Effects of high-intensity interval training on the vascular and autonomic components of the baroreflex at rest in adolescents

Ricardo Oliveira1, Alan Barker1, Sascha Kranen1, Florian Debras2, Craig Williams1

1 – Children's Health and Exercise Research Centre, Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

2 – Astrophysics Group, University of Exeter, EX4 4QL Exeter, UK; Ecole Normale Supérieure de Lyon, CRAL, UMR CNRS 5574, 69364 Lyon Cedex 07, France

Corresponding author:

Professor Craig A. Williams

Children's Health and Exercise Research Centre, Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St Luke's Campus, Exeter, EX1 2LU.

Tel: 44 (0)1392 724890: Fax: 44 (0)1392 724726: Email: C.A.Williams@exeter.ac.uk

Abstract

Purpose: In a sample of healthy adolescents, we aimed to investigate the effects of high intensity interval exercise (HIIE) training and detraining on baroreflex sensitivity (BRS) and its vascular and autonomic components at rest **Methods:** Nineteen volunteers were randomly allocated to: 1) four weeks HIIE training performed three times per week; or 2) a control (CON) condition with no intervention for the same duration as HIIE training. PRE, POST and following two weeks of detraining (DET) resting supine heart rate and blood pressure were measured and a cross-spectral method (LFgain) was used to determine BRS gain. Arterial compliance (AC) was assessed as the BRS vascular component. LFgain divided by AC (LFgain/AC) was used as the autonomic determinant of BRS. **Results:** HIIE training was completed with 100% compliance. HIIE did not change resting LFgain (P=0.66; effect size (ES)=0.21), AC (P=0.44; ES=0.36) or LFgain/AC (P=0.68; ES=0.19) compared to CON. **Conclusion:** Four weeks of HIIE training does not change BRS and its autonomic and vascular determinant in a sample of healthy adolescents at rest.

Key-words: Randomised controlled trial, exercise intensity, blood pressure, detraining, youth

Introduction

Elevated blood pressure is positively associated with atherosclerotic progression in healthy youth (22). A mechanism underpinning the development of hypertension is an impaired cardiac baroreflex sensitivity (BRS). In adults decreased BRS at rest has been shown to predict hypertension over five years (5), and BRS impairment measured using spontaneous indices is also observed in adolescents with pre-hypertension (6, 10, 11). Exercise training has been shown to improve BRS in healthy adults. In this population, increases in BRS were observed after 12-weeks of high-intensity interval exercise (HIIE) training (9) but not following moderate-intensity continuous training of similar duration (7, 20), indicating that the intensity of exercise may be an important determinant of BRS adaptations. However, the influence of HIIE training on BRS in adolescents is currently unknown.

To better understand the role of HIIE training on BRS in youth, the BRS gain can be reliably estimated as the autonomic and the vascular components (29). Although the effects of exercise on the autonomic and vascular determinants of BRS in youth remains unknown, several observations indicate that HIIE has potential to increase the BRS autonomic component. For example, cross-sectional associations between vigorous intensity physical activity and resting autonomic function in adolescents have been reported (30), and a previous investigation has demonstrated significant increases in cardiac autonomic function following two weeks of HIIE in normotensive adolescents (1). Moreover, it has been recently shown that the autonomic compared to the vascular determinant of BRS is more responsive to acute HIIE and hyperglycaemia in healthy adolescents (27, 28). Therefore, it can be reasoned that resting cardiac BRS may be improved, thus reflecting increases in its autonomic determinant following HIIE training.

In contrast, it should also be considered that improvements in resting cardiac BRS in adolescents, via augmented vascular component, may not be observed following HIIE training. The vascular component can be assessed using common carotid artery (CCA) compliance (29). In adults, 12 weeks of aerobic training improved resting cardiac BRS that was positively associated with increases in CCA compliance (23). However, in youth whether training can improve CCA distensibility is as yet unclear due to an

already elevated CCA distensibility in 12-years old adolescents compared adults aged 21 years (18). Indeed, a physiological ceiling effect may exist in healthy arteries impeding further adaptations to CCA compliance following training (25). Altogether, these results imply that the vascular component of BRS is unlikely to improve following training. Additionally, a better understanding of training effects can be achieved by the imposition of a detraining period. For example, in adolescents improvements in resting HRV and arterial function at 24 hours were reversed after 72 hours following HIIE training cessation (1). These data show that detraining following HIIE training may reverse putative training-induced adaptations to resting cardiac BRS in youth.

The aim of this study was to investigate in healthy adolescents the effects of four weeks of HIIE training and two weeks of detraining on resting cardiac BRS and its autonomic and vascular determinants. We hypothesised that HIIE training would improve cardiac BRS due to a significant increase in its autonomic component with no observable effects on the vascular component. We also reasoned that improvements in cardiac BRS after training would be lost following two weeks of training cessation in healthy adolescents.

