Effects of altitude on chronotype orientations in relation to cardiorespiratory and hematological quantities of college students in Ethiopia

Efrem Kentiba1,2*, Mala George3, Soumitra Mondal2, D. Mathi Vanan2

1 Department of Sports Science, Arba Minch College of Teachers’ Education, Arba Minch, Ethiopia, 2 Department of Sports Science, Mekelle University College of Natural and Computational Sciences, Mekelle, Ethiopia, 3 Department of Biochemistry, School of Medicine, Division of Biomedical Sciences, Mekelle University, Mekelle, Ethiopia

*efre89@gmail.com

Abstract

Background

The mechanism by which Ethiopians adapt to altitude is quite unique compared to other Highlanders with respect to increased oxygen saturation of hemoglobin. Although the effects of altitude on cardiorespiratory and hematological quantities on athletics performances are well known, but there is little information about its underlying effect on chronotype orientations.

Methods

In this cross-sectional study 60 male college students with mean age 20±1.3 years from high and low altitude regions living in a tropical setting in Ethiopia were included. The participants’ chronotype was determined using the self-administered Horne and Ostberg Morningness-Eveningness Questionnaires (MEQ). Measurements and estimations of hematological and cardiorespiratory parameters were performed from 7:00–9:00 AM, East African time zone, in order to minimize any variations that might occur in the course of the day. A multivariate binary logistic regression model was fitted to analyze the underlying chronotype predictors.

Results

28 (93.9%) of participants from high altitude were mainly intermediate type (I-type) dominant with (MEQ = 42–58). While, 16 (55.2%) of participants from low altitudes were morning type (M-type) dominant chronotype with (MEQ = 59–69). Our main finding confirmed that altitude is an independent predictor of chronotype orientations of the participants (p < 0.015). Thus, the results of the multivariate analysis seem to indicate that, participants from low and high altitudes may be uniquely oriented towards either M-type or I-type chronotype respectively (adjusted odds ratio [AOR] 4.772, 95% CI = 3.748–4618458). However, no significant
difference on cardiorespiratory and hematological quantities between I-type and M-type chronotype of students from low altitude living in the same setting was reported (p > 0.05).

Conclusion
Our finding, reported for the first time that, the human chronotype varies according to the altitude, with no underlying effect of cardiorespiratory and hematological quantities.

Introduction
Altitude adaptation in humans is an instance of evolutionary modification in certain populations, like the Tibetans, Andean inhabitants and Ethiopians [1]. This is perhaps due to their migration to the highland was relatively early [2]. The adaptation to high altitude among different Highlanders also arose independently of convergent evolution [3]. The analysis of specific genes that influence adaptation to high altitude seems to be expressed highly among Ethiopians compared to other Highlanders [4]. These genes are known to produce hypoxia induced factors known to regulate production of red blood cells and oxygen saturation of hemoglobin [5]. However, a sustained exposure to hypoxia may alter body composition [6], raise stress hormones [7], affects the volume of maximum oxygen consumption (VO\textsubscript{2}Max) and sleep patterns [8–10].

Further studies have highlighted differences between populations living in high and low altitudes based on morphophysiological characteristics [11,12]. Thus, people living at high altitudes have been reported to be thinner and shorter than those from the sea level. This is due to the effects of altitudes on oxygen saturation of hemoglobin [5], VO\textsubscript{2}Max, stress hormones [7] and blood compositions and circulation [13]. Hence, the irreversible, long-term physiological responses are associated with heritable behavioral changes in chronotype orientations [3,14,15]. Although the effects of altitude on cardiorespiratory and hematological quantities on athletics performances are well known [11–15], but there is little information about its underlying effect on chronotype orientations.

Chronotype refers to individual’s time-of-day preferences of activities that can be classified as “M = morning type”, “E = evening type” and “I = intermediate types” [16–18]. However, peoples’ preference of activity schedules may not be possible to match with their chronotype orientations [16,19,20]. This is in spite of the reported impact of a person’s chronotype orientation on their response to exercise [21,22], academic achievement and other activities [23–25]. Furthermore, recent studies have reported that the observed chronotype in athletes may be different to that of non-athletes [26–30] and varied either by longitudes or latitudes [31]. The underlying influence of longitudes and latitudes on chronotype orientations has been related to scotopic periods (i.e., the time interval between sunset and sunrise) and photopic periods (i.e., the time interval between sunrise and sunset) [32–34]. Therefore, the exposure to bright sunlight may affect the phase position of the main sleep episode, leading to different sleep-wake patterns [35].

