Serum cardiac markers are inversely associated with VO$_2$max of amateur athletes in response to endurance training adaptations

Gashaw Tesema, Mala George, Soumitra Mondal, D Mathivana

ABSTRACT

**Background** The influence of endurance training intensity and adaptation on serum cardiac markers is poorly understood and controversial; however, no enough data observed the association of serum cardiac markers with VO$_2$max. Therefore, we aimed to investigate whether serum cardiac markers are associated with maximum oxygen consumption (VO$_2$max) in response to 12-week endurance training on amateur athletes.

**Methods** 15 apparently healthy male amateur athletes with 19.47 ± 1.30 years of age were recruited and participated in endurance training with 70%–80% maximal heart rate intensity for 35 min per session for the first week and 2 min increments each week from the second to the last week for a period of 12 weeks. VO$_2$max and serum cardiac markers (lactate dehydrogenase [LDH], creatine kinase myocardial band [CK-MB] and cardiac troponin I [CTnI]) were assessed at the beginning of the training and after 12-week endurance training.

**Results** The result of CTnI indicated significantly (p < 0.01) and inversely (r = −0.466) correlated with VO$_2$max and CK-MB indicated significantly (p < 0.01) and inversely associated with VO$_2$max (r = −0.536) with moderate relationship. However, we did not find a significant association on LDH (p > 0.05) with VO$_2$max in response to endurance training adaptation.

**Conclusion** Our finding confirms our hypothesis that serum cardiac markers are inversely associated with estimated VO$_2$max in response to endurance training adaptation.

INTRODUCTION

Endurance training causes muscle damage, fatigue and muscle pain. This damage can be linked to large artery wall stiffening, cartilage and muscle damage, atrial fibrillation as well as left and right ventricular dysfunction and systemic inflammatory reaction resulting in elevated serum muscle damage markers. A study indicates seven continuous days of training did not increase incidences of muscle damage as compared with the group performed only one session of the training and this is attributed to adaptation of the stress caused by the training. Adaptations of training stress are best explained by maximum oxygen consumption (VO$_2$max) as the single indicator of cardiorespiratory fitness. In line with this, researchers suggested the time course of the training related to muscle fibre damage, pain, as well as creatine kinase (CK), cardiac troponin I (CTnI) and lactate dehydrogenase (LDH) release. However, there are no evidences that indicate the association of serum cardiac markers and VO$_2$max of amateur athletes in response to endurance training adaptation.

Moreover, studies reported serum cardiac markers (LDH, CK myocardial band [CK-MB] and CTnI) are not significantly correlated with VO$_2$max, a measure of endurance training adaptation, which is a more accurate and reproducible predictor of cardiovascular outcomes. Conversely, a study reported muscle damage markers are inversely related to VO$_2$max. Besides, the prevalence of elevated CTn is associated with inexperienced athletes than experienced athletes. This difference might strongly related to previous...
training experience\textsuperscript{12} and age\textsuperscript{10} of an athlete. Moreover, a 12-week soccer training with 80\% (maximal heart rate, HRmax) average intensity: reported increased VO\textsubscript{2}max and inversely decreased CK-MB at the end of the 12-week training.\textsuperscript{13} However, there is no enough data observed the association of serum cardiac markers with VO\textsubscript{2}max in response to 12-week endurance training adaptation of amateur athletes. Moreover, there is a lack of prospective studies that observe the relationship of high intensity endurance training to exercise-induced cardiac remodelling.\textsuperscript{14} Thus, since the athletes are exposed for elevated serum cardiac markers as a result of endurance training, it helps them to understand the level of serum markers in response to adaptation and preventing overtraining for monitoring fitness and enhancing cardiac health.

The aim of this study was to examine the association between serum cardiac markers and VO\textsubscript{2}max in response to chronic endurance training on amateur athletes. Thus, we have applied high intensity endurance training at 70\%–80\% HRmax with duration of 35 min per session for the first week and 2 min increments each week from the second to the last week for a period of 12 weeks. Consequently, we hypothesised that serum cardiac markers are inversely associated with estimated VO\textsubscript{2}max because of endurance training adaptation.

