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Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) participates in orchestrating the adaptive response to exercise. However, the importance of transient changes in circulating BDNF for eliciting whole-body and skeletal muscle exercise benefits in humans remains relatively unexplored. Here, we investigated effects of acute aerobic exercise and 3-month aerobic-strength training on serum, plasma and skeletal muscle BDNF in twenty-two sedentary older individuals (69.0 ± 8.0 yrs., 9 M/13F). BDNF response to acute exercise was additionally evaluated in young trained individuals (25.1 ± 2.1 yrs., 3 M/5F). Acute aerobic exercise transiently increased serum BDNF in sedentary (16%, $p = .007$) but not in trained elderly or young individuals. Resting serum or plasma BDNF was not regulated by exercise training in the elderly. However, subtle training-related changes of serum BDNF positively correlated with improvements in walking speed ($R = 0.59$, $p = .005$), muscle mass ($R = 0.43$, $p = .04$) and cognitive performance ($R = 0.41$, $p = .05$) and negatively with changes in body fat (R = -0.43, p = .04) and triglyceridemia (R = -0.53, $p = .01$). Individuals who increased muscle BDNF protein in response to 3-month training (responders) displayed stronger acute exercise-induced increase in serum BDNF than non-responders ($p = .006$). In addition, muscle BDNF protein content positively correlated with type II-to-type I muscle fiber ratio ($R = 0.587$, $p = .008$) and with the rate of post-exercise muscle ATP re-synthesis ($R = 0.703$, $p = .005$). Contrary to serum, acute aerobic exercise resulted in a decline of plasma BDNF 1 h post-exercise in both elderly-trained (−34%, $p = .002$) and young-trained individuals (−48%, $p = .034$). Acute circulating BDNF regulation by exercise was dependent on the level of physical fitness and correlated with training-induced improvements in metabolic and cognitive functions. Our observations provide an indirect evidence that distinct exercise-induced changes in serum and plasma BDNF as well as training-related increase in muscle BDNF protein, paralleled by improvements in muscle and whole-body clinical phenotypes, are involved in the coordinated adaptive response to exercise in humans.

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1. Introduction

Physical activity was shown to benefit the whole-body metabolic health and to improve brain functions in healthy adults (Smith et al., 2011) as well as in patients with preclinical and early-stage neurodegenerative diseases (Heyn et al., 2004). It was shown to affect morphology, metabolism and functional capacity of both skeletal muscle and brain regions important for memory, attention, processing speed, executive and motor functions (Barbieri et al., 2015; Erickson et al., 2011; Ngandu et al., 2015; Rosano et al., 2017). Regular physical activity, either alone or in the context of a complex lifestyle intervention, is an effective tool in the prevention and treatment of cognitive decline (Lautenschlager et al., 2008; Smith et al., 2011; van Uffelen et al., 2008), mainly by mediating the improvements in the whole-body metabolism, cardiovascular fitness, cerebral blood flow, or by changes in neurotrophic factors and modulation of neurotransmitter activity (Delezie and Handschin, 2018). The brain-derived neurotrophic factor (BDNF) is known to play a key role in learning and memory by regulating survival, growth and maintenance of neurons (Pedersen et al., 2009), processes underlying brain plasticity. A decrease in circulating BDNF is considered a biomarker of age-related cognitive decline (Gunstad et al., 2008; Komulainen et al., 2008). Interestingly, exercise has been shown to increase BDNF levels in brain of rats (Cotman and Berchtold, 2002; Vivar et al., 2013), and blocking the actions of BDNF in hippocampus hindered the beneficial effects of exercise on spatial memory in mice (Vaynman et al., 2004). Moreover, increased levels of circulating BDNF has been linked to improved cognitive functions in the elderly (Erickson et al., 2011; Gunstad et al., 2008; Komulainen et al., 2008; Rasmussen et al., 2009b; Tsai et al., 2018).

However, there is currently no consensus in literature whether to measure circulating BDNF in serum or plasma, which represent two distinct reservoirs of BDNF protein. The fact that the large amount of BDNF detected in serum is stored in platelets and released upon activation, makes serum BDNF levels a good marker of the long-term changes in BDNF production. On the other hand, plasma levels rather represent systemic pool of readily available bioactive BDNF (Serra-Millas, 2016).

Although brain is the main site of BDNF expression, non-neuronal tissues, e.g. endothelial cells, skeletal muscle and liver also express BDNF. BDNF was shown to regulate metabolism (Cao et al., 2010; Wang et al., 2007), angiogenesis (Kermani et al., 2005), myogenesis and muscle regeneration (Colombo et al., 2013), to improve peripheral glucose uptake/utilization (Suwa et al., 2010; Yamanaka et al., 2007b), diabetic hyperglycemia (Meek et al., 2013; Ono et al., 1997; Yamanaka et al., 2007a), skeletal muscle fatty acid oxidation (Matthews et al., 2009), and to protect from motor neuronal degeneration (Koliatsos et al., 1993). Brain represents a major contributor to circulating BDNF levels during exercise (Rasmussen et al., 2009b). A moderate increase of BDNF in peripheral circulation after regular and acute aerobic exercise is highlighted by recent meta-analyses (Dinoff et al., 2016; Szuhany et al., 2015), but a very limited number of studies analyzed the effects of exercise on BDNF in human skeletal muscle (Matthews et al., 2009; Walsh et al., 2015). An exercise-induced increase in muscle BDNF was shown to be involved in the autocrine/paracrine signaling supporting the skeletal muscle regeneration (Colombo et al., 2013), adaptation of muscle lipid metabolism and neuromuscular junction to exercise (Pedersen et al., 2009; Sakuma and Yamaguchi, 2011). The physiologically relevant contribution of muscle BDNF to the circulating pool has not been proven (Matthews et al., 2009).

The complex human studies focused on the synchronized effects of long-term exercise on the dynamics of BDNF regulation in serum, plasma and skeletal muscle are lacking. We therefore investigated (i) dynamics of the acute changes of plasma and serum BDNF in response to an acute bout of aerobic exercise in (i) sedentary elderly individuals, (ii) in the same individuals after 3-month of aerobic-strength exercise training and (iii) in healthy young trained volunteers as well as training-induced changes in the BDNF protein content in skeletal muscle of elderly individuals, in relation to the complex changes in the whole-body and muscle metabolism, physical fitness and cognitive functions.

2. Materials & methods

2.1. Ethical approval

All studies were approved by the ethics committee of the University Hospital Bratislava, Comenius University Bratislava and the Ethics Committee of the Bratislava Region Office and conform to the ethical guidelines of the Declaration of Helsinki 2000. All participants provided witnessed written informed consent prior to entering the study. Participants' health and capacity to undergo exercise intervention was assessed by physician/cardiologist. Clinical trials.gov registration number: NCT02253732.

2.2. Patients populations

Twenty-two sedentary elderly individuals (age 69 ± 8 yrs., 9 M/13F BMI 27.1 ± 3.2 kg/m^2, mean ± SD) were examined by a neurologist. Addenbrooke's cognitive examination (ACE-R, revised) was used to examine performance in various cognitive domains. In addition, healthy young individuals (age 25 ± 2 yrs., 3 M/5F, BMI: 22.4 ± 0.8 kg/m^2), who performed at least 1 h of moderate-to-high intensity aerobic exercise (60–80%VO_2max, ≥3 ×/week) for > 6 previous months were recruited.

2.3. Measurements of physical fitness and strength

Initial physical fitness and strength testing was performed at baseline (young and elderly volunteers) and after the completion of 3-month exercise training (elderly).

In the elderly, aerobic fitness was assessed by Rockport 1-mile walking test (1609 m) on a 400 m long track. Maximum heart rate (Polar RS300X, Finland) and time to completion (Witty, MicroGate, USA) were recorded and VO_2max was calculated (Kline et al., 1987; Krumpolec et al., 2017). Handgrip strength was measured (EH101, CAMRY, USA). Maximum isometric torque of knee extensors and flexors was assessed with a knee dynamometer (Science-to-practice S2P, Slovenia). Ambulatory physical activity was objectively measured by accelerometers during 7 consecutive days (HJ-720IT-E2, Omron, Japan), and subjectively assessed by Baecke's questionnaire (Baecke et al., 1982). Individuals with uncontrolled or late-stage cardiac/renal/liver/oncologic or other chronic diseases and regular use of pharmacotherapy were not eligible for the study.

Young trained participants underwent maximal aerobic capacity (VO_2max) testing by cycle spiroergometry as described in (Schon et al., 2019). Briefly, gas exchange was continuously measured (PowerCube - Ergo, Ganshorn Medizin Electronic GmbH, Germany) during an incremental exercise test on Corival cycle ergometer (Lode, Netherlands) with an initial load of 50 W, progressively increasing (25 W/min) towards maximum and followed by 6 min recovery period.