In our previous study, we reported that the chronotype preferences of college students may vary according to their altitude backgrounds [36]. However, the underlying parameter by which altitudes may influence chronotype orientations is still unknown. Hence, cross-sectional study design was used to assess cardiorespiratory and hematological quantities of 60 untrained male college students from high and low altitude living in tropical settings of Ethiopia. To associate the influence of altitude on chronotype orientations in relation to cardiorespiratory and
hematological quantities, a multivariate binary logistic regression analysis was used. Thus, the outcome of this study may be important to exercise physiologists, coaches and athletes while scheduling training and competition for participants from either high or low altitude backgrounds living in tropical settings.

Materials and methods

Study setting and ethical approval

Ethical approval was obtained from Mekelle University College of Health Sciences; Health Research Ethics Review Committee (HRERC) with Ref. ERC 1078/2017 dated 26/06/2017 and conducted in accordance with the declaration of Helsinki. In addition a written consent to participate in the study was obtained from participants. The study was conducted in Arba Minch town South-West Ethiopia, with an elevation of 1286 meters above sea level [37,38], which is categorized as low altitude tropical setting [39]. It is located at 6.0206˚N and 37.5641˚E, latitude and longitude coordinates with an average annual temperature of 25.2˚C, (average high and low temperature of 28.7˚C and 21.8˚C respectively) [36].

Study design and participants

A cross-sectional study design was used, to assess the chronotype, cardiorespiratory and hematological quantities of 60 male college students with mean age 20±1.3 years from high and low altitude regions living in tropical settings of Ethiopia (Fig 1). The number of participants was based on a formula for sample size of the mean [40]; assuming 95% confidence interval, 5% margin of error and 0.1 standard deviation obtained from previous studies [41,42]. A self-reported demographic questionnaire was used to determine, among other things the places of their origin and growth before joining the college within the study setting. Therefore, based on the altitude classification by the Canadian Academy of Sport and Exercise Medicine (CASEM) [39], participants who originated and grew up from areas between 500–2000 meters above sea level were grouped under low altitude backgrounds. While those from areas above 3000 meters above sea level were grouped under high altitude backgrounds [36,39]. However, participants who reported to be from neither high nor low altitude backgrounds were excluded from the study. Based on the information obtained, participants were grouped either to high (n = 30) or low altitude backgrounds (n = 29) with (n = 1) missing. Then after, data about chronotype orientations, hematological and cardiorespiratory parameters were obtained. The participants’ chronotype was determined using the self-administered Horne and Ostberg Morningness-Eveningness Questionnaires (MEQ) [43].

Procedures

All students participating in the study had no any preceding intense exercise, declared as healthy and in good physical condition. Measurements and estimations of hematological and cardiorespiratory parameters were performed from 7:00–9:00 AM, East African time zone, in order to minimize any variations that might occur in the course of the day [41,44–47]. \( \text{VO}_{2\text{max}} \) as an indicator of cardiorespiratory endurance (CRE) [48], was estimated using maximal exercise test (20 meter multistage shuttle run test) or beep test [49–50]. The test has been shown to be an accurate method to estimate \( \text{VO}_{2\text{Max}} \) in young adults with \( r = 0.9 \)[51]. The test was employed as outlined by the American College of Sports Medicine [52]. Generally, it was taken at 8.5 km/hr (level 1) and increased by 0.5km/hr at each level. The result was obtained by using online beep test calculator (BTC) based on the number of shuttles attained at each level. The calculator appears to be accurate within 0.1 ml/kg/min of the published values [53].
Blood sampling was done according to the procedures explained by Simundic et al. 2017. Participants were left to sit for 15 minutes prior to sampling. 5 ml venous blood was drawn from the ulnar vein of the non-dominant hand using a 20 G x 1½”–0.9 x 40 mm syringe after application of a tourniquet and cleansing the site. The blood was introduced into a tube with Ethylene Di-amine Tetra Acetate (EDTA) to determine the concentration of erythrocytes, leukocytes and thrombocytes using a hematology analyzer (BC-3000Plus Mindray Medical, Andheri East, Mumbai, Maharashtra India) [54].

**Data analysis**

All data were tested for normality using the Pearson normality test. Descriptive statistics were expressed either as mean ± standard deviation or frequency (proportion) for continuous and categorical variables respectively. To associate the influence of altitude on chronotype
orientations in relations to cardiorespiratory and hematological parameters, a multivariate binary logistic regression analysis was conducted. Independent sample t-test was used to compare the mean morningness-eveningness questionnaire results (MEQR), V\textsubscript{o\textsubscript{2}}Max, body mass index (BMI) and hematological parameters between students from high and low altitude backgrounds. Similarly, we used the above test to compare MEQR of V\textsubscript{o\textsubscript{2}}Max and hematological quantities between intermediate type (I-type) and morning type (M-type) chronotype orientations of students from low altitude backgrounds. All statistical analyses were performed using IBM-SPSS version 20 (IBM, Armonk, NY, United States of America). All reported p-values are two tailed and confidence intervals are calculated at 5% alpha value.

