European Journal of Applied Physiology (2019) 119:2167–2176
https://doi.org/10.1007/s00421-019-04202-w

ORIGINAL ARTICLE

Twelve weeks of resistance training does not influence peripheral levels of neurotrophic growth factors or homocysteine in healthy adults: a randomized-controlled trial

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Received: 29 April 2019 / Accepted: 29 July 2019 / Published online: 1 August 2019

Abstract

Introduction There is growing evidence for a preventative effect of resistance training on cognitive decline through physiological mechanisms; yet, the effect of resistance training on resting growth factors and homocysteine levels is incompletely understood. This study aimed to investigate the effect of intense resistance training, for 12 weeks, on changes in peripheral growth factors and homocysteine in late middle-aged adults.

Methods 45 healthy adults were enrolled into the single-site parallel groups' randomized-controlled trial conducted at the Department of Exercise Science, Strength and Conditioning Laboratory, Murdoch University. Participants were allocated to the following conditions: (1) high-load resistance training ($n = 14$), or (2) moderate-load resistance training ($n = 15$) twice per week for 12 weeks; or (3) non-exercising control group ($n = 16$). Data were collected from September 2016 to December 2017. Fasted blood samples were collected at baseline and within 7 days of trial completion for the analysis of resting serum brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1, vascular endothelial growth factor, and plasma homocysteine levels.

Results No differences in baseline to post-intervention change in serum growth factors or plasma homocysteine levels were observed between groups. A medium effect was calculated for BDNF change within the high-load condition alone (+ 12.9%, $g = 0.54$).

Conclusions High-load or moderate-load resistance training twice per week for 12 weeks has no effect on peripheral growth factors or homocysteine in healthy late middle-aged adults.

Trial registration Australian New Zealand Clinical Trials Registry: ACTRN12616000690459.

Keywords Strength training · Cognitive decline · Growth factors · Homocysteine · Preventative medicine

Abbreviations

ANCOVA Analysis of covariance
ANOVA Analysis of variance

Communicated by William J. Kraemer.

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APOE	Apolipoprotein E
BDNF	Brain-derived neurotrophic factor
ELISA	Enzyme-linked immunosorbent assay
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor-binding protein 3
IPAQ	International Physical Activity Questionnaire
MoCA	Montreal Cognitive Assessment
1RM	One-repetition maximum
VEGF	Vascular endothelial growth factor

Introduction

Resistance training is essential to maintaining physical function and independence as we age (Elsawy and Higgins 2010), yet, data supporting the benefits of continuous resistance training on brain and cognitive (e.g., memory, learning, awareness) health remain limited. Mechanistically, the influence of resistance exercise on cognitive function appears to be mediated by elevated expression and production of key growth factors (Cotman and Berchtold 2002): specifically, brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) (Fabel et al. 2003; Nagahara et al. 2009; Sonntag et al. 1999). In young adults, high volumes (i.e., total sets × repetitions) of intense (i.e., maximal effort) resistance exercise has been shown to transiently increase the peripheral concentrations of BDNF (Marston et al. 2017a), VEGF (Ross et al. 2014), and IGF-1 (Kraemer et al. 1990). However, the influence of chronic (i.e., weeks or months) resistance training on these growth factors in middle-aged and older adults is inconsistently reported. Several studies describe no increase in resting levels of BDNF (Fragala et al. 2014; Levinger et al. 2008), IGF-1 (Ogawa et al. 2010), or VEGF (Ogawa et al. 2010), whilst research that has demonstrated pre-to-post-intervention changes in resting levels of BDNF (Forti et al. 2015) and IGF-1 (Cassilhas et al. 2007; Tsai et al. 2015), has used high physiological intensity (i.e., the demand associated with performing an entire session) with large training volumes (e.g., 180–240 total repetitions per session), similar to acute study findings (Kraemer et al. 1990; Marston et al. 2017a; Ross et al. 2014). In contrast to beneficial neurotrophic factors, resting extracellular levels of the amino acid homocysteine increase with age (Herrmann et al. 1999) and are associated with cognitive decline through greater oxidative stress and vascular dysfunction (Obeid and Herrmann 2006). Whilst there is inconsistent evidence on the influence of resistance training on homocysteine (Vital et al. 2016), long-term intensive resistance training appears effective at minimizing extracellular homocysteine levels (Tsai et al. 2015; Vincent et al. 2003). Although the mechanisms remain unclear, it is likely that mechanical loading (Bamman et al. 2001; Goldspink 1999; Hoier and Hellsten 2014; Matthews et al. 2009), hormonal and metabolic adaptations (Deminice et al. 2016; Jones and Clemmons 1995; Kraemer and Ratamess 2005), elevated blood flow (Fujimura et al. 2002; Kraemer and Ratamess 2005) amongst many other factors are important mediators of resistance training-induced changes in growth factors and homocysteine.

