Title: The impact of an endurance training programme on exercise-induced cardiac biomarker release

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Running head: Endurance training and cardiac biomarker

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ABSTRACT

We evaluated the influence of a 14 week endurance running programme on the exercise-induced release of high-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP). Fifty-eight untrained participants were randomised to supervised endurance exercise (14 weeks, 3-4 days/week, 120-240 min/week, 65-85% of maximum heart rate) or a control group. At baseline and after the training programme hs-cTnT and NT-proBNP were assessed before and 5 min, 1 h, 3 h, 6 h, 12 h, and 24 h after a 60 min maximal running test. Before training hs-cTnT was significantly elevated in both groups with acute exercise ($P < 0.0001$) with no between group differences. There was considerable heterogeneity in peak hs-cTnT concentration with the upper reference limit exceeded in 71% of the exercise tests. After training both baseline and post-exercise hs-cTnT were significantly higher compared to pre-training and the response of the control group ($P = 0.008$). Acute exercise led to a small but significant increase in NT-proBNP but this was not mediated by training ($P = 0.121$). In summary, a controlled endurance training intervention resulted in higher pre- and post-exercise values of hs-cTnT with no changes in NT-proBNP.

Keywords: hs-cTnT, NT-proBNP, exercise, endurance training

INTRODUCTION

Exercise promotes the elevation of serum troponins (cTnI and cTnT) and N-terminal pro-BNP (NT-proBNP), which are accepted parameters for the identification of cardiac damage and dysfunction. The post-exercise values of these biomarkers are often higher than the population's upper reference limit (URL; 27, 30).

The clinical significance of the increase in cardiac-specific biomarkers after exercise in healthy subjects is unclear. Consequently, it is of interest to determine what personal or environmental factors mediate the exercise-induced release of cardiac biomarkers. One specific issue of interest has been the importance of training status. To date cross-sectional studies have been inconsistent. Neilan et al. (20) reported higher values for cTnT and NT-proBNP in less well-trained runners after a marathon and this has been supported by others (5, 15, 19, 21, 39). Conversely, others have found no association between training status and biomarker release (4, 9, 27, 30, 38), and recent controlled and field based studies have even suggested higher values of hs-cTnT in those with high levels of training (unpublished, Legaz-Arrese et al., 24). Data for NT-proBNP are also equivocal with respect to training status (8, 25, 27, 30, 38).

These discrepancies may be largely based on the limitations associated with cross-sectional studies and the lack of control in field-based competitive studies as well as the number and timing of blood draws post-exercise. To date there are no longitudinal training interventions that have assessed exercise-induced cardiac biomarker release before and after an intervention. Consequently, we employed a randomized, controlled trial to investigate the effects of a 14-week endurance running training programme, compared to a control group with no intervention, on the release of high-sensitivity cTnT (hs-cTnT) and NT-proBNP consequent to a 60 min running time trial. We

hypothesized that training would have no significant effect on exercise-induced release of cardiac biomarkers.

METHODS

Participants and Design

An invitation to participate in the study was sent to men and women (n = 323) between 18 and 45 years of age from two University Faculties and a local company (Fig. 1). Sixty-nine potentially eligible participants responded and requested more information and all provided written informed consent after the protocol was explained to them. The Committee on Biomedical Ethics of the Aragon Government approved this study.

Exclusion criteria were a significant personal or early family history of cardiovascular disease and/or abnormal electrocardiogram (ECG) at baseline examination. Further we excluded those already pursuing vigorous level of physical activity according to the self-administered, long format of the International Physical Activity Questionnaire (IPAQ) (3). The Spanish version (22) of the revised Physical Activity Readiness Questionnaire (PARQ) (35) was also administered to identify persons at risk for adverse events while exercising. Overall, 11 participants were excluded from the study because they performed vigorous physical activity on a regular basis according to the IPAQ and the remaining 58 participants were randomly assigned to either a training group or a control group according to a computer-generated randomization list (ratio 1.5:1). Seven participants in the training group (3 did not complete the exercise test; 4 did not attend<90% of training sessions) and 5 participants in the control group (2 did not complete the exercise test; 3 did not complete blood sampling) were not included in the final analysis. At completion of the study 28 subjects from the training group (22 men,

6 women) and 18 subjects from the control group (14 men, 4 women) were included in the intervention analysis.

Experimental procedures

All measurements were taken by examiners blinders to group assignment. During the first laboratory visit, subjects underwent anthropometric assessment. Body height was measured to the nearest 0.1 cm (SECA 225, SECA, Hamburg, Germany). Body mass was determined to the nearest 0.05 kg (SECA 861, SECA, Hamburg, Germany). Skinfold thickness was measured at the triceps, subscapular, supraspinal, abdominal, and front thigh and medial calf locations using Holtain skinfold callipers (Holtain, Crosswell, Crymych, UK). The fat percentage was obtained by the equation of Juhasz (41).