2.4. Exercise intervention protocol

Standardized acute exercise test (40 min at 65–75% HRmax and pedaling frequency of 60/min) was performed on a stationary cycle on a separate day during initial phenotyping phase, while the maximal and average power output was recorded. The test was repeated after 3-month training intervention at the identical heart rate and pedaling frequency, without controlling the power output. Participants were asked to refrain from moderate and vigorous physical activity and alcohol consumption within 48 h before the exercise test. In the morning of the testing day, after an overnight (10 − 12 h) fasting, the forearm

vein was cannulated, followed by a 30 min rest period before first baseline blood draw ("pre"). Next, participants were fed one banana (100 g) prior to the exercise test. Blood samples were also taken immediately after the test completion and after 1 h recovery period ("post"/"1 h rest").

Three-month combined training was supervised by professional trainers (Faculty of Physical Education and Sports, Commenius University in Bratislava) and consisted of 1x1h-session of low-impact aerobic focused on the development of coordination and balance, and 2x1h-sessions of combined aerobic-resistance exercise weekly. Aerobic training consisted of walking or Nordic walking for 20 min (1750–2100 m distance, 70% VO_2max). In case of bad weather, step-aerobic was performed indoor with a comparable intensity. Resistance training of major muscle groups (30 min) was initiated at 70% 1RM, progressively increasing number of repetitions (8–12 repetitions/3–4 series). Combined training was applied to maximize the effects of exercise in the elderly as described earlier (Sousa et al., 2014).

Young volunteers completed 90 min outdoor run at 75–80% HRmax (Polar RS300X, Finland), at average pace 6:08 min·km^{-1}. Details of the experimental protocol are described elsewhere (Schon et al., 2019). Blood was collected before, immediately after and 1 h after run. We have included population of young trained individuals (i) to show the age-related variability in the absolute levels of serum and plasma BDNF and (ii) to explore the age-related differences/similarities of the changes in circulating BDNF induced by acute exercise, and its relationship to the physical fitness.

2.5. Body composition and anthropometry

Body weight, composition (total/visceral fat, lean body mass - LBM) were determined with quadrupedal bioimpedance (BF-511, Omron). BMI was calculated from body weight and height and expressed in kg/m^2. Waist circumference was measured in the middle between the lower rib cage and the iliac crest.

2.6. Cognitive and motor testing

Addenbrooke's Cognitive Examination-Revised test (ACE-R) was used before and after training to target various cognitive domains (orientation, attention, memory, verbal fluency, language, visuospatial abilities). Maximum score of ACE-R was 100 points. Preferred (average of the 3 tests) and maximal (the best of the 3 tests) walking speed were assessed three times in a row on a 10 m track. These data were used in the association analyses with circulating BDNF.

2.7. Skeletal muscle biopsy

Biopsy of *m. vastus lateralis* was performed by Bergström technique under local anaesthesia in the fasted state at 08:30–10:00 h. Biopsy samples were taken at baseline and after 3-month exercise training (Kurdiova et al., 2014). Muscle samples were cut into smaller pieces, quickly cleaned from excessive blood, fat and connective tissue and immediately frozen (stored) in liquid nitrogen. For fiber-typing, muscle sample was shock-frozen in 3-methylbuthane cooled by liquid nitrogen, embedded in TissueTek O.C.T and stored at −80 °C. Myofibrillar ATPase activity was measured to determine muscle fiber-type (Krumpolec et al., 2017). It is important to note that this traditional fibertyping technique could not differentiate well between the fast (IIA, IIAB, IIB) fiber subtypes (Rivero et al., 1996), but it was sufficient for the purpose of this study.

2.8. Whole-body and muscle metabolism assessment

Oral glucose tolerance test was performed in the morning after an overnight fast both before and after exercise training intervention. Blood samples were collected from indwelling vein catheters (Surflo-W, Therumo) at baseline (0 min) and 30, 60, 90, 120 min after drinking of 75 g glucose solution. Samples were used to assess glucose, insulin, TAG and total/HDL-cholesterol levels in a certified laboratory (Alpha medical, Slovakia). Resting energy expenditure and metabolic substrate preference (respiratory quotient, RQ) were evaluated by indirect calorimetry in the fasted state (Ergostik, Geratherm Respiratory). Subset of participants underwent localized dynamic ^{31}P-MRS of *m. gastrocnemius medialis* (7 T MR system, Siemens Healthcare). Dynamics of the exercise-related changes in muscle concentrations of phosphorous metabolites were assessed during 6 min plantar flexion exercise at 30% of maximal voluntary contraction force (Trispect, Ergospect, Germany) and during the 6 min period of post-exercise recovery (Krumpolec et al., 2017; Valkovič et al., 2014). ^{31}P-MRS offers a rough estimate of the oxidative ATP synthesis rate. It is important to note that tau (τ, time course) of PCr recovery is highly dependent on the pH at the end of exercise (van den Broek et al., 2007), and that the calculation of Qmax does not account for the resting rate of ATP turnover (Boska, 1994; Kemp et al., 1993) as well as the facts that reduced oxygen availability during the exercise recovery period might influence the estimate of oxidative ATP synthesis rate (Korzeniewski and Zoladz, 2013).

2.9. Quantification of serum & plasma BDNF

Blood for BDNF and basic biochemical (TAG, glucose) analyses was collected during the acute exercise test day at baseline (pre-exercise), immediately post-exercise and 1 h after acute aerobic exercise test. Serum samples were incubated at RT for 30 min, centrifuged (1200 ×*g*/20 min/4 °C), while EDTA-plasma was immediately spun (1200 ×*g*/10 min/4 °C) and both were stored at −80 °C. Circulating BDNF was measured by ELISA (CYT306, EMD Millipore) using Synergy-H4-Hybrid (BioTek). Plasma samples were thawed on ice and centrifuged (10,000 ×*g*,/2.5 min/4 °C) prior to the assay (Cho et al., 2012). Intra-assay variability was 13.5% for serum and 11% for plasma. Inter-assay variability was 6.3%.

2.10. RNA isolation & quantification

Total skeletal muscle RNA was extracted from 20 to 30 mg of frozen tissue with phenol-chloroform method, using Qiazol (Qiagen, USA). Samples were DNAse treated (BioLabs, USA) and quantified with NanoDrop 2000c (Thermo Scientific, USA). RNA (500 ng) was reverse-transcribed to cDNA with High Capacity RNA-to-cDNA kit (Applied Biosystems, USA). Quantitative RT-PCR was performed using Fast SYBR Green (Thermo Scientific, USA) at ABI7900HT Fast Real-Time PCR System (Applied Biosystems, USA). Primers for BDNF (FWD:5′-GTGCC GAACTACCCAGTC-3′, REV:5′-TCGCCAGCCAATTCTCTT-3′), and housekeeping genes RPL13A (FWD:5′-GGACCGTGCGAGGTATGCT-3′, REV:5′-ATGCCGTCAAACACCTTGAGA-3′) and β2-microglobulin (FWD: 5′-CGCTCCGTGGCCTTAGC-3′, REV:5′-AATCTTTGGAGTACGCTGGAT AGC-3′) were used and ΔCt expression values were calculated.

2.11. Western blot

Muscle (20–30 mg) was homogenized in ice-cold RIPA buffer (200 µl) containing protease and phosphatase inhibitors (Roche, Switzerland; Sigma, USA). Lysis was performed by inversion mixing 2 h/4 °C with PTR-25 (Grant-bio, UK). Lysate was centrifuged (20 min/16000 ×*g*/4 °C), protein concentration was determined by BCA (Pierce, USA) and 30 µg of protein was loaded on 10% SDS-PAGE. Proteins were transferred to PVDF membrane (IPFL00010, Millipore, Germany). Anti-BDNF rabbit polyclonal antibody (sc:546, SantaCruz, USA) and anti-β-tubulin rabbit antibody (926-42211, LI-COR, USA) were used at 1:500 and 1:1000 dilution, respectively. Infrared-fluorescent IRDye® labeled secondary antibody (1:15000; 926-32211, LI-COR, USA) was used for visualization in Odyssey IR-imaging system (LI-COR, USA).

Table 1
Study population characteristics before & after 3-month aerobic-strength training.

Parameters	Baseline			After training		
	Study population (n = 22)	Women (*n* = 13)	Men (*n* = 9)	Study population (n = 22)	Women (n = 13)	Men (n = 9)
	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)	mean (SD)
Age [years]	69 (8)	70 (8)	67 (8)	69 (8)	70 (8)	67 (8)
BMI [kg/m^2]	27.1 (3.2)	27.0 (3.1)	27.3 (3.5)	26.9 (2.9)	26.9 (3.0)	26.8 (3.1)#
Waist circumference [cm]	96.3 (9.4)	95.6 (9.1)	97.3 (10.2)	93.5 (7.7)#	92.2 (6.9)	95.4 (8.8)
Body fat [%]	33.1 (10.4)	40.0 (5.6)	23.1 (7.1)***	31.2 (9.9)#	37.9 (4.4)#	21.6 (7.0)***#
Visceral adipose tissue [rel.u.]	10.7 (3.6)	9.8 (2.5)	12.0 (4.6)	10.4 (3.2)	9.5 (2.2)	11.6 (4.1)
Lean body mass [%]	28.6 (5.4)	25.0 (2.9)	33.7 (3.4)***	29.8 (4.9)#	26.4 (2.1)#	34.6 (3.6)***
ACE-R [score]	87 (8)	86 (9)	89 (8)	90 (7)#	90 (7)#	91 (7)
VO_2max [ml/min/kg]	30.5 (8.1)	26.5 (6.9)	36.9 (5.5)**	34.9 (9.5)#	29.9 (7.4)#	41.0 (8.7)**
Knee extension [N/m]	293.5 (123.3)	233.9 (122.0)	379.6 (59.1)**	325.0 (126.2)#	272.5 (121.7)#	397.7 (104.3)*

BMI - Body Mass Index; ACE-R - Addenbrooke's Cognitive Examination [revised version, max. Score 100]; Visceral adipose tissue - relative units [rel.u.] with total score of 30 (< 9: normal, 10–14: high, 15–30: very high fat content) - determined by bioimpedance; VO_2max - maximal aerobic capacity determined by bicycle spiroergometry;** $p < .01$, *** $p < .001$ compared to women; # effect of training /$p < .05$ vs. baseline, data are presented as mean (SD).