Although physiological intensity is identified as an important training parameter for enhancing growth factors (Knaepen et al. 2010), consideration should be given to the practicality of high-volume resistance training. Indeed, higher volume resistance training in older adults is associated with poorer training compliance (Mazzeo and Tanaka 2001), increased risk of overtraining, and greater time-burden (Hunter et al. 2004). Therefore, there is a need for further research that examines the influence of intensive resistance training on growth factors and homocysteine from a pragmatic standpoint. The purpose of this study was to explore the effects of 12 weeks of intensive, yet moderate volume (e.g., 100–120 repetitions per session), resistance training on change in serum concentrations of BDNF, IGF-1, VEGF, and plasma homocysteine, when compared to a non-exercising control group. The resistance training was either moderate-load (i.e., high physiological intensity) or high-load (i.e., moderate physiological intensity) as indicated by our previous works (Marston et al. 2017b). Furthermore, these training protocols represent traditional, yet ecologically valid, and theoretically work-matched, approaches to developing maximal muscle mass and strength, respectively. We hypothesized that a greater increase in serum BDNF, IGF-1, VEGF, and reduced plasma homocysteine would be observed within moderate-load and high-load resistance training groups when compared to the control group. Furthermore, we hypothesized that a greater magnitude of change would be apparent within the moderate-load group when compared to high-load resistance training, as an effect of greater physiological intensity.

Methods

Design

Healthy late middle-aged adults were recruited for this randomized-controlled trial to explore the influence of 12 weeks of resistance training on changes in physiological markers integral to cognitive health and function (Australian New Zealand Clinical Trials Registry: ACTRN12616000690459). Physiological markers were assessed at baseline and following 12 weeks of resistance training or a control condition. Participants attended a familiarization session, two baseline assessment sessions, and two post-intervention assessment sessions. Baseline assessments occurred within 10 days of

the familiarization session. Participants were allocated to one of three groups; (1) high-load resistance training (i.e., moderate physiological intensity), (2) moderate-load resistance training (i.e., high physiological intensity), or (3) a non-exercising control group. Post-intervention assessments were conducted within 7 days of intervention completion under the same conditions as the baseline assessments.

Participants

Forty-eight healthy males and females (range = 41–69 years) expressed interest in the study and were screened for eligibility (Fig. 1). Individuals were ineligible to participate if they were; (1) cognitively impaired as assessed by the Montreal Cognitive Assessment (MoCA; Nasreddine et al. 2005), (2) experiencing musculoskeletal disorders or injuries that would prevent resistance training, (3) categorized as high risk for adverse events by the Exercise and Sport Science adult pre-exercise screening tool (Coombes and Skinner 2014), or (4) involved in structured resistance training within the previous 6 months. All participants were deemed untrained in resistance training (American College of Sports Medicine Position Stand 2009). Two individuals did not meet the inclusion criteria of the study, and one individual declined participation. Thus, the cohort enrolled into the study consisted of 45 participants. The procedures of this study were approved by the institutional Human Research Ethics Committee and, therefore, were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This study complied with CONSORT guidelines. All data were collected at the Murdoch University Mind and Body laboratory.

Procedures

Familiarization

Participants were familiarized with the resistance exercises involved in the study. Instruction was given as to the correct technique for bench press, leg press, lat pulldown, and leg curl exercises, and participants were required to perform two-to-three sets of light-load repetitions for each exercise. Weights machine seating was adjusted for all machines to suit each participant, with adjustments kept consistent between maximal strength testing time-points and training periods. A fasted 4.0 ml venous blood sample was collected from the antecubital vein, prepared into 200 µl aliquots, and frozen at – 80 ° C for later Apolipoprotein E (APOE) genotyping.

