is not clear if the exercise induced increase of blood lactate concentration itself or other mechanisms are responsible for the blood BDNF increase. Based on previous studies we hypothesized that elevated blood lactate concentrations at rest would result in an increase of venous BDNF blood concentrations.

Eight male sport students with an average age, body mass and height of 25 (4) years, 80 (8) kg and 181 (5) cm respectively par-

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ticipated in this study. They were all regularly physically active in contact sports like judo and kickboxing with up to 3 trainings sessions per week. After being informed about the experimental protocol and the specific risks of the lactate infusion all subjects completed a medical examination and a physical activity questionnaire and afterwards gave their written consent to participate in the study. The study was approved by the local Ethics Committee.

The subjects were placed in a sitting position under controlled laboratory conditions (24 °C ambient temperature, 60% humidity). A 21 gouache needle placed in the right cubital vein was used for the infusion of a 4 molar sodiumlactate solution by a perfusor (Perfusor® Space, Firma Braun, Melsungen, Germany). The infusion was performed as an incremental design starting with an infusion rate of 0.01 ml/kgBW/min for the first three minutes and was increased every three minutes by 0.01 ml/kgBW/min up to the quantity of 0.08 ml/kg/min in the 24th minute. All together each subject received 4.2 mmol/infused. For safety reasons a physician with special skills in emergency medicine attended all experiments due to the potential risks of derailment of blood gas parameters. 2 out of 8 subjects reported temporary discomfort after hypermolar sodium-lactate infusion.

Venous blood samples from the right cubital vein were taken from an intravenous cannula before (pre) and immediately after (post) the infusion as well as in the 24th min (post 24) and the 60th min (post 60) of the postinfusion period. The blood samples were immediately centrifuged (3000 rpm, 10 min at 4 °C) and stored at −40 °C until analysis. Serum was analysed by enzyme immunoassay (ELISA) for BDNF (Catalog Number DBD00, R&D Systems, Minneapolis, USA) with assay sensitivity < 20 pg/ml and intra- and inter-assay coefficient (average CV of different concentrations) of variability of 5.0 and 9.0%, respectively. Platelets were analysed by Sysmex KX-21N (Sysmex, Norderstedt, Germany). Platelets were counted in 1 μl of whole blood with the DC (direct current) detection method. In our laboratory settings the reproducibility for PLT is 6.0% or less at the reliability level of 95%.

Blood gases (BG) and capillary blood lactate (La) were analysed from the arterialised (Finalgon, Boehringer Ingelheim, Germany) right and left ear-lobe before the test (pre), every three minutes directly before increasing the infusion rate (3, 6, 9, 12, 15, 18, 21), at the end of the infusion (post) and in the post infusions period until the 12th min (post 3, post 6, post 9, post 12) and after 24 (post 24) and 60 min (post 60). BG and La were analysed using "Osmetech OPTI CCA Opti 3" (Osmetech Inc., Roswell, USA) and BIOSEN C_line (EKF-diagnostic GmbH, Barleben, Germany), respectively (Fig. 1).

The statistical evaluation was performed with Statistica (Version 6.0, StatSoft, Tulsa, USA). Factorial analysis of variance was used to assess statistical differences with repeated measures (ANOVA, Newman–Keuls) Data is expressed as mean values (SD). The significance level for all analyses was set at $p < 0.05$.

La increased significantly ($p < 0.01$) from the beginning with every step until the end of the sodium-lactate infusion and reached almost baseline values at the end of the post infusion period ($p < 0.01$). The rise of La during the sodium-lactate infusion was accompanied by an increase of pH and BE ($p < 0.01$). In the post infusion period pH fell ($p < 0.01$) on values between minimum values at pre and maximum values at post 60, while BE values remained on maximum BE values at post. The BDNF increase after the sodium-lactate infusion was significant compared to baseline and post 24 and post 60 values ($p < 0.05$). Platelets remained unchanged throughout the entire examination ($p < 0.05$; Table 1).

The present examination revealed that lactate infusions at rest result in a significant increase of blood BDNF concentrations ($p < 0.05$) in young and healthy male. The maximum increases of lactate during the lactate clamp procedure in this study reached the predicted values up to 15 mmol l^{-1}. The magnitude of the increase depends on the amount of the infused sodium-lactate (Fig. 2).

