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Mechanisms of the adaptive reactions development in the students' body

SERGIY GUMENYUK[1], OLHA HULKA[1], HANNA SLOZANSKA[2], ROMAN GORBATIUK[3], PETRO LADYKA[4], KATERYNA OGNYSTA[5], IRYNA HRUBAR[4]

Abstract
Introduction. The article substantiates the importance of the mechanisms of adaptive reactions, which includes various functionally related subsystems. Aim of Study. The aim of the article is analysis of psychophysiological indicators, central hemodynamics and heart rate variability of students' organisms at the first year of study. Material and Methods. Contingent: students of University. The average age 17.5 ± 0.6 years. Methods for studying the properties of attention (tables Anfimov, Schulte), short-term arbitrary visual memory; 5-minute heart rate variability (HRV) records. Results. It is established that differences in mental functions are due to the specifics of educational process. High level of MVB, HR, SDNN and LF/HF ($p \leq 0.05$), in group I indicates increased hemodynamics and sympathicotonia. In the group II indicators point to optimal level of central hemodynamics and activation of the sympathetic link. Group III smallest LF/HF ($p \leq 0.05$) and normal hemodynamic characteristic. Group IV: lowest VLF and VLF%, the highest LF% and VEI – it's may be the result of reduced ergotropic resources under stress. Conclusions. It is proposed to evaluate the mechanisms of adaptive reactions development: optimal, compensatory, decompensatory, overstrain.

KEYWORDS: students, heart rate variability, central hemodynamics, psychophysiological indicators, functional systems, organism.

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Introduction

Development of highly sensitive registration methods and their processing means for obtaining diagnostic data can be used not only in clinical medical practice, but also for the purpose of diagnosis, prevention and correction of psychophysiological conditions of persons with different intensions [10, 19, 26].

Cardiac registration is very common in clinical practice and facilitates evaluation of cardiac rhythm indicators, investigation of structural defects of conduction, violation of the function of automaticity, etc. [3, 4, 20, 25].

The functional system theory, proposed by K. Anokhin and developed in the works of his followers [23, 27], shows that the human organism is a complex system of functionally connected physiological subsystems. Their concerted work depends on homeostasis. The interaction of the subsystems to maintain homeostasis is manifested in the electrical activity of the organism – electric biopotentials that serve as a source of information [4]. The internal structure of the system, its relationships and the level of functioning will depend on the external environment, in which the system is located [8]. Therefore, when analyzing subsystems and their connections, it is necessary to consider the structure of functions, which are formed under the influence of both internal and external factors.

The response to any stimuli is manifested in a change of the nervous system state, which is a component of the functional system of the organism as a whole. The analysis of mental functions, which are based on nervous processes, facilitates an assessment of how quickly the body reacts to the presented stimulus and how effectively it uses the received information to form a "reaction-response". Attention is not a self-sustaining mental process, but it is a necessary condition for the acquisition of knowledge and practical skills. Attention has a number of specific characteristics: volume, performance, persistence, switching, and distribution. According to scientists, studying the properties of attention makes it possible to assess an individual's ability to organize, regulate and control their own activities [8, 14, 18]. Memory involves processes of organizing and preserving past experiences. This allows memory to be used in the subsequent life situations, which are related to the acquisition of activity and learning experience [7, 15]. As the researchers claim, the registration of short-term memory indicators characterizes the state of higher nervous activity and working capacity of a person [18, 24].

Organism is a self-regulating system, represented by the extensive interaction of central and peripheral formations. These formations are components of an active complex with certain physiological properties and may include anatomical formations, combinations of humoral substances, while all components are united by selective interdependence and harmoniously support one another to obtain any adaptive effect in the organism [27]. Integral systemic reactions to the action of the environment are always specific; non-specific is only the adaptation that determines the manner of these reactions of the organism to the action of a stimulus [9].

The study of integrative indicators of the organism's functional state makes it possible to effectively evaluate the organization of different types of human activity [11, 15, 18]. The physiological side of any functional state finds reflection in changes of vegetative reactions. Deviations resulting from vegetative shifts are most clearly reflected in changes in the activity of the cardiovascular system [19, 23]. Regulation of blood circulation is a multi-circuit hierarchically organized system, in which the dominant role is determined by the urgent needs of organism. Changes in regulatory systems are preceded by hemodynamics, metabolic, and energy shifts. Determination of the state of the organism's regulatory systems and their deviation from normal functioning is an important prognostic criterion in assessing the course of adaptive reactions to environmental conditions [4].

The profession of a teacher is associated with constant stress. Teachers must work with pupils and parents. They need to constantly improve their skills and qualifications and be aware of all innovations. This is a strong load on the organism, which is manifested in changes of the nervous system state and vegetative functions [12, 23]. Establishing the mechanisms of stress reaction formation in students of pedagogical institutions is important for predicting the development of adaptive changes in the organism in the conditions of professional activity.