Results

No between-group differences (Age, height, body mass and BMI) existed at baseline, so the groups were well matched at entry level (Table 1). The chronotype preferences of participants from high compared to low altitudes indicated significant differences (P < 0.001). Thus, 28 (93.9%) of participants from high altitude were mainly (I-type) dominant chronotype (MEQ = 42–58). While 16 (55.2%) of participants from low altitudes were (M-type) dominant chronotype (MEQ = 59–69) (Figs 2–4).

The mean cardiorespiratory and hematological quantities of participants from high compared to low altitudes also indicated significant differences except for MCV and PLT (p < 0.05). However, there was no significant differences in the mean hematological and cardiorespiratory quantities between I-type and M-type chronotype orientation of participants from low altitude backgrounds (p > 0.05) Tables 1 and 2.

The predictor variables of chronotype orientation of students from varied altitude backgrounds are given in Table 3. The only significant predictor of the chronotype orientations was the altitude background of the participants (p < 0.015). Thus the results of the multivariate analysis seem to indicate that, participants from low and high altitudes may be uniquely

Table 1. Comparison of demographic, hematological, cardiorespiratory and chronotype orientations between participants from high and low altitudes (n = 59).

| Variables               | High Altitude Backgrounds (n = 30) | Low Altitude Backgrounds (n = 29) | p-value |
|-------------------------|------------------------------------|-----------------------------------|---------|
| Age                     | 19.93 ± 1.45                       | 20.28 ± 1.16                      | 0.319   |
| Height                  | 1.68 ± 0.05                        | 1.70 ± 0.08                       | 0.285   |
| Body mass               | 61.70 ± 4.70                       | 61.91 ± 4.82                      | 0.864   |
| BMI                     | 21.49 ± 1.95                       | 22.05 ± 1.57                      | 0.223   |
| Chronotype preferences  | I-type = 28 (93.9%)               | M-type = 16 (55.18%)             |         |
|                         | MEQR = 53.10 ± 4.94               | MEQR = 59.31 ± 5.83              | 0.001   |
| Cardiorespiratory Parameters (V\textsubscript{o\textsubscript{2}}Max) | 51.84 ± 7.64                                         | 47.25 ± 9.41                           | 0.044   |
| Hematological quantities |                                    |                                   |         |
| HGB in g/dL              | 16.43 ± 1.19                       | 15.69 ± 0.91                      | 0.010   |
| RBC in (x10\textsuperscript{6} / μL) | 5.33 ± 0.31                        | 5.15 ± 0.32                       | 0.034   |
| HCT in %                | 49.16 ± 2.96                       | 47.20 ± 2.49                      | 0.008   |
| MCV in fL               | 92.45 ± 3.69                       | 91.8 ± 3.17                       | 0.501   |
| PLT (x10\textsuperscript{3} / μL) | 234.37 ± 39.58                     | 254.06 ± 57.96                    | 0.132   |
| WBC (x10\textsuperscript{3} / μL) | 7.01 ± 2.00                         | 5.74 ± 0.70                       | 0.015   |

V\textsubscript{o\textsubscript{2}}Max = volume of maximum O\textsubscript{2} consumption in ml/(kg·min), MEQR = MEQ results, BMI = body mass index, HGB: hemoglobin count, RBC: red blood cell count, HCT: hematocrit, MCV: mean corpuscular volume, PLT: platelet, WBC: white blood cell count

https://doi.org/10.1371/journal.pone.0219836.t001
Discussion

This is the first study to our knowledge that investigated the effects of altitude on chronotype orientations in relation to cardiorespiratory and hematological quantities of students from high and low altitude backgrounds living in a tropical setting. Our main finding confirmed that altitude is an independent predictor of chronotype orientations of the participants [36].