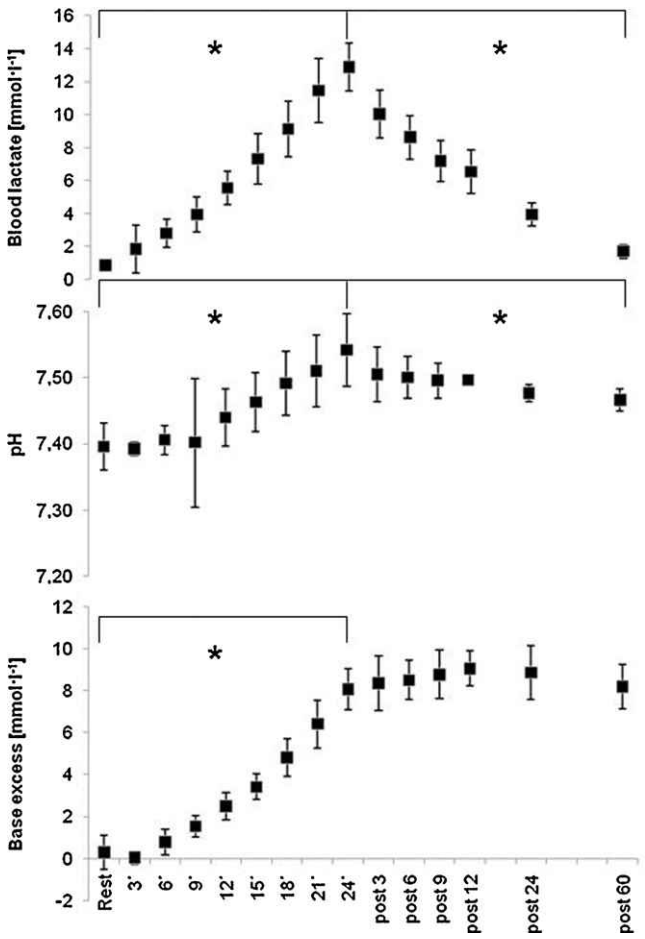

Fig. 1. Capillary blood lactate (La), base excess (BE) and pH values before (pre) and after (post) the sodium-lactate infusion and during the post infusion period. *La, BE and pH are significantly increased at post compared to pre post 60 values ($p < 0.01$). La and pH reached baseline values at post 60 ($p < 0.01$).

The infusion of sodium lactate may not be mixed up with the infusion of lactic acid. Sodium lactate itself is the salt of lactic acid and behaves as a base. Therefore, the infusion of sodium-lactate provides an increase of blood lactate without acidosis but with an alkalosis in the blood. Our present findings are in accordance with other lactate clamp study results [2,15,16,29]. As expected, the increase of lactate was accompanied by an increase of pH and an increase of the base excess ($p < 0.05$). In this regard, the increase of lactate during the lactate clamp procedure at rest differentiates markedly from the increase of lactate during high intensity exercise. All kind of exercises and even more high intensity exercise stresses anaerobic metabolic pathways, resulting in the production of lactic acid [24]. The accumulating blood lactate together with hydrogen ions and carbon dioxide demands puffer systems and results in a metabolic acidosis. These alterations are known to affect regulation mechanisms throughout the whole human organism.

In human studies, capillary blood lactate is usually used to determine the degree of the exercise intensity, suggesting that exercise with higher blood lactate concentrations results in higher BDNF

Table 1
Platelets values before (pre) and after (post) the sodium-lactate infusion and during the post infusion period. There were no significant differences ($p > 0.05$).

	Pre	Post	Post 24	Post 60
Platlets [10^9/l]	202 ± 31	191 ± 30	190 ± 19	194 ± 33

Data are presented as mean ± SD.