Aim of Study

The aim of the article is to analyze psychophysiological indicators, central hemodynamics and heart rate variability of students' organisms at the first year of study.

Materials and Methods

The research was conducted at the Ternopil Volodymyr Hnatiuk National Pedagogical University, Kremenets Taras Shevchenko Regional Academy of Humanities and Pedagogy, Drohobych Ivan Franko State Pedagogical University. Students of pedagogical universities acquire competencies that allow them to plan their future activities by profession. Therefore, future teachers feel a lot of stress in the conditions of educational activities at the university. First year students experience strong psycho-emotional stress because their educational activities have changed significantly: ways of processing information, acquiring professional skills and abilities. This is the reason for choosing this contingent to study the mechanisms of adaptive reaction development. The cohort of students consisted of first year students of different specialties, who showed differences in the content, training workload and motor mode: group I – Foreign Philology (n = 97), group II – Physical Education (n = 98), group III – Mathematics (n = 88), and group IV – Biology (n = 90). The present study was conducted on healthy volunteers. There were no pronounced chronic somatic diseases and physical disabilities in the sample studied (based on medical cards of the examined students). The average age of examined subjects was 17.5 ± 0.6 years old. The examination was carried out in stationary conditions from 8.00 to 13.00 o'clock with the same requirements [22].

Heart rate variability (HRV) is a marker that reflects autonomic regulation of cardiac cycle intervals. The use of HRV is a common method of diagnosing the functional state of the body, as it is sensitive to physiological and psychological changes [17]. Traditional data collection procedures for establishing short-term HRV in clinical or laboratory settings involve recording a 5-minute RR-interval. Research shows that recording short--term recordings (5 min) of heart rhythm is valid for data analysis data [2]. Because of the relative ease of recording, 5-minute measurements are widely used and are the most common source of published HRV [1-4, 6-8, 10, 11, 13, 14, 16, 20-24, 26].

The indexes of HRV were obtained from 5-minute cardiac interval records using a diagnostic computer system "Omega-M" to assess the functional state of the human organism (LLC "Dynamics" Research Laboratory, St. Petersburg, Russian Federation). The HRV indexes were obtained and analyzed: RRNN – average duration RR-intervals, (ms); SDNN – standard deviation of values of normal RR-intervals, (ms); RMSSD – square root of the mean of the squared difference of RR-intervals, (ms); CV – coefficient of variation, (%); NN50 – number of pairs of consecutive RR-intervals that differ by more than 50 ms; pNN50 – percentage of NN50 from the amount of all analyzed RR-intervals, (%); HRV-index – triangular index, (un.); HF, HF% – absolute and relative indexes of high-frequency oscillations (0.15-0.40 Hz), (ms^2); LF, LF% – absolute and relative values of the low-frequency component of the spectrum (0.04-0.15 Hz), (ms^2); VLF, VLF% – absolute and relative values of the very low--frequency component of the spectrum (0.003-0.04 Hz), (ms^2); LF/HF – indicator of vegetative balance between the activity of sympathetic and parasympathetic departments of the autonomic nervous system, (un.); TP – total spectrum power, (ms^2); Mo (mode) – value of RR-interval, which is most common in this dynamic range, (ms); AMo (amplitude of mode) – correlation of the amount of RR-intervals with the value Mo to the general amount of RR-intervals, (%); VS (variation span) – difference between maximum and minimum values of RR-intervals, (ms) [20, 22].

Based on the indicators of the dynamic series the indicators of cardiac activity are calculated by the diagnostic complex: VEI – vegetative equilibrium index, (un.); VRR – vegetative rate of rhythm, (un.); IARP – indicator of adequacy of regulatory processes, (un.); WI – workload index, (un.) [3, 4, 20].

Regulatory influences of central hemodynamics were evaluated according to indicators (which were obtained with a tonometer of the "Omega-M" diagnostic complex) of heart rate (HR), systolic pressure (SP), diastolic pressure (DP) and on their basis the following indexes were calculated: pulse pressure (PP), systolic blood volume (SBV), minute volume of blood (MVB), double result, or the Robinson index (DR) and adaptation potential (AP) [4, 19]. According to these data we can make conclusions concerning potential abilities of the human hemocirculatory apparatus and to assess the level of metabolic and energy processes in the organism [12, 18, 27].

To evaluate the psychophysiological functions the following were determined: amount, productiveness, stability of attention (Anfimov tables), switching of attention (red-black Schulte tables), distribution of attention; short-term visual memory for words, syllables, figures and numbers [18]. The use of these methods to determine the properties of attention and short-term memorization allows to characterize psychophysiological functions that are important for the professional development of future teachers and influence the formation of the organism's adaptive reactions [15, 17].