Maximal strength assessment

Maximal bench press, leg press, lat pulldown, and leg curl strength were determined via one-repetition maximum (1RM) within a single visit in order of mention. In preparation, a 5-min rowing ergometer general warm-up was performed at a self-selected intensity, followed by specific warm-up for each exercise (i.e., three sets at increasing

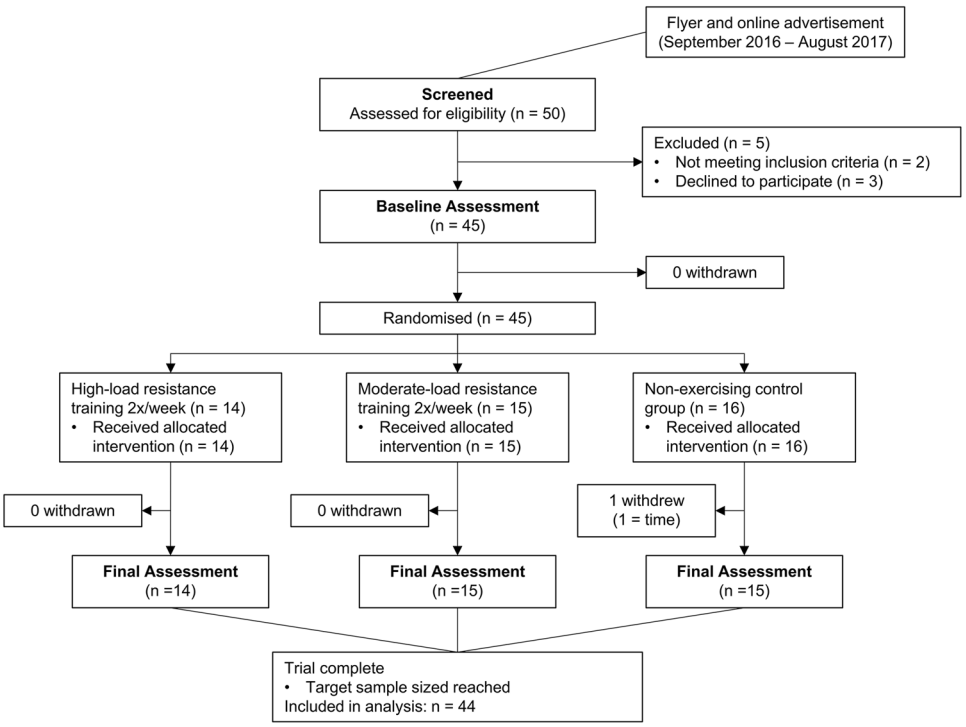

Fig. 1 CONSORT flow diagram

loads). To determine maximal strength, participants performed a single repetition at a pre-determined resistance through full range of motion. Resistance was adjusted until full range of motion was not achieved, exercise technique became compromised, or the assessor was required to terminate the attempt (Baechle and Earle 2008). A single rater conducted all 1RM assessments.

Body composition and general physical activity assessment

Total fat mass, total lean mass, and total appendicular lean mass were determined by dual-energy X-ray absorptiometry (Discovery™, Hologic®, Marlborough, MA, USA) at baseline and post-intervention.

Self-reported habitual physical activity levels (i.e., gardening, housework, work-related and recreational physical activity, etc.) were assessed using the long-form 7-day retrospective International Physical Activity Questionnaire (IPAQ) for all participants at baseline and post-intervention (Craig et al. 2003).

Venous blood sampling

Venous blood was collected at baseline and within 7 days of completing the 12-week intervention period. All participants fasted for 12 h overnight and were instructed to hydrate the morning of blood collection. Strenuous exercise was to be avoided the 24 h prior blood collection. Samples were collected in the morning between 6:00 a.m. and 9:00 a.m., with individual testing times remaining constant at baseline and follow-up. Venous blood was drawn using a 22-gauge needle into one 4.0 ml EDTA tube (Vacutainer®, Becton–Dickinson, USA), one 7.5 ml EDTA tube, and one 7.5 ml serum separation tube (S-Monovette®; Sarstedt, Nümbrecht, Germany), which was left upright at room temperature for 30 min to clot. 7.5 ml serum and EDTA tubes were centrifuged at 1800*g* for 15 min, and 1300*g* for 10 min, respectively. Serum and plasma supernatant were stored in 1.0 ml aliquots at – 80 °C for later analysis. Fresh whole blood from 4.0 ml EDTA tubes was refrigerated and analyzed for platelet count within 24 h.

Blood sample analysis

Serum samples, plasma samples, and assay kits were thawed at room temperature. Serum samples were analyzed for BDNF, IGF-1, and VEGF using standard ELISA techniques using commercial kits (DuoSet®, R&D Systems™, Minneapolis, USA). Serum insulin-like growth factor-binding protein 3 (IGFBP-3) was also quantified to indicate total IGF-1 bioavailability (Jones and Clemmons 1995). Serum samples were diluted at a ratio of 1:100 (BDNF), 1:10 (IGF-1), 1:500 (IGFBP-3), and undiluted (VEGF). The manufacturer declares intra-assay coefficients of variation of less than 10%. The plates were read on a FLUOstar® Optima reader system (BMG Labtech, Ortenberg, Germany). Five parameter logistic logarithmic equations were used to analyze the yield BDNF, IGF-1, IGFBP-3, and VEGF concentrations per well. Plasma samples were analyzed for homocysteine using a tabletop analyzer (COBAS INTEGRA® 400 Plus, Roche Diagnostics, Risch-Rotkreuz, Switzerland) as per the manufacturer's instructions.

