Cardio skeletal muscle stress markers responses against two different intensity endurance training zones in novice athletes

Gashaw Tesema[*,1], Tesfaye Desalegn[2], Haileyesus Gedefaw[3], Mala George[4]

[1,2,3]Bahir Dar University Sports Academy, Bahir Dar, Ethiopia
[4]Institute of Biomedical Sciences, University of Rwanda, Rwanda

Received: 13 December, 2023 **Accepted:** 24 October, 2024 **Published:** 10 January, 2025

Note:
This issue was initially scheduled for publication in October 2024 but was delayed due to an unforeseen internet shutdown in Bahir Dar.

ABSTRACT

This study aimed to investigate the effects of two different intensity endurance training zones on cardio skeletal stress markers in search of optimum training intensity. In this study, a completely randomized parallel-group study design was employed. Thirty healthy male young athletes aged between 18-25 years old were selected and randomly assigned to two intensity endurance exercise groups. Pre-training at the end of 1 week and 12-week endurance training cardio skeletal muscle stress markers were assessed for both intensity groups. The first group trained at 60-70% maximum heart rate of 45 min per session for the first week and 3-minute increments each week from the second to 12th weeks. The second intensity training was done at 70-80% maximum heart rate 30 min per session for the first week and 2-minute increments each week and ANOVA to analyze the data. There was a significant difference between pre to 1 and 12-week post-training tests of LDH ($p < .001$), CTnI ($p < .001$), and Ckmb ($p < .001$) with medium effect size in all cardio skeletal muscle stress markers for both intensity zones. Our findings confirmed that endurance training at both intensities with gradual increments in training duration reduces exercise-induced cardio skeletal muscle stress markers. However, the reduction rate is less in 70-80% HRmax intensity level than in 60-70% HRmax indicating changes in cardio skeletal muscle stress markers are not easily adaptable in higher intensities.

Keywords: Cardiac muscle markers; Endurance exercise; Skeletal muscle markers; Varied training durations; Young athletes
DOI: https://dx.doi.org/10.4314/ejst.v17i3.2.

INTRODUCTION

Serum levels of Cardio skeletal muscle markers are enzymes or proteins indicating the functional status of muscle tissues (Epstein *et al*. 1995). These are Creatine kinase, lactate dehydrogenase, troponin, aspartate aminotransferase, and carbonic anhydrase and they are the most useful serum markers of muscle injury observed in response to strenuous exercise (Brancaccio and Lippi, 2010). Previous studies reported increased cardio skeletal muscle markers as a result of high-intensity endurance exercise (Perry *et al*., 2008; Trapp *et al*., 2008). Generally, increasing training intensity during endurance

[*] Corresponding author: gashtesema@gmail.com

exercise has resulted in higher values than normal serum cardiac stress markers like cardiac troponin I (CTnI), creatine kinase (Ckmb), and lactate dehydrogenase (LDH), (Michael, 2002; Smith *et al*., 2004; Perry *et al*., 2008; Trapp *et al*., 2008). Severe metabolic stress due to high levels of physical exercise causes micro-injuries to the muscles and other tissues (Bessa *et al*., 2008; Kim *et al*., 2009). Most studies on the metabolic responses to prolonged endurance effort have observed metabolic waste product accumulations post-exercise and during the recovery period (Kratz *et al*., 2002; Suzuki *et al*., 2003; Wu *et al*., 2004). Besides, the optimum training intensity to elicit maximum cardio skeletal muscle markers adaptation during endurance exercise remains inconclusive (Nunes *et al*., 2003).

Likewise, increasing the duration of a training session during endurance exercise reported decreased levels of cTnI (Rifai *et al*., 1999). However, some studies reported that the duration of training has no inverse association with cTnI (Neilan *et al*., 2006; Scharhag *et al*., 2005; Scharhag *et al*., 2006). Although some studies have reported that high-intensity endurance training leads to transient elevations in lactate dehydrogenase (LDH) levels (Brancaccio and Lippi, 2010; Lippi *et al*., 2008), further investigation is needed to understand the implications of these findings. However, different studies find similar responses to LDH after different intensity endurance exercises (Bessa *et al*., 2016; Paschalis *et al*., 2007). It has been reported that long-term high-intensity endurance training of more than eight weeks has shown significant elevations of the cardio skeletal muscle marker (CKmb) (Brancaccio and Lippi, 2010; Lippi *et al*., 2008). In physically active and trained subjects Ckmb has been found elevated above the reference limit indicative of cardiac and skeletal muscle damage immediately after training (Marco *et al*., 2014). Conversely, researches that varying intensities of endurance exercise do not produce different responses in CK-MB levels (Paschalis *et al*., 2007; Bessa *et al*., 2016).