In the second and third visit to the laboratory two exercise sessions of, 30 and 45 min duration, were performed to accustom subjects to running and pacing exercise intensity on a treadmill. Participants refrained from strenuous exercise for 48 h prior to the fourth laboratory visit during which they performed a 60 min "all-out" running "time trial" on a treadmill (Run Exicite 700, Technogym, Italia). The same "resting" period was established post-training. Prior to the test, the subjects completed a self-paced 5-min warm-up (HR <130 beats/min). Pairs of subjects competed side-by-side to provide some motivation and competition and strong verbal encouragement was provided during the test. Subjects were constantly aware of the time and distance covered. Water intake was allowed *ad libitum*. HR was recorded continuously during the tests via a Polar HR monitor (Polar Electro Oy, Kempele, Finland). The distance run in each test was recorded every 10 min. Pacing strategy was defined as the mean running velocity every

10 min. Immediately after the test participants rated the test for perceived exertion (RPE) (1). Venous blood samples were taken before, immediately after (5 min), and 1, 3, 6, 12, and 24 h after exercise to assess serum hs-cTnT and NT-proBNP. All testing sessions occurred at 11:00 am in an air-conditioned laboratory with the temperature and relative humidity set at 20º C and 50%, respectively.

Exercise training

The 14-week endurance training programme followed a periodised progressive traditional design (10) consisting of five mesocycles with the programme divided into three training periods (Table 1). Briefly, in the preparatory period, the frequency and duration of training were increased and the intensity remained the same. In the first mesocycle of the competitive period, the duration and intensity were increased. The intensity continued to increase in the second mesocycle of this period, with a progressive decrease in the duration. In the taper period, the intensity was kept the same and duration was decreased. In all sessions, continuous running was performed on a flat course. From the second to the fifth mesocycle, sessions of higher and lower demand were rotated each week. Furthermore, regular stretching, core strengthening exercises and running drills were included in the sessions to minimise injury risk and improve running technique. Participants were provided with HR monitors. During each training session, intensities were individualised by prescribing training paces based on %HRmax according to different work areas (29). Each session was allowed a variation of ±3% of the %HRmax prescribed. All sessions were conducted in groups and were supervised by a coach. The control group was instructed not to change their habits regarding physical activities during the period and no subject performed vigorous levels of physical activity according to the IPAQ.

Blood Sampling Procedures

Blood samples were drawn by repetitive venipuncture from an antecubital vein and quickly centrifuged. The serum and plasma were drawn off and stored at −80°C for later analysis. hs-cTnT was measured quantitatively with the new high-sensitive enzyme immunoassay based on electrochemiluminescence technology using the Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). This assay has a range from 3 to 10,000 ng/l with a lower limit of the blank of 3 ng/L. The coefficient of variation at a mean hs-cTnT level of 13.5 ng/L is 5.2%. The upper reference limit for hs-cTnT, defined as the 99th percentile of healthy participants, was 14 ng/L (7). NT-proBNP was analyzed in the serum with an Elecsys proBNP electrochemiluminescent immunoassay on the Roche Elecsys 1010 (Roche Diagnostics, Lewes, United Kingdom) with an analytical range of 5–35,000 ng/L and intra- and interassay imprecisions of 0.7–1.6% and 5.3–6.6%, respectively (2). The URL for NT-proBNP was considered to be 125 ng/L (34).

Statistical analysis

Statistical analyses were performed using the IBM Statistical Package for the Social Sciences (IBM SPSS Statistics, v. 20.0 for WINDOWS). Cohort data are presented as the mean ± standard deviation unless otherwise stated. Kolmogorov-Smirnov tests were used to check for normal distribution and data for cTnI and NT-proBNP were log-transformed prior to statistical testing. 3-way analysis of variance (ANOVA) was conducted with two within-subject factors (1-blood sampling time: pre-exercise, and 5 min, 1, 3, 6, 12, and 24 h post-exercise; 2-training status: pre- vs. post-) and one

between-subject factor (group: control vs. training). Post-hoc Bonferroni tests were employed when appropriate. To support this analysis we performed 2-way ANOVAs on peak post-exercise hs-cTnT and peak NT-proBNP. Finally, for each participant in each trial we calculated a delta score for both biomarkers from pre to peak-post exercise.

The association between the exercise increase in both biomarkers and other relevant variables (e.g., baseline biomarker concentration, mean and max exercise HR) were assessed using bivariate Pearson's product-moment correlation coefficients. The differences were considered significant if $P < 0.05$.

RESULTS

Impact of exercise training on subject characteristics and exercise data

No between group differences existed at baseline so the groups were well matched at entry (Table 2). The training programme led to a decrease in weight and %fat ($P < 0.0001$). After training, despite RPE being unchanged, there was an increase in speed in the 60 min running time trial (range: 1-2.70 km/h; $P < 0.0001$) as well as an increase in mean HR and %HRmax. No change in these variables was noted in the control group. Pacing strategy was similar in all subjects and was not influenced by the training programme.

Impact of exercise training on hs-cTnT release

A significant main effect of sampling time was observed for hs-cTnT, with increases compared to pre-exercise at 5 min, 1 h, 3 h, 6 h, 12 h, and 24 h post-exercise ($P < 0.0001$) (Table 3). hs-cTnT was above the URL in 80% of participants. In most exercise tests, the maximum post-exercise value was observed at 3 h (83%) or 6 h (13%) and this

was largely consistent pre and post-training. Before the training programme, the two groups had similar pre-exercise (control group: median [range]; 3.0 [3.0-8.1]; training group: 3.6 [3.0-16.2] ng/L; P = 0.072) and peak post-exercise hs-cTnT (control group: 14.7 [9.7-65.0]; training group: 16.6 [4.9-140.7] ng/L; P = 0.919). A significant group x training interaction was observed (P = 0.008), but no significant interaction between sampling time x training x group (P = 0.125). A significant training x group interaction was also observed for peak post-exercise hs-cTnT (P = 0.002). After training, hs-cTnT was lower in the control group at both pre (control group: 3.2 [3.0-7.5]; training group: 5.1 [3.0-16.8] ng/L; P = 0.016) and peak post-exercise (control group: 17.8 [8.4-49.8]; training group: 27.7 [6.6-171.0] ng/L; P = 0.007).