Based on the cited studies [55–62] one might hypothesize that the effects of acclimatization on sleep are altitude dependent. Early, studies have suggested that a pronounced sleep fragmentation was a characteristic change occurring with exposure to hypoxia [63]. As suggested previously by Ashkenazi et al 1982, hypoxia may act as real phase-shift inducer of the circadian system [64]. This effect may be related to a delayed phase of sleep-wake cycle. Consequently, we found that participants from low and high altitudes living in a tropical setting may be uniquely oriented towards either be M-type or I-type chronotype respectively. Since, increase in sleep onset latency (SOL) after hypoxic exposure at high altitude seems to depend on the evening decline of core body temperature and plasma melatonin [65–67]. Therefore, our finding is consistent with the study by, Coste et al., 2004a that significant negative association between the SOL and the age-correlated Horne & Ostberg score reflects a close relation.

Fig 2. MEQ scores of students from high and low altitudes living in tropical settings.

https://doi.org/10.1371/journal.pone.0219836.g002
between an elevated morningness preference and short SOL. However, further studies are required to better quantify the effects of different levels of altitude on sleep in persons of both sexes and of various ages and to elucidate the underlying physiological mechanisms.

Most surprisingly, we did not report E-type dominant chronotype orientations, despite the young adults participating in the study. Considering that changes from M-type to E-type take place according to age, with higher prevalence of M-type occurring during childhood and higher prevalence of E-type during adolescence [23,68,69]. The adolescence age group is known to poorly tolerate altitude related stresses [8–10], which may affect their sleep patterns. Thus, in our study, the absence of E-type chronotype might be obscured by the unique evolutionary adaptation to altitude by the participants. Our finding further reported no significant difference on VO$_2$Max and hematological quantities between I-type and M-type chronotype among students from low altitude living in a tropical setting. However, previous studies reported a better VO$_2$max in E-types than M-types [68,70–72]. This might be reflective of better aerobic participation in energy metabolism during the evening time, leading to both mental and physical activeness among E-type than other chronotype [16,36,73]. Although our finding is not consistent with previous studies, but this might be due to absence of E-type among compared chronotype orientations. Furthermore, participants in our study are homogenous in their altitude backgrounds (low only) and may similarly adapt to increased blood plasma and oxygen volume.
Evidence supports the notion that performance can be improved if individuals are matched with their preferred chronotype [17,36]. Our finding that human chronotype varies according to altitude may be useful for exercise physiologists, coaches and athletes while either

![Fig 4. MEQ scores of students from low altitude backgrounds.](https://doi.org/10.1371/journal.pone.0219836.g004)

Table 2. Mean hematological and cardiorespiratory parameters between I-type and M-type chronotype orientations of participants from low altitudes backgrounds (n = 29).

| Variables                  | Low Altitude Backgrounds |   |   |   |
|----------------------------|--------------------------|---|---|---|
|                            | I-type (n = 13) (Mean ± SD) | M-type (n = 16) (Mean ± SD) | p-value |
| Cardiorespiratory parameters (Vo₂Max) | 49.09±10.09 | 45.75±8.86 | 0.352 |
| Hematological quantities  |  |  |  |
| HGB in g/dL                | 15.85±0.62 | 15.57±1.10 | 0.413 |
| RBC in (x10⁶ / μL)         | 5.17±0.30 | 5.13±0.34 | 0.757 |
| HCT in %                   | 47.47±1.75 | 46.97±3.01 | 0.600 |
| MCV in fl                  | 92.06±3.43 | 91.66±3.04 | 0.738 |
| PLT in (x10³ / μL)         | 246.53±6.23 | 260.18±66.87 | 0.538 |
| WBC in (x10³ / μL)         | 5.70±0.80 | 5.78±0.64 | 0.765 |

Vo₂Max = volume of maximum O₂ consumption in ml/(kg·min), HGB: hemoglobin count, RBC: red blood cell count, HCT: hematocrit, MCV: mean corpuscular volume, PLT: platelet, WBC: white blood cell count

[https://doi.org/10.1371/journal.pone.0219836.t002](https://doi.org/10.1371/journal.pone.0219836.t002)
scheduling training or competition for participants. Since, altitude effects on blood compositions and circulation may lead to an altered cardiorespiratory and hematological quantities [13]. Thus, the strength of this study is that we evaluated if the above underlying parameters of students from varied altitude background that might influence their chronotype orientations. However, students from high altitude background were majorly I-type dominant and we could not compare their chronotype orientation in relation to cardiorespiratory and hematological quantities. Furthermore, it would be interesting to assess the underlying effect of altitude on chronotype of participants from low living in high altitude in order to give bidirectional relationship. Whether our findings indicate the uniqueness of Ethiopians to altitude adaptation in tropical setting in relation to chronotype orientation needs further investigation.

**Conclusion**

Our finding, reported for the first time that, the human chronotype varies according to the altitude, with no underlying effects of cardiorespiratory and hematological quantities.