When choosing research methods, attention was paid to the fact that the selected methods did not take a lot of time, did not tire the participants, were simple and objective, adequately reflected the state of the body's functional systems under the action of the dominant factor, which is the educational process. The objectivity and reliability of the chosen methods is confirmed by a large number of scientific publications [1-4, 6, 7, 10, 12-14, 18, 21-24, 27].

Statistical processing of the results was performed using the Statistica 6.0 software package [5]. The normality of the distribution of the sample was determined by the Shapiro–Wilk test. Samples should be described by median and interquartile ranges (25th and 75th percentiles). The significance of differences between groups was determined using the non-parametric Kruskal–Wallis test to compare three or more unrelated groups [5, 16]. There factor analysis was conducted in each group. The principal component method and variation rotation of data were used for factor analysis [5]. From the data of the whole sample, the optimal values were determined for each indicator – these were the indicators of central hemodynamics and heart rate variability, which were within the norms [17, 20, 22], and psycho-physiological, which characterized high abilities of attention and memory (the biggest number of signs, the least time of doing test, etc.) [18]. The indicators of factor I of each group were compared to the optimal and deviations from them were presented in %. On the basis of obtained deviations characteristics of mechanisms of the development of adaptive reactions were established.

Ethical approval for the original studies and further ethical approval for the secondary data analysis were obtained from the Education's Research Ethics Committees of the Ternopil Volodymyr Hnatiuk National Pedagogical University (protocol No. 2 dated 05.09.22).

Results

Students of group I (Table 1) were characterized with the highest scores in evaluations of the amount of memory for words, syllables, group III – the highest speed of switching attention, memory for numbers and figures, group IV – the amount, efficacy and separation of memory that met the requirements of vocational training. The lowest indexes of psychophysiological functions were recorded among the students of group II [18].

The central hemodynamic indexes (Table 2) are the basis for the statement about strengthening of regulatory influences on the cardiovascular system of examined students from group I – the highest meaning MVB [6.0 (5.4; 6.6) l/min, $p \leq 0.05$] was caused by the significant input of HR [the greatest among the examined groups – 83 (74; 89) beats/min, $p \leq 0.05$)]. In group II MVB was formed because of the highest value of SBV [75.6 (70.1; 83.0) ml/min, $p \leq 0.05$]. Arterial pressure of the examined students from all the groups was within age norms [13]. The high value of SP in group II [131 (124; 138) mmHg, $p \leq 0.05$] can be explained by age peculiarities of functioning of the organism. Indicators of a satisfactory functioning of the hemodynamic apparatus of students from groups III and IV was confirmed by the indicators of HR, SP, DP, SBV, MVB (Table 2), which were within the norms [13, 17].

The indicators of AP and DR show no significant differences ($p \geq 0,05$) and indicated a satisfactory level

Table 1. Comparison of indicators of psychophysiological functions of students

	Indicators	group I (n = 97)	group II (n = 98)	group III (n = 88)	group IV (n = 90)
attention	amount, signs	778 (860; 903)*	662 (508; 828)*	737 (617; 865)*	**841 (720; 945)***
	stability, %	0,94 (0,91; 0,97)	0,93 (0,88; 0,96)	0,94 (0,92; 0,97)	0,94 (0,92; 0,98)
	productivity, signs	735 (660; 883)*	584 (448; 731)*	693 (543; 796)*	**786 (684; 858)***
	division, signs	19 (17; 23)*	19 (17; 20)*	20 (19; 21)*	**21 (18; 24)***
	switching, s	231 (216; 252)*	297 (263; 341)*	**230 (220; 261)***	256 (237; 280)*
memory	for syllables	**5 (4; 6)***	2 (2; 3)*	4 (3; 5)*	4 (3; 5)*
	for words	**8 (7; 8)***	7 (6; 8)*	8 (5; 9)*	7 (6; 9)*
	for numbers	6 (4; 7)*	6 (4; 7)*	**7 (4; 8)***	6 (5; 8)*
	for figures	6 (4; 7)*	5 (5; 6)*	**8 (7; 9)***	7 (5; 8)*

Note: Indicators that had the biggest differences between the groups are given in bold.
* differences between groups at $p \leq 0.05$ (Kruskal–Wallis criterion)