Apolipoprotein E genotyping

DNA was extracted from whole blood using QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany). Genotyping was performed as described previously (Porter et al. 2018a, b). Briefly, TaqMan® genotyping assays (Life Technologies, Carlsbad, CA, USA) were used to determine APOE genotype and were performed on a QuantStudio 12K Flex™ Real-Time-PCR system (Applied Biosystems, Foster City, CA, USA) using the TaqMan® GTXpress™ Master Mix (Life Technologies) methodology.

Randomization

Participants were enrolled into the study by the primary author, and randomly allocated by a third party to one of three experimental groups using minimization randomization software (QMinim) following baseline assessment. Randomization was stratified for age, sex, and APOE genotype (i.e., carrier of at least one APOE ε4 allele, or carriage of no ε4 alleles).

Exercise intervention

Participants attended the Murdoch University Strength and Rehabilitation Laboratory in training groups to perform sessions of either high-load or moderate-load resistance training twice per week for 12 weeks. High-load and moderate-load protocols were theoretically work-matched a priori. Participants allocated to the control group performed no resistance training, and were encouraged to maintain their usual lifestyle and dietary habits. The high-load group performed five sets of five repetitions of bench press, leg press, lat pulldown, and leg curl at a weight intensity of 85% of 1RM, and rested for 180 s between sets. The moderate-load group performed three sets of ten repetitions at a weight intensity of 70% of 1RM, and rested for 60 s between sets. Rest between exercises was 120 s for both resistance training groups. Training sessions commenced with a 5-min rowing ergometer warm-up at a self-selected intensity, and a set of ten repetitions at 50% of 1RM prior to each exercise. Resistance was progressively increased by 2.5–5% per week or per individual tolerance, but no greater than 10% per week.

Statistical analysis

Baseline characteristics across groups were compared using a one-way analysis of variance (ANOVA) for continuous variables, and Chi-square for categorical variables. Change scores from baseline to post-intervention were calculated for BDNF, IGF-1, IGF-1/IGFBP-3-binding ratio, VEGF, homocysteine, maximal muscle strength, body composition, and IPAQ scores, and differences across groups were assessed using an analysis of covariance (ANCOVA), including the baseline value of each variable as a covariate. Platelet count was incorporated as a covariate for the analysis of BDNF and VEGF given evidence that these growth factors can be released from platelets (Fujimura et al. 2002; Selheim et al. 2002). Five participants had undetectable levels of VEGF at baseline and post-intervention; thus, a total to ten samples were not included in the analysis (high-load = 0, moderate-load = 6, control = 4). A Sidak adjustment was applied for multiple pairwise *post-hoc* comparisons. Statistical analyses were performed with SPSS analytical software (Version 24, IBM®, New York, USA) with a significance of $p \leq 0.05$. Effect size estimates (Hedge's g) were calculated to represent the magnitude of difference between groups or time-points, and were interpreted as small ($g < 0.50$), medium ($g = 0.50$–0.79) or large ($g \geq 0.80$) (Cohen 1988). All data are presented as mean ± SD unless otherwise noted. Data were analyzed in 2018.

Results

Adherence to the training intervention was 93.5% for high-load (1.6 ± 1.2 unattended sessions) and 95.8% for moderate-load (1.0 ± 1.1 unattended sessions). Training compliance at attended sessions was 99.7% for high-load (7.1 ± 18.2 uncompleted repetitions) and 99.3% for moderate-load (4.3 ± 9.6 uncompleted repetitions). There were no reported side-effects or adverse events as a result of the training intervention. A total of 44 of the 45 enrolled participants completed the post-intervention testing.