Methods

Participants

Twenty-one male adolescents volunteered to take part in this study. Participants were recruited using a convenient sample from local secondary schools. Assemblies were conducted to explain the risk, benefits and the protocol of the study. At the end of each assembly, envelopes containing the study details were distributed. A total of 70 envelopes were delivered to potential participants from which 21 were returned. All 21 volunteers who returned the envelopes were enrolled in the study and randomly allocated to either a control (CON) or a HIIE training group. Health screening questionnaires were completed before participation, and all volunteers were free of any contraindications affecting the cardiac autonomic and vascular systems, such as asthma, congenital heart disease, and hypertension (35). All adolescents who volunteered and their parents/carers provided signed assent and consent

forms, respectively. All procedures performed were approved by a local ethics committee (Ref No: 161207/B/02) and the study conformed to the standards set by the Declaration of Helsinki.

Study design

Participants performed four visits to the laboratories consisting of:

Visit 1 (familiarisation): Participants were familiarised to the procedures of the study followed by measurements of stature and body mass, body fat percentage (BF%), and maximum aerobic speed (MAS). To determine MAS for prescription of the HIIE training, a 20 m shuttle run test was performed. For this, participants ran back and forth to cones set 20 m apart with the speed guided using a pre-recorded audio cue. Speed increased by 0.5 $km \cdot h^{-1}$ at the end of each stage (19). The 20 m shuttle run test was chosen because it provides a reliable and valid assessment of the MAS (36) to guide all training intensity during HIIE training intervention. Heart rate (HR) was monitored (Polar Team2, Polar, Kempele, Finland) and maximum effort was considered when participants achieved a HR within 90% of age predicted maximum (i.e. 220 – age), displayed signs of subjective fatigue, and an unwillingness to continue the test despite strong verbal encouragement. For descriptive purposes, body fat percentage was determined using air displacement plethysmography (BodPod®, Concord, California, USA) and participants received a package containing adapted drawings of pubic hair development for self-assessment of maturity status (26).

Visit 2 (PRE): This visit took place 2-10 days following visit 1. Following an overnight fast, participants were transported to the laboratory with a car and completed baseline measures between 8-9 a.m. Before visit 2, participants were instructed to avoid formal exercise in the 48-hParticipants were fitted with a three-lead ECG and a finger cuff (Finometer PRO, Netherlands). The BRS protocol started after 10-min of supine rest in a temperature (21-24 ºC) and light controlled room. The BRS protocol consisted of the following: 1) two automatic measurements of brachial blood pressure were conducted using the return-to-flow method with a Finometer to obtain a brachial reconstructed blood pressure assessment (8); this device has been validated to monitor blood pressure in children (32); 2) after calibration, images of the

common carotid artery (CCA) were recorded for 15 cardiac cycles; and 3) participants were instructed to pace their breathing frequency at 12 cycles per min for five minutes (38-40). This breathing frequency was chosen because it increases autonomic modulation in adolescents (40), and we have recently shown using phase and coherence that the feedback nature of BRS is measured using this protocol (28). The procedures were completed in the described order and lasted ~20 min (including the 10-min rest preceding the protocol).

Visit 3 (POST): This visit took place four weeks following visit 2 and the training intervention. The procedures of visit 3 were identical to visit 2. To avoid possible effects of detraining, or the acute influences of the last training session, Visit-3 took place 48-h following the last training session for the HIIE condition. To match the time elapsed between data collection for the HIIE condition, Visit-3 for CON was always completed 48-h later and following four weeks after completion of Visit-1.

Visit 4 (detraining – DET): This visit took place two weeks following visit 3 for both CON and HIIE groups. This visit was identical to visits 2 and 3.

Group allocation

Group allocation was conducted by two researchers and participants were not present. The allocation procedures were completed following Visit-1. Participants were randomly allocated to either CON or HIIE group. For this, a simple randomisation was conducted by drawing 21 identical cards from a closed container. The cards were blindly assigned to each participants' codes that were inside 21 shuffled opaque envelopes. The group assignment was revealed after randomisation took place and participants and parents contacted to arrange Visit-2. Due to the nature of the intervention, participants were not blind to the conditions. Researchers were however, blinded for data handling and statistics for which code numbers were used.

Training intervention

Participants allocated to the HIIE group performed three training sessions per week for four weeks providing a total of 12 HIIE sessions. The HIIE sessions were performed in the morning at the school

sports hall. For HIIE sessions 1-6, participants performed eight bouts, for HIIE sessions 7-9, participants performed 10 bouts, and for HIIE sessions 10-12, participants performed 12 bouts of 1-min running, each interspersed by 75 s of recovery. During the 1-min running, participants continuously ran between two cones set apart to allow the speed to match participants 90% of MAS (i.e. the distance between the cones varied between participants). To pace individual speeds, at every six seconds (i.e. 10 times per minute) a sound cue was emitted to which participants should be at their individual cone. During recovery, participants performed one bout at ~ 4 $km \cdot h^{-1}$ between the cones and remained passive for the remaining 75 s of the recovery period. All training sessions were preceded by a 1-min warm up performed at 6-km/h. For the CON group, no intervention was performed. All participants in the present investigation kept their usual physical activity, exercise and physical education routines.