**Supporting information**

S1 File. Raw data (Demographic and cardiorespiratory quantities).
(XLSX)

S2 File. Raw data (Hematological quantities).
(XLSX)

**Acknowledgments**

During this study the corresponding author was Exercise Physiology PhD candidate at Mekelle University. We would like to thank Mekelle University, Arba Minch College of Teachers’
Education and all students participated in the study. Also, we would like to express our sincere gratitude to the staffs involved in the fieldwork.

**Author Contributions**

*Conceptualization:* Efrem Kentiba, Mala George, Soumitra Mondal, D. Mathi Vanan.

*Data curation:* Efrem Kentiba, Soumitra Mondal.

*Formal analysis:* Efrem Kentiba, D. Mathi Vanan.

*Funding acquisition:* Efrem Kentiba.

*Investigation:* Efrem Kentiba.

*Methodology:* Efrem Kentiba, Mala George, Soumitra Mondal, D. Mathi Vanan.

*Project administration:* Efrem Kentiba.

*Resources:* Soumitra Mondal.

*Supervision:* Mala George.

*Validation:* Mala George, Soumitra Mondal, D. Mathi Vanan.

*Visualization:* Mala George.

*Writing – original draft:* Efrem Kentiba, Mala George.

*Writing – review & editing:* Efrem Kentiba, Mala George, Soumitra Mondal, D. Mathi Vanan.

**References**

1. Windsor JS, Rodway GW. Altitude. Postgr Med J. 2007; 83:148–51.

2. Pleurdeau D. Human technical behavior in the African Middle Stone Age: The lithic assemblage of Porc-Epic Cave (Dire Dawa, Ethiopia). African Archaeol Rev. 2006; 22(4):177–97.

3. Scheinfeld LB, Soi S, Thompson S, Ranciaro A, Woldemeskel D, Beggs W et al. Genetic adaptation to high altitude in the Ethiopian highlands. Genome Biol. 2010; 13(1).

4. Alkorta-Aranburu G, Beall CM, Witonsky DB, Gebremedhin A, Drgbasdrg B Prichard JK. The genetic architecture of adaptations to high altitude in Ethiopia. PLOS Genet. 2010; 8(12).

5. Van Patot MC, Gassmann M; Gassmannv an Patot MC GMG. Hypoxia: adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2α. High Alt Med Biol. 2011; 12(2):157–67. [https://doi.org/10.1089/ham.2010.1099 PMID: 21718164](https://doi.org/10.1089/ham.2010.1099 PMID: 21718164)

6. Kayser B, Verges S. Hypoxia, energy balance and obesity: From pathophysiological mechanisms to new. Obes Rev. 2013;1–14.

7. Sørensen B, Weber ROYE. Effects of oxygenation and the stress hormones adrenaline and cortisol on the viscosity of blood from the trout oncorhynchus mykiss. J Exp Biol. 1995; 198:953–9. PMID: 9318749

8. West JB. Review The Physiological Basis of High-Altitude Diseases OF. Ann Intern Med. 2004; 141:789–800. [https://doi.org/10.7326/0003-4819-141-10-200411160-00010 PMID: 15545679](https://doi.org/10.7326/0003-4819-141-10-200411160-00010 PMID: 15545679)

9. San T, Polat S, Cingi C, Eskiiizmir G, Oghan F, Cakir B. Effects of High Altitude on Sleep and Respiratory System and Theirs Adaptations. Hindawi Publ Corp Sci J. 2013;1–7.

10. Nussbaumer-Ochsner Yvonne, Schuepfer Nicole, Siebenmann Christoph, Maggiorini Marco and KEB. High altitude sleep disturbances monitored by actigraphy and polysomnography. High Alt Med Biol. 2011; 12(3):229–36. [https://doi.org/10.1089/ham.2010.1073 PMID: 21962066](https://doi.org/10.1089/ham.2010.1073 PMID: 21962066)

11. Morfofisiol P, Atencional C. Effects of High Altitude on Morphophysiological Patterns, Perception and Attention Capacity in Students from Putre (3500 m. a. s. l.) and Arica (2 m. a. s. l.), Chile. Int J Morphol. 2014; 32(2):593–8.