Baseline characteristics are detailed in Table 1. As expected, no differences in the distribution of age ($p = 0.28$), *APOE* ε4 allele carriers ($\chi^2 = 0.14$), or sexes ($\chi^2 = 0.21$) between groups. Serum BDNF levels were

Table 1 Baseline characteristics of high-load (HL), moderate-load (ML), and non-exercising control (CON) groups

Variable	CON ($n = 15$) Mean (SD)	HL ($n = 14$) Mean (SD)	ML ($n = 15$) Mean (SD)	Difference (p)
Age (year)[a]	59.1 (7.4)	55.2 (6.8)	58.0 (5.5)	0.28
APOE ε4, % (n)[a]	46.6 (7)	42.8 (6)	40.0 (6)	0.93
Female [(n) %][a]	80.0 (12)	85.7 (12)	80.0 (12)	0.90
Height (cm)	164.2 (9.3)	166.2 (4.6)	167.0 (9.0)	0.64
Mass (kg)	73.4 (13.3)	71.5 (13.0)	69.2 (12.0)	0.66
BMI (kg•m^{-2})	27.3 (4.4)	25.9 (4.2)	24.9 (4.1)	0.30
Serum BDNF (pg ml^{-1})	28,307.3 (7313.8)	22,325.2 (5623.9)	22,272.8 (6125.1)	**0.03**
Serum VEGF (pg ml^{-1})	116.0 (138.2)	94.7 (70.6)	77.7 (69.2)	0.63
Serum IGF-1 (pg ml^{-1})	1311.7 (932.0)	1973.4 (1710.1)	1525.4 (799.8)	0.39
Serum IGF-1/IGFBP-3, ratio	0.043 (0.030)	0.058 (0.044)	0.050 (0.027)	0.57
Plasma homocysteine (μmol L^{-1})	10.1 (3.4)	10.7 (2.7)	10.8 (2.2)	0.80
Platelets (10^9 L^{-1})	286.0 (62.8)	242.9 (44.8)	250.0 (64.7)	0.13
1RM bench press (kg)	29.6 (12.2)	31.3 (10.8)	30.3 (14.7)	0.93
1RM leg press (kg)	114.2 (43.8)	107.0 (40.7)	102.7 (36.5)	0.73
1RM lat pulldown (kg)	38.2 (11.1)	37.1 (9.5)	39.5 (15.3)	0.87
1RM leg curl (kg)	49.5 (16.2)	48.4 (15.4)	44.2 (16.2)	0.64
Total fat mass (kg)	24.6 (8.0)	22.6 (7.1)	21.5 (7.8)	0.54
Total lean mass (kg)	42.8 (8.7)	42.8 (7.9)	41.8 (9.2)	0.93
Appendicular lean mass (kg)	19.6 (5.0)	19.4 (4.0)	19.1 (5.2)	0.96
IPAQ MET (min week^{-1})	3073.8 (1621.3)	3858.3 (2270.3)	3006.3 (2340.4)	0.51

Differences across groups were determined by ANOVA for continuous variables and Chi-square for categorical variables. Bold indicates statistical significance ($p < 0.05$)

1RM one-repetition maximum, *APOE* Apolipoprotein E, *BDNF* brain-derived neurotrophic factor, *BMI* body mass index, *IGF-1* insulin-like growth factor 1, *IGFBP-3* insulin-like binding protein 3, *IPAQ* International Physical Activity Questionnaire, *MET* metabolic equivalent of task, *SD* standard deviation, *VEGF* vascular endothelial growth factor

