

A preliminary investigation of acute exercise intensity on memory and BDNF isoform concentrations

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Abstract:

Little is known about the biological mechanisms underlying the beneficial effect of acute exercise on memory or the influence of single nucleotide polymorphisms (SNPs) on this effect. Brain-derived neurotrophic factor (BDNF) is a putative biological mechanism, and while findings from human studies are equivocal, they have neglected to assess how exercise affects individual BDNF isoform (proBDNF, mBDNF) concentrations in serum or the influence of the *BDNF* val66met SNP on BDNF isoform concentrations. Therefore, the objective of this study was to conduct an exploratory assessment of the effect of acute exercise intensity on memory performance and BDNF isoform concentrations relative to carrier status of the *BDNF* val66met SNP met allele and to provide guidance for future, fully-powered trials. Memory and BDNF isoform concentrations were assessed in three exercise groups (light intensity, vigorous intensity, and non-exercise) relative to *BDNF* met carrier status. Analyses revealed that BDNF isoform concentrations and memory were differentially affected by exercise intensity and *BDNF* met carrier status. Vigorous intensity exercise increased mBDNF, and *BDNF* met carriers had lower mBDNF concentration. Light intensity exercise improved memory, and over 24 h, memory was worse for *BDNF* met carriers. Implications from this work will help direct future mechanistic studies of the exercise-memory relationship.

Keywords: exercise | cognition | genetics

Article:

Introduction

It is well established that acute exercise improves cognitive performance (Chang, Labban, Gapin, & Etnier, 2012; Lambourne & Tomporowski, 2010), with evidence of higher intensities eliciting greater benefits (Etnier et al., 2016; Hötting, Schickert, Kaiser, Röder, & Schmidt-Kassow, 2016; Winter et al., 2007). However, little is known about the mechanisms or influence of genetic variation. Gaining this insight will help researchers develop exercise protocols aimed at improving cognitive performance.

Brain-derived neurotrophic factor (BDNF) is vital for learning and memory (Cirulli, Berry, Chiarotti, & Alleva, 2004; Mu, Li, Yao, & Zhou, 1999) and is a potential mechanism of the effect of exercise on memory (Etnier et al., 2016; Griffin et al., 2011; Piepmeier & Etnier, 2014; Winter et al., 2007). While evidence suggests that acute exercise increases BDNF concentrations (Szuhany, Bugatti, & Otto, 2015), with higher intensities inducing larger increases (Piepmeier & Etnier, 2014), research investigating the relation between acute exercise-induced BDNF and cognitive performance is equivocal (Etnier et al., 2016; Ferris, Williams, & Shen, 2007; Griffin et al., 2011; Lee et al., 2014; Skriver et al., 2014; Tonoli et al., 2015; Tsai et al., 2014; Winter et al., 2007). This may be due to methodological differences related to the particular BDNF assay. In past studies, researchers have failed to provide adequate justification for the selection of enzyme-linked immunosorbent assays (ELISAs). This is an important short-coming because product data sheets for commercially available ELISAs indicate differences in the BDNF isoforms assessed. Due to differences in the basic form and function of BDNF, these methodological differences hinder interpretation of past studies.

As with all neurotrophins, BDNF is first expressed as an immature isoform (proBDNF) before being enzymatically modified into a mature isoform (mBDNF). The BDNF isoform-dependent signalling pathway leads to dichotomous molecular effects. While proBDNF stimulates apoptosis and long-term depression (i.e. LTD: reduced postsynaptic sensitivity to a presynaptic stimulus), mBDNF stimulates neurogenesis and long-term potentiation (i.e. LTP: enhanced postsynaptic sensitivity to a presynaptic stimulus). Thus, the isoform directs BDNF's effect on the form (apoptosis/neurogenesis) and function (LTD/LTP) of the brain. ELISAs used in previous studies of acute exercise and memory either did not differentiate, or had cross-reactivity between BDNF isoforms (Piepmeier & Etnier, 2014). Thus, the effects of acute exercise on BDNF isoforms remains poorly understood.

To date, only one study has explored the effect of acute exercise on BDNF isoforms. Results from Brunelli et al. (2012) showed that acute exercise affected BDNF levels in an isoform-specific and intensity-dependent manner. While exercise increased proBDNF levels, mBDNF decreased following a maximum intensity condition and increased following a sub-maximal condition, suggesting that mBDNF may be particularly responsive to exercise intensity.

Further, it is also unknown if *BDNF* genotype moderates the effect of acute exercise on BDNF isoforms. The val66met single nucleotide polymorphism (SNP) has been shown to affect memory and BDNF concentrations (Egan et al., 2003; Hariri et al., 2003). This SNP produces three possible allele pairs (genotypes): (1) val homozygous (val/val), (2) val heterozygous (val/met), and (3) met homozygous (met/met). *BDNF* genotype has been related to cognitive

performance (Erickson et al., 2013; Moreau, Kirk, & Waldie, 2017) and brain activation (Egan et al., 2003; Hariri et al., 2003), but no study has assessed its effect on BDNF isoforms.