Peak post-exercise hs-cTnT was weakly associated with pre-exercise hs-cTnT only when all exercise tests were combined (r = 0.358, P < 0.0001). In the training group, the change in pre to peak post-exercise hs-cTnT were not associated with changes in 60 min time trial speed (r = -0.290, P = 0.886) or changes in mean HR (r = 0.079, P = 0.689). In the control group, a significant positive association was noted between pre and peak post-exercise hs-cTnT obtained at the two running time trials (r = 0.610, P = 0.007; r = 0.742, P < 0.0001) suggesting some consistency/reliability in baseline and exercise hs-cTnT response. Interestingly, despite intervention related changes in pre and peak post-exercise hs-cTnT in the training group, the between test correlation for baseline and peak post-exercise hs-cTnT were also significant (r = 0.585, P = 0.001; r = 0.608, P = 0.001).

Impact of exercise training on NT-proBNP release

There was increase in NT-proBNP from pre-exercise to 5 min, 1 h, 3 h, 6 h, 12 h, and 24 h post-exercise (P < 0.0001; Table 4). In 15% of participants NT-proBNP values

exceeded the URL. Differences in the magnitude of peak data were accompanied by variable kinetics of NT-proBNP during recovery with the peak post-exercise values observed at 0 h (38%), 1 h (13%), 3 h (4%), 6 h (10%), 12 h (26%), and 24 h (9%). Prior to the intervention pre-exercise (control group: median [range]; 20.9 [7.1-87.7]; training group: 20.4 [8.4-123.4] ng/L; $P = 0.685$) and peak post-exercise values of NT-proBNP (control group: 40.6 [16.4-169.3]; training group: 43.1 [17.4-234.5] ng/L; $P = 0.910$) were similar between groups. The training programme had no effect on pre- or post-exercise NT-proBNP level ($P = 0.121$).

In all tests peak post-exercise level of NT-proBNP was strongly associated with pre-exercise concentration ($r > 0.90$). In the training group, NT-proBNP data were not associated with changes in 60 min time trial speed ($r = -0.061$, $P = 0.758$) or mean HR ($r = 0.015$, $P = 0.940$). Similar to hs-cTnT the pre and peak post-exercise data for NT-proBNP were strongly correlated in the two time trials undertaken suggesting a high level of consistency in biomarker data (resting: control group, $r = 0.899$, $P < 0.0001$, training group: $r = 0.694$, $P < 0.0001$; peak post-exercise: control group: $r = 0.874$, $P < 0.0001$, training group: $r = 0.694$, $P < 0.0001$).

DISCUSSION

The main findings of this study are that (1) a single 60-min bout of "all-out" running resulted in a significant increase in the both hs-cTnT and NT-proBNP with substantial variability in individual peak post-exercise data; (2) 14 weeks of endurance training resulted in higher pre-exercise and post-exercise values of hs-cTnT when compared to baseline testing and the control group; (3) endurance training had no impact on post-exercise NT-proBNP or on the post-exercise kinetics of either biomarkers; and (4) pre-

and peak post-exercise levels of both biomarkers were strongly correlated between both tests suggesting high reproducibility in biomarker response to exercise.

hs-TnT and NTproBNP release

This study confirms that the exercise-induced release of hs-cTnT and NT-proBNP is not exclusive to ultra-endurance effort. Between study differences in exercise duration and/or the use of the new high sensitive assay for cTnT could explain why the percentage of subjects with cTn values above the URL was greater in this study (60 min) than after a 30 min run (33). The release of NT-proBNP is largely associated with exercise duration and/volume (30, 31) with little influence exerted by exercise intensity (12, 30, 31). A shorter, 60 min run, could explain a lower percentage of subjects exceeding the URL of NT-proBNP in the current study.

Our results also confirm marked individual variability in both biomarkers (16, 38). The high individual variability in the peak post-exercise response of hs-cTnT could not be explained by any control variable in this or other studies (38). This suggests that some currently unknown physiological factors make participants more or less likely to release cTn in response to exercise.

Individual variability in the response to exercise of NT-proBNP was strongly related to the variability in pre-exercise values (11, 12, 23, 31). Despite this the current study observed a high degree of within subject consistency in peak post-exercise hs-cTnT release in both groups over their 2 trials, suggesting that hs-cTnT release is quite a robust phenomenon within any participant and that persists even after a training intervention that may alter the actual peak value of hs-cTnT. Whilst this supports some recent work with hs-cTnT (37, 40) it contradicts other field work that proposed that the

cTn (17, 18, 26) and NT-proBNP (37) response to exercise is random and not reproducible. Study to study differences are most likely due to methodological issues such as assay precision, environmental and exercise control as well as more frequent sampling. High reproducibility in the peak post-exercise values of NT-proBNP also confirms previous work (23, 26). On-going work should determine the causes of the high between subject variability in quantitative cTn and NT-proBNP release.

