Endurance training enhances BDNF release from the human brain

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Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknobt B, Adser H, Jakobsen AH, Pilegaard H, Nielsen HB, Secher NH. Endurance training enhances BDNF release from the human brain. Am J Physiol Regul Integr Comp Physiol 298: R372–R377, 2010.—Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic family of proteins and facilitates neurogenesis, neuroprotection, neuroregeneration, cell survival, synaptic plasticity, as well as formation, retention, and recall of memory (23). BDNF is produced both in the central nervous system and in other tissues, including the vascular endothelium, and it is stored in platelets (12, 43). A particularly high expression of BDNF mRNA is found in the hippocampus and in the cerebral cortex (42), and attenuated expression of BDNF mRNA in the hippocampus may constitute a pathogenic factor common to Alzheimer’s disease and major depression (40). Accordingly, these patients demonstrate low circulating BDNF levels (14, 28) and elevated blood glucose reduces plasma BDNF in patients with type-2 diabetes, and we have demonstrated cerebral output of BDNF in resting healthy humans (16).

Treatment with antidepressant medication upregulates BDNF mRNA in the rat hippocampus (33), and because physical exercise is considered to improve cognitive function by promoting neurogenesis (30), endurance training may also be effective in improving cognitive function in major depression and Type 2 diabetes. Acute exercise increases hippocampal BDNF production in rats (25), and prolonged exercise training increases hippocampal BDNF mRNA expression in rats to the extent induced with the administration of antidepressant drugs (33). In healthy humans, short-term exercise increases the circulating BDNF level (7), and the contribution from the brain to BDNF in the systemic circulation is enhanced after prolonged exercise, possibly as a result of its release from the hippocampus, cortex, and cerebellum since BDNF mRNA expression in mouse hippocampus and cortex is elevated in response to a single bout of exercise (32). However, neither 12 wk of strength nor endurance training alters plasma BDNF levels (35), and increased cardio-respiratory fitness and habitual exercise have been associated with low levels of circulating BDNF (5), although that is not a consistent finding (44).

One explanation for contrasting results may be that BDNF levels in blood samples obtained from a vein in, e.g., an arm may not represent changes in the release of BDNF from the brain. We evaluated the release of BDNF from the brain by arterial and internal jugular venous catheterization in healthy males randomized to either 3 mo of endurance training or sedentary living to evaluate the hypothesis that endurance training would increase the release of BDNF from the brain. To further specify the site of potential increased BDNF production, mice were randomly assigned to either 5 wk of exercise training or a control period and a following evaluation of hippocampal and cerebral cortex BDNF mRNA expression.

MATERIALS AND METHODS

Human study. Twelve sedentary male subjects participated in this study after providing written informed consent as approved by the local ethical committee (H-KF-2006–6443) in accordance with the Declaration of Helsinki. The subjects were included in the study on the basis of the following criteria: no use of medication, normal levels of fasting plasma glucose (≤5.6 mM), and arterial blood pressure (<130/85 mmHg, systolic/diastolic, respectively) with no predisposition to Type 2 diabetes. To elicit a substantial effect of endurance training, the subjects were selected to be physically inactive as assessed by no involvement in regular physical training as judged by interview and a questionnaire and to demonstrate a maximal oxygen uptake (VO2max) lower than 45 ml O2·kg−1·min−1. For purposes unrelated to the present report, we were also interested in the effect of training on fat metabolism, and the subjects had to be overweight (body mass index 25–30 kg/m2) with a percentage of body fat above 25%. Upon inclusion in the study, the subjects were randomly as-
Table 1. Physical characteristics of 12 subjects before and after 3 mo of endurance training (n = 7) or sedentary living (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Training Group (n = 7)</th>
<th>Control Group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>Baseline</td>
<td>Three-Month Follow-Up</td>
</tr>
<tr>
<td>Height, cm</td>
<td>29 ± 6</td>
<td>31 ± 7</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>90.1 ± 7.7</td>
<td>85.6 ± 6.9*</td>
</tr>
<tr>
<td>Fat %</td>
<td>29.6 ± 1.6</td>
<td>24.0 ± 0.8*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3 ± 0.6</td>
<td>26.0 ± 0.7</td>
</tr>
<tr>
<td>VO₂max, l/min</td>
<td>3.4 ± 0.4</td>
<td>4.1 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05 vs. baseline.

signed to either endurance training or to a control group. Accordingly, seven subjects trained (29 ± 6 years, 90 ± 8 kg, and 181 ± 6 cm) and five subjects served as controls (31 ± 7 years, 96 ± 8 kg, and 184 ± 5 cm; means ± SD).