Gaps in the literature concerning the effect of (1) acute exercise on BDNF isoform concentrations and memory performance, and (2) *BDNF* genotype motivated this study. The objective of this study was to conduct an exploratory assessment of the effect of acute exercise on memory performance and BDNF isoform concentrations relative to *BDNF* val66met genotype and to provide guidance for future trials. A randomized control trial (RCT) was used to assess differences between light intensity exercise, vigorous intensity exercise, and a non-exercise control group. It was hypothesized that (1) acute exercise would induce an intensity-dependent change in BDNF isoform concentrations and memory, with higher intensities eliciting higher mBDNF concentrations and better memory, and (2) memory and BDNF isoform concentrations would differ based on *BDNF* genotype, with met allele carriers having lower concentrations of mBDNF and worse memory.

Methods

Participants

Participants consisted of twenty-nine adult males ($M = 21.69$ years, Range: 18–29). To obtain a homogenous sample for this preliminary study, women were not included. Participants completed the American Heart Association/American College of Sport Medicine (ACSM) Health/Fitness Facility and Pre-participation Screening Questionnaire to ensure they met ACSM guidelines as “low risk” (Pescatello, Arena, Riebe, & Thompson, 2013). The study protocol was approved by the University’s institutional review board and informed written consent was obtained prior to data collection.

Design

As memory was the primary outcome, a randomized between-subjects design was used to reduce learning effects that may have biased results.

Exercise protocol

Participants attended two sessions between 6:00 am and 12:00 pm, separated by at least 72 h, at approximately the same time of day for a given participant. Participants arrived having fasted (10–12 h) to reduce the influence of diet on BDNF (Araya & Orellana, 2008).

Session one. Participants completed a demographic questionnaire, were fitted with a heart rate (HR) monitor, and height and weight were assessed. Participants sat on a reclined seat, an intravenous catheter (IV) was placed (e.g. antecubital), and the first (max.pre) of four blood samples was obtained. Next, participants mounted the cycle, were instructed in the use of the Rating of Perceived Exertion (RPE) scale, fitted with the metabolic cart’s facemask, and sat quietly for five minutes (resting HR and VO_2).

We modified the submaximal YMCA cycle ergometer protocol (Golding & Myers, 1989) into a graded maximal exercise test to volitional exhaustion (MAX). The MAX test stages were as follows: (1) “warm-up” – 3.5-minutes (25 watts), cadence: at least 50RPM; (2) “test period” (100 watts), increasing 50-watts every two minutes, cadence: at least 50RPM; (3) “cool-down” – 5-minutes (25 watts), cadence: self-selected. HR and RPE were assessed every minute during stages 1 and 2, and every two minutes during stage 3. Water was available ad libitum.

Immediately following the MAX test, the second (max.post) blood sample was obtained. Participants then completed the Pittsburgh Sleep Quality Index, as sleep quality may influence cognitive performance, a 2-week physical activity history, and viewed the documentary “Planet Earth” until the end of the session. The final blood samples were obtained at post 30-minutes (max.post.30) and 60-minutes (max.post.60).

Personalized intensity levels. Individualized VO_2 reserve was calculated as follows: $VO_2\text{reserve} = VO_2\text{max} - VO_2\text{resting}$. $VO_2\text{max}$ was determined when at least two of three criteria had been reached (Pescatello et al., 2013): (1) $RER > 1.1$, (2) plateau in VO_2 , (3) $RPE > 17$. $VO_2\text{peak}$ was used when $VO_2\text{max}$ was not achieved ($n = 4$). Intensity was calculated following ACSM definitions: light = 35% $VO_2\text{reserve}$ ($\pm 5\%$) and vigorous = 85% $VO_2\text{reserve}$ ($\pm 5\%$) (Pescatello et al., 2013). The cycle’s resistance was determined by referencing the watts cycled during the MAX test that corresponded to the participant’s $VO_2\text{reserve}$ at the assigned exercise intensity. HR corresponding to the participant’s $VO_2\text{reserve}$ was used to confirm achievement of ACSM’s definition of intensity as listed above.

Randomization. A list of conditions (light, vigorous, or non-exercise) was created (random number generator, Random.org) and participants’ group assignment was determined by their position on this list.

Session two. As with session one, participants wore a HR monitor, an IV was placed, and the first blood sample was obtained (pre). Next, participants were informed of their group assignment.

The exercise session was as follows: (1) “warm-up” – 5 min (25 watts), increasing each minute (assigned intensity was reached by minute-4), cadence: at least 50RPM; (2) “exercise period” – 25 min (assigned intensity), cadence: at least 50RPM, if participants could not maintain the required cadence, watts were reduced until the participants could sustain cadence, HR was maintained at the assigned intensity; (3) “cool-down” – 5-minutes (25 watts), cadence: self-selected. HR and RPE were assessed every minute during warm-up and cool-down, and every five minutes during the exercise period. The control group sat on the cycle (35 min) and viewed “Planet Earth”, and HR was assessed at the same intervals as the exercise group. Water was available ad libitum for all groups. Immediately upon finishing either the cool-down or control, the second blood sample was obtained (post). Participants then performed the cognitive tasks. Next, participants viewed “Planet Earth” until the end of the session. The final blood samples were obtained 30 (post.30) and 60 (post.60) minutes following the post draw.