Kinetics of hs-TnT and NTproBNP release

This study provided a comprehensive analysis of individual post-exercise kinetics for hs-cTnT and NT-proBNP. For hs-cTnT, most subjects showed the peak post-exercise value at 3 h which is consistent with the only previous hs-cTnT study (38). Several studies have considered it sufficient to take a single blood sample 3-4 h post-exercise to record the post-exercise cTn peak (6, 21, 37). This viewpoint is only partially supported by the results of this study as 15% of subjects obtain the peak post-exercise value 6 h after the 60 min run. The discrepancy between studies might be attributed to differences in assay precision and the number of blood sample points after the start of exercise.

Importantly, the kinetics data from the current study is somewhat different to that observed for cTn in acute myocardial infarction (36). At 24 h post-exercise all hs-cTnT values were close to pre-exercise levels. In addition, the increase in hs-cTnT occurred in the absence of clinical signs and symptoms. This suggests that post-exercise hs-cTnT level may be reflect a physiological, rather than pathological, response to exercise stimulus. The hypothesis that has been proposed is that endurance exercise causes an increase in membrane permeability due to the physiological stress placed on the cell, inducing a transient cytosolic leakage due to membrane damage, rather than cardiomyocyte necrosis (32) although this requires empirical support.

The post-exercise kinetics of NT-proBNP has received less attention (38). Our data suggest that the kinetics for NT-proBNP are more diverse than for hs-cTnT with an incomplete recovery to baseline at 24 hr post-exercise. The elevation in NT-proBNP at 24 h could reflect a temporary reduction in kidney function and changes in cardiac function (38), but this requires further study.

Effect of exercise training on hs-TnT and NTproBNP values

Our main objective was to determine whether the hs-cTnT and NT-proBNP response to exercise is mediated by a change in training status. We observed higher baseline and peak post exercise values for hs-cTnT after 14 weeks of running training that also differentiated this group from a control cohort. This supports some previous cross-sectional data from athletes of different training status (unpublished, Legaz-Arrese et al., 24) but does contradict past field-based studies of runners with higher and lower self-report training volume (e.g. 20). The assay precision and the method used to quantify the level of training may also be a confounding factor in the relationship observed in previous studies. In the largest case series data in marathoners by Fortescue et al. (5) noted that the runners with less prior experience in marathon running were more likely to have cTnT increases. They also found no relationship between race time and an increase in cTnT, suggesting that the number of previous marathons may not be the most appropriate way to quantify the current level of training. Furthermore, the authors indicated no significant relationship between the release of cTnT and average training pace or average miles run per week during the last 3 months. In the same direction, a multiple regression analysis demonstrated marathon experience was a significant predictor of post-marathon hs-cTnT (19). Once again, the authors did not establish a relationship between marathon time and the release of hs-cTnT and did not

gather other data on the level of training of the athletes. The association observed by Nie et al. (21) and Tian et al. (39) between the number of years of training and cTnT or cTnI release after a half-marathon in adolescent subjects was weak and could have been influenced by the maturity status of the adolescents. Moreover, in neither of these studies did the authors observe a relationship for other indicators of the level of training, such as weekly training distance. Finally, using a prospective-study design, Mehta et al. (15) revealed that average miles run per week in the last 3 years (an indicator of previous training experience) was negatively associated with post-marathon cTnI release. No association, however, was observed for the current training status (miles run per week in the last 4 month) or for the race time.

Training resulted in a performance improvement in the 60 min running time trial at the same time as baseline and peak post-exercise hs-cTnT were increased. It was, therefore, somewhat surprising to see performance and hs-cTnT changes were not strongly associated. Whether this reflects a limited range of change in performance data or a lack of a systematic effect of training status change is difficult to determine. In field-based studies, where numerous factors are not controlled, and performance and biomarker differences between groups may be more extensive a closer association between training status and hs-cTnT release may be more apparent.

A number of theories could be proposed to explain higher hs-cTnT after training. Although speculative, higher pre-exercise and post-exercise hs-cTnT values after training could be a consequence of an adaptive process occurring in the heart due to the exercise training stimulus. Heart size is associated with performance (14), and its adaptation is greater in less trained subjects (13). Recent work from Saravia et al. (24) have suggested a link between higher levels of inflammation and increased cTn in faster

marathon runners. Future work should evaluate these hypotheses in appropriate research designs.

We observed that training had no influence on the value of NT-proBNP or on the kinetics of either biomarker. Few studies have established the relationship between the level of training and the exercise-induced release of NT-proBNP. Like Neilan et al. (20), Serrano-Ostáriz et al. (30) in road cyclists and Hermann et al. (8) in marathoners also observed a weak relationship between the level of training and the release of NT-proBNP. This relationship disappeared when differences between athletes in the duration of effort were controlled. In other recent studies the relationships have not been significant (12, 23, 27, 28, 38).