12. Wilson M. H.; Newman S. & Imray CH. The cerebral effects of ascent to high altitudes. Lancet Neurol. 2009; 8(2):175–91. [https://doi.org/10.1016/S1474-4422(09)70014-6 PMID: 19161909](https://doi.org/10.1016/S1474-4422(09)70014-6 PMID: 19161909)
13. Kaur C. & Ling EA. Blood brain barrier in hypoxic-ischemic conditions. Curr Neurovasc Res. 2008; 5 (1):71–81. PMID: 18289024

14. Koskenvuo M, Hublin C, Partinen M. Heritability of diurnal type: a nationwide study of 8753 adult twin pairs. J Sleep Res. 2007; 16:156–62. https://doi.org/10.1111/j.1365-2869.2007.00580.x PMID: 17542945

15. Krieger E, Pedrazzoli M, Vallada H, Pereira AC. Distribution and heritability of diurnal preference (chronotype) in a rural Brazilian family-based cohort, the Baependi study. Sci Rep. 2010; 5:1–6.

16. Adan A, Archer SN, Hidalgo MP, Di Milia L, Natale V, Randler C. Circadian typology: A comprehensive review. Chronobiol Int. 2012; 29(9):1153–75. https://doi.org/10.3109/07420528.2012.719971 PMID: 23004349

17. Hines CB. Time-of-Day Effects on Human Performance. Cathol Educ A J Inq Pract Artic. 2013; 7 (3):390–413.

18. Gupta Omji, Patel Hrishikesh, AK Pati RV. Sports Chronobiology: circadian Rhythms in Psychological Physiological and Physical Performances. Asian Man. 2011; 5(1):40–42.

19. Goldstein D, Hahn CS, Hasher L, Wiprzycka UJ ZP. Time of day, intellectual performance, and behavioral problems in morning versus evening type adolescents: Is there a synchrony effect? Pers Ind Diff. 2007; 42:431–40.

20. Giannotti F, Cortesi F, Sebastiani T, Ottaviano S. Circadian preference, sleep and daytime behaviour in adolescence. J Sleep Res. 2002; 11(3):191–9. PMID: 12220314

21. Rossi A, Formenti D, Calogiuri G, Weydahl A. The effect of chronotype on psychophysiological responses during aerobic self-paced exercises. Percept Mot Ski. 2015; 121(3):1–16.

22. Capp TA. Review: Time of Day Effect on Athletic Performance: J Strength Cond Res. 1999; 13(4):412–21.

23. Da A, Sa M. Wakefulness-Sleep Patterns and their Relationship to Practicing of Sports, Quality of Sleep and Academic Performance in School Students. Austin J Sleep Disord. 2017; 4(2):1–5.

24. Hidalgo MP, Camozzato A, Cardoso L, Preussler C.E. Nunes R. Tavares et al. Evaluation of behavioral states among morning and evening active healthy individuals. Brazilian J Med Biol Res. 2002; 35:837–42.

25. Facer-childs E, Brandstaetter R. The Impact of Circadian Phenotype and Time since Awakening on Diurnal Performance in Athletes. Curr Biol Elsevier. 2015; 25(4):518–22.

26. Silva A, Queiroz SS, Winckler C. Sleep quality evaluation, chronotype, sleepiness and anxiety of Para- lympic Brazilian athletes. Br itish J Sport Med. 2012; 46(2):150–54.

27. Kunorozva L, Stephenson KJ, Rae DE RL. Chronotype and PERIOD3 variable number tandem repeat polymorphism in individual sports athletes. Chronobiol Int. 2012; 29(8):1004–10. https://doi.org/10.3109/07420528.2012.719966 PMID: 22971169

28. Henst RH, Jaspers RT, Roden LC RD. A chronotype comparison of South African and Dutch marathon runners: the role of scheduled race start times and effects on performance. Chronobiol Int. 2015; 32 (6):858–68. https://doi.org/10.3109/07420528.2015.1048870 PMID: 26102236

29. Rae DE, Stephenson KJ RL. Factors to consider when assessing diurnal variation in sports performance: the influence of chronotype and habitual training time-of-day. Eur J Appl Physiol. 2015; 115 (6):1339–49. https://doi.org/10.1007/s00421-015-3109-9 PMID: 25631930

30. Lastella M, Roach GD, Halson SL SC. The chronotype of elite athletes. J Hum Kinet. 2016; 54:219–25. https://doi.org/10.1515/hukin-2016-0049 PMID: 28031772

31. Randler C. Morningness - Evenningness Comparison in Adolescents from Different Countries around the World. Chronobiol Int. 2008; 25(6):1017–28. https://doi.org/10.1080/07420520802551519 PMID: 19005902

32. Natale V AA. Season of birth modulates the morningness-eveningness preference. Neurosci Lett. 1999; 274:139–41. https://doi.org/10.1016/s0304-3940(99)00672-2 PMID: 10553957