Endurance training is accompanied by an increased volume of oxygen (VO2), which may alter cardiac and skeletal muscle stress markers (Mastaloudis *et al*., 2001; Vider *et al*., 2001; Palmer *et al*., 2003). However, this phenomenon cannot occur with low training intensity (<50% VO2max). Previous studies involving the intensification of training in already well-trained athletes have shown unclear results (Seiler and Tønnessen, 2009; Seiler, 2010). Although studies have found no significant differences related to varying durations and intensities of training (John *et al*., 2003), there remains a lack of research specifically designed to investigate the effects of different intensity training across varied durations. Therefore, in this study, we grouped participants into two different intensity zones; 60% to 70% maximum heart rate (HRmax) for 45 min per session for the first group and 70% to 80% HRmax for 30 min per session for the second group, each exercising 3 days per week. Duration of training was increased from the 2nd to the 12th week by 3 and 2 min for the first and second groups, respectively. The findings may help to establish at which intensity zones the participants have better cardio-skeletal muscle markers adaptation.

METHODS

Figure 1. Sample size determination procedure (F tests - ANOVA: Repeated measures within factors; Analysis: A priori: Computed required sample size; Input: Effect size f=0.28; α err prob = 0.05; Power (1-β err prob); = 0.90; Number of groups = 2; Number of measurements = 3; Corr among rep measures; = 0.5; Non sphericity correction ε = 1)

This study was conducted in Bahir Dar, located about 578 km north-northwest of Addis Ababa-Ethiopia. It has an altitude of 1,840 meters above sea level and within latitude and longitude of 11°36′N 37°23′E coordinates. While the annual average temperature is 25-32^{o}c, with an average humidity of 58% (Haile 2009).

In This study, a completely randomized parallel-group study design was employed. Accordingly, the 30 subjects participating in this study, were randomly placed into two different intensity endurance exercise groups. Pre-training serum cardio skeletal muscle stress markers were assayed for both intensity exercise groups. The First-intensity group trained at 60-70% HRmax 45 min per session for the 1st week and 3-minute increments each week from the 2nd to 12th weeks. The second intensity training group was done at 70-80% HRmax 30 min per session for the 1st week and 2-minute increments each week from the 2nd to 12th weeks. At the end of 1 week (between the training test) and 12 weeks endurance training (post-test) was assessed.

Study population

Thirty healthy novice young athletes aged between 18-25 years old were recruited from Fasilo high school students. Only male athletes were included in the study to maintain the homogeneity of the study population. Consequently, a validated physical activity readiness questionnaire was used to evaluate conditions that may prohibit participants from practicing endurance training (Shephard, 2014).

Inclusion and exclusion criteria

Volunteer young male junior athletes between the ages of 18 and 25 years old were selected. This is because the physical performance of men above 25 years begins to decline. However, the performance of youngsters below the age of 18 years is in the process of developing, thus many factors may introduce bias to the final results. Subjects with normal body mass index were included this is due to the physiological differences between underweight and overweight. Subjects who reported health problems (heart issues like chest pain during exercise) and physical conditions (loss of balance, unconsciousness, and bone or joint problems) and were taking antihypertensive 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 cardiorespiratory biomarkers (Leel *et al*., 2013; Matthew 2014).

Sample size and sampling techniques

The sample size was determined using G*Power 3.1 software (Faul et al., 2007) based on a priori sample size analysis for F tests of repeated measure ANOVA. We adopted power in 0.90, α = 0.05, a correlation coefficient among repeated measures = 0.5, non-sphericity correction of 1, and an effect size of 0.28. From these values, a total sample size of n = 30 subjects was calculated at 120 total population as follows (Figure 1).

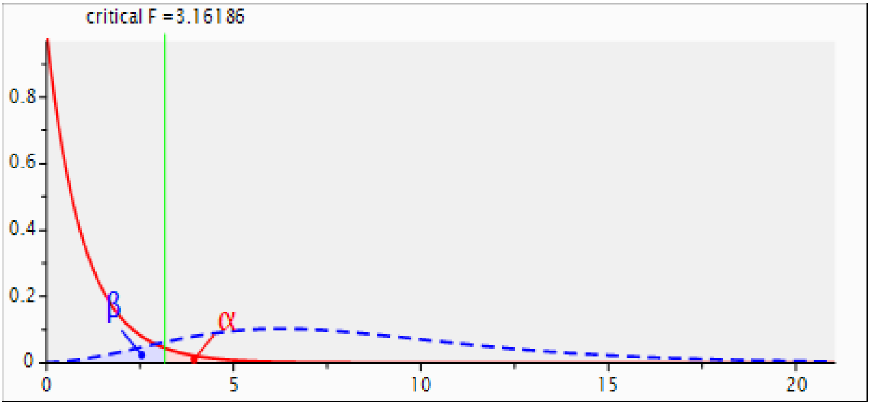

Figure 1. Sample size determination procedure (F tests - ANOVA: Repeated measures within factors; Analysis: A priori: Computed required sample size; Input: Effect size f=0.28; α err prob = 0.05; Power (1-β err prob); = 0.90; Number of groups = 2; Number of measurements = 3; Corr among rep measures; = 0.5; Non sphericity correction ε = 1)