2.12. Statistical analysis

Changes of circulating BDNF were analyzed by two-way ANOVA with repeated measures (RM) with time-points of acute exercise (pre/post/1 h rest) as factor #1 and 3-month exercise training as factor #2. Differences in BDNF in young individuals were analyzed by one-way ANOVA with repeated measures. Multiple comparison analysis was performed with Tukey's HSD (SigmaStat 3.5, Systat, USA). Pearson's correlation was used (JMP 4.0.2, SAS, USA). Data are presented as mean ± SD, $p < .05$ was considered significant.

3. Results

3.1. Clinical characteristics of the study population

Three-month combined aerobic-resistance intervention to slightly overweight sedentary population of individuals in their late sixties and early seventies reduced abdominal adiposity and percentage of body fat independent on gender (Table 1). Exercise training increased lean body mass, VO_2max, maximal voluntary contraction force of knee extension and Adenbrooke's Cognitive Examination (ACE-R) score in the entire population and these effects were more pronounced in females (Table 1). In this manuscript, we used clinical characteristics exclusively to explore the associations with systemic (serum & plasma) and muscle BDNF.

3.2. Effects of acute and regular exercise on serum and plasma BDNF

An acute bout of aerobic exercise induced a moderate transient increase of serum BDNF in sedentary elderly individuals, which was followed by a decline to baseline after 1 h of exercise recovery (pre: 19.2 ± 5.1 ng/ml, post: 22.2 ± 6.5 ng/ml, 1 h rest: 17.2 ± 5.4 ng/ml) (Fig. 1A). Combined training blunted the acute exercise-related regulation of serum BDNF in the elderly (pre:20.4 ± 5.6 ng/ml, post: 22.1 ± 5.5 ng/ml, 1 h rest: 20.2 ± 6.1 ng/ml) (Fig. 1A) and an acute effect of aerobic exercise on serum BDNF was neither found in young trained individuals (Fig. 4). Three-month of aerobic-strength training did not induce any changes in resting serum BDNF or in the peak post-exercise serum BDNF levels (Fig. 1A). Two-way ANOVA with RM showed highly significant effect of an acute exercise bout on serum BDNF ($F_{(2,42)} = 14.309$, $p < .001$), while training ($F_{(1,21)} = 2.019$, $p = .17$) and training × acute exercise interaction ($F_{(2,42)} = 2.393$, $p = .104$) remained non-significant.

No significant regulation of plasma BDNF was detected in sedentary elderly in response to acute exercise bout (Fig. 1B; pre:6.6 ± 3.4 ng/ml, post:6.9 ± 3.3 ng/ml, 1 h rest:6.2 ± 3.6 ng/ml). However, a

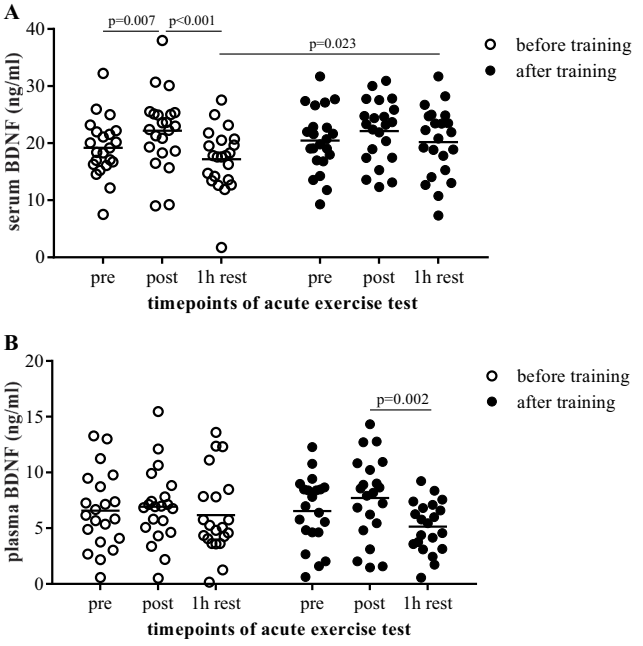

Fig. 1. The effect of acute aerobic exercise and 3-month combined aerobic-resistance exercise training on serum (**A**) and plasma (**B**) BDNF in elderly individuals (n = 22, 9 M/13F). For BDNF analyses in plasma, one male individual was excluded. Statistics: two-way RM ANOVA with Tukey post-hoc test.

significant effect of acute exercise was detected in trained elderly ($F_{(2,40)} = 5.666$, $p = .007$). Specifically, one-hour post-exercise recovery was paralleled by 34% decline of plasma BDNF in trained elderly (Fig. 1B, pre:6.5 ± 3.1 ng/ml, post:7.7 ± 3.7 ng/ml, 1 h rest: 5.1 ± 2.3 ng/ml) as well as in trained young individuals (Fig. 4). Three-month combined training did not regulate resting plasma BDNF in the sedentary elderly population (Fig. 1B). Training and training × acute exercise factor interaction remained non-significant ($F_{(1,20)} = 0.020$, $p = .888$; $F_{(2,40)} = 1.368$, $p = .266$, respectively).

Training-related regulation of resting serum BDNF was negatively correlated with training-induced changes in body fat and fasting serum triglycerides ($p = .012$) and positively with changes of muscle mass (Fig. 2 A-C). Borderline correlation was observed between changes in BDNF and visuospatial abilities (ACE-R, $p = .056$) (Fig. 2D). Training-related changes of serum BDNF measured at baseline and immediately post-exercise were positively correlated with preferred walking speed ($p = .005$ and $p = .002$, Fig. 2E, F). In addition, greater training-