33. Mongrain V, Paquet J DM. Contribution of the photoperiod at birth to the association between season of birth and diurnal preference. Neurosci Lett. 2006; 406:113–16. https://doi.org/10.1016/j.neulet.2006.07.002 PMID: 16889896

34. Brockmann PE, Gozal D, Villarroel L, Damiani F, Cajoche C, Brockmann PE, et al. Geographic latitude and sleep duration: A population-based survey from the Tropic of Capricorn to the Antarctic Circle. Chronobiol Int. 2017; 34(3):373–81. https://doi.org/10.1080/07420528.2016.1277735 PMID: 28128998

35. Leocadio-miguel MA, Louzada FM, Duarte LL, Areas RP, Alam M, Freire MV, et al. Latitudinal cline of chronotype. Sci Rep. 2017; 7(June):2–7.
36. Kentiba Efrem, Mondal S, Mathivanan D, George M. Chronotype preferences of college students from varied altitude backgrounds in Ethiopia. Chronobiol Int. 2018; 35(12):1742–7. https://doi.org/10.1080/07420528.2018.1501054 PMID: 30067391

37. CSA. Central Statistical Agency [Ethiopia] and ICF International. Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: CSA and ICF; 2012.

38. FMHO. Health Sector Development Program IV (2010-11-2014/15). Addis Ababa, Ethiopia. Ministry of Health; 2010.

39. Koehle MS, Cheng I, Sporer B. Canadian Academy of Sport and Exercise Medicine Position Statement: Athletes at High Altitude. Clin J Sport Med. 2014; 24:120–7. https://doi.org/10.1097/JSM.000000000000024 PMID: 24569430

40. Israel GD. Sampling The Evidence Of Extension Program Impact. Program Evaluation and Organizational Development. IFAS, Univ Florida PEOD-5. 1992;

41. Ammar A, Chtourou H, Trabelsi K, Padulo J, Abed K El, Hoekelman A, et al. Temporal specificity of training: intra-day effects on biochemical responses and Olympic- Weightlifting performances. J Sports Sci. 2015; 33(4):358–68. https://doi.org/10.1080/02640414.2014.944559 PMID: 25117722

42. Boussetta N, Abdelmalek S, Aloui K, Souissi N. The effect of air pollution on diurnal variation of performance in anaerobic tests, cardiovascular and hematological parameters, and blood gases on soccer players following the Yo–Yo Intermittent Recovery Test Level-1. Chronobiol Int. 2017a; 35:1–18. https://doi.org/10.1080/07420528.2017.1374966

43. Östberg HJ and. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms 2008 Version. Int J Chronobiol. 1976; 4(January):97–100.

44. Boussetta N, Davison MCFRCR, James GDF. Diurnal physiological and immunological responses to a 10 - km run in highly trained athletes in an environmentally controlled condition of 6˚C. Eur J Appl Physiol. 2017; 117(1):1–6. https://doi.org/10.1007/s00421-016-3489-5 PMID: 27830328

45. Boussetta N, Abdelmalek S, Aloui K, Souissi N. The effect of air pollution on diurnal variation of performance in anaerobic tests, cardiovascular and hematological parameters, and blood gases on soccer players following the Yo–Yo Intermittent Recovery Test Level-1. Chronobiol Int. 2017b; 35:1–18. https://doi.org/10.1080/07420528.2017.1374966

46. Brisswalter J, Bieuzen F, Giacomoni M, Tricot V and Falgairette G. Morning-to-evening differences in oxygen uptake kinetics in short-duration cycling exercise. Chronobiol Int. 2007; 24(3):495–506. https://doi.org/10.1080/07420520701420691 PMID: 17612947

47. Loftin M, Heusel L, Bonis M, Carlisle L SM. Comparison of Oxygen Uptake Kinetic and Oxygen Deficit in Severely Overweight and Normal Weight Adolescent Females. J Sport Sci Med. 2005; 4:430–6.

48. Jørgensen T., Andersen L. B., Froberg K., Maeder U., et al. Position statement: Testing physical condition in a population: how good are the methods?. Eur J Sport Sci. 2009; 9:257–67.

49. Leger LA, Mercier D, Gadoury C LJ. The multistage 20 metre shuttle run test for aerobic fitness. J Sport Sci. 1988; 6:93–101.

50. ACSM R. ACSM’s Foundations of Strength Training and Conditioning. New Jersey: Lippincott Williams & Wilkins; 2012. 514 p.