Laboratory procedures

5mL blood samples were collected just before training (pretest), at the end of 1 (between training test), and 12 weeks (posttest) endurance training. Post-training samples were collected after 4 hours immediately the final training time at the end of 1 and 12 weeks training. Since the peak cardio-metabolic markers are achieved 3-4 hours after training time (Tian *et al*., 2012; Legaz *et al* 2015). Blood sample was collected from an antecubital vein using Vacutainer Serum Separator Tube (SST) comprising blood clotting accelerant gel. The serum was separated by centrifugation of the blood sample at 4000 rpm (revolution per minute) for 3 minutes and stored at -20 °C until analysis. The levels of lactate dehydrogenase (LDH) were measured using a spectrophotometric assay with a BS-2E chemistry analyzer according to the guidelines of the International Federation of Clinical Chemistry (Beckman Coulter, Krefeld, Germany). While, the levels of cardiac troponin I band (cTnI) and creatine kinase myocardial band (CK –MB) were measured by chemiluminescence immunoassay using Maglumi 800 fully automated chemiluminescence immunoassay analyzer (CLIA) via Shenzhen new industries biomedical engineering Co., Ltd. (snibe) protocol. We checked the quality of the data in the laboratory test with its control. As a result LDH results 458 U/L with a control range 357 – 513 U/L, CTnI results 5.048 ng/mL with a control range 4.28 - 7.96 ng/mL and

Ckmb results 73.92 ng/mL with a control range 52.9 - 98.3 ng/mL. Therefore, all the results are within their international quality control (IQC) ranges.

Data analysis

To compare the effects of high and moderate-intensity endurance training cardio skeletal muscles stress markers of athletes; one-way and repeated measure analysis of variance (ANOVA) was carried out using IBM-SPSS version 20. Post-hoc Bonferroni tests were used when appropriate. All statistical interpretations were seen at ($p \leq 0.05$) level of significance.

Ethical consideration

Ethical permission was obtained from the Research Ethics Review Committee of Mekelle University College of Health Science as conformed to the 1975 Declaration of Helsinki with Ref. ERC 1079/2017 dated 26/06/2017. Written consent was obtained from the participants and they were aware of the aim of the study. Involvements in this study were purely a voluntary activity and their right not to take part was respected. Issues of confidentiality and anonymity were also maintained.

RESULTS

There was a significant difference between pre, 1, and 12-week post-training, tests on the concentration of cardio skeletal muscle stress markers at 60-70%HRmax intensity zone (Table 1).

Table 1. Effects of twelve-week endurance training at 60-70% HRmax intensity on cardio skeletal stress markers

Variables	**Time**	***F***	***p*-value**
LDH	Sphericity Assumed	9.338	.001
	Greenhouse-Geisser	9.338	.002
CTnI	Sphericity Assumed	12.968	.000
	Greenhouse-Geisser	12.940	.000
CKmb	Sphericity Assumed	18.094	.000
	Greenhouse-Geisser	18.094	.000

LDH = Lactate dehydrogenase, CTnI = Cardiac troponin I, Creatine kinase myocardial band.

This indicates that the concentration of cardio skeletal muscle stress markers after 1 week showed a significant elevation as compared to pretest and post-training tests. Therefore, a significant value of LDH, CTnI, and Ckmb ($p < .001$) with medium effect size have been observed in all cardio skeletal muscle stress markers. Cardio skeletal muscle stress marker between the three tests showed a significant increase of the LDH level at 1-week training compared with pre-test values ($p = 0.007$) indicating 1-week endurance training exposed athletes to elevated concentrations of LDH than pre-training value. Interestingly, we observed a significant difference in CTnI levels, both at 1-week training (*p* = 0.001) and posttests (*p* = 0.033) compared to the pretest test value. This implies that 1-week endurance training showed higher concentration in CTnI than pre and post-tests. In addition, a significant difference in Ckmb levels was observed between the pretest and after 1 week (*p* = 0.001), pre and post-test (*p* = 0.005), and after 1 week and post-test (*p* = 0.048) (Table 2).

Table 2. Comparisons between pre-, after one week, and posttests of cardio skeletal muscle stress markers after 12 endurance training at 60-70%hrmax intensity zone

(I) Time	(J) Time	Mean difference (I-J)	Std. Error	*p*-Value[b]	95% Confidence interval for difference[b]	
					Lower Bound	Upper Bound
Measure: LDH						
1	2	-109.800*	29.475	.007	-189.905	-29.695
	3	-52.000	20.147	.065	-106.755	2.755
2	1	109.800*	29.475	.007	29.695	189.905
	3	57.800	25.763	.125	-12.219	127.819
3	1	52.000	20.147	.065	-2.755	106.755
	2	-57.800	25.763	.125	-127.819	12.219
Measure: Ctni						
1	2	-0.334*	.066	.001	-.513	-.154
	3	-0.147	.067	.138	-.330	.036
2	1	0.334*	.066	.001	.154	.513
	3	0.187*	.064	.033	.013	.360
3	1	0.147	.067	.138	-.036	.330
	2	-0.187*	.064	.033	-.360	-.013
Measure: Ckmb						
1	2	-2.283*	.461	.001	-3.536	-1.030
	3	-1.464*	.376	.005	-2.486	-.442
2	1	2.283*	.461	.001	1.030	3.536
	3	0.819*	.299	.048	.005	1.633
3	1	1.464*	.376	.005	.442	2.486
	2	-0.819*	.299	.048	-1.633	-.005

Based On Estimated Marginal Means
*. The Mean Difference Is Significant At The .05 Level. 1 = Pre-Training Test, 2 = After 1 Week Test; B. Adjustment For Multiple Comparisons: Bonferroni, 3 = Post-Training Test