METHODS

Study area
This study was conducted in Bahir Dar, located about 578 km north-northwest of Addis Ababa-Ethiopia. It has an altitude of 1840 m above the sea level and within latitude and longitude of 11°36'N 37°23'E coordinates. While the annual average temperature is 25°C–32°C, with an average humidity of 58\%.\textsuperscript{15}

Study population
In all, 15 apparently healthy male amateur moderately trained athletes within 18–25 years of age were selected randomly from Bahir Dar University sport academy students. The sample size was based on similar studies done before using the following equations which considers 80\% power and p≤0.05, where n=sample size needed, $\sigma^2$ T is variance of the treatment, $\sigma^2$ R is variance of the reference, $\delta$ is the maximum percent of no clinical importance, CVT is the coefficient of variations of the treatment and CVR is the coefficient of variations of the reference:

$$n = \frac{(z_0+z_\alpha/2)^2 (\sigma^2_T+\sigma^2_R)}{(\delta/^2)/CVR^2)}$$

Consequently, a validated physical activity readiness questionnaire\textsuperscript{17} was used to evaluate conditions that may prohibit participants from practising in endurance training. Therefore, participants that reported problems in their health (heart dysfunction and chest pain during exercise) and physical conditions (loss of balance, consciousness and bone or joint problem) and are currently taking anti-hypertensive drugs were excluded from the study. In addition, smokers and alcoholics were also excluded from participating in the study due to reported influences on both metabolic and physical performance biomarkers.\textsuperscript{18 19}

Study design
A single group pre–post design was used in this study. In all, 15 subjects selected and placed in high intensity endurance training. VO\textsubscript{2}max and serum markers of muscle damage were assessed at the beginning of the training and after 12-week endurance training. High intensity endurance training was done at 70\%–80\% HRmax 35 min per session for the first week and 2 min increments each week from the second to the last week for a period of 12 weeks.

Training protocol and ethical approval
This field-based study conducted based on the protocol specified here. One professional athletics coach assigned to the experiment. Five minutes of warming up and stretching exercise are given at the beginning of the training. The participants were performed at 70\%–80\% HRmax, a continuous endurance running in the open field track for 35 min per session for the first week and 2 min increments each week from the second to the last week. The training intensity was monitored with polar heart rate monitor throughout the entire session. The heart rate monitor adjusted to the designed percent maximum heart rate. Therefore, the beep sound of the heart rate monitor guided us if it is below and above the designed % HRmax to adjust the heart rate drift throughout the training. Five minutes of cooling down training were also given at the end of each training time for 3 days per week and it continued for 12 weeks aimed to observe chronic effects of the training on serum cardiac markers and VO\textsubscript{2}max. Participants were instructed not to participate in other exercise training and to continue the usual habit of nutrition throughout the experiment.

Ethical approval was obtained from Institutional Research Ethics Review Committee of Mekelle University conformed to the 1975 Declaration of Helsinki. Written consent was delivered to the participants and they were informed about the objectives of the study. Participation in this study was voluntary and their right not to participate was respected. Confidentiality and anonymity were also ensured.

Assessment of VO\textsubscript{2}max
VO\textsubscript{2}max of subjects was estimated using 3 min step test. Subjects stepped up and down a 16.5 inch stepping bench with a rate of 96 steps per min for 3 min in time of metronome beep sound. Five seconds after exactly 3 min pulse was taken from radial artery with heart rate monitor tied around the wrist. Finally, maximal aerobic power (VO\textsubscript{2}max) was calculated using the equation VO\textsubscript{2}max=111.33 – (0.42×maximum pulse measured 5
s after stepping for 3 min) and reported in (mL/kg/min).

**Assays of serum cardiac markers**

Blood samples of 5 mL were collected just before training (pretest) from each participant. Then, post-training samples collected 4 hours after the final training session at the end of 12-week endurance training. Since the peak cardiometabolic markers have been achieved 3–4 hours after training time. Blood was taken from an antecubital vein using vacutainer serum separator tube containing blood clotting accelerant gel. The serum was separated by centrifugation of the blood sample at 4000 rpm (revolution per minute) for 3 min and stored at −20°C until analysis. The level of LDH in U/L was measured using a spectrophotometric assay with BS-2E chemistry analyzer according to the guidelines of the International Federation of Clinical Chemistry (Beckman Coulter, Krefeld, Germany). The levels of cTnI band in ng/mL and CK-MB in ng/mL were measured by chemiluminescence immunoassay using Maglumi 800 fully automated chemiluminescence immunoassay analyzer.