For all training sessions, HR was monitored (Polar team 2) and internal training load calculated using the Edward's training impulse (TRIMP) method (2). The time spent in five different HR zones was multiplied by 1-5, respectively. The zones were calculated as 1=50-60%; 2=60-70%; 3=70-80%; 4=80-90%; and 5=90-100% of peak HR obtained during the shuttle run test. This was used as a descriptive measurement of the participants' internal training load during the HIIE sessions and is presented in Table 1.

Baroreflex sensitivity

The BRS analysis procedures for the present study were performed according to previous paediatric work by our group (27-29) and others (3, 18) using validated (31), and reliable (29) methods. ECG and BP were recorded simultaneously at 1000 Hz (PowerLab, ADInstruments) and RR intervals and systolic blood pressure (SBP) data extracted and saved for later analysis. Ectopic beats were automatically identified and linear interpolation with a low filter was applied when <3% error was present (Kubios v3.0) (33). Systolic blood pressure and RR intervals were visually checked before data analysis. Integrated gain (LFgain) of BRS was determined from the final five minutes of the BRS protocol. For this purpose, beat-to-beat RR interval and brachial reconstructed systolic blood pressure were interpolated at 2 Hz, de-trended using a linear function and filtered using a Butterworth filter set to 0.95

Hz. A Fast-Fourier Transformation was then applied using a Welch method to obtain the power spectrum in the low frequency band (LF = 0.04 – 0.15 Hz) and a cross-spectral transfer function was then calculated to evaluate baroreflex gain, defined as the average of the cross-spectrum divided by the power spectrum of systolic blood pressure in the range where the coherence was > 0.5, hence expressed in $ms \cdot mmHg^{-1}$. LFgain was calculated using a laboratory devised programme in Python (https://www.python.org).

Vascular and autonomic determinants

For determination of the vascular and autonomic determinants of BRS, we used a previously described reliable protocol in adolescents (29). CCA images were recorded ~ 2 cm distal from the carotid bulb using a high-resolution (~ 13 MHz) linear array transducer (Apogee, 1000, SIUI, China). The images were obtained over 15 cardiac cycles recorded at 15 frames per second. Subsequently, CCA images were analysed using validated wall tracking software (Carotid Analyzer - Medical Imaging Applications LLC) (21) for determination of diastolic lumen diameter and systolic lumen diameter. The average of 3-7 cardiac cycles with clear definitions of the near and far intra-media thickness was used. During the 15 cardiac cycles, beat-to-beat brachial reconstructed blood pressure (8) was averaged and used to determine pulse pressure. The vascular components of BRS were determined according to previously published literature (17):

Arterial compliance – AC ($\mu m \cdot mmHg^{-1}$) = $\Delta D/PP$

Where ΔD is systolic lumen diameter minus diastolic lumen diameter, and pulse pressure is the obtained pulse pressure;

The autonomic and vascular determinants of BRS were determined according to a previous study (18). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

182 **Autonomic modulation**

183 Heart rate variability (HRV) was obtained in the five minutes when BRS was measured according to
184 published guidelines (34). For this, a Fast Fourier Transformation was applied and the area under the
185 low (HF: 0.15 to 0.50 Hz) and high frequency (HF: 0.15 to 0.50 Hz) were calculated in absolute units
186 (ms^2). The relative contribution of HF and LF were also obtained and expressed in normalised units
187 (nu), and the LF/HF ratio calculated. HF is known to reflect parasympathetic modulation whilst LF
188 reflects overall autonomic modulation (34). Finally, to avoid a possible saturation effect following
189 aerobic training (13), HF was divided by HR to obtain an autonomic modulation measure normalized
190 by HR.

191 **Statistical analyses**

192 Based on the between-day coefficient of variation of the LFgain/AC of 20% a sample size of 9
193 participants would be required to obtain a large effect size with a power of 0.80 and an alpha of 0.05,
194 as previously discussed (29).

195 Data are presented as mean and standard deviation unless otherwise stated. Normal distribution was
196 investigated using Shapiro Wilk's test and log transformation performed when appropriate. To compare
197 the effects of training on the resting (Baseline) measures, a series of univariate analysis was performed.
198 For this, delta changes (POST-PRE) were calculated and inserted in the model as the dependent variable.
199 Group (HIIE or CON) was inserted as fixed factor and the baseline measures (PRE) used as covariate
200 to control for baseline differences between the groups. Results from univariate analysis are presented
201 as mean and standard error adjusted to the corresponding PRE values. The group effect was then
202 obtained, and effect sizes calculated for the between groups comparisons after adjustments for the
203 baseline values. Effect sizes were interpreted as <0.2 (trivial), ≥0.2 (small), ≥0.5 (moderate) and ≥0.8
204 (large) (4). To compare the effects of detraining, a similar approach was used only when a training effect
205 was obtained. For this delta changes (DET-POST) were inserted as dependent variable, group as fixed
206 factor, and the POST measures as covariate. SPSS was used for all analysis, and $P<0.05$ was considered
207 statistically significant.