Limitations, future research and implications of this study

We selected previously untrained subjects because of the difficulty in establishing both control periods and marked training change in athletes. For this reason, our work was limited to assessing the response of the release of cardiac biomarkers after an effort of relatively short duration (60 min). Our results, and their clinical impact, cannot be directly extrapolated to the effects of much more demanding training programmes such as those performed by elite athletes competing in marathons, road cycling, and triathlons. Many field-based studies, however, recruit amateur athletes who present with biomarker responses similar to that observed in the current study and these athletes represent a much bigger and diverse proportion of mass-participation endurance events than elite athletes. The clinical importance therefore is more likely to be related to the amateur athlete presenting after an endurance event with a raised cardiac biomarker. Clearly appropriate “work-up” of any elevated hs-cTnT is important but this must also recognize prior exercise as a key component.

The relationship between the exercise-induced release of cardiac biomarkers and the factors determinants of running performance (e.g. VO_{2max}) might be useful to evaluate high individual variability in biomarker release. Future work should also recruit a larger sample of female athletes and address the issue of whether the phase of the menstrual cycle could mediate biomarker release.

In conclusion, a 14-week endurance running training programme led to higher pre-exercise and peak post-exercise values of hs-cTnT but did not change NT-proBNP or the kinetics of either biomarker. The change in hs-cTnT after training was not directly related to changes in performance or the cardiovascular stress of a 60 min exercise stimulus after the intervention. The kinetics of hs-cTnT appearance suggests that the exercise-induced release is a physiological process with no known clinical consequences. Clinicians should be aware that the release of cardiac biomarkers is not exclusive to long-term strenuous efforts and values above the URL for hs-cTnT can be observed in most subjects.

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Figure Legends

Fig. 1. The flow of participants throughout the trial.

Fig. 2. Individual data points of hs-cTnT (ng/L) in the control group [*(a)* baseline, *(b)* post-training] and in the training group [*(c)* basaline, *(d)* post-training] before exercise (PRE) and 5 min, 1 h, 3 h, 6 h, 12 h, and 24 h (0HR, 1HR, 3HR, 6HR, 12HR, 24HR, respectively) after a 60 min maximal running trial. The horizontal dotted line is the upper reference limit (99th percentile) at 14 ng/L.

Table 1. Characteristics of the training programme

Period	Mesocycle	Week	Session 1 (min-%HRmax)	Session 2 (min-%HRmax)	Session 3 (min-%HRmax)	Session 4 (min-%HRmax)	Duration/week (min)	Intensity/week (%HRmax)
Preparatory	1	1	40 min-65%	40 min-65%	40 min-65%		120	65
		2	40 min-65%	40 min-65%	40 min-65%		120	65
		3	50 min-65%	50 min-65%	50 min-65%		150	65
		4	50 min-65%	50 min-65%	50 min-65%		150	65
	2	5	50 min-65%	40 min-65%	50 min-65%	40 min-65%	180	65
		6	50 min-65%	40 min-65%	50 min-65%	40 min-65%	180	65
Competition	3	7	60 min-70%	40 min-65%	60 min-70%	40 min-65%	200	65-70
		8	60 min-70%	40 min-65%	60 min-70%	40 min-65%	200	65-70
		9	70 min-75%	40 min-70%	70 min-75%	40 min-70%	220	70-75
		10	80 min-75%	40 min-70%	80 min-75%	40 min-70%	240	70-75
	4	11	70 min-80%	40 min-75%	70 min-80%	40 min-75%	220	75-80
		12	60 min-85%	40 min-80%	60 min-85%	40 min-80%	200	80-85
Taper	5	13	50 min-85%	40 min-80%	50 min-85%	40 min-80%	180	80-85
		14	40 min-85%	40 min-80%	40 min-85%	40 min-80%	160	80-85

Table 2. Subject characteristics and exercise data Pre and Post training

	Control group (n = 18)		Exercise group (n = 28)	
	Pre	Post	Pre	Post
Subject characteristics				
Age	30.6 ± 8.7	--------	29.9 ± 9.9	--------
Height, m	1.76 ± 0.09	--------	1.76 ± 0.06	--------
Weight, kg	69.9 ± 9.4	69.1 ± 9.8*	71.1 ± 8.2	67.9 ± 8.5*†
Body fat, %	11.6 ± 4.6	11.1 ± 4.8*	11.8 ± 4.4	9.9 ± 4.4*†
Maximum HR, bpm	187 ± 11	187 ± 11	189 ± 8	188 ± 8
60 min performance				
Velocity, km/h	10.5 ± 1.1	10.7 ± 0.9	10.4 ± 1.1	12.1 ± 0.9*†
Mean HR, bpm	161 ± 11	163 ± 11	160 ± 11	166 ± 11*†
% HRmax	86 ± 3	87 ± 4	85 ± 4	88 ± 4* †
RPE	8.5 ± 0.8	8.8 ± 0.8	8.4 ± 0.8	8.7 ± 1.1

Values are presented as means ± SD. *significantly different from pre-training; †significantly different from control group at Post training.