Pretesting. Graded exercise on a cycle ergometer (Ergometrix 800S, Ergo-line, Bitz, Germany) was used to assess VO₂ max. To familiarize the subjects to ergometer cycling, they carried out two bouts of maximal exercise prior to the first determination of VO₂ max. Cycling began at 75 W and after 4 min with the workload increased by 25 W per minute until exhaustion. Pulmonary ventilation, oxygen uptake (VO₂), and exhalation of carbon dioxide (VCO₂) were registered every 10th second by an online gas analyzing system (Oxycon Pro, Jaeger, Würzburg, Germany). Heart rate (HR) was monitored by telemetry (WearLink 31 transmitter, Polar Electro, Kempele, Finland). The criteria used to ascertain that the subjects reached VO₂ max were a leveling off in VO₂ with increasing workload and a respiratory exchange ratio > 1.14. Body composition, fat mass, lean body mass, and total body mass were assessed by dual-energy X-ray absorptiometry scanning (Prodigy Bone Densimeter System, GE Lunar, Madison, WI; and Lunar Prodigy Advance and enCORETM 2006 software ver. 10.50.080).

Endurance training. The control subjects were asked to continue their sedentary lifestyle, but they were on a diet aiming at creating a negative energy balance of ~600 kcal/day and reported to the laboratory for determination of VO₂ max before and after the intervention period. The training group carried out daily endurance-type exercise aimed to establish a similar negative energy balance. Each training session lasted ~60 min or until the target energy expenditure was reached and included mainly cycling, but the subjects were also allowed to run, swim, or use a rowing ergometer to create some variation and thereby maintain compliance. The exercise intensity was on average ~70% of maximal HR equivalent to ~65% of VO₂ max. All sessions were supervised for the first 2–3 wk by an exercise physiologist, and the subjects wore the HR monitor during the sessions to verify that the required energy expenditure and exercise intensity were achieved. The training intensity was adjusted to changes in VO₂ max after 6 wk of training.

Procedures. On the days of the study, the subjects had no restrictions in diet but abstained from strenuous physical activity on the previous day. Upon arrival to the laboratory at 8:00 AM, the subjects were placed in a hospital bed and tilted slightly head-down. Under local anesthesia (lidocaine, 2%) and guided by ultrasound, a catheter (1.6 mm; ES-04706; Arrow International, Reading, PA) was inserted retrograde in the right internal jugular vein and advanced to the bulb of the vein at the base of the skull. Thus, samples were considered to represent blood leaving the brain with a small contribution from cerebrospinal fluid drained to the sinus sagittalis. A second catheter (1.1 mm) was inserted in the left brachial artery. After catheterization, the subjects were supine and recovered for 1 h before exercise to offset any perturbations in brain activity caused by “arousal” and nociceptive stimuli (37).

Experimental protocol. To evaluate brain metabolism during exercise, the subjects performed ergometer cycling (Ergomedic 874E; Monarch, Stockholm, Sweden) before and after the intervention. At the first visit to the laboratory, the subjects cycled for 5 min at a light intensity, whereafter the workload was increased to represent 70% VO₂ max. That intensity was maintained for 15 min, and blood samples were obtained simultaneously from the brachial artery and the right internal jugular vein after 5, 10, and 15 min. The subjects then recovered for 30 min until they carried out incremental cycling. Subjects cycled for 4 min at 60%, 70%, 80%, 90%, and 100% of VO₂ max, and each 4-min bout was separated by 6 min of recovery with blood samples obtained at the end of each workload. This exercise protocol was repeated after 3 mo, except that the trained subjects cycled for 30 min with the first 15 min adjusted to the pretraining VO₂ max (same absolute intensity) and the last 15 min adjusted to an intensity that corresponded to 70% VO₂ max (same relative intensity).

The 3 mo follow-up was placed 48 h after the last training session to avoid any exercise effects on BDNF release.