Besides, only 20% of the participants' CTnI were above the URL of 0.1ng/ml concentration during the pretest. However, after 1week, the CTnI level was raised to 100% above URL and after 12 week post-training test, the participants' CTnI level decreased to 66.6% above URL indicating that the increased CTnI concentration after 1 week of training above URL might be a poor adaptation to endurance training. However, the decreased concentration of CTnI after 12 weeks of endurance training is indicative of adaptation to training stress (Figure 2). Similarly, 6.6% of the participants' LDH were above the URL of 198 IU/l level during the pretest. Consequently, after 1 and 12-week post-training tests, the participants' LDH level was raised to 66.6% and decreased to 46.6% above URL respectively (Figure 3). Indicative of poor training adaptation at 1 week and after 12 weeks of training the reduction of the concentration of these markers could be an indicator of adaptation to training stress resulting in less muscle cell damage. Interestingly, the participant’s Ckmb level was below the URL of 5 ng/ml at the pretest and post-training tests (Figure 4).

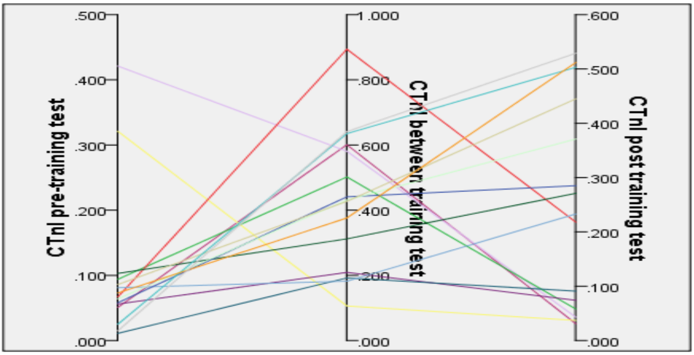

Figure 2. Patterns of CTnI concentration at pre-test, after 1 week, and post-test

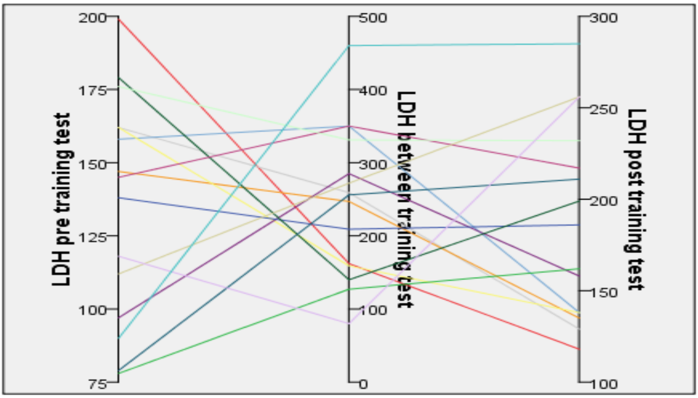

Figure 3. Patterns of LDH concentration at pre-test, after 1 week, and post-test

There was a significant difference between the pretest to 1 and 12-week post-training, tests on the concentration of cardio skeletal muscle stress markers at 70-80% HRmax intensity zone (Table 3). This indicates that the concentration of cardio skeletal muscle stress markers after 1 week showed a significant elevation as compared to pretest and post-training tests. Therefore, a significant value of LDH, CTnI, and Ckmb ($p <. 001$) with medium effect size has been observed in all cardio skeletal muscle stress markers.

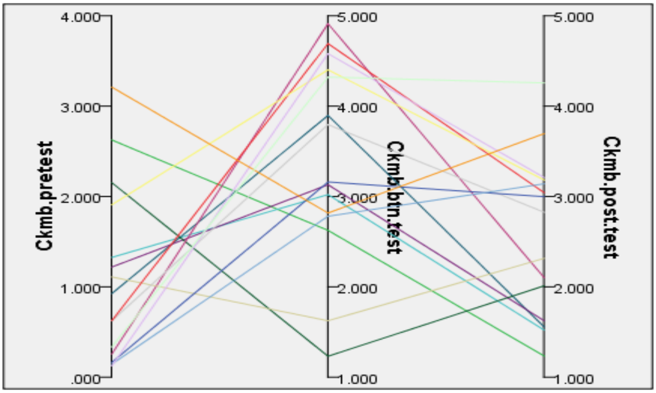

Figure 4. Patterns of Ckmb concentration at pretest, after 1 week, and post-test

Table 3. Effects of twelve-week endurance training at 70-80%HRmax intensity on cardioskeletal stress markers

Source	Time	Type III Sum of Squares	Df	Mean Square	F	*p*-value	ηp^2
LDH	Sphericity Assumed	106322.8	2.0	53161.4	13.0	.000	.482
	Greenhouse-Geisser	106322.8	1.5	69846.9	13.0	.000	.482
CTnI	Sphericity Assumed	1.8	2.0	.9	14.7	.000	.513
	Greenhouse-Geisser	1.8	1.6	1.1	14.7	.000	.513
Ckmb	Sphericity Assumed	41.8	2.0	20.9	12.3	.000	.468
	Greenhouse-Geisser	41.8	1.6	26.5	12.3	.001	.468

ηp^2 = Partial Eta Squared (effect size), df = degree of freedom, F = F value, sig = p value,
LDH = Lactate dehydrogenase, CTnI = Cardiac troponin I, Creatine kinase myocardial band.