51. Ramsbottom R, Brewer J, Williams C. A progressive shuttle run test to estimate maximal oxygen uptake r. Br J Sports Med. 1988; 22(4):141–4. https://doi.org/10.1136/bjsm.22.4.141 PMID: 3228681

52. Simundic AM, Bolenius K, Cadamuro J, Church St, Cornes MP, et al. EFLM Recommendation for venous blood sampling. European Federation of Clinical Chemistry and Laboratory Medicine. 2017 Vol. VI.

53. Stowhas AC, Grimm M SK, Tesler N, Achermann P, Huber R, Kohler M BK. Latshang TD, Lo Cascio CM, Are nocturnal breathing, sleep, and cognitive performance impaired at moderate altitude (1,630–2,590 m) ? Sleep. 2011; 36:1969–76. https://doi.org/10.5665/sleep.3242 PMID: 22433773

54. Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C MM, KE. B. Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. Sleep. 2012; 35:419–23. https://doi.org/10.5665/sleep.1708 PMID: 22379248

55. Randler C, Prokop P, Sahu S, Haldar P. Sleep–wake measures from Germany, India and Slovakia. Int J Psychol. 2014;1–9. https://doi.org/10.1002/ijp.12035

56. Tonetti L., Sahu S., & Natale V. Circadian preference in Italy and India: A comparative study in young adults. Pers Individ Dif. 2012; 53:355–8.
59. Roenneberg T., Kumar C. J., & Merrow M. The human circadian clock entrains to sun time. Curr Biol. 2007; 17:44–45.

60. Xu X, Liu X, Ma S, Xu Y, Xu Y, Guo X, et al. Association of melatonin production with seasonal changes, low temperature, and immuno-responses in hamsters. Molecules. 2018; 23(3):1–12.

61. Smith CS, Folkard S, Schmiedera RA, Parra LF, Spelten E, Almiral H, et al. Investigation of morning-evening orientation in six countries using the preferences scale. Personal Individ Differ Elsevier. 2002; 32:949–68.

62. Tseng CH, Lin FC, Chao HS, Tsai HC, Shiao GM CS. Impact of rapid ascent to high altitude on sleep. Sleep Breath. 2015; 19:819–26. https://doi.org/10.1007/s11325-014-1093-7 PMID: 25491080

63. Anholm JD, Powles AC, Downey R 3rd, Houston CS SJ, Bonnet MH CA. Operation Everest II: arterial oxygen saturation and sleep at extreme simulated altitude. Am Rev Respir Disease. 1992; 145:817–26.

64. Ashkenazi I. E., Ribak J., Avgar D. M. and Klepfish A. Altitude and hypoxia as phase shift inducers. Aviat Sp Environ Med. 1982; 53:342–6. PMID: 7200769

65. Coste O., Beaumont M., Batéjat D., Van Beers P., Charbuy H and, Touitou Y. Hypoxic depression of melatonin secretion after simulated long duration flight in man. J Pineal Res. 2004a; 37:1–10. https://doi.org/10.1111/j.1600-079X.2004.00128.x PMID: 15230862

66. Coste O., Beaumont M., Batéjat D., Van Beers P. and Touitou Y. Prolonged mild hypoxia modifies human circadian core body temperature and may be associated with sleep disturbances. Chronobiol Int. 2004b; 21:417–31.

67. Coste O., Van Beers P., Bogdan A., Charbuy H. and Touitou Y. Hypoxic alteration of cortisol circadian rhythm in man after simulation of a long duration flight. Steroids. 2005; 70:803–10. https://doi.org/10.1016/j.steroids.2005.05.003 PMID: 16019044

68. Van D, Pivovarni P. Identification of chronotype and diurnal performance. Slovak J Sport Sci. 2016; 1 (1):1–8.

69. Till Roenneberg, Kuehnle Tim, Pramstaller Peter P, Ricken Jan, Havel Miriam, Guth Angelika, et al. A marker for the end of adolescence. Curr Biol Elsevier. 2004; 14:1038–9.

70. Vitale JA, Bjoerkesett E, Campana A, Panizza G, Weydahl A. Chronotype and response to training during the polar night: Int J Circumpolar Health. 2017; 76:1–11.

71. Hill DW, Cureton KJ, Collins MA. Diurnal variations in responses to exercise of “morning types” and “evening types.” J Sport Med Phys Fitness. 1988; 28:213–9.

72. Roden LC, Rae DE. Impact of chronotype on athletic performance: Current perspectives. ChronoPhysiology Ther. 2017; 7:1–6.

73. Safari S, Nejati MM, Akaberi S, Eghbalmoghaniou A. Effect of time of day on aerobic responses with high intensity exercise in volleyball players. Eur J Exp Biol. 2012; 2(4):872–4.