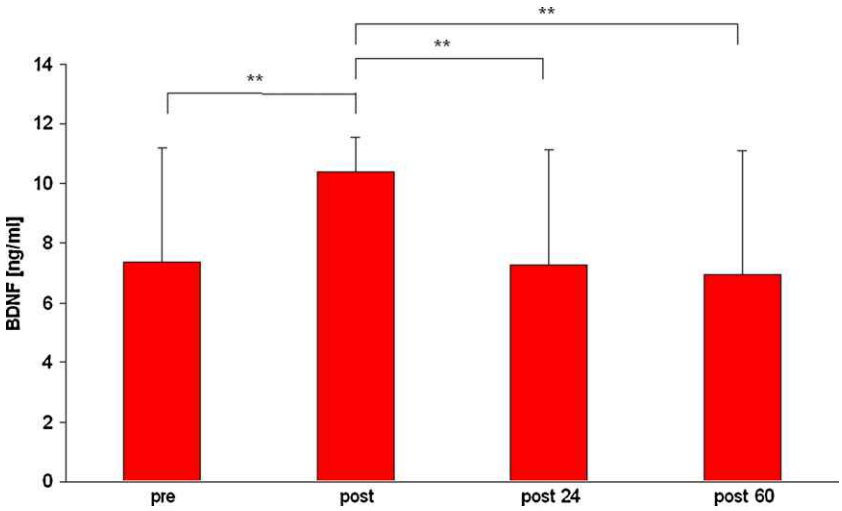

Fig. 2. BDNF values before (pre) and after (post) the sodium-lactate infusion and during the post infusion period. *BDNF is significantly increased at post compared to pre and post 24 and post 60 values ($p < 0.05$).

plasma concentrations. 30 min of moderate PA are an adequate stimulus for serum BDNF increases in multiple sclerosis patients and healthy sedentary controls [8]. In athletes, moderate exercise is not an adequate stimulus to increase BDNF blood concentrations, but in the same subjects a ramp test to physical exhaustion induces significant BDNF elevation [28]. As well, high intensity short-term exercise (15 min step-exercise) increases blood BDNF in healthy human subjects [30]. Ferris et al. [7] examined one group with either a high (10% above the ventilatory threshold) or a low intensity (20% below the ventilatory threshold), whereas only high intensity enhanced BDNF blood concentrations. Ferris et al. [7] proposed that the magnitude of the increase of BDNF is exercise intensity dependent. Accordingly, higher blood lactate concentrations will result in higher BDNF blood concentrations. Even though it is not clear, if the increase of BDNF has to be attributed to the lactate per se or rather to other exercise accompanied parameters such as pH or blood gases, it is conceivable that lactate operates as a pseudo-hormone. The brain can utilise lactate originating from the muscles via an active astrocyte-neurone shuttle [22]. Noakes et al. [20] suggested that lactate might be a peripheral exercise signal which regulates the mass of skeletal muscle used during exercise by the recruitment of motoneurones [18–20]. It is not obligatory necessary that lactate regulates the BDNF blood concentration in the skeletal muscle, where BDNF is produced to a minor degree [13]. However the lactates potential hormonal promoter function enables lactate to support the production of BDNF at its major secretion area during exercise in the central nervous system [12,27].

The circulating BDNF most likely originates largely from the brain, while only one quarter arises from peripheral sources [13]. Potential sources for the peripheral BDNF are skeletal muscles, endocrine organs, glands and blood cells [1,12,17,25]. The content of BDNF in glands is rather small [1,11,17,25]. Matthews et al. [13] suggested from in vitro studies that the BNDF from muscle cells is not released into the circulation. Nevertheless platelets also contain BDNF and it is conceivable that a disturbance of the blood gases results in a damage of platelets with a subsequent release of BDNF. However, our data did not show any significant changes of the platelet count. Independent from the morphological integrity of the platelets it is possible that BDNF can be released by an activation [26,34] through an intracellular rise of Ca^{2+} concentration [3,31]. However, our data are not appropriate to support this mechanism.

In summary recent data provided evidence that blood BDNF seems to increase with the exercise intensity in humans, which is accompanied by metabolic acidosis and hyperlactatemia. The lactate clamp procedure provides an increase of blood lactate without acidosis but rather alkalosis. This study suggests that blood lactate per se has potential influence on the regulation of BDNF blood concentrations. Considering the mentioned potential risks of the lactate clamp procedure, this method seems to be a powerful tool to examine BDNF responses on lactate in healthy humans.

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