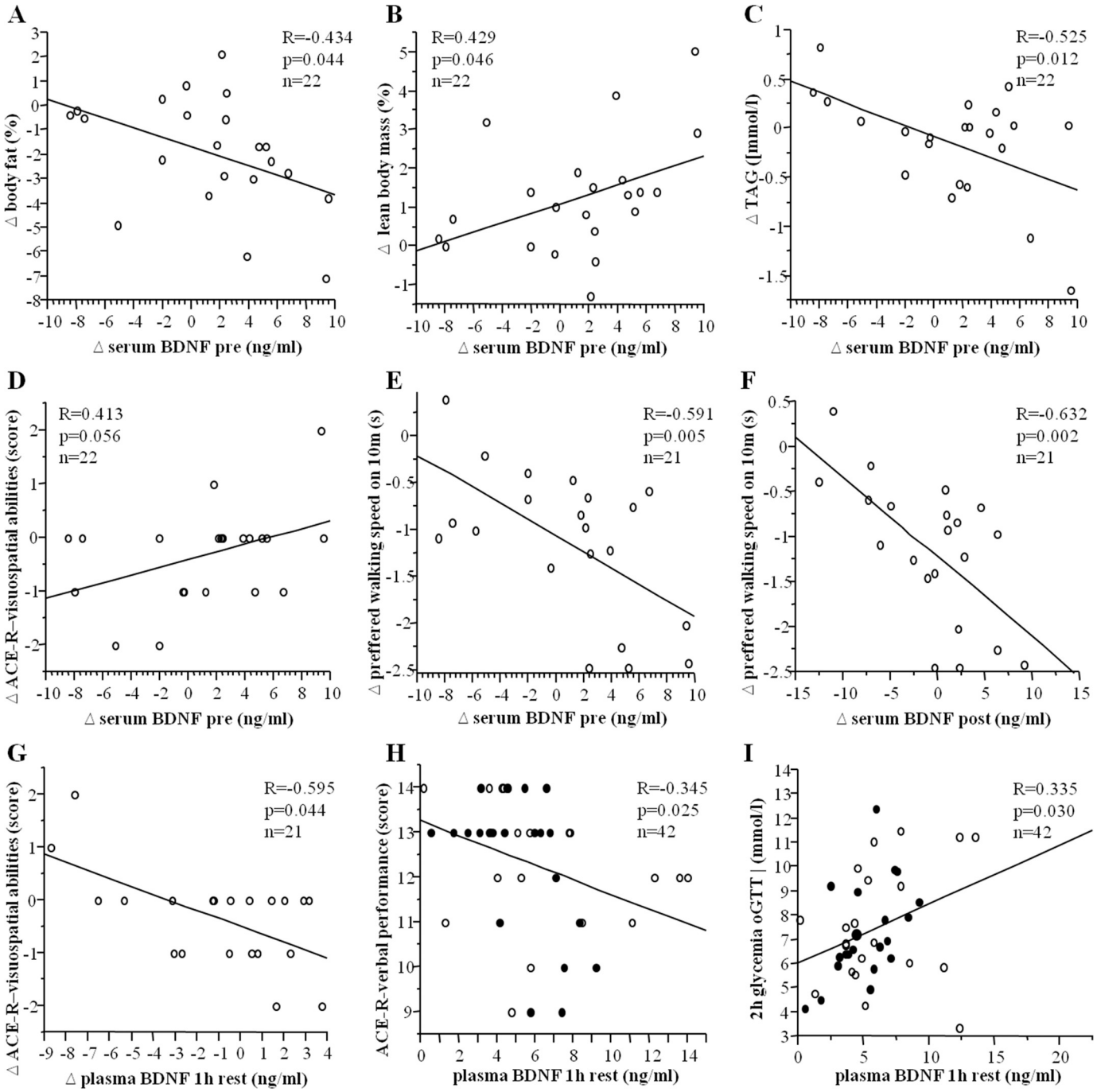

Fig. 2. Exercise training-induced regulation of serum (**A-F**) and plasma (**G-I**) BDNF correlates with improvements of metabolic functional and cognitive parameters in the elderly. Figures H and I show absolute values of plasma BDNF (○ - before training, ● - after training). Effect of training - delta (Δ) is calculated as [post-training values minus pre-training values]. Δ serum BDNF pre: training-induced change of resting serum BDNF; Δ serum BDNF post: training-induced change of serum BDNF measured immediately after the exercise bout; Δ plasma BDNF 1 h rest: training-induced change of plasma BDNF measured 1 h after the exercise bout. ACE-R - Addenbrooke's cognitive examination-revised test, TAG - fasting triglyceridemia.

induced decrease of plasma BDNF detected 1 h post-exercise inversely correlated with training-associated improvement of visuospatial abilities (p = .004, Fig. 2G). Moreover, lower plasma concentration of BDNF detected 1 h post-exercise was associated with better verbal performance in ACE-R (p = .025, Fig. 2H), as well as with better glucose tolerance (2 h glycemia oGTT, p = .030, Fig. 2I).

3.3. Exercise-induced changes in muscle BDNF protein parallel structural, functional and metabolic adaptive response in skeletal muscle

Our data suggest that the exercise-related changes of muscle BDNF protein might contribute to the adaptive changes of skeletal muscle to regular exercise as it positively correlated with the ratio of fast-to-slow muscle fibers, as well as with the rate of post-exercise muscle ATP re-synthesis (Qmax) (Fig. 3D,E). Next, we compared individuals who increased muscle BDNF protein content in response to exercise training (responders, n = 7) with those who did not (non-responders, n = 5;

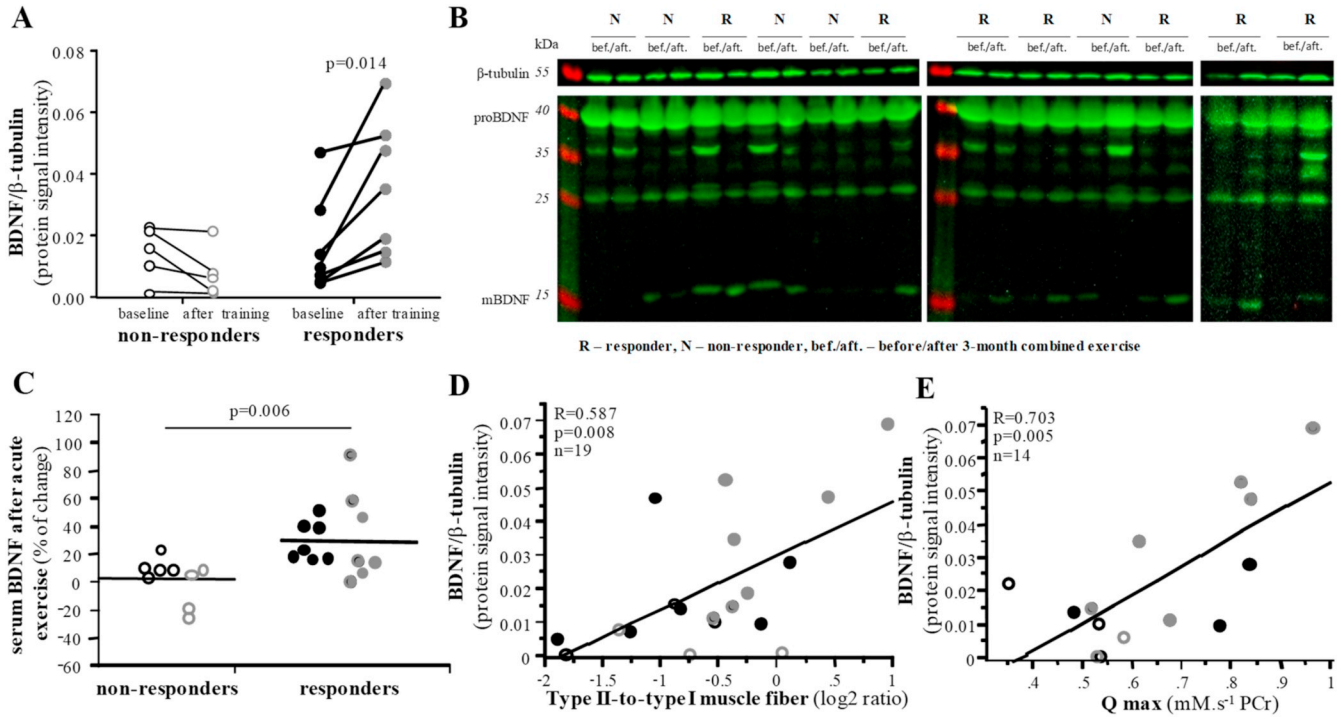

Fig. 3. The effect of 3-month combined aerobic/strength training on BDNF protein content in human skeletal muscle (*m. vastus lateralis*). Variability in muscle BDNF protein response to exercise training in elderly was used to discriminate the two distinct groups of patients - responders (n = 7, 3 M/4F, black circles) and non-responders (n = 5, 1 M/4F, open circles) **(A)**. Western blot analysis of mature and proBDNF with β-tubulin as a housekeeping protein (N/R non-responder/responder, **(B)**). Responders differed from non-responders in the capacity of aerobic exercise to acutely increase serum BDNF **(C)**. Muscle BDNF protein content correlated with the ratio of fast-to-slow muscle fibers **(D)** and with the maximum oxidative flux (Qmax, ^{31}P-MRS) of *m. gastrocnemius medialis* **(E)**. Gray color represents post-training values. Statistics: *t*-test (A:paired, C:non-paired), bivariate correlation analysis.

Fig. 3A,B) and observed that responders were characterized by stronger acute increase of serum BDNF immediately after the exercise bout performed in sedentary elderly just prior entering the exercise-training program (Fig. 3C).

3.4. Plasma BDNF levels decrease in response to acute exercise in young trained individuals

We have used this small population of young trained individuals to validate acute responses of serum and plasma BDNF to an intensive aerobic exercise. Detailed characteristics of the study population could be found elsewhere (Schon et al., 2019). Resting serum but not plasma BDNF appeared to be higher in young physically active individuals than in the elderly (Figs. 1 & 4). Moreover, similar to exercise-trained elderly, serum BDNF was not regulated by acute aerobic exercise (pre: 37.66 ± 9.93, post: 38.29 ± 12.17, 1 h rest: 37.36 ± 5.47; Fig. 4) and plasma BDNF was decreased after 1 h exercise-recovery in young trained individuals (pre: 6.29 ± 3.58, post: 5.87 ± 2.89, 1 h rest: 3.04 ± 1.75, Fig. 4), confirmed both by one-way repeated measures ANOVA ($F_{(2,12)} = 3.112$, $p = .082$) and a paired t-test ($p = .034$).

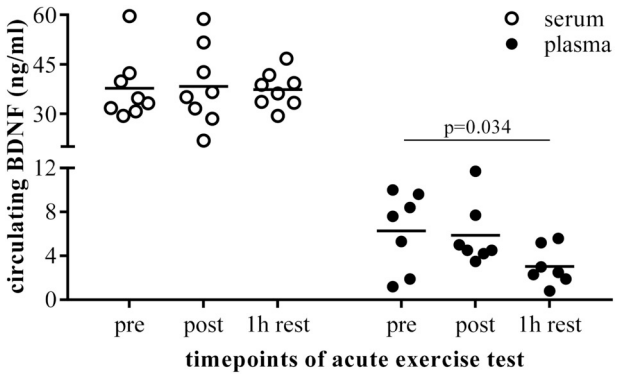

Fig. 4. Effect of an acute bout of aerobic exercise (90 min run) on serum and plasma BDNF in eight young healthy physically active individuals (3 M/5F). One volunteer was excluded from the analysis of plasma BDNF due to non-detectable values 1 h post-exercise. Statistics: one-way RM ANOVA, paired t-test.