Results

From the initial 21 participants, two dropped out after visit 2 for reasons unrelated to the study (one participant dropped out due to illness and the other for personal reasons). The final sample size included in the analysis was 19. Participants' descriptive characteristics are presented in Table 2. Training compliance was 100% for the HIIE sessions and no adverse effects were reported. Table 1 shows a constant increase in training load and average HR in each training session over the four weeks.

HIIE training and detraining on resting measurements

Changes in resting BRS and its autonomic and vascular determinants are presented in Figure 1. There were no effects of HIIE training for LFgain (adjusted change in means CON=-0.01, HIIE=1.4 $ms \cdot mmHg^{-1}$; *P*=0.66; ES=0.21), AC (CON=2.4, HIIE=0.9 $\mu m \cdot mmHg^{-1}$; *P*=0.44; ES=0.36), and LFgain/AC (CON=-0.02, HIIE=0.09 $ms \cdot \mu m^{-1}$; *P*=0.68; ES=0.19).

Figure 1 here

Resting cardiac autonomic modulation and blood pressure is presented in Table 3. There were no effects of training on vagal related HRV measures: HF (adjusted change in means CON=-0.01, HIIE=0.13 ms^2; *P*=0.58; ES=0.26), HF/HR (CON=0.001, HIIE=0.011 $ms^2 \cdot bpm^{-1}$; *P*=0.20; ES=0.61). Similarly, no effects between groups for changes in HR (CON=1, HIIE=-4 bpm; *P*=0.10; ES=0.80), SBP (CON=-2.3, HIIE=-2.4 mmHg; *P*=0.97; ES=0.01), or DBP (CON=1.8, HIIE= -3.7 mmHg; *P*=0.20; ES=0.62) were observed.

Discussion

This is the first randomised controlled trial to investigate the effects of four weeks of HIIE training and detraining on BRS and its autonomic and vascular determinants in healthy adolescents. The main findings of the present study were: 1) there was no effect of HIIE training on resting cardiac BRS and its autonomic and vascular determinants; and 2) because no effect of training was observed, detraining did not influence any of the outcomes.

Our present findings showed that resting BRS does not change after four weeks of HIIE training in healthy adolescents. The lack of adaptations following HIIE training in the present investigation is an interesting observation given previous work in adults that reported improvements in BRS following training. For instance, significant BRS improvements were observed after 12-weeks of repeated sprint training in overweight adults (9), and one investigation including older adults (56 years old), increases in BRS were observed following 12 weeks of aerobic training at 65% of maximal aerobic capacity (23). Several differences exist between the present study and the cited literature, which may explain the different results. For example, the training characteristics such as intensity (i.e. moderate, and sprints) and duration (12-weeks) were different than that used in the present investigation. Moreover, the participants in the cited investigations were elderly (23) and overweight adults (9) who present lower BRS compared to young healthy adolescents (16, 18). Indeed, LFgain in the present investigation (CON = 23.1 ± 10.7; HIIE = 21.8 ± 6.12 $ms \cdot mmHg^{-1}$ at PRE) is higher compared to data in 12-years old adolescents (e.g. ~ 8 $ms \cdot mmHg^{-1}$) (18) but comparable to adolescents of similar age (3) and previous work from our group (27-29). These studies and the present investigation indicate that in contrast to our hypothesis, BRS does not improve with HIIE training in healthy adolescents. Future studies are encouraged to investigate the effects of HIIE training on BRS in adolescents with conditions known to decrease BRS such as hypertension and obesity (11).

A novel aspect of the present investigation was to investigate the influence of HIIE training on the autonomic and vascular determinants of BRS. Regarding the vascular component, no effects of HIIE were observed following 4-weeks of training. This finding is different to training studies involving adults when improvements in BRS and AC have been reported. For example, Monahan, Tanaka (24) showed a positive association between increases in CCA compliance and increases in BRS following training in healthy adults. The lack of increases in CCA compliance in the current study shows that HIIE training does not alter this parameter at rest, and corroborates the recent hypothesis of a ‘ceiling effect’ in arteries of healthy adolescents, which present an optimal CCA compliance (25).

No significant improvements were observed in the autonomic determinant of BRS estimated as the LFgain/AC. Although no investigation in adolescents exists to compare our findings, a cross-sectional investigation involving older adults has shown no effect of training status on the autonomic determinant of BRS (23). Conversely, other cross-sectional data highlights that the autonomic determinant of BRS is higher in participants with a higher training status (15). Contrasting the present investigation with the cited literature is challenging due to the cross-sectional design of the cited studies (15, 23), the study population (i.e. adults, older adults) (23), and the methods used to measure the autonomic component of BRS (i.e. vasoactive drugs, Valsalva manoeuvre, and spontaneous indices) (15, 23).