In a comparative analysis of the three tests in cardio skeletal muscle stress marker at 70-80%HRmax intensity zone, LDH showed a significant increase at the end of 1 week (p = 0.003) and 12-week post-training tests (p = 0.03) compared to pre-test values indicating the observed elevation of LDH after 1-week training showed a significant decrease to training adaptation after 12-week endurance training. Similarly, we observed a significant increase in CTnI levels between both pretest to 1-week training (p = 0.001) and 1 week to posttests (p = 0.022) but there was no significant difference between pre and post-training tests. This implies that CTnI concentration after 12 weeks of endurance training becomes similar to the concentration level at pre-training, a test indicating training adaptation can reduce the serum CTnI level. In addition, a significant difference in Ckmb levels was observed between the pretest and after 1 week (p = 0.001). However, we did not observe significant differences between the pre and post-test as well as between after 1 week and the post-test (Table 4).

Table 4. Comparisons between pre-, after-week, and posttests of cardio skeletal muscle stress markers after 12 endurance training at 70-80% HRmax Intensity zone.

(I) Time	**(J) Time**	**Mean difference (I-J)**	**Std. error**	***p*-value[b]**	**95% CI [b]**	
					Lower bound	**Upper bound**
Measure: LDH						
1	2	-118.600*	28.662	.003	-196.496	-40.704
	3	-50.200*	17.186	.034	-96.908	-3.492
2	1	118.600*	28.662	.003	40.704	196.496
	3	68.400*	22.729	.028	6.628	130.172
3	1	50.200*	17.186	.034	3.492	96.908
	2	-68.400*	22.729	.028	-130.172	-6.628
Measure: CTnI						
1	2	-.476*	.094	.000	-.730	-.222
	3	-.147	.066	.132	-.327	.033
2	1	.476*	.094	.000	.222	.730
	3	.329*	.105	.022	.043	.615
3	1	.147	.066	.132	-.033	.327
	2	-.329*	.105	.022	-.615	-.043
Measure: Ckmb						
1	2	-2.361*	.364	.000	-3.351	-1.370
	3	-1.111	.460	.090	-2.362	.141
2	1	2.361*	.364	.000	1.370	3.351
	3	1.250	.578	.145	-.320	2.820
3	1	1.111	.460	.090	-.141	2.362
	2	-1.250	.578	.145	-2.820	.320

Based on estimated marginal means

*The mean difference is significant at the .05 level. b. Adjustment for multiple comparisons: Bonferroni.

In addition, 33% of the participants' CTnI was above URL of 0.1 ng/ml concentration during the pretest, after 1 week it increased to 100% above URL and after the 12-week post-training test, the participants' CTnI level decreased to 80% above URL. Indicating that increased CTnI concentration after 1 week of training above URL might be indicative of muscle cell damage as a result of poor adaptation to endurance training. However, the decreased concentration of CTnI after 12 weeks of training indicates adaptation to training stress (Figure 5). Similarly, 33% of the participants' LDH were above the URL of 198 IU/l level during the pretest. Consequently, after 1 week it increased to 86% above URL and after a 12-week post-training test, the participants' LDH level decreased to 73% above URL (Figure 6). This is an indicator of poor training adaptation at 1 week and less muscle cell damage as a result of adaptation to training after 12 weeks. Interestingly, the participant's Ckmb level was below the URL of 5ng/ml at the pretest and post-training tests. However, the Ckmb level was increased to 20% above URL after 1 week of training test an indicator of less training adaptation (Figure 7).

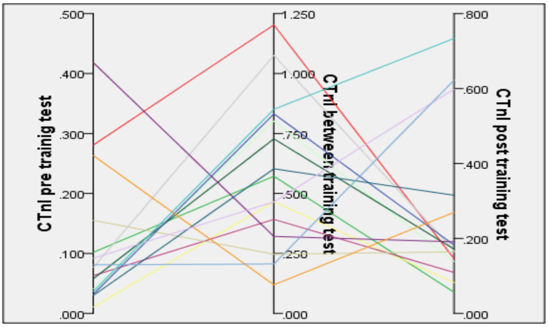

Figure 5. Patterns of CTnI concentration at pre test, after one week, and post-test

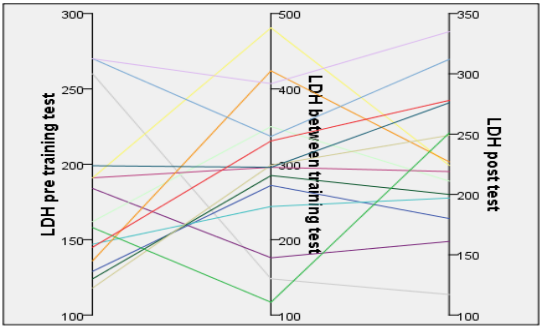

Figure 6. Patterns of LDH concentration at pretest, after one week, and post-test

DISCUSSION

This study aimed to evaluate cardio-metabolic responses to 12-week endurance training (60-70%) HRmax and (70-80%) HRmax intensity zones by continuous variation of the duration of exercise in young moderately trained athletes. Our main finding shows a reduction in CTnI level to 66.6% URL post-12-week exercise at 60-70% HRmax and 80% URL 70-80% HRmax intensity level. And the reduction in the LDH level to 46.6% URL post 12 weeks of exercise at 60-70% HRmax and 73% URL 70-80% HRmax intensity level.