4. Discussion

Brain derived neurotrophic factor (BDNF) is one of the crucial mediators of exercise-induced adaptive response in both the brain and periphery. We have explored the complexity of the exercise-induced responses related to BDNF in serum, plasma and skeletal muscle and linked the observed changes in BDNF with the parallel exercise-related changes in metabolic, cognitive and muscle functional state in the elderly. We have reproduced the response to acute aerobic exercise in young healthy population and explored predictive value of the serum BDNF response in sedentary seniors for the adaptive response of muscle structural and metabolic characteristics to regular exercise.

4.1. Why should we measure both serum and plasma BDNF

Assessment of BDNF in both plasma and serum can shed some light on the regulation of distinct circulating pools of BDNF which have distinct biological half-life and availability. Moreover, there is no consensus whether serum or plasma BDNF is more suitable biomarker of age-related cognitive and/or metabolic decline. Recent meta-analyses indicated that effects of exercise training on plasma and serum BDNF are not statistically different (Dinoff et al., 2016; Szuhany et al., 2015), however this might not always be the case. We know that brain is the primary (70–80%) but not the sole source of circulating BDNF at rest as well as during exercise (Rasmussen et al., 2009b). We have to consider the fact that BDNF protein released into systemic circulation could be

taken up and stored by platelets (Fujimura et al., 2002; Yamamoto and Gurney, 1990). Serum BDNF level therefore reflects the entire circulating blood pool of BDNF including that released from the activated platelets and could - albeit with limitations - mirror both the long-term and acute changes in BDNF production and release. Plasma BDNF rather represents the biologically available pool of BDNF, which is not safeguarded by platelets. It has to be noted that large inter and intra-individual variability of plasma BDNF in humans exists and it could be caused by the dynamics of BDNF release, uptake and degradation. Previous reports indicated a broad range of 10 to 200-times lower concentrations of BDNF in plasma than in serum. Level of disparity between the plasma and serum BDNF is also evidenced by the fact that the overall correlation coefficient between the two circulating BDNF pools ranges between 0.2 and 0.7 (Karege et al., 2005; Serra-Millas, 2016). In our study, plasma concentration of BDNF was in average 3-times lower than that found in serum, and plasma BDNF and serum levels correlated at neither resting/fasting state, nor when taken immediately and/or 1 h post-exercise. The differences and possible distinct biological significance of serum and plasma BDNF remain to be elucidated. Interestingly, elderly participants in our study had lower serum BDNF concentrations than healthy young individuals, but at the same time their plasma BDNF levels were comparable. This could be at least in part related to aging-associated platelet counts decrease (Balduini and Noris, 2014; Segal and Moliterno, 2006). Platenik et al. showed that the reduced plasma BDNF levels in patients with Alzheimer's disease were due to a reduced platelet number and not due to reduced platelet BDNF content (Pláteník et al., 2014).

4.2. Effects of an acute bout of aerobic exercise on circulating BDNF in the elderly

We have observed that acute aerobic exercise elicited a transient increase of serum BDNF in sedentary elderly individuals. However, this effect was blunted by 3-month aerobic-resistance training. Results of studies examining effects of acute aerobic exercise on serum BDNF are relatively consistent. Acute exercise-induced elevations of serum BDNF were extensively reported in metabolically healthy populations (Cho et al., 2012; Ferris et al., 2007; Goekint et al., 2008; Griffin et al., 2011; Heyman et al., 2012; Nofuji et al., 2012; Rojas Vega et al., 2006, 2012; Schmolesky et al., 2013). Both Gold and Castellano investigated effects of acute exercise (bicycling) in middle-aged to elderly patients with multiple sclerosis and observed either an acute exercise-induced increase followed by a decrease to baseline levels at 30 min post-exercise (Gold et al., 2003), or no alterations of serum BDNF, but a significant decrease 2 h post-exercise (Castellano and White, 2008).

In our work, acute aerobic exercise failed to increase plasma BDNF in sedentary and trained elderly (40 min bicycling) and no increase was found in young healthy trained participants after 90 min submaximal running exercise. There are reports indicating no effects of acute exercise (aerobic or resistance) on plasma BDNF in healthy young to middle-aged adults (Correia et al., 2010; Nofuji et al., 2012; Seifert et al., 2010). Rasmussen showed that internal jugular venous plasma BDNF increases only after long-term intense exercise - 4 h ergometer rowing at workload 10–15% below the lactate threshold (Rasmussen et al., 2009). Zoladz and colleagues described an acute increase of plasma BDNF in thirteen young healthy physically active men who participated in 5-week moderate intensity endurance training. This, however, was not present prior to training. It is important to note that increase of plasma BDNF in this study was determined as a difference between pre-exercise plasma BDNF level and that measured at the peak of maximal incremental exercise test (30 W per 3 min increments until exhaustion) (Zoladz et al., 2008). In our study, we observed a steep decline of plasma BDNF 1 h post-exercise in both physically trained elderly and in young healthy individuals. This suggests that exercise training with the capacity to increase aerobic fitness induces adaptive mechanisms favoring post-exercise down-regulation of BDNF in plasma. It is plausible to speculate that increased BDNF tissue uptake following an acute exercise bout can contribute to the integration of the systemic adaptive response to exercise.

4.3. Effects of 3-month aerobic-resistance training on circulating BDNF in the elderly

Here we observed that resting serum BDNF was not regulated by 3-month exercise training in the elderly. Several other exercise intervention studies with elderly participants showed no alterations of resting serum BDNF in response to 3-month resistance or aerobic training (Forti et al., 2014; Maass et al., 2016), some even observed that 1-year aerobic training intervention was not effective in modulating circulating BDNF (Erickson et al., 2011). However, others reported that 3–6 months of combined aerobic-resistance exercise intervention elicited an increase of resting serum BDNF in healthy middle-aged and elderly women (Cho et al., 2014; Vedovelli et al., 2017). This could be due to the differences in metabolic state or to the nature, intensity and duration of the training intervention.

Moreover, we observed that 3-month combined exercise training had no effect on venous plasma BDNF in the elderly. Similarly, unaltered venous plasma levels were reported after 8-week aerobic training in middle-aged patients with multiple sclerosis (Schulz et al., 2004). Study examining the effects of 16-week multimodal exercise training program in older women reported an increase in plasma BDNF (Vaughan et al., 2014), similar to 10-week progressive dynamic resistance-training program of knee extensors and flexors performed three times per week in pre-frail elderly women population (Coelho et al., 2012), and increase in plasma BDNF was also found after 5-weeks of moderate intensity endurance training in young healthy and physically active men (Zoladz et al., 2008). It is plausible to think that this discrepancy reflects the fact that blood taken from the antecubital vein does not reflect the changes of brain BDNF production and release, and largely depends on the activation state of platelets (BDNF reservoir), plasma BDNF levels thus being highly variable (Seifert et al., 2010). Seifert and colleagues clearly showed that although resting arterial plasma BDNF was not affected by 3-month aerobic training in healthy males, jugular venous plasma BDNF, representing the overall brain output, was substantially increased (Seifert et al., 2010).

4.4. Skeletal muscle BDNF expression and adaptive response of muscle to regular exercise

We observed that individuals who responded to 3-month exercise training with an increase of muscle BDNF protein content had also higher peak serum BDNF levels detected immediately post-exercise and lower score in cognitive test (ACE-R, unpublished observation). To date, there is no evidence of muscle BDNF contribution to circulating pool (Matthews et al., 2009). However, positive associations between muscle BDNF protein and fast-to-slow muscle fiber ratio and/or muscle mitochondrial oxidative flux (rate of post-exercise ATP re-synthesis) assessed by ^{31}P-MRS indicate that muscle BDNF protein might be related to the exercise-induced changes in muscle histomorphological and metabolic characteristics. The animal studies clearly support our observations by showing that BDNF mimetic compound enhances skeletal muscle mitochondrial biogenesis by affecting AMPK-CREB-PGC1α axis (Wood et al., 2018). BDNF increased fatty acid oxidation in C2C12 and L6 myotubes and in rat skeletal muscle via regulating the energy sensor, AMP kinase (Matthews et al., 2009). Despite the fact that the evidence on the effects of chronic exercise on muscle BDNF is largely limited to animal models (Cuppini et al., 2007; Kim et al., 2015; Lee et al., 2017), it clearly indicates that energy metabolism fueling the mitochondrial fatty acid oxidation is linked with the actions of BDNF.

The limited number of human studies focus on the effect of acute exercise. Matthews and Walsh have previously reported contradictory results on the effects of acute exercise on muscle BDNF (Matthews et al.,

2009; Walsh et al., 2015). Matthews demonstrated an increase of muscle BDNF mRNA, as well as delayed increase of BDNF protein after acute bicycling (120 min at 60% VO_2max) in young physically active volunteers. On the other hand, Walsh et al. observed no change of muscle BDNF mRNA in healthy young men following high intensity interval bicycling (Walsh et al., 2015). Our results clearly show that training-induced changes in muscle BDNF protein in the elderly are associated with adaptive changes in muscle structural and functional phenotypes, supporting the role of BDNF in integrating exercise-induced response in skeletal muscle.