In the present study, no effects of HIIE intervention was noted on resting autonomic modulation measured via HRV, which corroborates with the lack of improvements on the autonomic determinant of BRS. This is a surprising finding, as in a sample of similar healthy adolescents, two weeks of HIIE caused significant increases in vagal-related HRV (1). Although a lack of a control group impedes conclusions about the training effect in the previous work, a possible explanation for the differences between our present findings and Bond, Cockcroft (1), may lie in the likely presence of saturation of vagal modulation in the present study. A saturation represents a HR point at which no more improvements in HRV can be observed (14). In our present investigation, a trend was observed for a decrease in resting HR with a large effect size between CON and HIIE (P=0.10; ES=0.80). We further normalised HF according to HR to decrease the saturation effects and a moderate effect size, although not significant, was then observed between HIIE and CON (ES=0.61). It is recognised that 24 hours HRV analysis is usually required to obtain a measurement of saturation (13); however the lack of improvements in HRV in the present investigation should be interpreted with caution. Finally, it is currently unknown whether increases in vagal modulation would reflect a better cardiac BRS.

Several strengths of the present investigation should be noted including the randomised controlled trial design. Similarly, compliance with the HIIE training was excellent (100%) and we carefully controlled exercise intensity using direct measures of internal responses to exercise (i.e. TRIMP), which was progressively increased (see Table 1). We also performed a comprehensive analyses of the autonomic

283 and vascular determinants of the BRS using reliable methods (29). Another strength of the present
284 investigation was the timing between the end of HIIE training and the post-training measurements. To
285 avoid possible detraining or acute effects of the last bout on the autonomic and arterial systems,
286 participants were tested 48-h after the last training session.

287 Several limitations are worth reporting. For example, the convenience sampling approach limits the
288 findings to a specific sample of adolescents in terms of fitness levels. For example, using reference
289 values Tomkinson et al., (2017) (37) all but one of the participants had MAS > than the 60th percentile
290 (CON: p90 n=2, p80 n=1, p70 n=3, p60=2 and p20=1; HIIE: p90 n=3, p80 n=3, p70=3, and p60=1).
291 However, as most of participants in our sample had fitness levels >p60 for their age the influence of
292 fitness cannot be properly addressed. Moreover, due to the lack of fitness assessment following the
293 training intervention it is not possible to know whether training increased fitness and whether fitness
294 could act as a potential mediator of BRS changes with training. Similarly, we could not totally control
295 for the exercise activities undertaken by the participants outside the CON and HIIE interventions. It is
296 likely that participants were involved in some exercise routines, which increased the overall training
297 load during the four weeks and a ceiling effect was likely present for the adaptations of the autonomic
298 system, as previously described (12). Another limitation is that for the measures of arterial compliance
299 BP was not obtained at the CCA which may not adequately represent the distensile force experienced
300 by the baroreceptors located in the carotid bulb. Finally, caution should be taken when interpreting the
301 results due to the small sample size in the present study, which is powered (80%) to find a significant
302 (alpha 0.05) for large effect sizes.

303 **Conclusions**

304 A four-week HIIE intervention did not change resting BRS and its autonomic and vascular determinants
305 in a sample of healthy adolescents. Our findings highlight that in healthy adolescents, any training
306 benefits derived from HIIE does not change the mechanisms of beat-to-beat control of BP at rest.
307 Investigations using other training strategies (i.e. > 4 weeks) and targeted groups (i.e. obese or pre-
308 hypertensive) are warranted.

References

1. Bond B, Cockcroft EJ, Williams CA, Harris S, Gates PE, Jackman SR, et al. Two weeks of high-intensity interval training improves novel but not traditional cardiovascular disease risk factors in adolescents. Am J Physiol. 2015;309(6):H1039-47.

2. Borresen J, Lambert MI. The quantification of training load, the training response and the effect on performance. Sports Med. 2009;39(9):779-95.

3. Chirico D, Liu J, Klentrou P, Shoemaker JK, O'Leary DD. The effects of sex and pubertal maturation on cardiovagal baroreflex sensitivity. J Pediatr. 2015;167(5):1067-73.

4. Cohen J. Statistical Power Analysis for the Behavioral Sciences: Academic Press; 1977. 567 p.

5. Ducher M, Fauvel JP, Cerutti C. Risk profile in hypertension genesis: A five-year follow-up study. Am J Hypertens. 2006;19(8):775-80.

6. Fitzgibbon LK, Coverdale NS, Phillips AA, Shoemaker JK, Klentrou P, Wade TJ, et al. The association between baroreflex sensitivity and blood pressure in children. Appl Physiol Nutr Metab. 2012;37(2):301-7.

7. Goldberg MJ, Boutcher SH, Boutcher YN. The effect of 4 weeks of aerobic exercise on vascular and baroreflex function of young men with a family history of hypertension. J Hum Hypertens. 2012;26(11):644-9.

8. Guelen I, Westerhof BE, van der Sar GL, van Montfrans GA, Kiemeneij F, Wesseling KH, et al. Validation of brachial artery pressure reconstruction from finger arterial pressure. J Hypertens. 2008;26(7):1321-7.