[a]Included as stratifying variables for randomization by minimization

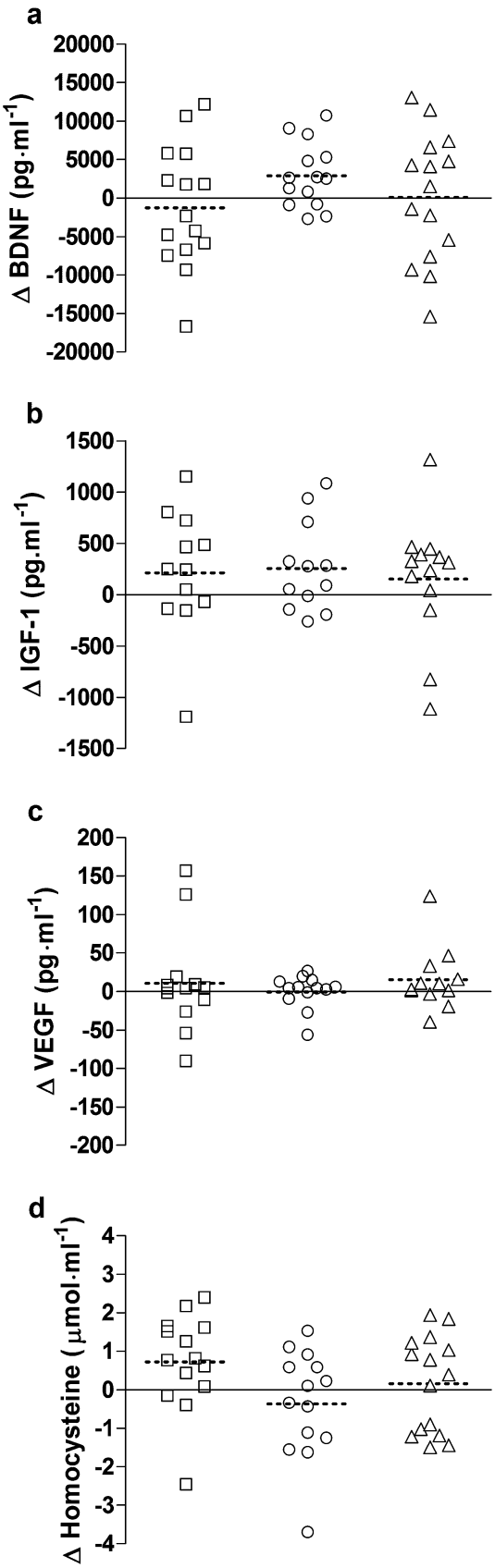

Fig. 2 Individual baseline to post-intervention change about the mean (dashed lines) for **a** brain-derived neurotrophic factor (BDNF), **b** insulin-like growth factor 1 (IGF-1), **c** vascular endothelial growth factor (VEGF), and **d** homocysteine in control (unfilled square), high-load (unfilled circle), and moderate-load (unfilled triangle) groups

significantly different between groups at baseline; however, post hoc comparisons indicated trend-level differences in the control group when compared to high-load ($p = 0.07$) and moderate-load ($p = 0.06$) groups. There were no differences in baseline 1RM strength for bench press, leg press, lat pulldown, or leg curl across groups. There were no differences in total fat mass, total lean mass, or total appendicular lean mass between groups at baseline.

The values for change in maximal strength are reported in detail elsewhere (Marston et al. 2019). Pre-to-post-intervention change in 1RM strength was greater ($p < 0.01$) in moderate-load (change: bench press = 25.0 ± 12.0%; leg press = 35.3 ± 13.9%, lat pulldown = 15.5 ± 9.0%, leg curl = 27.8 ± 15.7%), and high-load (change: bench press = 33.1 ± 11.6%; leg press = 40.7 ± 23.4%, lat pulldown = 23.3 ± 10.5%, leg curl = 17.5 ± 11.2%) groups for all exercises when compared to the control group (change: bench press = − 0.2 ± 6.2%; leg press = 5.5 ± 5.0%, lat pulldown = − 0.6 ± 7.8%, leg curl = − 0.4 ± 9.5%). There were no differences observed for change in 1RM strength for any exercises between high-load and moderate-load groups. The values for change in body composition are reported elsewhere (Marston et al. 2019). Baseline-to-post-intervention change was not different between groups in total fat mass ($p = 0.87$), total lean mass ($p = 0.51$), or appendicular lean mass ($p = 0.34$).

Self-reported habitual physical activity levels were not different between groups at baseline (Table 1). Baseline-to-post-intervention change in physical activity was not different ($p = 0.63$) between high-load (− 666.4 ± 284.1 MET-min•week^{-1}), moderate-load (− 325.6 ± 272.3 MET-min•week^{-1}), or control (− 342.9 ± 271.9 MET-min•week^{-1}) groups.

Baseline-to-post-intervention change in levels of BDNF, IGF-1, VEGF, and homocysteine are represented in Fig. 2. No group differences were observed in baseline-to-post-intervention change in serum BDNF ($p = 0.56$; Fig. 2a), IGF-1 ($p = 0.49$; Fig. 2b), VEGF ($p = 0.75$; Fig. 2c), or plasma homocysteine ($p = 0.12$; Fig. 2d). Change in IGF-1/IGFBP-3-binding ratio was not different between groups ($p = 0.99$). An increase from baseline to post-intervention in mean serum BDNF was observed within the high-load group, with a medium-effect size (+ 12.9%; $g = 0.54$).

Discussion

The purpose of this study was to examine the influence of intensive and ecologically valid moderate-load (i.e., high physiological intensity) and high-load (i.e., moderate physiological intensity) resistance training for 12 weeks on change in resting growth factors and homocysteine levels in healthy late middle-aged adults. We hypothesized that 12 weeks of intensive resistance training would increase serum BDNF, IGF-1, VEGF, and reduced plasma homocysteine when compared to the control group. Furthermore, as an effect of greater physiological intensity, we hypothesized that a greater magnitude of change would be observed within the moderate-load when compared to high-load resistance training group. Regardless of physiological intensity, resistance training resulted in no significant change to fasted resting serum BDNF, IGF-1, VEGF, or plasma homocysteine.