4.5. Acute response of serum BDNF is associated with changes in adiposity, cognition and metabolism

Our results show that the acute exercise-related changes of serum BDNF in the elderly correlate negatively with training-induced changes in body fat and fasting triglycerides, and positively with training-induced changes in muscle mass, preferred walking speed, oxidative fiber size and visuospatial cognitive abilities. Available evidence suggests that BDNF has a broad spectrum of functions in exercise-induced plasticity both in the periphery and the brain (Marosi and Mattson, 2014). It can influence cognition, mood, as well as the whole body and skeletal muscle metabolism. BDNF has the capacity to increase insulin sensitivity and mitochondrial biogenesis, thus enhancing glucose and fatty acid metabolism, and protecting us against the development of chronic metabolic and neurodegenerative diseases (Marosi and Mattson, 2014). Our findings support the role of BDNF in orchestrating the whole-body and muscle-specific adaptive response to exercise training in humans, where the available evidence is very scarce.

4.6. Limitations and future directions

The size of the study population limits the statistical power of our analyses. Another limitation is associated with the fact that major part of circulating BDNF is stored in platelets (Fujimura et al., 2002). One could speculate that the exercise-induced increase in serum BDNF is caused at least in part by an increase in platelet count/reactivity or by changes in the blood volume (Pareja-galeano et al., 2015; Winter et al., 2007; Yarrow et al., 2010). We acknowledge that presented data on circulating BDNF were not adjusted for the possible confounding factors such as platelet count, hematocrit or PF4 (marker of platelet reactivity) (Baker et al., 2010), but this will be considered in our future work.

5. Conclusion

Presented work showed that measuring BDNF in serum and plasma is important to evaluate its complex regulation by acute and regular exercise. Our observations indicate that transient, dynamic regulation of circulating BDNF with each bout of exercise likely contributes to the integrated adaptive response to regular exercise training despite the fact that resting circulating BDNF levels remain unchanged. We propose that the transient increase of serum BDNF in sedentary elderly and a subsequent decrease in plasma BDNF observed 1 h post-exercise in trained elderly and young individuals represent specific triggers, physiological role of which would need to be evaluated. Acute circulating BDNF regulation by exercise was dependent on the level of physical fitness and correlated with training-induced improvements in metabolic and cognitive functions. Our observations provide an indirect evidence that distinct exercise-induced changes in serum and plasma BDNF as well as training-related increase in muscle BDNF protein, paralleled by improvements in muscle and whole-body clinical phenotypes, are involved in the coordinated adaptive response to exercise in humans.

Authors contributions

Project conception (BU, JU), design (BU, JU, TC-L) & coordination of the studies (BU, JU); clinical examinations & tissue sampling (DM, JU, BU, LS, MS, SS, VT, PK, PT); exercise intervention design & implementation (JC, MV, MiS); MR measurements (LV, MaK); histological examinations (ZK, JU); muscle molecular analyses and ELISA (DM); manuscript writing (DM, JU, BU) and critical reading (TC-L).

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Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Baecke, J., Burema, J., Frijters, J., 1982. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am. J. Clin. Nutr. 36, 936–942.
Baker, L.D., Frank, L.L., Foster-schubert, K., Pattie, S., Wilkinson, C.W., Mctiernan, A., Plymate, S.R., Mehta, P.D., Craft, S., 2010. Effects of aerobic exercise on mild cognitive impairment. Arch. Neurol. 67, 71–79. https://doi.org/10.1001/archneurol.2009.307.Effects.
Balduini, C.L., Noris, P., 2014. Platelet Count and Ageing. 120. pp. 8–10. https://doi.org/10.3324/haematol.2014.106260.
Barbieri, E., Agostini, D., Polidori, E., Potenza, L., Guescini, M., Lucertini, F., Annibalini, G., Stocchi, L., De Santi, M., Stocchi, V., 2015. The pleiotropic effect of physical exercise on mitochondrial dynamics in aging skeletal muscle. Oxidative Med. Cell. Longev. 2015, 917085. https://doi.org/10.1155/2015/917085.
Boska, M., 1994. ATP production rates as a function of force level in the human gastrocnemius/soleus using 31P MRS. Magn. Reson. Med. 32, 1–10.
Cao, L., Liu, X., Lin, E.D., Wang, C., Choi, E.Y., Riban, V., Lin, B., During, M.J., 2010. Environmental and genetic activation of a brain-adipocyte BDNF/ leptin axis causes cancer remission and inhibition. Cell 142, 52–64. https://doi.org/10.1016/j.cell.2010.05.029.Environmental.
Castellano, V., White, L.J., 2008. Serum brain-derived neurotrophic factor response to aerobic exercise in multiple sclerosis. J. Neurol. Sci. 269, 85–91. https://doi.org/10.1016/j.jns.2007.12.030.
Cho, H., Kim, J., Kim, S., Son, Y.H., Lee, N., Jung, S.H., 2012. The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO 2max performance in healthy college men. Neurosci. Lett. 519, 78–83. https://doi.org/10.1016/j.neulet.2012.05.025.
Cho, H.C., Kim, J.K., Lee, N.J., Kim, S.Y., Yoon, N.K., 2014. Effects of combined exercise on cardiovascular risk factors and serum BDNF level in mid-aged women. J. Exerc. Nutr. Biochem. 18, 61–67.
Coelho, F.M., Pereira, D.S., Lustosa, L.P., Silva, J.P., Dias, J.M.D., Dias, R.C.D., Queiroz, B.Z., Teixeira, A.L., Teixeira, M.M., Pereira, L.S.M., 2012. Physical therapy intervention (PTI) increases plasma brain-derived neurotrophic factor (BDNF) levels in non-frail and pre-frail elderly women. Arch. Gerontol. Geriatr. 54, 415–420. https://doi.org/10.1016/j.archger.2011.05.014.
Colombo, E., Bedogni, F., Lorenzetti, I., Landsberger, N., Previtali, S.C., Farina, C., 2013. Autocrine and immune cell-derived BDNF in human skeletal muscle: implications for myogenesis and tissue regeneration. J. Pathol. 231, 190–198. https://doi.org/10.1002/path.4228.
Correia, P.R., Pansani, I.A., Machado, I.I.F., Andrade, I.M., Carlos, I.A., Fulvio, I., Ricardo, I.I., Arida, M., Scorza, A., Abra, I.I.E., 2010. Acute strength exercise and the involvement of small or large muscle mass on plasma brain-derived neurotrophic factor levels. Clinics 65, 1123–1126. https://doi.org/10.1590/S1807-59322010001100012.
Cotman, C.W., Berchtold, N.C., 2002. Exercise : a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25, 295–301.
Cuppini, R., Sartini, S., Agostini, D., Guescini, M., Ambrogini, P., Betti, M., Bertini, L., Vallasciani, M., Stocchi, V., 2007. BDNF expression in rat skeletal muscle after acute or repeated exercise. Arch. Ital. Biol. 145 (2), 99–110.
Delezie, J., Handschin, C., 2018. Endocrine crosstalk between skeletal muscle and the