9. Heydari M, Boutcher YN, Boutcher SH. High-intensity intermittent exercise and cardiovascular and autonomic function. Clin Auton Res. 2013;23(1):57-65.

10. Honzikova N, Fiser B. Baroreflex sensitivity and essential hypertension in adolescents. Physiol Res. 2009;58(5):605-12.

11. Honzikova N, Zavodna E. Baroreflex sensitivity in children and adolescents: physiology, hypertension, obesity, diabetes mellitus. Physiol Res. 2016;65(6):879-89.

12. Iwasaki K, Zhang R, Zuckerman JH, Levine BD. Dose-response relationship of the cardiovascular adaptation to endurance training in healthy adults: how much training for what benefit? J Appl Physiol. 2003;95(4):1575-83.

13. Kiviniemi AM, Hautala AJ, Makikallio TH, Seppanen T, Huikuri HV, Tulppo MP. Cardiac vagal outflow after aerobic training by analysis of high-frequency oscillation of the R-R interval. Eur J Appl Physiol. 2006;96(6):686-92.

14. Kiviniemi AM, Hautala AJ, Seppanen T, Makikallio TH, Huikuri HV, Tulppo MP. Saturation of high-frequency oscillations of R-R intervals in healthy subjects and patients after acute myocardial infarction during ambulatory conditions. Am J Physiol. 2004;287(5):H1921-7.

15. Komine H, Sugawara J, Hayashi K, Yoshizawa M, Yokoi T. Regular endurance exercise in young men increases arterial baroreflex sensitivity through neural alteration of baroreflex arc. J Appl Physiol. 2009;106(5):1499-505.

16. Kornet L, Hoeks AP, Janssen BJ, Houben AJ, De Leeuw PW, Reneman RS. Neural activity of the cardiac baroreflex decreases with age in normotensive and hypertensive subjects. J Hypertens. 2005;23(4):815-23.

17. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588-605.

18. Lenard Z, Studinger P, Mersich B, Kocsis L, Kollai M. Maturation of cardiovagal autonomic function from childhood to young adult age. Circulation. 2004;110(16):2307-12.

19. Liu NY, Plowman SA, Looney MA. The reliability and validity of the 20-meter shuttle test in American students 12 to 15 years old. Res Q Exerc Sport. 1992;63(4):360-5.

20. Loimaala A, Huikuri H, Oja P, Pasanen M, Vuori I. Controlled 5-mo aerobic training improves heart rate but not heart rate variability or baroreflex sensitivity. J Appl Physiol. 2000;89(5):1825-9.

21. Mancini GB, Abbott D, Kamimura C, Yeoh E. Validation of a new ultrasound method for the measurement of carotid artery intima medial thickness and plaque dimensions. Can J Cardiol. 2004;20(13):1355-9.

22. McGill HC, McMahan CA, Zieske AW, Malcom GT, Tracy RE, Strong JP, et al. Effects of nonlipid risk factors on atherosclerosis in youth with a favorable lipoprotein profile. Circulation. 2001;103(11):1546-50.

23. Monahan KD, Dinenno FA, Seals DR, Clevenger CM, Desouza CA, Tanaka H. Age-associated changes in cardiovagal baroreflex sensitivity are related to central arterial compliance. Am J Physiol. 2001;281(1):H284-9.

24. Monahan KD, Tanaka H, Dinenno FA, Seals DR. Central arterial compliance is associated with age- and habitual exercise-related differences in cardiovagal baroreflex sensitivity. Circulation. 2001;104(14):1627-32.

25. Montero D, Breenfeldt-Andersen A, Oberholzer L, Haider T. Effect of Exercise on Arterial Stiffness: Is There a Ceiling Effect? Am J Hypertens. 2017;30(11):1069-72.

26. Morris NM, Udry JR. Validation of a self-administered instrument to assess stage of adolescent development. J Youth Adolesc. 1980;9(3):271-80.

27. Oliveira R, Barker AR, Debras F, O'Doherty A, Williams CA. Mechanisms of blood pressure control following acute exercise in adolescents: Effects of exercise intensity on haemodynamics and baroreflex sensitivity. Exp Physiol. 2018;103(8):1056-66.

28. Oliveira RS, Barker AR, Debras F, Kranen SH, Williams CA. Effects of exercise intensity on vascular and autonomic components of the baroreflex following glucose ingestion in adolescents. Eur J Appl Physiol. 2019;119(4):867-78.

29. Oliveira RS, Barker AR, Debras F, O'Doherty A, Williams CA. Reliability of autonomic and vascular components of baroreflex sensitivity in adolescents. Clin Physiol Funct Imaging. 2018;38(6):986-93.