**Data analysis**

Analysis of the data obtained from the study inferential statistics was employed. To evaluate whether or not the data are normally distributed, the Kolmogorov-Smirnov and Shapiro-Wilk normality tests were applied. To identify the presence of any significant relationships between variables, Pearson’s correlation analysis was used. IBM-SPSS V.20 packages were used to analyse the data. Variables were expressed as Pearson’s correlation coefficient (r) and probability value (p). In all cases, P<0.05 was accepted significant.

**RESULTS**

The Kolmogorov-Smirnov and Shapiro-Wilk’s normality tests indicate (p>0.05) and visual inspection of their histograms, normal Q–Q plots and box plots showed that the data were approximately normally distributed. General characteristics of participants in mean±SD were body mass in kg (53.96±5.67) and body mass index in kg/m² (19.47±1.30), height in metres (1.67±0.07), age in years (19.47±1.30).

Serum cardiac markers correlation results with estimated VO2max in response to 12-week endurance training are shown in table 1. Value of cTnI with 12-week endurance training was significantly (p<0.01) and inversely correlated with estimated VO2max (r=−0.466) indicating low relationship. Moreover, CK-MB was statistically significant (p<0.01) and inversely associated with estimated VO2max (r=−0.536) with moderate relationship. Furthermore, cTnI and LDH showed significant (p<0.05) and positive association with CK-MB. However, we did not find a significant association of LDH with VO2max (r=−0.23) and cTnI in response to endurance training adaptation in amateur athletes.

**DISCUSSION**

The purpose of this study was to examine the association between serum cardiac markers and VO2max in response to chronic endurance training on amateur athletes so as to consider cardiac health. The main findings of the study support our hypothesis that serum cardiac markers are inversely associated with estimated VO2max in response to endurance training adaptation. Consequently, serum cardiac markers (cTnI and CK-MB) in response to 12-week endurance training indicated a statistically significant (p<0.01) association with estimated VO2max. However, we did not find a significant association on LDH (p>0.05) with estimated VO2max in response to endurance training adaptation.

Ctn and VO2max are important factors in the study of cardiac muscle damage markers and cardiorespiratory fitness. Consequently, our study confirmed a statistically significant and inverse associations of cTnI with estimated VO2max. Consistently, our study reported cTnI is inversely related to VO2max. The reason might be adaptation of the stress caused by chronic endurance training. Conversely, a study reported cTnI has no relation with VO2max. This disparity could be attributed to training experiences of the athletes.

Recent evidences reported increased VO2max and inversely decreased CK-MB at the end of the 12-week training with 80% (HRmax) average intensity. It supports our finding of CK-MB is significantly and inversely associated with estimated VO2max in response to endurance training adaptation. Similarly, a study reported an inverse relationship between cardiac markers and VO2max. On the contrary, CK-MB is not significantly correlated with VO2max. The variation of results between studies might be training experience of athletes and age.

Changes in serum cardiac muscle damage markers can be influenced by gender, age, different training intensity, training method differences and in this study, we used calculated or estimated VO2max rather than measured VO2max which was the limitation of our study.
design. Thus, further study is needed regarding the fore
mentioned points. Nevertheless, we can see training
experience effect with gradual increment in training
duration for 12 weeks improved VO$_2$\textsubscript{max} and reduced
serum cardiac markers. Therefore, these factors should
be considered in designing training to improve cardiorespiratory fitness and maintain cardiovascular health.

CONCLUSION

In conclusion, serum cardiac markers, CTnI band and
CK-MB, are significantly and inversely associated with
estimated VO$_2$\textsubscript{max} in amateur athletes in response to
12-week endurance training. These findings add to
the growing body of evidence linking between cardiac
damage markers to cardiorespiratory fitness. Indicating
gradual adaptation reduces serum cardiac marker
concentration after exercise.

Acknowledgements The authors thank Mekelle University and Mizan-Tepi
University for their support.

Contributors I am the contributor of this work.

Funding The authors have not declared a specific grant for this research from any
funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Open access This is an open access article distributed in accordance with the
Creative Commons Attribution 4.0 Unported (CC BY 4.0) license, which permits
others to copy, redistribute, remix, transform and build upon this work for any
purpose, provided the original work is properly cited, a link to the licence is given,
and indication of whether changes were made. See: https://creativecommons.org/
licenses/by/4.0/.