Exercise measures & equipment. HR was assessed using Polar monitors (Polar, USA). RPE was assessed using the Borg RPE scale. Cycling was performed on a LODE Corival Recumbent

Cycle-Ergometer (Lode BV, Groningen, The Netherlands). Gas exchange was measured with a SensorMedics metabolic cart (Vmax, SensorMedics, Yorba Linda, CA) and was used to assess resting and maximal aerobic capacity (i.e. $VO_{2\text{resting}}$, $VO_{2\text{max}}$; 30-second intervals). $VO_{2\text{resting}}$ was calculated by averaging VO_2 levels from minutes two to five during the 5-minute rest period.

Blood sampling and BDNF assessment protocol

Blood was collected in serum separator tubes, allowed to clot for at least 20–30 min at room temperature, centrifuged (3000 rpm) for 20 min, aliquoted into samples (500–1000 ml), and stored in a -80°C freezer (Tuck et al., 2009). During a two-day period, within four months of sample collection, ELISAs were performed in duplicate, with all samples from a given participant analyzed on the same plate (intra-assay CV = 7.44), following recommended procedures (Aviscera Bioscience INC).

Genotyping protocol

Buccal cells were used for analyses of the *BDNF* val66met SNP to identify participants as met allele carriers (met/met, val/met) or non-carriers (val/val). Buccal swabs were stored in a -80°C freezer prior to being sent to the University's Core Laboratory for analysis.

Cognitive performance protocol

The order of cognitive tasks was constant: (1) Rey Auditory Verbal Learning Task (RAVLT) Trials 1–7, (2) spatial memory task. Participants then sat on the cycle and viewed “Planet Earth” until the end of the session. Trial 8 (RAVLT) was performed 30 min following Trial 7, and 24-hour recall/recognition was performed by phone the next day.

RAVLT. Assesses learning, delayed recall/recognition, and retention (Schmidt, 1996). The learning trials (Trials 1–5) required participants to listen to and recall a 15-item word list (list A). Trial 6 required participants to listen to and recall a second 15-item word list (list B). Participants were asked to immediately recall list A again (Trial 7), as well as following a 30-minute (Trial 8) and 24-hour (Trial 9) delay. At 24-hours, participants were also read a larger list of words (Lists A, B, and 20 new words), and were asked to assign each word to its appropriate list (24-hr recognition). Correctly recalled/recognized words were measured as follows: learning (sum of Trials 1–5), second list recall (Trial 6), immediate recall (Trial 7), 30-min recall (Trial 8), 24-hr recall (Trial 9), 24-hr recognition, retention (calculated as: $[\text{immediate recall} \div \text{highest score from Trials 1–5}] \times 100$), 30-min retention (calculated as: $[\text{30-min recall} \div \text{highest score from Trials 1–5}] \times 100$), and 24-hr retention (calculated as: $[\text{24-hr recall} \div \text{highest score from Trials 1–5}] \times 100$).

Spatial memory. A 32-trial computerized task (E-Prime 2.0) required participants to remember the shape and location of an object on a three-by-three grid. This style of task has previously observed age-related differences in memory (Mitchell, Johnson, Raye, & D'Esposito, 2000; Mitchell, Johnson, Raye, Mather, & Esposito, 2000). Each trial (Figure 1) consisted of an observation cue (“object + location”), followed by the sequential presentation of three object-

location combinations, a blank screen, and a recognition cue (“READY”). Next, an object-location combination was presented. Participants identified if the combination had been previously viewed (50% of trials) or was new (50% of trials), using the “P” and “Q” keys respectively. Lastly, a blank screen was presented (inter-trial interval). Outcomes were accuracy (correct trials ÷ total trials) and reaction time (average time [ms] to respond).

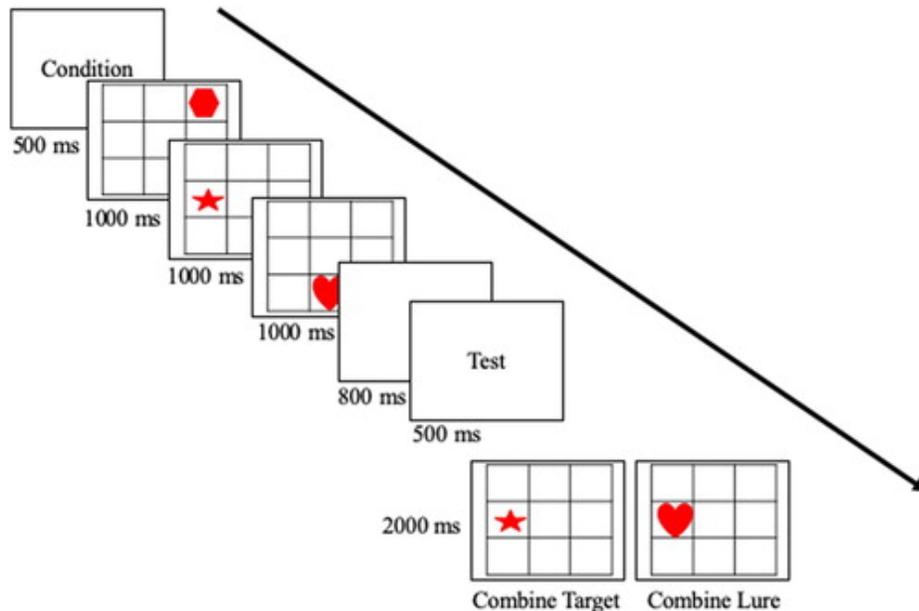


Figure 1. Relational memory trial.