We confirmed the finding by Serrano *et al*. (2009) that pre- to after 1-week training CTnI level was higher than the population upper reference limit (URL). It is important to note that CTnI levels do exceed the URL after exercise training due to a rise in core body temperature (Louie *et al*., 2013; Li *et al*., 2018). Consequently, in agreement with previous studies, CTnI levels were 60% above URL after 12 weeks post-training (Scharhag *et al*. 2005; Serrano *et al*., 2009; Serrano *et al*., 2011).

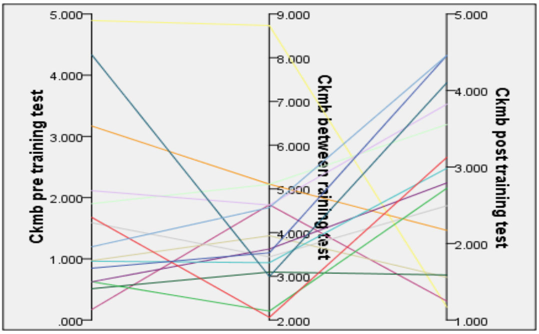

Figure 7. Patterns of Ckmb concentration at pretest, after one week, and post-test

Our participants were from an active population known for long-distance walking and running as part of their daily life as early as childhood, hence easily adaptable to the training stress (Scott *et al*, 2003). Therefore, cTnI levels above URL might be indicative of poor adaptation to endurance training (Scharhag *et al*. 2005; Neilan *et al*., 2006). Furthermore, elevated CK and LDH above URL after exercise training have been reported (Paschalis *et al*., 2007). Our findings showed that LDH levels were reduced to 46.6% above URL after 12 weeks post-training activity at 60-70% HRmax, indicative of less muscle cell damage hence a better adaptation to training.

Moreover, our result indicates that cardio skeletal muscle stress markers at 60-70% HRmax showed lesser concentration than 70-80% HRmax intensity level during post-training. The increase in cardio skeletal muscle stress markers might be due to the intensity level of the training. A similar study reported that the magnitude of cardio skeletal muscle stress markers is low or insignificant if the exercise intensity and duration are moderate (Middleton *et al*., 2008). However, we confirmed that 80% of participants' CTnI levels were above URL at 70-80% HRmax intensity after a 12-week post-training test as compared to 60-70% HRmax intensity level. Consistently, it was reported that cardiac troponin was reduced as event duration increased and had lower training intensity in endurance athletes (Shave *et al*., 2007; Serrano *et al*., 2011).

Besides, LDH levels increased 80% above URL after 1 week as exercise induced a significant increase in LDH (Mena *et al*., 1996). However, it reduced to 73 % above URL after 12 weeks post-training activity at 70-80% HRmax intensity indicative of adaptation to training. Consistently to our findings, the degree of increase depends on the intensity difference and duration of the training (Munjal *et al*., 1993). Therefore, our finding favors 60-70% HRmax with a longer duration for young athletes than 70-80% HRmax intensity level with a shorter duration. Prolonged and intensive endurance training activities elevate cardio-skeletal muscle stress markers concentration and remained increased for 2 weeks (Kobayashi *et al*., 2005) resulting the increased cardio-skeletal muscle stress markers and reduction in cardiac function (Neilan *et al*., 2006; La

Gerche *et al*., 2008). Therefore, the adaptation of training could be affected by intensive endurance training.

CONCLUSION

Our findings confirmed that endurance training at 60-70% maximum heart rate and 70-80% HRmax with gradual increment in training duration reduces exercise-induced cardio skeletal muscle stress markers in young moderately trained athletes. However, the reduction rate is less in 70-80% HRmax intensity level than in 60-70% HRmax indicating changes in cardio skeletal muscle stress markers are not easily adaptable in higher intensities. Therefore, we recommend a 60-70% HRmax intensity zone with a gradual increment in training duration for young novice athletes rather than a 70-80% HRmax intensity zone to promote safe training adaptation.

ACKNOWLEDGEMENTS

It is our pleasure to take this opportunity to thank all the Fasilo High School students who took part in our study. Their readiness to participate in the study has been tangible to this study, and their active participation has added immeasurable value to this concept. Further, we would also like to express our gratitude to Mekele University for their considerable contribution. The support of the university in terms of resources, guidance, and expertise has been really useful in making the objectives of our research achievable. The completion of this study would not have been possible without these efforts, and we genuinely appreciate everyone's engagement and commitment to our collaboration.

REFERENCES

Barnes, M.J. (2014). Alcohol: impact on sports performance and recovery in male athletes. *Sports Medicine* **44**(7): 909–919.

Bessa, A., Oliveira, V.N., De Agostini, G.G., Oliveira, R.J.S., Oliveira, A.C.S., White, G., Wells, G., Teixeira, D.N.S., Mineiro, U and Espindola, F.S. (2016). Exercise intensity and recovery: biomarkers of injury, inflammation, and oxidative stress. *Journal of Strength and Conditioning Research* **30**: 311–319.