Greater peripheral BDNF at rest is associated with enhanced neural growth and maintenance (Mattson et al. 2004), and slower cognitive decline in individuals with neurodegenerative disease (Laske et al. 2011); however, BDNF levels decline with age (Lommatzsch et al. 2005; Ziegenhorn et al. 2007). The use of exercise, both aerobic (Zoladz et al. 2008) and resistance (Coelho et al. 2012), has been suggested as a therapeutic method to increase peripheral BDNF; thus, slowing or stopping its decline. Within the current study, we observed no change in resting BDNF after 12 weeks of either a moderate-load, high physiological intensity (three sets of ten repetitions at 70% of 1RM, 60 s recovery between sets) or high-load, moderate physiological intensity (five sets of five repetitions at 85% of 1RM, 180 s recovery between sets) resistance training program in healthy older adults. Previously, Coelho et al.(2012) observed a 65.2% increase in plasma BDNF levels in pre-frail and frail older women following 10 weeks of knee flexion and knee extension exercise. Although not significant, we observed a 12.9% ($g = 0.54$) increase in resting BDNF in participants undertaking the high-load intervention. The difference in the magnitude of BDNF change between our study and that of Coelho et al. (2012) could be attributed to the differing baseline levels of BDNF. Currently, the normal range of peripheral levels of resting BDNF is unclear (e.g., ≈1500–30,900 pg•ml^{-1}) (Knaepen et al. 2010); yet, BDNF is typically 14-fold greater in serum than in plasma (Yoshimura et al. 2010). Whilst we utilized serum samples in the current study, when we used previous evidence (Yoshimura et al. 2010) to estimate baseline *plasma* BDNF levels for our study, they were purportedly more than 350% greater than those reported for the cohort recruited by Coelho and associates (our study: control = 2021.9 pg•ml^{-1}; moderate-load = 1590.9 pg•ml^{-1}; high-load = 1594.7 pg•ml^{-1}; compared to 353.3 ± 300.4 pg•ml^{-1} for Coelho et al.(2012)). It is possible that participants with already higher levels of BDNF at baseline (e.g., the participants within the current cohort) are less likely to demonstrate large-magnitude changes than those with lower levels. It is probable that the divergent age groups assessed, as well as assay-specific variations, account for the differences in baseline BDNF levels observed here compared to Coelho et al. However, since the majority of evidence in middle-aged and older adults remains inconclusive (Forti et al. 2015; Fragala et al. 2014; Levinger et al. 2008; Pereira et al. 2013), further study is warranted to explore the influence of intense resistance training on resting BDNF.

Greater peripheral IGF-1 concentrations at rest are associated with greater total cerebral volume (Westwood et al. 2014), and predict later-life cognitive function (Okereke et al. 2006). Consistent with a previous study (i.e., 10-week study) (Goekint et al. 2010), we observed no differences between groups for the change in serum IGF-1 following 12 weeks of intense resistance training. Although intensive, it is possible that total training volume (i.e., sets × repetitions) per session within the current study (moderate-load = 120 total repetitions; high-load = 100 total repetitions) was not high enough to stimulate greater IGF-1 expression. Indeed, previous studies that have reported increased IGF-1 levels in young and older adults prescribed intensive resistance training of 180 total repetitions or greater per session (Borst et al. 2001; Cassilhas et al. 2007; Tsai et al. 2015). As already discussed, however, high-volume resistance training in older adults is associated with poorer training compliance and greater risk of overtraining (Hunter et al. 2004; Mazzeo and Tanaka 2001). Therefore, an effective training stimulus that enhances resting levels of IGF-1 (i.e., highly intensive and high-volume) is likely to be an ineffective long-term training approach for older adults outside of a controlled laboratory environment.

As a potent stimulator of endothelial cell growth (Ferrara et al. 2003), VEGF is a downstream mediator of neurogenesis that promotes cognitive health (Fabel et al. 2003; Wang et al. 2011). In the current study, serum VEGF levels from baseline to post-intervention were not different between conditions. Few other studies have examined the resting VEGF response following longitudinal resistance training; therefore, our findings are adding to a small body of literature. Our findings are consistent with a single study observing no changes in plasma VEGF following 12 weeks of resistance training undertaken at least once per week in older females (Ogawa et al. 2010). However, load-intensity and inter-set recovery duration were not reported by the authors making it difficult to appraise the total training stimulus (Ogawa et al. 2010). Using evidence available from acute resistance