384 30. Oliveira RS, Barker AR, Williams CA. Cardiac Autonomic Function, Cardiovascular Risk and
385 Physical Activity in Adolescents. Int J Sports Med. 2018;39(2):89-96.
386 31. Robbe HW, Mulder LJ, Ruddel H, Langewitz WA, Veldman JB, Mulder G. Assessment of
387 baroreceptor reflex sensitivity by means of spectral analysis. Hypertension. 1987;10(5):538-43.
388 32. Tanaka H, Thulesius O, Yamaguchi H, Mino M, Konishi K. Continuous non-invasive finger
389 blood pressure monitoring in children. Acta Paediatr. 1994;83(6):646-52.
390 33. Tarvainen MP, Niskanen J-P, Lipponen JA, Ranta-aho PO, Karjalainen PA. Kubios HRV –
391 Heart rate variability analysis software. Comput Methods Programs Biomed. 2014;113(1):210-20.
392 34. Task-Force. Heart rate variability: standards of measurement, physiological interpretation, and
393 clinical use. Circulation. 1996;93(5):1043-65.
394 35. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate
395 variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141(2):122-31.
396 36. Tomkinson GR, Lang JJ, Blanchard J, Léger LA, Tremblay MS. The 20-m Shuttle Run:
397 Assessment and Interpretation of Data in Relation to Youth Aerobic Fitness and Health. Pediatr Exerc
398 Sci. 2019;31(2):152-63.
399 37. Tomkinson GR, Lang JJ, Tremblay MS, Dale M, LeBlanc AG, Belanger K, et al. International
400 normative 20 m shuttle run values from 1 142 026 children and youth representing 50 countries. British
401 Journal of Sports Medicine. 2017;51(21):1545-54.
402 38. Tzeng YC. The Role of Ultrasonography in the Assessment of Arterial Baroreflex Function. In:
403 Ainslie PN, editor. Applied Aspects of Ultrasonography in Humans: InTech; 2012.
404 39. Tzeng YC, Sin PY, Lucas SJ, Ainslie PN. Respiratory modulation of cardiovagal baroreflex
405 sensitivity. J Appl Physiol (1985). 2009;107(3):718-24.
406 40. Williams CA, Lopes P. The influence of ventilatory control on heart rate variability in children.
407 J Sports Sci. 2002;20(5):407-15.

408

Figure legends

Figure 1: Individual data of baroreflex sensitivity and its autonomic and vascular determinants at baseline, post training, and at detraining.

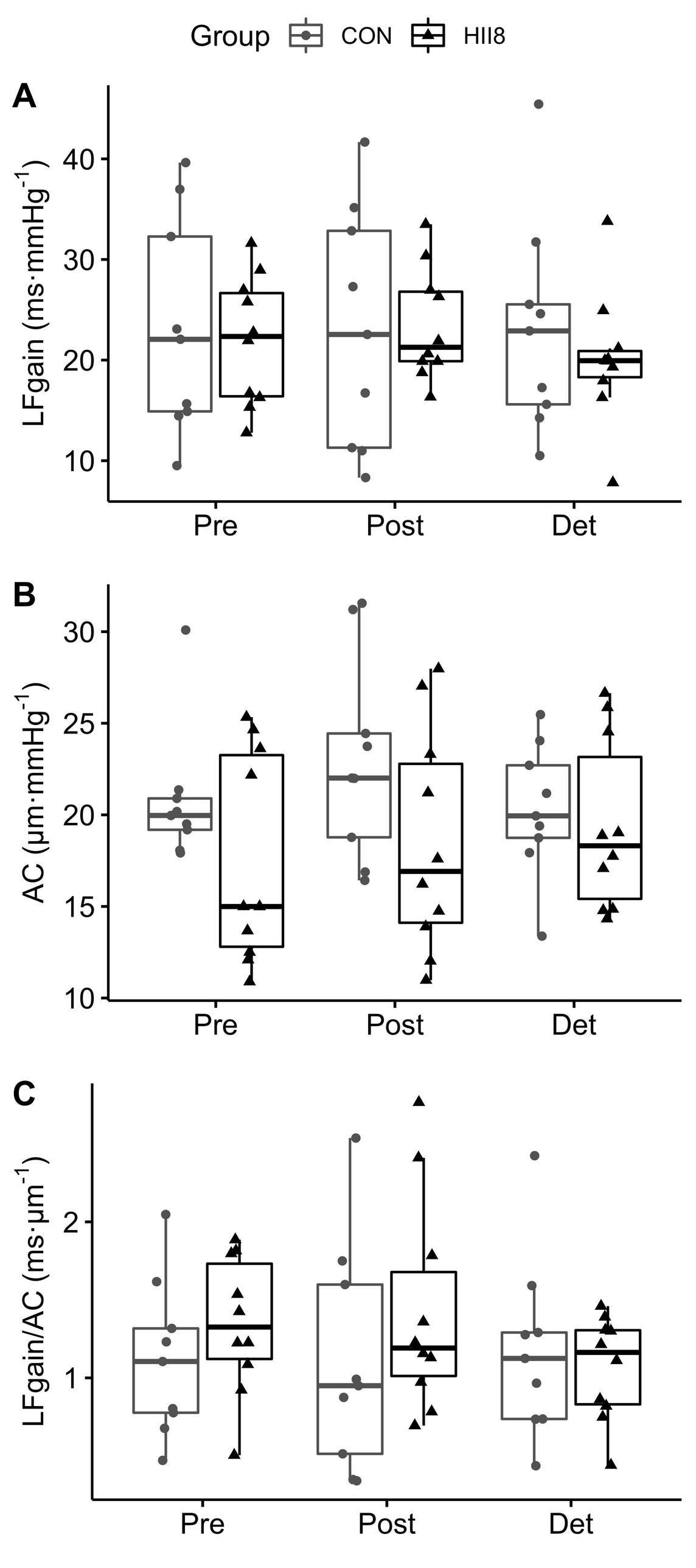
Group
CON
HII8
A
LFgain (ms·mmHg-1)
40
30
20
10
Pre
Post
Det
B
AC (μm·mmHg-1)
30
25
20
15
10
Pre
Post
Det
C
LFgain/AC (ms·μm-1)
2
1
Pre
Post
Det