Bessa, A., Oliveira, V.N., De Agostini, G.G., Oliveira, R.J.S., Oliveira, A.C.S., White, G., Wells, G., Teixeira, D.N.S and Mineiro, U. (2008). High-intensity ultraendurance promotes early release of muscle injury markers. *British Journal of Sports Medicine* **42**: 889–893.

Brancaccio, P., Lippi, G and Maffulli, N. (2010). Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine* **48**: 757–767.

Epstein, Y., Shani, Y., Meckel, Y., Zakin, V., Berman, A., Sheffer, H and Heled, Y. (1995). Clinical significance of serum creatine phosphokinase activity levels following exercise. *Israeli Journal of Medical Sciences* **31**: 698–699.

Faul, F., Erdfelder, E., Lang, A-G and Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods* **39**(2): 175–191.

Haile, Z. (2009). Assessment of climate change impact on the net basin supply of Lake Tana water balance. *International Institute for Geo-Information Science and Earth Observation*, Enschede, The Netherlands.

John, M., Bess, H., Kara, I., Melissa, N and Wei, L. (2003). Effect of exercise duration and intensity on weight loss in overweight sedentary women. *Journal of the American Medical Association* **290**: 1323–1330.

Kim, Y., Choi, S., Kim, D., Lee, S., Park, H and Jeong, J. (2009). Changes in serum cartilage oligomeric matrix protein (COMP), plasma CPK and plasma Hs-CRP in relation to running distance in a marathon (42.195 km) and an ultra-marathon (200 km) race. *European Journal of Applied Physiology* **105**: 765–770.

Kobayashi, Y., Takeuchi, T., Hosoi, T., Yoshizaki, H and Loeppky. J.A. (2005). Effect of a marathon run on serum lipoproteins, creatine kinase, and lactate dehydrogenase in recreational runners. *Research Quarterly for Exercise and Sport* **76**: 450–455.

Kratz, A., Lippi, G., Montagnana, M., Salvagno, G.L and Zaninotto, M. (2002). Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *American Journal of Clinical Pathology* **118**: 856–863.

La Gerche, A., Connelly, K.A., Mooney, D.J., MacIsaac, A.I and Prior, D.L. (2008). Biochemical and functional abnormalities of left and right ventricular function after ultra-endurance exercise. *Heart* **94**: 860–866.

Lee1, K.C and Wen-Dien Chang, W.D.. (2013). The effects of cigarette smoking on aerobic and anaerobic capacity and heart rate variability among female university students. *International Journal of Women's Health* **5**(1): 667–679.

Legaz-Arrese, A., Terreros-Blanco, J.L and Bocos-Terraz, P. (2015). Impact of an endurance training program on exercise-induced cardiac biomarker release. *American Journal of Physiology – Heart and Circulatory Physiology*, **308**: 100–107.

Li, Q., Sun, R., Lyu, H., Shen, D., Hu, Q., Wang, H., Wang, N., Yan, J and, Wang, J. (2018). Effects of different core temperatures after heat stroke on serum inflammatory cytokines and multiple organ dysfunction syndrome in rats. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*, **30**(5): 439–443.

Lippi, G., Schena, F., Montagnana, M and Salvagno, G.L. (2008). Influence of acute physical exercise on emerging muscular biomarkers. *Clinical Chemistry and Laboratory Medicine*, **46**: 1313–1318.

Louie, R.F., Ferguson, W.J., Curtis, C.M., Vy, J.H., Tang, C.S and Kost G.J. (2013). Effects of environmental conditions on point-of-care cardiac biomarker test performance during a simulated rescue: implications for emergency and disaster response. *American Journal of Disaster Medicine* **8**(3): 205–212.

Mastaloudis, A., Moser, R and and Ring, S. (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radical Biology and Medicine* **31**(7): 911–922.

Mena, P., Maynar, M and Campillo, J.E. (1996). Changes in plasma enzyme activities in professional racing cyclists. *British Journal of Sports Medicine* **30**: 122–124.

Michael, G. (2002). Biochemical and immunological markers of over-training. *Journal of Sports Science and Medicine* **1**: 31–41.

Middleton, N., George, K.P., Whyte, G.P., Shave, R., Gaze, D and Collinson, P. (2008). Cardiac troponin T release is stimulated by endurance exercise in healthy humans. *Journal of the American College of Cardiology* **52**: 1813–1814.

Munjal, D.D., McFadden, J.A., Matix, P.A., Coffman, K.D and Cattaneo, S.M. (1993). Changes in serum myoglobin, total creatine kinase, lactate dehydrogenase, and creatine kinase MB levels in runners. *Clinical Biochemistry* **16**: 195–199.

Neilan, T.G., Januzzi, J.L., Lee-Lewandrowski, E., Ton-Nu, T.Tand Yoerger, D.M. (2006). Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston Marathon. *Circulation* **114**: 2325–2333.

Nunes, J.P.L., Mota Garcia, J.M., Farinha, R.M.B, Silva, J.C., Magalhães, D., Pinheiro, L.V and Lima, C.A. (2003). Cardiac troponin I in aortic valve disease. International Journal of Cardiology **89**: 281–285.