brain. Front. Neurol. 9, 698. https://doi.org/10.3389/fneur.2018.00698.
Dinoff, A., Herrmann, N., Swardfager, W., Liu, C.S., Sherman, C., Chan, S., Lanctot, K.L., 2016. The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): a Meta-analysis. PLoS One 11, e0163037. https://doi.org/10.1371/journal.pone.0163037.
Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. PNAS 108, 3017–3022. https://doi.org/10.1073/pnas.1015950108.
Ferris, L., Williams, J., Shen, C., 2007. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. Med. Sci. Sports Exerc. 39, 728–734.
Forti, L.N., Njemini, R., Beyer, I., Eelbode, E., Meeusen, R., Mets, T., Bautmans, I., 2014. Strength training reduces circulating interleukin-6 but not brain-derived neurotrophic factor in community-dwelling elderly individuals. Age 36, 9704.
Fujimura, H., Altar, C.A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J., Sun, B., Tandon, N.N., 2002. Brain derived neurotrophic factor is stored in human platelets and release by agonist stimulation brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb. Haemost. 87, 728–734.
Goekint, M., Heyman, E., Roelands, B., Njemini, R., Bautmans, I., Mets, T., Meeusen, R., 2008. No influence of noradrenaline manipulation on acute exercise-induced increase of brain-derived neurotrophic factor. Med. Sci. Sports Exerc. 40, 1990–1996. https://doi.org/10.1249/MSS.0b013e31817eee85.
Gold, S.M., Schulz, K., Hartmann, S., Mladek, M., Lang, U.E., Hellweg, R., Braumann, K., Heesen, C., 2003. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. J. Neuroimmunol. 138, 99–105. https://doi.org/10.1016/S0165-5728(03)00121-8.
Griffin, É.W., Mullally, S., Foley, C., Warmington, S.A., O'Mara, S.M., Kelly, Á.M., 2011. Physiology & Behavior Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. Physiol. Behav. 104, 934–941. https://doi.org/10.1016/j.physbeh.2011.06.005.
Gunstad, J., Benitez, A., Smith, J., Glickman, E., Spitznagel, M.B., Alexander, T., Juvancic-Heltzel, J., Murray, L., 2008. Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. J. Geriatr. Psychiatry Neurol. 21, 166–170. https://doi.org/10.1177/0891988708316860.
Heyman, E., Gamelin, F.X., Goekint, M., Piscitelli, F., Roelands, B., Leclair, E., Di Marzo, V., Meeusen, R., 2012. Intense exercise increases circulating endocannabinoid and BDNF levels in humans — possible implications for reward and depression. Psychoneuroendocrinology 37, 844–851. https://doi.org/10.1016/j.psyneuen.2011.09.017.
Heyn, P., Abreu, B.C., Ottenbacher, K.J., 2004. The effects of exercise training on elderly persons with cognitive impairment and dementia: a meta-analysis. Arch. Phys. Med. Rehabil. 85, 1694–1704. https://doi.org/10.1016/j.apmr.2004.03.019.
Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J., Bertschy, G., 2005. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. Biol. Psychiatry 57, 1068–1072. https://doi.org/10.1016/j.biopsych.2005.01.008.
Kemp, G.J., Taylor, D.J., Thompson, C.H., Hands, L.J., Rajagopalan, B., Styles, P., Radda, G.K., 1993. Quantitative Analysis by 31P Magnetic Resonance Spectroscopy of Abnormal Mitochondrial Oxidation in Skeletal Muscle during Recovery from Exercise. 6. pp. 302–310.
Kermani, P., Rafii, D., Jin, D.K., Whitlock, P., Schaffer, W., Chiang, A., Vincent, L., Friedrich, M., Shido, K., Hackett, N.R., Crystal, R.G., Rafii, S., Hempstead, B.L., 2005. Neurotrophins promote revascularization by local recruitment of TrkB + endothelial cells and systemic mobilization of hematopoietic progenitors. J. Clin. Invest. 115, 653–663. https://doi.org/10.1172/JCI200522655.
Kim, H., So, B., Son, J.S., Song, H.S., Oh, S.L., Seong, J.K., Lee, H., Song, W., 2015. Resistance training inhibits the elevation of skeletal muscle derived-BDNF level concomitant with improvement of muscle strength in zucker diabetic rat. J. Exerc. Nutr. Biochem. 19, 281–288.
Kline, G., Porcari, J., Hintermeister, R., Freedson, P., Ward, A., McCaron, R., Ross, J., Rippe, J., 1987. Estimation of VO2max from a one-mile track walk, gender, age, and body weight. Med. Sci. Sports Exerc. 19, 253–259.
Koliatsos, V.E., Clatterbuck, R.E., Winslow, J.W., Cayouette, M.H., Prices, D.L., 1993. Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. Neuron 10, 359–367. https://doi.org/10.1016/0896-6273(93)90326-M.
Komulainen, P., Pedersen, M., Hänninen, T., Bruunsgaard, H., Lakka, T.a., Kivipelto, M., Hassinen, M., Rauramaa, T.H., Pedersen, B.K., Rauramaa, R., 2008. BDNF is a novel marker of cognitive function in ageing women: the DR's EXTRA study. Neurobiol. Learn. Mem. 90, 596–603. https://doi.org/10.1016/j.nlm.2008.07.014.
Korzeniewski, B., Zoladz, J.A., 2013. Slow VO2 off-kinetics in skeletal muscle is associated with fast PCr off-kinetics—and inversely. J. Appl. Physiol. 115, 605–612. https://doi.org/10.1152/japplphysiol.00469.2013.
Krumpolec, P., Vallova, S., Slobodova, L., Tirpakova, V., Vajda, M., Schon, M., Klepochova, R., Janakova, Z., Straka, I., Sutovsky, S., Turcani, P., Cvecka, J., Valkovic, L., Tsai, C., Krssak, M., Valkovic, P., Sedliak, M., Ukropcova, B., Ukropec, J., 2017. Aerobic-strength exercise improves metabolism and clinical state in Parkinson's disease patients. Front. Neurol. 8, 1–12. https://doi.org/10.3389/fneur.2017.00698.
Kurdiova, T., Balaz, M., Vician, M., Maderova, D., Vlcek, M., Valkovic, L., Srbecky, M., Imrich, R., Kyselovicova, O., Belan, V., Jelok, I., Wolfrum, C., Klimes, I., Krssak, M., Zemkova, E., Gasperikova, D., Ukropec, J., Ukropcova, B., 2014. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J. Physiol. 592, 1091–1107. https://doi.org/10.1113/jphysiol.2013.264655.
Lautenschlager, N., Cox, K., Flicker, L., 2008. Effect of physical activity on cognitive function in older adults at risk for alzheimer disease: a randomized trial. JAMA 300, 1027–1037.
Lee, H.W., Ahmad, M., Wang, H., Leenen, F.H.H., 2017. Effects of exercise training on brain-derived neurotrophic factor in skeletal muscle and heart of rats post myocardial infarction. Exp. Physiol. 102, 314–328. https://doi.org/10.1113/EP086049.
Maass, A., Düzel, S., Brigadski, T., Goerke, M., Becke, A., Sobieray, U., Neumann, K., Lövdén, M., Lindenberger, U., Bäckman, L., Braun-Dullaeus, R., Ahrens, D., Heinze, H.J., Müller, N.G., Lessmann, V., Sendtner, M., Düzel, E., 2016. Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. Neuroimage 131, 142–154. https://doi.org/10.1016/j.neuroimage.2015.10.084.
Marosi, K., Mattson, M., 2014. BDNF mediates adaptive brain and body responses to energetic challenges. Trends Endocrinol. Metab. 25, 89–98.
Matthews, V.B., Åström, M.B., Chan, M.H.S., Bruce, C.R., Krabbe, K.S., Prelovsek, O., Åkerström, T., Yfanti, C., Broholm, C., Mortensen, O.H., Penkowa, M., Hojman, P., Zankari, A., Watt, M.J., Bruunsgaard, H., Pedersen, B.K., Febbraio, M.A., 2009. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia 52, 1409–1418. https://doi.org/10.1007/s00125-009-1364-1.
Meek, T.H., Wisse, B.E., Thaler, J.P., Guyenet, S.J., Matsen, M.E., Fischer, J.D., Taborsky, G.J., Schwartz, M.W., Morton, G.J., 2013. BDNF action in the brain attenuates diabetic hyperglycemia via insulin-independent inhibition of hepatic glucose production. Diabetes 62, 1512–1518. https://doi.org/10.2337/db12-0837.
Ngandu, T., Lehtisalo, J., Solomon, A., Levälahti, E., Ahtiluoto, S., Antikainen, R., Bäckman, L., Hänninen, T., Jula, A., 2015. A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial. Lancet 385, 2255–2263. https://doi.org/10.1016/S0140-6736(15)60461-5.
Nofuji, Y., Suwa, M., Sasaki, H., Ichimiya, A., Nishichi, R., Kumagai, S., 2012. Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects. J. Sports Sci. Med. 11, 83–88.
Ono, M., Ichihara, J., Nonomura, T., Itakura, Y., Taiji, M., Nakayama, C., Noguchi, H., 1997. Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. Biochem. Biophys. Res. Commun. 238, 633–637. https://doi.org/10.1006/bbrc.1997.7220.
Pareja-galeano, H., Alis, R., Sanchis-gomar, F., Cabo, H., Cortell-ballester, J., Gomez-cabrera, M.C., Lucia, A., Viña, J., 2015. Methodological considerations to determine the effect of exercise on brain-derived neurotrophic factor levels. Clin. Biochem. 48, 162–166. https://doi.org/10.1016/j.clinbiochem.2014.11.013.
Pedersen, B.K., Pedersen, M., Krabbe, K.S., Bruunsgaard, H., Matthews, V.B., Febbraio, M.A., 2009. Role of exercise-induced brain-derived neurotrophic factor production in the regulation of energy homeostasis in mammals. Exp. Physiol. 94, 1153–1160. https://doi.org/10.1113/expphysiol.2009.048561.
Plášteník, J., Fišar, Z., Buchal, R., Jirák, R., Kitzlerová, E., Zvěřová, M., Raboch, J., 2014. GSK3β, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 50, 83–93. https://doi.org/10.1016/j.pnpbp.2013.12.001.
Rasmussen, P., Brassard, P., Adser, H., Pedersen, M.V., Leick, L., Hart, E., Secher, N.H., Pedersen, B.K., Pilegaard, H., 2009. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Exp. Physiol. 94, 1062–1069. https://doi.org/10.1113/expphysiol.2009.048512.
Rivero, J.L., Talmadge, R.J., Edgerton, V.R., 1996. Correlation Between Myofibrillar ATPase Activity and Myosin Heavy Chain Composition in Equine Skeletal Muscle and the Influence of Training. pp. 207.
Rojas Vega, S., Strüder, H.K., Wahrmann, B.V., Schmidt, A., Bloch, W., Hollmann, W., 2006. Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. Brain Res. 1121, 59–65. https://doi.org/10.1016/j.brainres.2006.08.105.
Rojas Vega, S., Hollmann, W., Wahrmann, B.V., Strüder, H.K., 2012. pH buffering does not influence BDNF responses to exercise. Int. J. Sports Med. 33, 8–12.
Rosano, A.C., Guralnik, J., Pahor, M., Glynn, N.W., Newman, B., Ibrahim, T., Erickson, K., Cohen, R., Elizabeth, C., Maccloud, R.L., Aizenstein, H.J., Guralnik, J., Pahor, M., Glynn, N.W., Newman, B., Ibrahim, T., Erickson, K., Cohen, R., Shaaban, C.E., Rebecca, L., 2017. Hippocampal response to a 24-month physical activity intervention in sedentary older adults. Am. J. Geriatr. Psychiatry 25, 209–217. https://doi.org/10.1016/j.jagp.2016.11.007.
Sakuma, K., Yamaguchi, A., 2011. The recent understanding of the Neurotrophin ' s role in skeletal muscle adaptation. J. Biomed. Biotechnol. 2011, 1–12. https://doi.org/10.1155/2011/201696.
Schmolesky, M., Webb, D., Hansen, R., 2013. The effects of aerobic exercise intensity and duration on levels of brain- derived neurotrophic factor in healthy men. J. Sports Sci. Med. 12, 502–511.
Schon, M., Janakova, Z., Kosutzka, Z., Nemec, M., Tomkova, M., Jackova, L., Maderova, D., Slobodova, L., Valkovic, P., Ukropec, J., Ukropcova, B., 2019. Effects of running on adiponectin, insulin and cytokines in cerebrospinal fluid in healthy young individuals. Sci. Rep. 9, 1959. https://doi.org/10.1038/s41598-018-38201-2.
Schulz, K., Gold, S.M., Witte, J., Bartsch, K., Lang, U.E., Hellweg, R., Braumann, K., Heesen, C., 2004. Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. J. Neurol. Sci. 225, 11–18. https://doi.org/10.1016/j.jns.2004.06.009.
Segal, J.B., Moliterno, A.R., 2006. Platelet Counts Differ by Sex, Ethnicity, and Age in the United States. https://doi.org/10.1016/j.annepidem.2005.06.052.
Seifert, T., Brassard, P., Wissenberg, M., Rasmussen, P., Nordby, P., Stallknecht, B., Adser, H., Jakobsen, A.H., Pilegaard, H., Nielsen, H.B., Secher, N.H., 2010. Endurance