REFERENCES

1. Lee YH, Lee YH, Kim CK. Biomarkers of muscle and cartilage
damage and inflammation during a 200 Km run. Eur J Appl Physiol
2007;99:443–7.
2. Lieber RL, Shah SFJ. Cytoskeletal disruption after eccentric
contraction-induced muscle injury. Clin Orthop Relat Res
2002;403:S90–9.
3. Kim HJ, Lee YH, Kim CK, et al. Changes in serum cardiac
oligomeric matrix protein (COMP), plasma CKP and plasma hs-CRP
in relation to running distance in a marathon (42.195 Km) and an
ultra-marathon (200 Km) race. Eur J Appl Physiol 2009;105:765–70.
4. Mascia G, Perrotta L, Galanti GPL. Atrial fibrillation in athletes. Int J
Sport Med 2013;34:379–84.
5. Wallberg L, Mikael Mattsson C, Enqvist JK, et al. Plasma IL-6
concentration during ultra-endurance exercise. Eur J Appl Physiol
2011;111:1081–8.
6. Chen TC, Hsieh SS. Effects of a 7-day eccentric training period
on muscle damage and inflammation. Med Sci Sports Exerc
2001;33:1732–8.
7. Evans GF, Haller RG, Wyrick PS, et al. Submaximal delayed-onset
muscle soreness: correlations between MR imaging findings and
Clinical measures. Radiology 1998;208:815–20.
8. Ponte AD, Giovanelli N, Antonutto G. Changes in cardiac and
muscle biomarkers following an uphill-only marathon. Res Sport
Med An Int J 2017;26:1–12.
9. Myers J, Gullestad L, Vagelos R, et al. Hemodynamic, and
cardiopulmonary exercise test determinants of survival in
patients referred for evaluation of heart failure. Ann Intern Med
1998;129:286–93.
10. Manfredi TG, Fielding RA, O’Reilly KP, et al. Plasma creatine kinase
activity and exercise-induced muscle damage in older men. Med Sci
Sports Exerc 1991;23:1028–34.
11. Shave R, George KP, Atkinson G, et al. Exercise-induced cardiac
troponin T release: a meta-analysis. Med Sci Sport Exerc
2007;39:2099–106.
12. Mehta R, Gaze D, Mohan S, et al. Post-exercise cardiac troponin
release is related to exercise training history. Int J Sports Med
2012;33:333–7.
13. Bekris E, Giannis S, Sampanis M, et al. The effect of soccer training
on biochemical indices of young soccer players during the game
season. Phys Train 2012.
14. La Gerche A, Gerche L. The potential cardiotoxic effects of exercise.
Can J Cardiol 2016;32:421–8.
15. Haile Z. Assessment of climate change impact on the net Basin
supply of Lake Tana water balance. The Netherlands: International
Institute for geo-information science and earth observation
ENSCHENDE, 2009.
16. Thalheimer W, Cook S. How to calculate effect sizes from published
research articles: a simplified methodology. Work Res 2002.
17. Warburton DER, Jamnik VK, Bredin SSD. The physical activity
readiness questionnaire for everyone (PAR-Q+) and electronic
physical activity readiness medical examination (ePARmed-X+). Heal
Fit J Canada 2011;4:3–23.
18. Lee C-L, Chang W-D. The effects of cigarette smoking on aerobic
and anaerobic capacity and heart rate variability among female
university students. Int J Womens Health 2013;5:687–79.
19. Barnes MJ. Alcohol: impact on sports performance and recovery in
male athletes. Sports Med 2014;44:909–19.
20. Marquaria R, Aghema PRE. Determination of maximal oxygen
consumption in man. J Appl Physiol 1965;20:1070–3.
21. Tian Y, Nie J, Huang C, et al. The kinetics of highly sensitive cardiac
troponin T release after prolonged treadmill exercise in adolescent
and adult athletes. J Appl Physiol 2012;113:418–25.
22. Legaz-Arrese A, Lopez-Laval I, George K, et al. Impact
of an endurance training program on exercise-induced
cardiac biomarker release. Am J Physiol Heart Circ Physiol
2015;308:H913–20.
23. Razli M, Yap W. Power comparisons of some selected normality
tests. in: proceedings of the regional conference on statistical
sciences 2010:126–38.
24. Shapiro SS, Wilk MB. An analysis of variance test for normality
(complete samples). Biometrika 1965;52:591–611.