Palmer, F., John, D., Andrew, S., Blake, R and Williams, M. (2003). Influence of vitamin C supplementation on oxidative and salivary IgA changes following an ultramarathon. *European Journal of Applied Physiology* **89**: 100–107.

Paschalis, V., Giakas, G., Baltzopoulos, V., Jamurtas, A.Z., Theoharis, V., Kotzamanidis, C and Koutedakis, Y. (2007). The effects of muscle damage following eccentric exercise on gait biomechanics. *Gait and Posture* **25**: 236–242.

Perry, C.G.R., Heigenhauser, G.J.F., Bonen, A and Spriet, L.L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism* **33**(6): 1112–1123.

Rifai, N., Douglas, P.S., O'Toole, M., Rimm, G and Ginsburg, G.S. (1999). Cardiac troponin T and I, echocardiographic [correction of electrocardiographic] wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman triathlon. *American Journal of Cardiology* **83**: 1085–1089.

Romagnoli, M., Alis, R., Aloe, R., Salvagno, G.L., Basterra, J., Galeano, H.P and Gomar, F.S.. (2014). Influence of training and a maximal exercise test on analytical variability of muscular, hepatic, and cardiovascular biochemical variables. *Scandinavian Journal of Clinical and Laboratory Investigation* **74**: 192–198.

Scharhag, J., Herrmann, M., Urhausen, A., Haschke, M., Kindermann, W and Herrmann, W. (2005). Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. *American Heart Journal* **150**: 1128–1134.

Scharhag, J., Urhausen, A., Schneider, G., Herrmann, M., Schumacher, K., Haschke, M., Krieg, A., Meyer, T., Herrmann, W and Kindermann, W. (2006). Reproducibility and clinical significance of exercise-induced increases in cardiac troponins and N-terminal pro-brain natriuretic peptide in endurance athletes. *European Journal of Cardiovascular Prevention and Rehabilitation* **13**: 388–397.

Scott, R.A., Georgiades, E., Wilson, R.H., Goodwin, W.H., Wolde, B and Pitsiladis, Y.P. (2003). Demographic characteristics of elite Ethiopian endurance runners. *Medicine and Science in Sports and Exercise* **35**(10): 1727–1732.

Seiler, S and Tønnessen, E. (2009). Intervals, thresholds, and long slow distance: the role of intensity and duration in endurance training. *Sportscience* **13**: 32–53.

Seiler, S. (2010). What is best practice for training intensity and duration distribution in endurance athletes? *International Journal of Sports Physiology and Performance* **5**: 276–291.

Serrano-Ostáriz, E., Legaz-Arrese, A., Terreros-Blanco, J.L., López-Ramón, M., Bocos-Terraz, P., Cremades-Arroyos, D., Carranza-García, L.E and Izquierdo-Álvarez, S. (2009). Cardiac biomarkers and exercise duration and intensity during a cycle-touring event. *Clinical Journal of Sports Medicine* **19**: 293–299.

Serrano-Ostariz, J.L., Terreros-Blanco, A., Legaz-Arrese, K., George, R., Shave, P., Bocos-Terraz, S., Izquierdo-Álvarez, J.L., Bancalero, J.M., Echavarri, J.M and Quilez, J., T. (2011). The impact of exercise duration and intensity on the release of cardiac biomarkers. *Scandinavian Journal of Medicine and Science in Sports* **21**: 244–249.

Shave, R., George, K.P., Atkinson, G., Hart, E., Middleton, N., Whyte, G., Gaze, D and Collinson, P.O. (2007). Exercise-induced cardiac troponin T release: a meta-analysis. *Medicine and Science in Sports and Exercise* **39**: 2099–2106.

Shephard, R.J. (2014). Physical activity participation, clearance, and prescription. *The Health and Fitness Journal of Canada* **7**(1).

Smith, J., Thomas, J.R., Wilson, M Harris, S. (2004). Effects of prolonged strenuous exercise (marathon running) on biochemical and hematological markers used in the investigation of patients in the emergency department. *British Journal of Sports Medicine* **38**(3): 292–294.

Suzuki, K., Yamaguchi, M., Tanaka, H and Nakamura, T. (2003). Impact of competitive marathon race on systemic cytokine and neutrophil responses. *Medicine and Science in Sports and Exercise* **35**: 348–355.

Tian, Y., Jinlei, N., Chuanye, H and George, K.P. (2012). The kinetics of highly sensitive cardiac troponin T release after prolonged treadmill exercise in adolescent and adult athletes. *Journal of Applied Physiology* **113**: 418–425.

Trapp, E.G., Chisholm, D.J., Freund, J and Boutcher, S.H. (2008). The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *International Journal of Obesity* **32**(4): 684–691.

Vider, J., Sniadecki, M., Kowalski, A and Piotrowski, J. (2001). Acute immune response in respect to exercise-induced oxidative stress. *Pathophysiology* **7**: 263–270.

Wu, H.J., Zhang, L., Chen, Y and Xu, W. (2004). Effects of 24 h ultra-marathon on biochemical and hematological parameters. *World Journal of Gastroenterology* **10**: 2711–2714.