Statistical analysis

Analyses were performed using SPSS (V. 24.0) and SAS (V. 4). Where appropriate, Mauchly’s test of sphericity was utilized to ensure sphericity assumptions were met, and a Huynh-Feldt adjustment was used for degrees of freedom if assumptions were not met.

Sample characteristics. Separate one-factor analyses of variance (ANOVAs) assessed differences in age, BMI, VO_{2max} , PA behaviour, and sleep quality between groups. A chi-square analysis assessed differences in *BDNF* genotype between groups.

Exercise intensity confirmation. HR and RPE were assessed to confirm intensity differences between groups. A 3 by 4 (Group: light intensity, vigorous intensity, control X Time: resting, warm-up, treatment, cool-down) mixed ANOVA assessed differences in HR, and a 2 by 4 (Group: light intensity, vigorous intensity X Time) mixed ANOVA assessed differences in RPE between exercise groups. Resting measures of HR and RPE consisted of single data points, while measures during 5-minute warm-up, 25-minute treatment, and 5-minute cool-down each consisted of averages of five data points.

BDNF isoform concentrations. Were log transformed to stabilize variance. To assess the effects of exercise intensity and *BDNF* genotype on the changes in BDNF over time, we fit separate linear mixed models for proBDNF and mBDNF. The models each included fixed effects for Group (light intensity, vigorous intensity, control), Time (pre, post, post.30, and post.60), their

interaction, *BDNF* genotype (carrier, non-carrier), the interaction between Group and *BDNF* genotype, the interaction between Time and *BDNF* genotype, and random intercepts and slopes over time for each subject. We estimated differences in changes over time between groups, along with 95% confidence intervals, using appropriately specified linear contrasts of the model parameters. Secondly, we explored the potential for effect moderation by *BDNF* genotype by including appropriate 2- and 3-way interaction fixed effects in the model.

Memory performance. To assess the effects of acute exercise on memory, separate 2 by 3 (*BDNF* genotype X Group) ANOVAs were performed to assess outcomes from the RAVLT (learning, second list recall, and 24-hr recognition) and spatial memory task (accuracy and reaction time). As with *BDNF*, a mixed model approach was used to analyze the effect of exercise intensity on changes in memory over time between groups and also between carriers and non-carriers. Separate models were fit for memory recall (immediate recall, 30-min recall, 24-hr recall) and memory retention (retention, 30-min retention, 24-hr retention). The fixed effects portions of these models were identical to those for *BDNF*, but random effects included only random intercepts for each subject.

BDNF isoform concentration & RAVLT correlations. Correlation analyses were performed to examine the potential link between both absolute concentrations of *BDNF* and changes in *BDNF* and memory. *BDNF* outcomes were post, post.30, post.60, *BDNF* Change (calculated as [post – pre] / pre), and maintenance of *BDNF* (calculated as: [post.60 – post] / post); and RAVLT outcomes were learning, second list recall, delayed recall, 30-min recall, 24-hr recall, 24-hr recognition, retention, 30-min retention, 24-hr retention. While it is important to interpret results against an adjusted alpha ($\alpha = .05 / 72 = .001$), given the exploratory nature of this study, we argue that a preliminary examination of correlations with $p < 0.05$ is warranted, not to make inferences to the population, but to aid in the development of future hypotheses.

Results

Sample characteristics

There were no significant differences in sample characteristics between groups, p 's > 0.05 (see Table I).

Table I. Sample characteristics.

Variable	Total (N=29)		Control condition (N=11)		Light condition (N=8)		Vigorous condition (N=10)	
	Mean	Min, Max	Mean	Min, Max	Mean	Min, Max	Mean	Min, Max
Age	21.69	18.00, 29.00	21.18	18.00, 28.00	22.37	18.00, 28.00	21.70	19.00, 29.00
BMI	25.89	18.79, 42.56	26.39	18.79, 42.56	27.54	22.94, 37.04	24.01	19.80, 28.82
PA Hist	7.10	0.86, 17.69	8.75	0.86, 17.69	5.79	1.43, 9.20	6.34	0.86, 10.30
Sleep	5.03	2.00, 9.00	5.64	2.00, 9.00	5.00	3.00, 7.00	4.40	2.00, 9.00
VO ₂ max	37.72	26.40, 48.50	36.08	26.40, 42.60	37.80	27.90, 46.80	39.46	28.90, 48.50
Met status	N		N		N		N	
No	18		6		4		8	
Yes	11		5		4		2	

BMI = body mass index; PA Hist = 2-week physical activity history displayed as METS; Sleep = Sleep Quality (Pittsburgh Sleep Index); Met Status = *BDNF* val66met genotype (val/val = No; val/met, met/met = Yes).