Table 3. hs-cTnT concentrations after 60 min of high-intensity running Pre and Post training

	Control group (n = 18)		Exercise group (n = 28)		ANOVA *P*-values	
	Pre	Post	Pre	Post		
Pre-exercise	3.0 (3.0-8.1) (0)	3.2 (3.0-7.5) (0)	3.6 (3.0-16.2) (4)	5.1 (3.0-16.8) (7)	Time	<0.0001
5 min	5.1 (3.0-10.8) (0)	5.3 (3.3-12.2) (0)	6.8 (3.0-39.4) (14)	9.8 (3.0-28.7) (14)	Training	0.003
1 h	9.5 (5.2-23.7) (11)	9.6 (3.8-22.3) (17)	10.0 (3.0-36.5) (25)	13.6 (6.1-67.9) (46)	Group	0.073
3 h	14.2 (9.7-57.2) (50)	16.6 (8.4-49.8) (67)	16.1 (4.9-140.7) (64)	26.3 (6.6-171.0) (89)	Time x Group	0.512
6 h	13.0 (7.0-65.0) (44)	13.4 (5.7-41.2) (50)	15.2 (3.5-97.7) (54)	18.9 (6.4-156.0) (79)	Training x Group	0.008
12 h	6.7 (3.7-58.1) (11)	8.3 (4.0-24.7) (22)	9.2 (3.0-39.1) (29)	11.0 (4.6-68.5) (32)	Time x Training	0.123
24 h	4.3 (3.0-33.2) (11)	4.9 (3.0-12.5) (0)	6.3 (3.0-19.3) (7)	6.7 (3.0-36.2) (11)	Time x Training x Group	0.125

Data are median (range) with the percentage of subjects with hs-cTnT values exceeding the URL in parentheses.

Table 4. NT-proBNP concentration after 60 min of high-intensity running Pre and Post training

	Control group (n = 18)		Exercise group (n = 28)		ANOVA *P* -value	
	Pre	Post	Pre	Post		
Pre-exercise	20.9 (7.1-87.7) (0)	18.7 (6.0-121.0) (0)	20.4 (8.4-123.4) (0)	21.1 (6.7-73.0) (0)	Time	<0.0001
5 min	36.0 (11.0-169.3) (6)	36.3 (9.8-215.2) (6)	34.9 (11.1-215.7) (7)	40.7 (9.3-142.0) (7)	Training	0.299
1 h	28.4 (11.2-147.5) (11)	30.8 (10.4-196.7) (11)	32.9 (12.5-234.5) (7)	36.0 (10.9-178.6) (7)	Group	0.896
3 h	26.7 (9.9-139.3) (6)	29.1 (9.1-187.4) (6)	27.2 (12.6-207.7) (4)	33.5 (11.1-204.7) (7)	Time x Group	0.431
6 h	31.6 (12.3-148.4) (17)	29.6 (9.9-142.7) (6)	31.3 (11.9-135.0) (4)	31.9 (17.0-127.5) (4)	Training x Group	0.121
12 h	29.5 (16.4-158.9) (17)	33.1 (10.8-121.2) (0)	29.3 (9.1-103.4) (0)	35.1 (16.0-105.3) (0)	Time x Training	0.207
24 h	26.4 (10.5-99.7) (0)	27.1 (10.5-100.4) (0)	24.1 (9.1-55.9) (0)	26.7 (11.4-103.8) (0)	Time x Training x Group	0.540

Data are median (range) with the percentage of subjects with NT-proBNP values exceeding the URL in parentheses.

Fig. 1. The flow of participants throughout the trial.