Measurements. Blood samples were drawn into glass tubes containing EDTA and immediately spun at ~2,600 g for 15 min at 4°C. Afterward, plasma was centrifuged again at ~7,500 g for 10 min at 4°C to secure platelet removal and immediately stored at ~80°C. Plasma concentration of BDNF was measured in duplicate by ELISA (R&D Systems, Minneapolis, MN, USA), according to manufacturer’s guidelines and expressed as the mean.

Mean flow velocity of the proximal segment of the left middle cerebral artery (MCA Vmean) was monitored by transcranial Doppler sonography through the temporal ultrasound window at a depth of 48–60 mm (Multidop X, DWL, Sipplingen, Germany). Once the optimal signal-to-noise ratio was obtained, the probe (2-MHz and 20 mm in diameter) was mounted on a headband, and an acoustic coupling was secured by adhesive ultrasonic gel (Tensive, Parker Laboratories, Orange, NJ). MCA Vmean was calculated from the integral of the maximal frequency Doppler shifts over one heartbeat, and 30-s averages were calculated. Transcranial Doppler is justified as a measure of changes in global cerebral blood flow, as increases in MCA Vmean during exercise parallel the inflow from the internal carotid artery (11), the “initial slope index” of the 133Xenon clearance determined cerebral blood flow (13), and the regional cerebral blood flow measurements determined by positron emission tomography (31). The global cerebral blood flow was calculated from changes in MCA Vmean using 46 ml·100 g⁻¹·min⁻¹ as an estimation of the resting level (21).

Mouse study. Mice were assigned to a training group (n = 8) or a control group (n = 8). All mice were acclimatized to a treadmill (Model exer-4 treadmill; Columbus Instruments, Columbus, OH) by 10 min of running on 2 days (10% slope and for each day the speed was increased). The training group exercised on the treadmill (10%...
Table 3. Arterial and internal jugular venous BDNF concentrations and jugular venous–arterial concentration difference of BDNF at rest and during exercise before and after 3 mo of endurance training (n = 7) or sedentary living (n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Rest)</th>
<th>Baseline (Exercise)</th>
<th>Three-Month Follow-Up (Rest)</th>
<th>Three-Month Follow-Up (Exercise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial BDNF, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training group</td>
<td>1.2±0.6</td>
<td>2.4±1.3†</td>
<td>1.0±0.3</td>
<td>2.0±0.9‡</td>
</tr>
<tr>
<td>Control group</td>
<td>0.9±0.3</td>
<td>1.6±0.6‡</td>
<td>1.1±0.1</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>Internal jugular venous BDNF, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training group</td>
<td>2.5±2.4</td>
<td>4.4±2.4†</td>
<td>5.5±2.3*‡</td>
<td>5.9±3.9†</td>
</tr>
<tr>
<td>Control group</td>
<td>1.2±0.5</td>
<td>1.3±0.3</td>
<td>2.2±1.1</td>
<td>3.6±0.8</td>
</tr>
<tr>
<td>JV-a difference of BDNF, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training group</td>
<td>1.3±2.3</td>
<td>2.4±2.7†</td>
<td>4.5±2.4*‡</td>
<td>4.2±4.0*‡</td>
</tr>
<tr>
<td>Control group</td>
<td>0.3±0.2</td>
<td>-0.1±0.4</td>
<td>1.0±2.2</td>
<td>2.9±2.5</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. BDNF, brain-derived neurotrophic factor. *P < 0.05 vs. baseline, †P < 0.05 vs. control group, ‡P < 0.05 vs. rest. Jv-a, jugular venous–arterial.

RESULTS

Human study. With an 88 ± 6% compliance to the training program, both the training and the control group demonstrated a weight loss, but only the training group reduced the percentage of body fat (P < 0.05; Table 1). Endurance training caused a ~25% increase in VO2 max (P < 0.05), and the maximal workload increased ~20% (from 302 ± 45 to 365 ± 39 W; P < 0.05), whereas VO2 max remained unchanged for the control group. MCA Vmean at rest was not significantly different between baseline, and the 3-mo follow-up within both groups of subjects and increased ~15% from rest to exercise (P < 0.05; Table 2).