Exercise intensity manipulation check

There was a significant time by group interaction for both measures of intensity (HR: $F_{2,181,52.342} = 114.97, p < 0.001, \text{partial } \eta^2 = 0.91$; RPE: $F_{1,16} = 14.88, p = 0.001, \text{partial } \eta^2 = 0.60$). The time X group interaction for HR shows that those in the vigorous group had greater HR during warm-up (vigorous: $M = 120.20, SD = 9.83$; light: $M = 96.05, SD = 10.59$; control: $M = 64.29, SD = 8.74$), treatment (vigorous: $M = 166.16, SD = 10.48$; light: $M = 119.02, SD = 12.69$; control: $M = 63.53, SD = 10.60$), and cool-down (vigorous: $M = 120.84, SD = 10.44$; light: $M = 94.18, SD = 11.93$; control: $M = 68.26, SD = 10.68$) compared to other groups, and those in the light group had greater HR during warm-up, treatment, and cool-down compared to the control group. The time X group interaction for RPE shows that those in the vigorous group had greater RPE during warm-up (vigorous: $M = 10.76, SD = 1.31$; light: $M = 8.45, SD = 0.70$), treatment (vigorous: $M = 17.62, SD = 1.30$; light: $M = 12.03, SD = 1.20$), and cool-down (vigorous: $M = 9.28, SD = 1.08$; light: $M = 8.03, SD = 1.28$) compared to the light group.

BDNF isoform concentrations

See Table II for means and standard deviations.

proBDNF. Mean concentrations of proBDNF did not significantly change across time or between groups ($p > 0.05$). All interactions were non-significant ($p > 0.05$).

mBDNF. Mean mBDNF concentrations in the vigorous group changed significantly from pre to post.60 compared to the control group ($M \text{ diff.} = 0.713, 95\% \text{ CI} [0.218, 1.208], p = 0.006$) (Figure 2). Additionally, *BDNF* met allele carriers had lower average mBDNF concentrations which reached significance at post.30 ($M \text{ diff.} = -0.632, 95\% \text{ CI} [-1.197, -0.067], p = 0.029$).

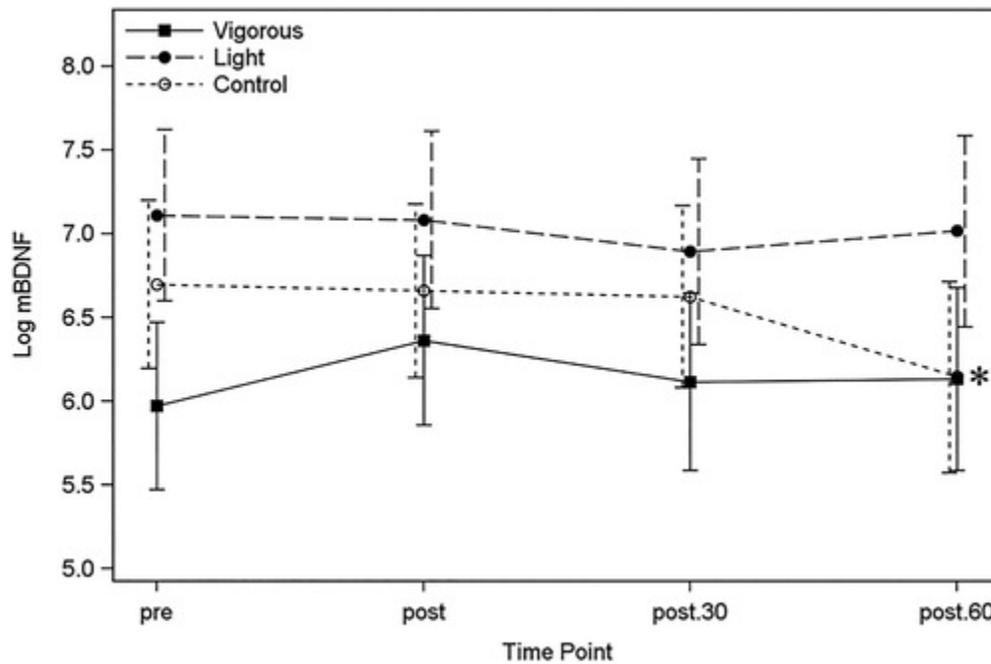


Figure 2. Mean mBDNF isoform concentrations and 95% confident intervals.

Table II. BDNF isoform and memory outcome means & standard deviations.