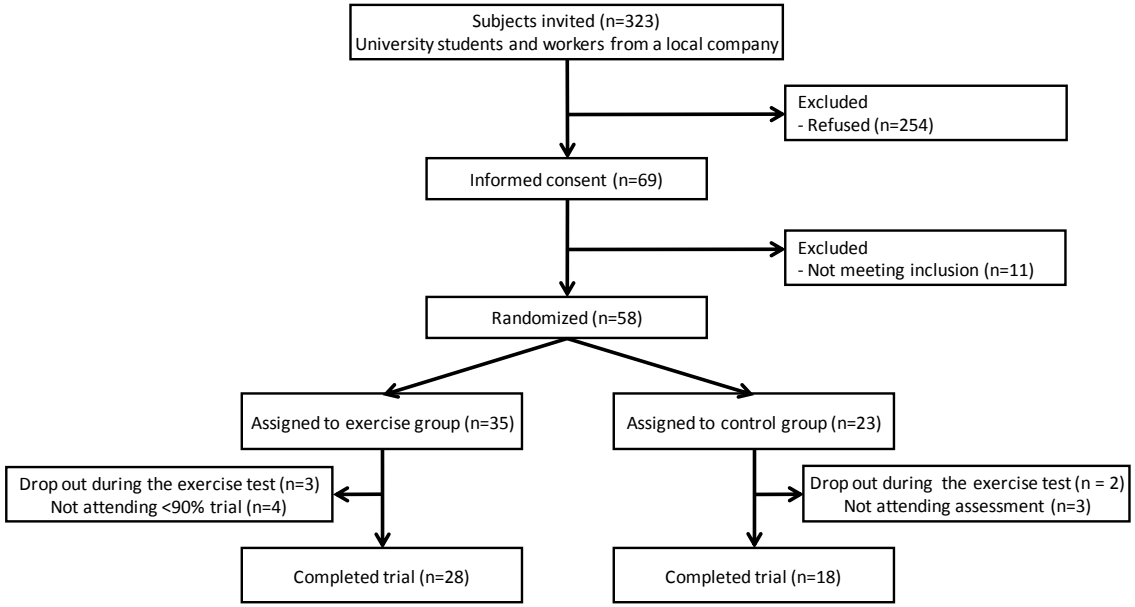
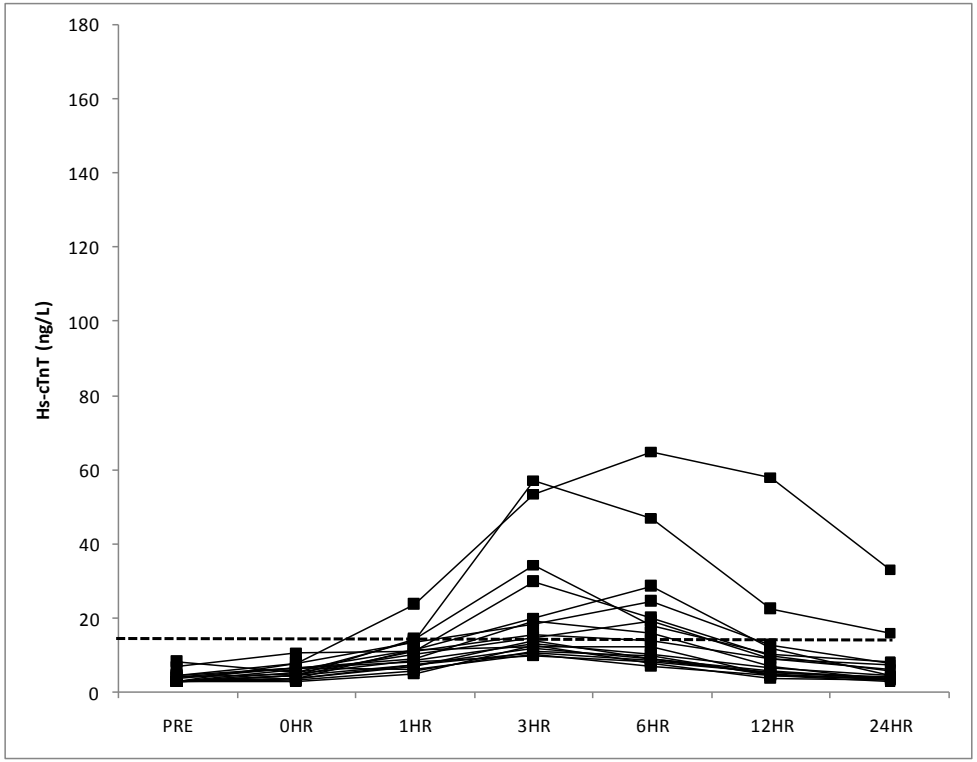


Fig. 2. Individual data points of hs-cTnT (ng/L) in the control group [*(a)* baseline, *(b)* post-training] and in the training group [*(c)* basaline, *(d)* post-training] before exercise (PRE) and 5 min, 1 h, 3 h, 6 h, 12 h, and 24 h (0HR, 1HR, 3HR, 6HR, 12HR, 24HR, respectively) after a 60 min maximal running trial. The horizontal dotted line is the upper reference limit (99th percentile) at 14 ng/L.

(a)