training enhances BDNF release from the human brain. Am. J. Phys. Regul. Integr. Comp. Phys. 298, R372–R377. https://doi.org/10.1152/ajpregu.00525.2009.
Serra-Millas, M., 2016. Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? World J. Psychiatry 6, 84–101. https://doi.org/10.5498/wjp.v6.i1.84.
Smith, P.J., Blumenthal, J.A., Hoffman, B.M., Strauman, T.A., Welsh-bohmer, K., Jeffrey, N., Sherwood, A., 2011. Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. Psychosom. Med. 72, 239–252. https://doi.org/10.1097/PSY.0b013e3181d14633.Aerobic.
Sousa, N., Mendes, R., Abrantes, C., Sampaio, J., Oliveira, J., 2014. Effectiveness of combined exercise training to improve functional fitness in older adults : a randomized controlled trial. Geriatr Gerontol Int 14, 892–898. https://doi.org/10.1111/ggi.12188.
Suwa, M., Yamamoto, K., Nakano, H., Sasaki, H., Radak, Z., Kumagai, S., 2010. Brain-derived neurotrophic factor treatment increases the skeletal muscle glucose transporter 4 protein expression in mice. Physiol. Res. 59, 619–623.
Szuhany, K., Bugatti, M., Otto, M., 2015. A meta-analytic review of the effects of exercise on brain- derived neurotrophic factor. J. Psychiatr. Res. 60, 56–64. https://doi.org/10.1016/j.jpsychires.2014.10.003.A.
Tsai, C., Ukropec, J., Ukropcová, B., Pai, M., 2018. An acute bout of aerobic or strength exercise speci fi cally modi fi es circulating exerkine levels and neurocognitive functions in elderly individuals with mild cognitive impairment. Neuroimage Clin. 17, 272–284. https://doi.org/10.1016/j.nicl.2017.10.028.
Valkovič, L., Chmelík, M., Just, I., Jakubová, M., Christina, M., Krumpolec, P., Tu, M., Bogner, W., Meyerspeer, M., Ukropec, J., Frollo, I., Ukropcová, B., Trattnig, S., Kršňák, M., 2014. Depth-resolved surface coil MRS (DRESS) - localized dynamic 31 P-MRS of the exercising human gastrocnemius muscle at 7 T †. NMR Biomed. 27, 1346–1352. https://doi.org/10.1002/nbm.3196.
van den Broek, N.M.A., De Feyter, H.M.M.L., de Graaf, L., Nicolay, K., Prompers, J.J., 2007. Intersubject differences in the effect of acidosis on phosphocreatine recovery kinetics in muscle after exercise are due to differences in proton efflux rates. Am. J. Phys. Cell Phys. 293, C228–C237. https://doi.org/10.1152/ajpcell.00023.2007.
van Uffelen, J.G.Z., Chinapaw, M.J.M., van Mechelen, W., Hopman-Rock, M., 2008. Walking or vitamin B for cognition in older adults with mild cognitive impairment? A randomised controlled trial. Br. J. Sports Med. 42, 344–351.
Vaughan, S., Wallis, M., Polit, D., Steele, M., Shum, D., Morris, N., 2014. The effects of multimodal exercise on cognitive and physical functioning and brain-derived neurotrophic factor in older women : a randomised controlled trial. Age Ageing 43, 623–629.
Vaynman, S., Ying, Z., Gomez-Pinilla, F., 2004. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur. J. Neurosci. 20, 2580–2590. https://doi.org/10.1111/j.1460-9568.2004.03720.x.
Vedovelli, K., Giacobbo, B.L., Correa, M., Wieck, A., Argimon, I., Bromberg, E., 2017. Multimodal physical activity increases brain-derived neurotrophic factor levels and improves cognition in institutionalized older women. GeroScience 39, 407–417.
Vivar, C., Potter, M.C., Van Praag, H., 2013. All about running: synaptic plasticity, growth factors and adult hippocampal neurogenesis. Curr. Top. Behav. Neurosci. 15, 189–210. https://doi.org/10.1007/7854.
Walsh, J.J., Edgett, B.A., Tschakovsky, M.E., Gurd, B.J., 2015. Fasting and exercise differentially regulate BDNF mRNA expression in human skeletal muscle. Appl. Physiol. Nutr. Metab. 40, 96–98. https://doi.org/10.1139/apnm-2014-0290.
Wang, C., Bomberg, E., Billington, C., Levine, A., Kotz, C.M., 2007. Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate. Am. J. Phys. Regul. Integr. Comp. Phys. 293, 992–1002. https://doi.org/10.1152/ajpregu.00516.2006.
Winter, B., Breitenstein, C., Mooren, F.C., Voelker, K., Fobker, M., Lechtermann, A., Krueger, K., Fromme, A., Korsukewitz, C., Floel, A., Knecht, S., 2007. High impact running improves learning. Neurobiol. Learn. Mem. 87, 597–609. https://doi.org/10.1016/j.nlm.2006.11.003.
Wood, J., Tse, M.C.L., Yang, X., Brobst, D., Liu, Z., Pang, B.P.S., Chan, W.S., Zaw, A.M., Chow, B.K.C., Ye, K., Lee, C.W., Chan, C.B., 2018. BDNF mimetic alleviates body weight gain in obese mice by enhancing mitochondrial biogenesis in skeletal muscle. Metabolism. 87, 113–122. https://doi.org/10.1016/j.metabol.2018.06.007.
Yamamoto, H., Gurney, M.E., 1990. Human platelets contain brain-derived neurotrophic factor. J. Neurosci. 10, 3469–3478.
Yamanaka, M., Itakura, Y., Tsuchida, A., Nakagawa, T., Noguchi, H., Taiji, M., 2007a. Comparison of the antidiabetic effects of brain-derived neurotrophic factor and thiazolidinediones in obese diabetic mice. Diabetes Obes. Metab. 9, 879–888. https://doi.org/10.1111/j.1463-1326.2006.00675.x.
Yamanaka, M., Tsuchida, A., Nakagawa, T., Nonomura, T., Sugaru, E., Noguchi, H., Taiji, M., 2007b. Brain-derived neurotrophic factor enhances glucose utilization in peripheral tissues of diabetic mice. Diabetes Obes. Metab. 9, 59–64. https://doi.org/10.1111/j.1463-1326.2006.00572.x.
Yarrow, J.F., White, L.J., McCoy, S.C., Borst, S.E., 2010. Training augments resistance exercise induced elevation of circulating brain derived neurotrophic factor (BDNF). Neurosci. Lett. 479, 161–165. https://doi.org/10.1016/j.neulet.2010.05.058.
Zoladz, J.A., Pilc, A., Majerczak, J., Grandys, M., Zapart-Bukowska, J., Duda, K., 2008. Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men 1. J. Physiol. Pharmacol. 59, 119–132.