Variable	Carrier	Control condition	Light condition	Vigorous condition
		Mean(SD)	Mean(SD)	Mean(SD)
<i>proBDNF</i>				
Pre	total	5.77(1.32)	5.33(0.99)	5.83(1.14)
	Y	5.92(1.72)	4.90(0.59)	6.20(1.28)
	N	5.57(0.76)	5.77(1.20)	5.64(1.11)
Post	total	5.69(1.27)	5.33(0.98)	5.73(0.97)
	Y	5.92(1.72)	4.90(0.59)	6.20(1.38)
	N	5.57(0.76)	5.77(1.20)	5.63(1.11)
Post.30	total	5.75(1.24)	5.36(0.91)	5.79(1.07)
	Y	5.92(1.72)	4.90(0.59)	6.20(1.38)
	N	5.57(0.76)	5.77(1.20)	5.63(1.11)
Post.60	total	5.80(1.17)	5.35(0.90)	5.86(1.06)
	Y	5.92(1.72)	4.90(0.59)	6.20(1.38)
	N	5.57(0.76)	5.77(1.20)	5.63(1.11)
<i>mBDNF</i>				
Pre	total	6.62(0.79)	7.03(0.57)	5.88(0.47)
	Y	6.04(0.30)	7.36(0.48)	5.70(0.27)
	N	7.34(0.56)	6.77(0.54)	5.95(0.54)
Post	total	6.54(0.93)	6.99(0.76)	6.25(0.41)
	Y	5.86(0.93)	7.25(0.94)	6.18(0.04)
	N	7.37(0.67)	6.80(0.67)	6.28(0.50)
Post.30	total	6.55(0.91)	6.80(0.86)	5.99(0.62)
	Y	6.06(0.79)	6.78(0.75)	5.56(0.19)
	N	7.16(0.70)	6.82(1.04)	6.16(0.66)
Post.60	total	6.09(0.82)	6.90(0.66)	6.00(0.53)
	Y	5.55(0.47)	7.42(0.17)	5.66(0.20)
	N	6.77(0.61)	6.51(0.63)	6.13(0.58)
<i>RAVLT</i>				
Learning	total	47.44(5.25)	52.00(6.32)	48.80(7.67)
	Y	45.60(6.47)	49.50(3.32)	45.00(4.24)
	N	49.75(2.22)	54.50(8.10)	49.75(8.24)
Novel	total	5.67(1.50)	7.50(1.60)	5.60(1.43)
	Y	5.40(1.52)	6.25(0.96)	6.00(1.41)
	N	6.00(1.63)	8.75(0.96)	5.50(1.51)
Im. Recall	total	10.56(2.07)	11.13(2.23)	9.90(2.23)
	Y	10.40(2.07)	9.75(1.50)	10.50(0.71)
	N	10.75(2.36)	12.50(2.08)	9.75(2.49)
Recall.30	total	10.33(2.35)	10.13(3.04)	9.20(3.23)
	Y	9.80(2.59)	8.25(2.36)	7.50(0.71)
	N	11.00(2.16)	12.00(2.58)	9.63(3.50)
Recall.24	total	7.67(2.56)	8.00(3.82)	7.40(2.59)
	Y	6.40(2.30)	6.00(3.37)	6.50(0.71)
	N	9.25(2.06)	10.00(3.46)	7.63(2.88)
Recog.24	total	29.33(4.58)	32.25(4.10)	28.30(4.52)
	Y	29.20(5.54)	31.25(1.50)	28.50(3.54)
	N	29.50(3.87)	33.25(5.85)	28.25(4.95)
Im. Ret	total	72.76(38.08)	82.97(14.06)	78.49(10.23)
	Y	91.35(12.15)	73.49(10.18)	83.98(0.91)
	N	57.27(46.35)	92.45(10.88)	77.12(11.13)
Ret.30	- total	70.86(37.44)	75.56(21.75)	72.32(18.15)

Variable	Carrier	Control condition	Light condition	Vigorous condition
		Mean(SD)	Mean(SD)	Mean(SD)
	Y	85.64(16.11)	62.09(16.99)	59.94(2.27)
	N	58.55(46.85)	89.03(18.21)	75.42(19.18)
Ret.24	total	51.99(29.63)	59.24(25.96)	58.48(16.10)
	Y	55.15(12.59)	45.19(25.59)	52.24(8.61)
	N	49.36(40.13)	73.29(19.79)	60.04(17.58)
<i>RMT</i>				
Accuracy	total	0.85(0.08)	0.92(0.09)	0.88(0.09)
	Y	0.84(0.09)	0.88(0.12)	0.84(0.09)
	N	0.88(0.07)	0.95(0.05)	0.89(0.10)
RT	total	938.30(171.93)	919.00(154.14)	1010.128(176.61)
	Y	875.38(72.50)	867.91(168.92)	877.22(86.89)
	N	1016.95(238.69)	970.09(141.20)	1043.36(180.87)

Memory performance

Learning. Learning did not significantly differ between groups, and all interactions were non-significant ($p > 0.05$).

Second-list recall. Performance was significantly different between groups ($F_{2,21} = 4.29, p = 0.027, \text{partial } \eta^2 = .29$), and a Bonferroni post-hoc analyses revealed that those in the light intensity group ($M = 7.5, SD = 1.6$) recalled significantly more words compared to those in the vigorous intensity group ($M = 5.6, SD = 1.43, p = 0.027$) or control group ($M = 5.67, SD = 1.5, p = 0.039$). All interactions were non-significant ($p > .05$).

24hr Recognition. Recognition did not significantly differ between groups, and all interactions were non-significant ($p > 0.05$).