exercise studies, it is possible that high-repetition (i.e., ≥ 15 repetitions), lower load (i.e., 60–65% of 1RM), and short-recovery protocols influence VEGF levels by maximizing local muscle hypoxia and other factors (Ross et al. 2014). In the current investigation, it is possible the repetition ranges (i.e., 5–10 repetitions per set) and inter-set recovery durations, in combination, were inadequate to maximize VEGF expression and, in-turn, influence VEGF levels. However, careful consideration should be given to the benefits of high-repetition training in ageing populations. Strength declines by 1.4% per year from the age of 50 (Hurley and Roth 2000), representing a major factor affecting physical function. For instance, declining maximal muscle strength is indicated as a key risk factor for falls in ageing populations (Pijnappels et al. 2008). High-repetition resistance training at lower loads is less effective at developing maximal muscle strength and muscle hypertrophy when compared to lower-repetition, higher load resistance training (Campos et al. 2002), potentially limiting its practicality in an ageing population.

Finally, homocysteine is normally metabolized to the anti-oxidant glutathione, yet, accumulates with age (Herrmann et al. 1999), with hyperhomocysteinemia associated with increased risk of developing neurodegenerative disease (Clarke et al. 1998). Elevated homocysteine concentrations inhibit vascular tone, promote vascular dysfunction, and are associated with poorer cognitive function (Mattson and Shea 2003; Seshadri et al. 2002). We observed no change in plasma homocysteine following 12 weeks of moderate-load or high-load resistance training when compared to the control group. Our findings are consistent with a previous study reporting no change in serum homocysteine following 16 weeks of resistance training three times per week (Vital et al. 2016). Indeed, mean baseline homocysteine values reported by Vital et al. (2016) for the exercise group (12.5 ± 4.6 μmol·L^{-1}) were similar to the values observed in the moderate-load (10.8 ± 2.2 μmol·L^{-1}) and high-load (10.7 ± 2.8 μmol·L^{-1}) groups in the current study. Given that normal physiological concentrations of homocysteine are between 5.0 and 14.0 μmol·L^{-1} in adults (Ferri 2017), it is possible that there is no additive benefit of resistance training in individuals with already healthy homocysteine levels (Vital et al. 2016). Nevertheless, despite physiologically normal baseline homocysteine levels (low-intensity = 8.5 ± 2.4 μmol·L^{-1}; high-intensity = 9.2 ± 2.6 μmol·L^{-1}), Vincent et al. (2003) reported significant reductions in homocysteine (− 5.3%; $g = 0.20$–0.30) following 6 months of low- and high-intensity resistance training. These findings suggest that, in combination with higher extracellular homocysteine concentration at baseline, longer interventions may be necessary for exercise-induced homocysteine reductions.

We acknowledge a number of limitations within the current study. The use of a small sample size ($n = 14$–15 per group) and the recruitment of healthy participants may influence the generalizability of our findings. The measurement of BDNF, IGF-1, IGFBP-3, and VEGF using commercially available ELISA kits in the current study was associated with a moderate degree of variability and a small number of undetectable samples. Nevertheless, undetectable samples that were reanalyzed were found to remain outside detectable limits, and thus, we are confident in the validity of our biomarker measurements.

Conclusions

In healthy late middle-aged adults, ecologically valid and intensive moderate-load and high-load resistance training twice per week for 12 weeks does not significantly influence serum BDNF, IGF-1, VEGF, or plasma homocysteine. Although intensity is likely key to enhancing growth factor levels at rest, it is possible that highly intense protocols that are high in volume are necessary to enhance growth factors; however, this may not be a realistic method of resistance training in ageing adults. Furthermore, VEGF may only be responsive to high-repetition (i.e., > 15 repetitions), short-recovery resistance training; yet, this training method is less effective at developing muscular strength, an important consideration for maintaining health and independence in older adults. It remains unclear what resistance-training variables influence homocysteine; however, evidence from longer resistance-training interventions (i.e., > 6 months) indicates that changes in homocysteine levels occur slowly.

Acknowledgements The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. No external funding was utilized to conduct this research.

Author contributions KJM, BMB, SRRS, and JJP were involved in conception of the study. KJM and SYMT collected the data. KJM, SB, LW, and SML analyzed the data. All authors were involved in data interpretation, drafting of the manuscript, and approving the final version.

Funding BMB is supported by the NHMRC National Institute of Dementia Research (GNT1097105). SRRS is supported by a Bright-Focus Foundation Fellowship. RNM is the Founder and owns stock in Alzhyme.

Compliance with ethical standards

Conflict of interest All other authors declare no conflict of interest in the development and completion of this study.

Informed consent Participants were provided with written documentation of the possible risks and benefits related to their participation in this study and signed informed consent was obtained in writing.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Murdoch University Human Research Ethics Committee (#2016/052) and have, therefore, been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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