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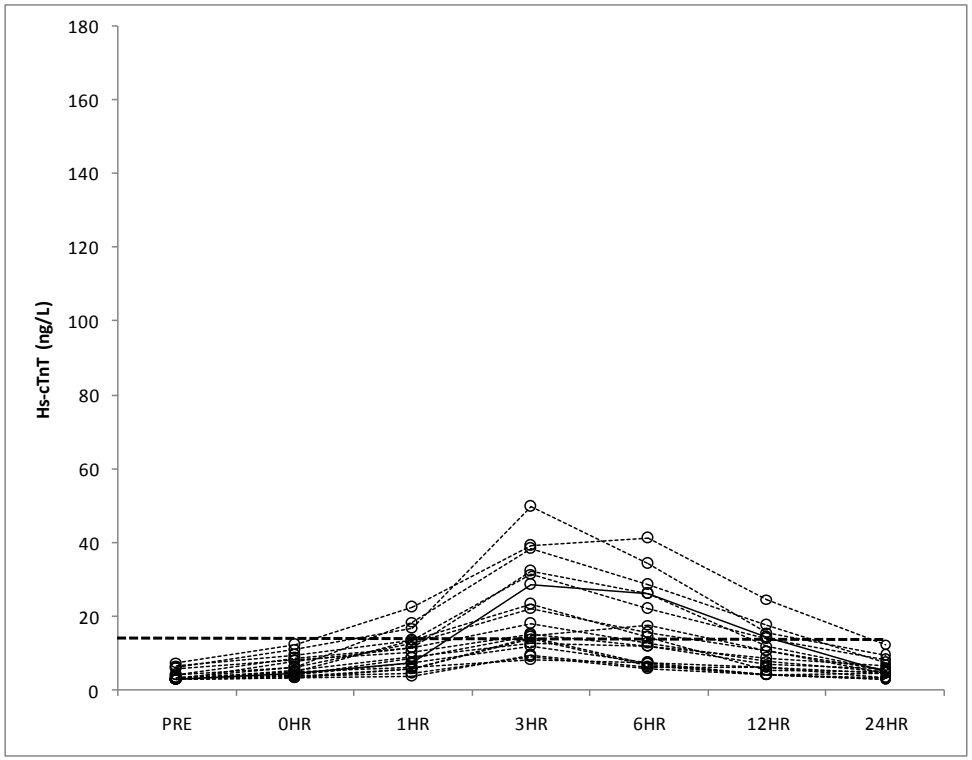
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Hs-cTnT (ng/L)
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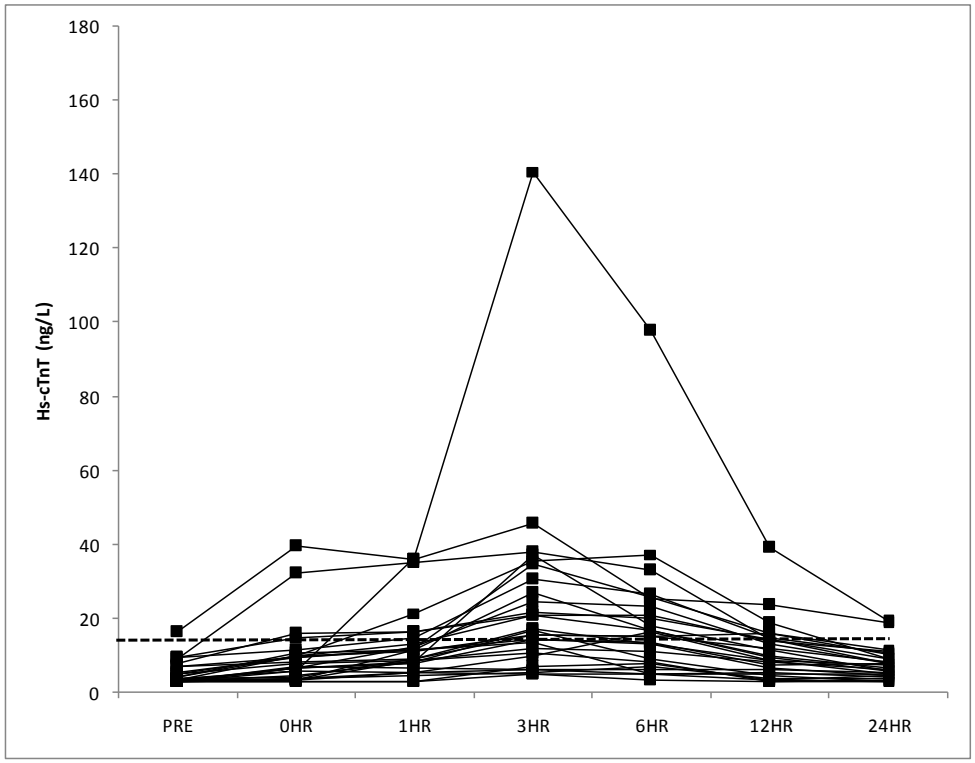
6HR
12HR
24HR

(c)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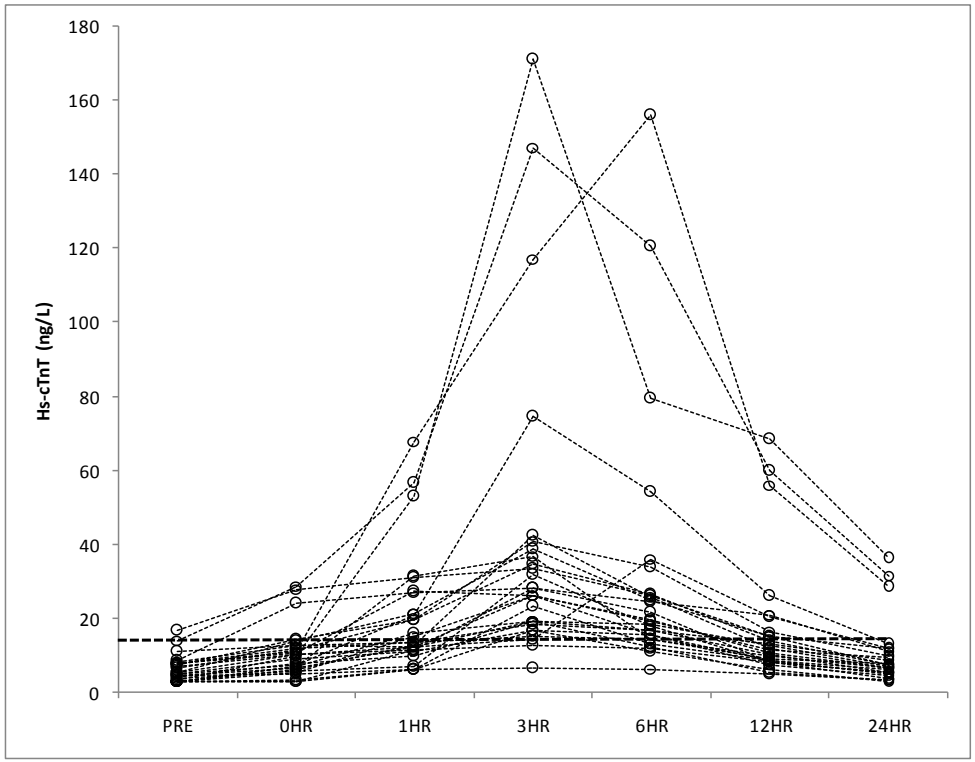
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