BDNF release from the brain at rest. The resting arterial BDNF level was not affected by 3 mo of endurance training or by sedentary living (Table 3), but training increased the jugular venous BDNF level (P < 0.05) and to a higher level than in the control group (P < 0.05), in which there was no change. Consequently, the release of BDNF from the brain was enhanced by 3 mo of endurance training (P < 0.05; Fig. 1) both compared with the baseline level and to the control group (P < 0.05).

Fig. 1. Brain release of brain-derived neurotrophic factor (BDNF) at rest and during exercise before and after 3 mo of endurance training (n = 7) or sedentary living (n = 5). After endurance training, the brain released more BDNF compared with baseline and also compared with 3 mo with sedentary living. At baseline, the training group released more BDNF during exercise than the control group, but 3 mo of endurance training did not increase the release of BDNF from the human brain during exercise. *P < 0.05 vs. baseline, †P < 0.05 vs. control group. Values are expressed as means ± SE.
**BDNF release from the brain during exercise.** There were no changes in the arterial BDNF level during exercise from baseline to the 3-mo follow-up. However, the arterial BDNF level was elevated during exercise compared with rest ($P < 0.05$) both before and after training. For the control group, this exercise-induced increase in arterial BDNF was only evident at baseline. The jugular venous BDNF level did not change with exercise-induced increase in arterial BDNF was only evident at baseline. The jugular venous BDNF level did not change with either training or sedentary living, but the BDNF level was higher in the training group than in the control group ($P < 0.05$), both before and after training. For the control group, this level was elevated during exercise compared with rest ($P < 0.05$; Fig. 1). In the cerebral cortex, the BDNF mRNA level was not significantly elevated by training ($14 \pm 1\%$) but was comparable to that in the hippocampus of the untrained mice.

**DISCUSSION**

Three months of endurance training increased the release of BDNF from the human brain at rest and although acute exercise per se did not influence the release of BDNF, the arterial BDNF level was elevated during exercise compared with rest. As evaluated in mice, increased expression of BDNF mRNA in the hippocampus rather than in the cerebral cortex appears to be responsible for the training-induced increase of the BDNF release from the brain. The higher mRNA expression in the cerebral cortex in the untrained mice suggests that training does not induce an additional increase in the BDNF level in this brain region or that there is no room for training-induced improvement.

The finding that the resting arterial BDNF level did not change significantly from baseline to the 3-mo follow-up is in agreement with a study that demonstrated unaltered venous plasma levels following 12 wk of strength or endurance training in healthy humans (35) and following 8 wk of aerobic training in patients suffering from multiple sclerosis (36). However, resting venous BDNF levels following 5 wk of endurance training has been reported, and it may be elevated in athletes compared with untrained individuals (44). Peripheral venous blood sampling may, however, blunt the contribution from the brain to the measured BDNF concentration, and that could explain inconsistencies in results (5, 26). Alternatively, it may be that a lower BDNF level in well-trained subjects is a reflection of an elevated resting cortisol level (19) since cortisol inhibits hippocampal BDNF production (34). In the present study, blood samples were obtained from the internal jugular vein with the catheter advanced to the bulb of the vein. Because BDNF crosses the blood-brain barrier in both directions (27) and the influence of platelets to the measured values is considered minimal, the increase in internal jugular venous BDNF concentration following endurance training, most likely, reflects increased release from the brain. A contribution from the vascular endothelium, however, cannot be ruled out (16).

Although there was a release of BDNF from the brain during acute exercise, it was not larger than at rest, and we could not demonstrate a significant training-induced increase in the release of BDNF from the brain during exercise. One study has reported increased cerebral release of BDNF following prolonged exercise and differences in exercise intensity and/or the

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**Table 4. Release of BDNF from the human brain at different exercise intensities before and after 3 mo of endurance training ($n = 7$) or sedentary living ($n = 5$)**