Memory recall. Change in memory over time did not significantly differ as a function of group ($p > 0.05$). However, compared to non-carriers, *BDNF* met allele carriers experienced significant decreases in memory recall from Trial 7 to Trial 8 ($M \text{ difference} = -1.446, 95\% \text{ CI} [-2.668, -0.225], p = 0.021$) and from Trial 8 to 24-hr Recall ($M \text{ difference} = -1.875, 95\% \text{ CI} [-3.096, -0.654], p = 0.003$).

Memory retention. Change in memory retention over time did not significantly differ as a function of group. However, compared to non-carriers, *BDNF* met allele carriers experienced significant decreases in memory retention from Trial 7 to Trial 8 ($M \text{ difference} = -11.15, 95\% \text{ CI} [-20.95, -1.357], p = 0.027$) and from Trial 8 to 24-hr Retention ($M \text{ difference} = -18.56, 95\% \text{ CI} [-28.36, -8.764], p < 0.001$).

Spatial memory. Accuracy and reaction time did not significantly differ between groups, and all interactions were non-significant ($p > .05$).

BDNF isoform concentration & memory correlations

Many correlations achieved $p < .05$. Of note, proBDNF at post ($r = -0.54, p < 0.01$), post.30 ($r = -0.48, p = 0.01$), and post.60 ($r = -0.46, p = 0.02$) was negatively correlated with learning,

and proBDNF at post was negatively correlated with 24-hr recognition ($r = -0.46, p = 0.02$). Maintenance of mBDNF was negatively correlated with 24-hr recall ($r = -0.44, p = 0.03$), 30-minute retention ($r = -0.42, p = 0.04$), and 24-hr retention ($r = -0.51, p = 0.01$). All other correlations were non-significant.

Discussion

The study objective was to assess the effect of acute exercise on memory and BDNF isoform concentrations relative to *BDNF* genotype.

Past studies show that acute exercise increases BDNF concentrations (Ferris et al., 2007; Griffin et al., 2011; Szuhany et al., 2015; Winter et al., 2007) and improves cognition (Chang et al., 2012; Etnier et al., 1997; Etnier, Labban, Piepmeier, Davis, & Henning, 2014; Kamijo et al., 2009; Labban & Etnier, 2011; Lambourne & Tomporowski, 2010; Pesce, Crova, Cereatti, Casella, & Bellucci, 2009; Piepmeier et al., 2015; Roig, Nordbrandt, Geertsen, & Nielsen, 2013). However, compare findings to past literature is impeded by the lack of past isoform-specific research. One study has explored the effects of acute exercise on BDNF isoforms; but no studies have done so with proBDNF assessed from serum or relative to *BDNF* genotype. Brunelli et al. (2012) observed isoform-specific and exercise-intensity-dependent changes in BDNF levels. While isoform levels increased following exercise, at 60-minutes only mBDNF levels either increased or decreased depending on exercise intensity (submaximal or maximal, respectively) (Brunelli et al., 2012). Since proBDNF isoform levels were obtained from cells using molecular methods and not from serum, we cannot directly compare our proBDNF findings. However, both the current study and Brunelli et al. (2012) suggest that mBDNF in serum may be selectively affected by exercise intensity.

BDNF concentrations and exercise intensity

Exercise intensity may differentially affect isoform concentrations in blood serum. While exercise did not significantly affect proBDNF concentrations, vigorous exercise increased mBDNF concentrations. These findings are consistent with a review that showed a dose-response relationship between exercise intensity and post-exercise BDNF concentrations, with greater intensities generating greater BDNF concentrations (Knaepen, Goekint, Heyman, & Meeusen, 2010). Further, the review concluded that low to moderate intensity acute exercise is less effective at increasing BDNF concentrations in healthy adults. While additional research is needed, it is plausible that past findings of acute exercise-induced BDNF concentrations were driven by mBDNF. Our findings expand our understanding of the intensity-dependent nature of exercise-induced BDNF and show that mBDNF, and not proBDNF, is both responsive and sensitive to exercise intensity. This implies that acute exercise may affect a discrete BDNF-signalling pathway (e.g. mBDNF – TrkB) and may be used to develop future exploration. Namely, acute exercise may affect memory through increased long-term potentiation. Smallwood et al. (2015) observed that participants with greater physical activity levels experienced increased long-term potentiation. These findings provide insight for future research into the acute exercise-mBDNF-LTP relationship.

BDNF genotype and BDNF concentrations

As with exercise intensity, *BDNF* genotype showed an isoform-specific effect. Our hypothesis was supported in that mBDNF concentrations were lower for carriers. Genotype did not significantly affect proBDNF concentrations. As this is the first study to assess the effect of *BDNF* genotype on exercise-induced concentrations of BDNF, more work is needed to replicate and extend these findings.

Memory and exercise intensity

Light intensity exercise increased second word list recall. This is a novel finding as the typical intent of Trial 6 is to inhibit mental rehearsal of List A prior to completing Trial 7. The reset-of-encoding hypothesis states that directed forgetting may “reset” one’s encoding ability of subsequent lists, thus reducing cognitive load and improving memory, and studies have employed electroencephalography to explore the alpha and theta oscillations as potential mechanisms for the reset-of-encoding hypothesis (Bäumel, Hanslmayr, Pastötter, & Klimesch, 2008; Pastötter, Schicker, Niedernhuber, & Bäumel, 2011). Our findings suggest that light intensity exercise may produce a similar reset-of-encoding. This is important because it provides a known theory, literature base, and proposed mechanisms as foundations on which to establish future mechanistic investigations.