Table 1: Mean and standard deviation of the observed training load and heart rate profile during the 12 training sessions

Training session	Internal training Load (AU)	Average HR (bpm)	Average HR (% of max)	Peak HR (bpm)	Peak HR (% of max)
First	72.3±8.6	157±12	76.9±4.6	197±9	96.8±2.2
Second	75.2±9.2	161±11	79.1±3.3	199±11	97.9±2.5
Third	75.1±8.5	162±10	79.4±3.5	198±6	96.7±2.7
Fourth	75.5±8.9	161±10	79.2±3.7	196±8	96.2±2.8
Fifth	73.7±7.7	159±7	77.9±3.2	196±7	96.1±2.4
Sixth	70.3±8.7	158±9	77.6±3.6	194±7	95.1±2.7
Seventh	87.1±7.8	161±7	78.9±3.0	196±7	96.1±2.2
Eighth	81.4±6.9	158±8	77.5±3.1	193±8	94.9±2.7
Ninth	81.7±9.8	155±11	75.9±3.8	193±9	94.7±3.1
Tenth	101.3±7.7	160±8	78.0±2.8	197±9	95.9±2.8
Eleventh	99.6±11.4	159±10	77.0±3.7	194±7	94.5±2.1
Twelfth	98.8±9.4	159±8	78.0±2.9	194±7	95.3±1.7

HR: heart rate. Bpm: beats-per-minute

Table 2: Participants characteristics.

	Pre		Post		Detraining	
	CON (n=9)	HIIE (n=10)	CON (n=9)	HIIE (n=10)	CON (n=9)	HIIE (n=10)
Stature (cm)	164.1±9.8	159.3±8.6	166.6±10.4*	161.1±8.7*	166.6±10.3*	161.1±8.7*
Body Mass (kg)	50.1±8.8	44.4±6.2	50.2±8.7	45.0±6.2	50.8±8.8	45.2±6.1
Fat mass (%)	21.6±7.5	18.0±7.3	21.9±9.1	18.4±6.2	21.3±7.9	17.6±6.4
Tanner	1=0 2=1 3=4 4=4 5=0	1=1 2=4 3=1 4=3 5=0	–	–	–	–
MAS ($km \cdot h^{-1}$)	11.8±0.9	12.2±0.5	–	–	–	–
Age (y)	13.2±0.5	13.3±0.5				

HIIE: high-intensity exercise. CON: control. MAS: maximal aerobic speed. *P<0.05 compared to PRE.

Table 3: Mean and standard deviation of autonomic modulation at pre, post and detraining for both groups.

	Pre		Post		Detraining	
	CON (n=9)	HIIE (n=10)	CON (n=9)	HIIE (n=10)	CON (n=9)	HIIE (n=10)
HR (bpm)	63±6	60±8	62±8	57±5	60±7	59±7
HF (ln)	8.7±0.9	8.6±0.8	8.7±0.9	8.7±0.8	8.7±0.8	8.7±0.8
HF adjusted HR (a.u.)	0.14±0.03	0.14±0.02	0.14±0.03	0.16±0.02	0.15±0.03	0.15±0.02
LF (ln)	7.3±0.5	7.0±0.7	7.7±1.03	7.4±0.7	7.8±0.6	7.4±0.8
Total power (ln)	16.0±1.3	15.6±1.4	16.4±1.9	16.2±1.2	16.5±1.3	16.1±1.4
HF (nu)	79.6±7.2	81.6±10.7	72.7±9.7	76.5±11.8	70.3±9.6	76.2±11.3
LF (nu)	20.3±7.3	18.1±10.7	27.2±9.8	23.4±11.9	29.6±9.7	23.5±11.4
LF/HF (a.u.)	0.26±0.10	0.25±0.19	0.40±0.19	0.34±0.24	0.44±0.18	0.34±0.23
SBP (mmHg)	111.1±6.3	111.8±7.1	108.9±11.0	109.3±7.5	110.2±12.3	110.2±11.0
DPB (mmHg)	64.9±8.2	60.1±7.3	65.9±11.9	57.2±7.1	60.7±7.9	61.8±8.1

CON: Control group. HIIE: Training group. HR: heart rate. HF: high frequency. LF: low frequency.