<table>
<thead>
<tr>
<th>BDNF Release, ng·100 g$^{-1}$·min$^{-1}$</th>
<th>Rest</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>58±106</td>
<td>213±143</td>
<td>242±250</td>
<td>145±263</td>
<td>51±200</td>
<td>111±117</td>
</tr>
<tr>
<td>Three-month follow-up</td>
<td>206±108*†</td>
<td>110±142</td>
<td>152±195</td>
<td>210±174</td>
<td>183±299</td>
<td>289±369</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12±17</td>
<td>93±121</td>
<td>−21±54</td>
<td>−30±40</td>
<td>13±29</td>
<td>0±12</td>
</tr>
<tr>
<td>Three-month follow-up</td>
<td>48±101</td>
<td>−4±16</td>
<td>188±303</td>
<td>232±413</td>
<td>257±388</td>
<td>−45±26</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. *$P < 0.05$ vs. baseline; †$P < 0.05$ vs. control group.
duration of exercise may be of importance (7, 32). The training group had a higher internal jugular venous BDNF concentration during exercise compared with the control group both at baseline and at the 3-mo follow-up, possibly because of the large interindividual differences in BDNF levels (22, 35). The production and secretion of BDNF during exercise may be regulated differently than at rest, and it may be that exercise modifies the uptake and release mechanisms in the central nervous system or in the peripheral storage and release systems (41).

At baseline, the arterial, but not internal jugular venous BDNF level was higher during exercise than at rest in both groups of subjects, but at the 3-mo follow-up, only the training group increased the arterial BDNF level from rest to exercise. The increased arterial BDNF level during exercise may be expected, because BDNF levels increase following 10 min of maximal exercise (44), and the absence of an increase in the internal jugular venous BDNF level may indicate fast metabolism of BDNF and/or loss of BDNF in the capillaries.

Low BDNF mRNA and protein levels in the brain are reported in several patient groups (6, 24, 28), and serum BDNF levels are reduced in patients with Alzheimer’s disease, major depression, and panic disorder (14, 28, 39). Conversely, when patients are treated with antidepressants both hippocampal BDNF mRNA expression and serum BDNF levels increase (3, 38). The present study confirms findings demonstrating increased BDNF mRNA expression in the hippocampus in rats following exercise training (1, 9, 15), and the endurance training-induced increase in BDNF release from the brain at rest suggests that exercise may be neuroprotective and important for maintaining neuronal health and survival in humans suffering from neurological and psychiatric diseases and diseases related to obesity and physical inactivity (16). Thus, plasma BDNF increases during exercise in patients with multiple sclerosis, major depression, and panic disorder (8, 10, 39).

In moderately depressed patients, the plasma BDNF level decreased toward baseline 30 min after exercise, but it was elevated again after 60 min, indicating upregulation of BDNF synthesis (10) in accordance with an elevated BDNF mRNA expression in the mouse cerebellum, hippocampus, and cortex 24 h following an acute exercise bout (32).

Methodological considerations. We acknowledge that the study suffers from a small sample size. Furthermore, generalization to the population may be affected by the fact that the subjects that were selected were males, sedentary, and overweight. In humans, plasma BDNF demonstrates a large variability, and other factors than neurological diseases may influence BDNF levels. We did not screen the subjects for depression besides that they were not taking any medication, and the training group likely experienced improved quality of life that may have influenced the resting measurements. Furthermore, a negative association between BDNF and age, plasma cholesterol, weight gain, and hyperglycemia are evident in humans (18). Although these differences were minimized by inclusion of a homogenous group of subjects, the internal jugular venous BDNF level was elevated in the training group during exercise rather than at rest before the intervention. Because both groups of subjects experienced a weight loss of similar magnitude, a possible confounding effect of weight should be minimal although the training group reduced their percentage of body fat to a larger extent than the control group. Accordingly, increased resting BDNF levels could be influenced by a lower leptin level (2). In addition, a circadian variation has been reported in BDNF mRNA expression in the rat hippocampus in parallel with plasma cortisol (34), but blood samples were obtained at the approximately same hour during the day. Finally, we measured BDNF levels in the right internal jugular vein into which the cerebral hemispheres are most likely to drain. Given the highly asymmetric drainage of the cerebral venous sinus (17), an association between the increased BDNF mRNA level in the hippocampus seen in the mice study and the increased BDNF release from the human brain should be made with caution.

Perspectives and Significance

Taken together, endurance training increased the expression of BDNF mRNA in mice hippocampus rather than in the cortex and also the BDNF release from the human brain. Exercise promotes cardiovascular and musculoskeletal health, and this study adds that regular physical activity may be important for maintenance and improvement of brain health and, thereby, supports exercise as a coadjuvant to the treatment of various neurological diseases, including Alzheimer’s disease, major depression, and for the treatment of type-2 diabetes patients.

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GRANTS

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DISCLOSURE

The authors declare no conflicts of interest.

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