The non-significant memory findings are inconsistent with past studies by Etnier et al. (2014, 2016). This may be due to differences in study design: (a) modality (treadmill or Progressive Aerobic Cardiovascular Endurance Run), (b) intensity (ventilatory threshold \pm 20% or maximal), (c) characteristics (adult, adolescent, males, females), and (d) design (within-subjects). However, meta-analytic support for these factors are equivocal. While Lambourne and Tomporowski (2010) showed larger cognitive benefits for cycling compared to running, Roig et al. (2013) showed that modality moderates the effects of acute exercise on short- and long-term memory. Further, Chang et al. (2012) illustrated that acute exercise decreases RAVLT performance. Thus, research is needed into how factors such as these may account for conflicting findings.

Spatial memory was not affected by exercise. These findings may be due to the tool (sensitivity, number of trials, validity for population), the timing of the task (after the RAVLT), or alternatively that acute exercise does not affect spatial memory in young, physically active males. Findings from Chang et al. (2012) suggest that task timing is vital, and our participants may have been outside the window of cognitive benefit (11–20 min). Future studies should use spatial/relational memory tools shown to be sensitive in exercise/fitness paradigms such as those performed with adolescents (Chaddock et al., 2010; Chaddock, Hillman, Buck, & Cohen, 2011), young adults (Schwarb et al., 2017), and older adults (Erickson et al., 2011).

BDNF genotype and memory

Supporting our hypothesis, results showed the influence of genotype on memory over time, with carriers having significantly worse memory compared to non-carriers. However, spatial memory was not affected by *BDNF* genotype. This may be due to limitations of the task as described above. *BDNF* genotype has been shown to moderate the effect of exercise on executive function

in a cross-sectional study with middle-aged adults (Erickson et al., 2013) and a chronic exercise paradigm with children (Moreau et al., 2017). We believe this is the first acute exercise study of the effect of *BDNF* genotype on memory with young adults. Future studies may consider taking genetic variation into account.

Correlations between BDNF concentrations and memory

Negative correlations were observed between proBDNF and learning across the three post-exercise time points, as well as between maintenance of mBDNF and 30-minute retention, 24-hr recall, and 24-hr retention. It appears that while higher concentrations of proBDNF are detrimental to learning, sustained concentrations of mBDNF are detrimental to long-term memory. To our knowledge, these represent the first isoform-specific correlations with memory in an exercise paradigm. However, these results are in contrast to past non-isoform-specific work showing significant positive correlations between BDNF and memory in young adult males (Lee et al., 2014; Skriver et al., 2014; Winter et al., 2007) and non-significant correlations between BDNF and executive function in young adult males and females (Ferris et al., 2007), between BDNF and reaction time in young adult men (Tsai et al., 2014), and between BDNF and reaction time in older men and women with mild cognitive impairment (Tsai, Ukropec, Ukropcová, & Pai, 2018). Yet, as noted, the purpose of the correlation analyses in our study were to facilitate the development of future hypotheses. These findings provide rationale for future explorations of the relations between BDNF isoforms, learning, and memory. For instance, investigating differences in correlations between BDNF isoform concentrations and multiple cognitive domains (memory, executive function, processing) in a single study would improve our understanding. Further, we suggest that future investigations include populations other than young, healthy adults, instead focusing on populations that are experiencing or at risk of cognitive impairment (e.g. cancer survivors, older adults).

Conclusion

We believe this is the first study to assess the effect of acute exercise on memory and BDNF isoform concentrations relative to *BDNF* genotype. Work is needed to replicate the following findings: (1) support of the reset-of-encoding hypothesis, (2) BDNF isoform-specific correlations with memory, and (3) poorer memory for *BDNF* met carriers irrespective of exercise condition. Exploratory correlational analyses suggest that future investigation of exercise-induced BDNF isoform concentrations and memory is necessary. This novel isoform-specific study produced questions relating to the role of BDNF in the acute exercise-memory relationship. Namely, our findings of greater mBDNF concentrations following vigorous intensity exercise and better memory performance following light intensity exercise are counter to BDNF as a proposed mechanism of the acute exercise-memory relation (Piepmeier & Etnier, 2014). Further investigations will help elucidate this seemingly paradoxical finding. For instance, it is plausible that the role of BDNF as a mediator of the exercise-memory relationship is moderated by intervention duration (acute, chronic). A physiological advantage of maintaining proBDNF, the molecular precursor for mBDNF, at a relatively stable concentration regardless of exercise is also plausible. Namely, to ensure the availability of provisions to create exercise-induced increases in mBDNF.

Limitations

Statistical power is a limitation in small exploratory studies. Therefore, results provide suggestions for future studies rather than definite truths. The sample's low aerobic fitness (mean = 37.72) limits generalizability, and restricting recruitment to men limits our ability to assess sex-based interactions. Future, fully powered studies are needed to replicate our findings.

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