Table 2. Comparison of indicators of central hemodynamic system of students

Indicators	group I (n = 97)	group II (n = 98)	group III (n = 88)	group IV (n = 90)
SP, mmHg	123 (118; 131)*	131 (124; 138)*	124 (118; 136)*	123 (119; 133)*
DP, mmHg	74 (63; 78)	71 (64; 79)	75 (72; 87)	78 (69; 81)
HR, beats/min	**83 (74; 89)***	77 (65; 82)*	79 (72; 89)*	80 (76; 97)*
SBV, ml/min	73.3 (65.7; 78.5)*	**75.6 (70.1; 83.0)***	67.1 (62.1; 71.8)*	68.2 (63.5; 71.9)*
MVB, l/min	**6.0 (5.4; 6.6)***	5.7 (5.2; 6.0)*	5.3 (5.0; 5.8)*	5.7 (5.1; 6.1)*

Note: SP – systolic pressure, DP – diastolic pressure, HR – heart rate, SBV – systolic blood volume, MVB – minute volume of blood. Indicators that had the biggest differences between the groups are given in bold.
* differences between groups at $p \leq 0.05$ (Kruskal–Wallis criterion)

of adaptation [25] with reduced functional abilities of the systolic heart work [17]. Adaptive reactions in the organism were formed at low levels of energy-exchange processes, which may be the result of the inclusion of central hemodynamics in the compensatory mechanisms.

Students from group I were characterized by the indicators of HRV (Table 3), that is evidence of the activity of the sympathetic regulation link. The highest results for SDNN among the examined students should have shown at the predominance of the vagus influence on the rhythm of the heart [7, 22]. However, the obtained results are consistent with those reported by other scientists: an increase of SDNN in the conditions of acute stress was associated with a decrease of the respiratory rate [24]. The spectral indicator of vegetative balance LF/HF was 2.43 (1.18; 3.64) un., which indicates the predominance of the sympathetic link of regulation [22]. The indicator of VLF% went beyond the norm [20] and amounted to 50 (40; 60) %. As is noted in research papers, low HRV values are associated with stress responses and increased sympathicotonia [7]. WI showed no high values [20]. Such discrepancies between the data of regulatory reactions can be explained by the formation of decompensatory mechanisms under the influence of training intension and age-related changes in the organism.

The examined students from group II were characterized by indicators of HRV, which were within the norm [20, 22]. The spectral indicator of vegetative balance (LF/HF) indicated the predominance of activity of the sympathetic

Table 3. Comparison of indicators of HRV of students

Indicators	group I (n = 97)	group II (n = 98)	group III (n = 88)	group IV (n = 90)
Mo, ms	720 (680; 800)	720 (610; 860)	760 (680; 840)	680 (640; 760)
AMo, %	25.68 (22.97; 27.21)*	**25.32 (22.28; 40.18)***	30.34 (22.37; 35.99)*	30.07 (26.78; 34.34)*
RRNN, ms	723 (662; 791)	711 (629; 796)	754 (672; 828)	743 (619; 780)
SDNN, ms	**63.3 (55.0; 70.1)***	60.1 (36.5; 68.7)*	49.7 (42.1; 74.5)*	56.0 (46.1; 62.9)*
CV, %	8.8 (8.0; 9.9)*	6.6 (5.8; 8.7)*	7.5 (5.3; 9.2)*	7.9 (6.0; 9.3)**
HRV-index, un.	15 (12; 15)*	**17 (14; 19)***	13 (10; 16)*	13 (11; 15)*
HF, ms	628 (192; 1016)	586 (213; 1040)	797 (273; 1297)	562 (256; 901)
LF, ms^2	891 (675; 1590)	630 (457; 1966)	720 (379; 1094)	1020 (592; 1402)
VLF, ms^2	1771 (1355; 2267)*	902 (500; 1432)*	756 (594; 1232)*	854 (612; 1616)*
LF/HF, un.	**2.43 (1.18; 3.64)***	2.21 (1.14; 3.20)*	**0.88 (0.63; 2.39)***	2.05 (0.66; 3.16)*
TP, ms^2	3596 (2724; 4480)	2600 (1236; 4611)	2098 (1865; 4925)	2803 (2042; 3157)
HF%	18 (8; 25)	21 (13; 30)	31 (12; 43)	19 (11; 43)
LF%	29 (23; 39)*	31 (24; 40)*	27 (24; 35)*	**39 (29; 52)***
VLF%	**50 (40; 60)***	45 (43; 56)*	41 (29; 53)*	**33 (24; 47)***
VEI, un.	80.2 (72.8; 105.6)*	95.6 (71.5; 218.4)*	117.9 (73.6; 164.0)*	**122.7 (91.4; 157.0)***
VRR, un.	0.43 (0.38; 0.45)*	0.30 (0.28; 0.37)*	0.36 (0.27; 0.40)*	0.37 (0.29; 0.43)*
WI, un.	62.6 (50.6; 86.9)	59.5 (39.6; 156.0)	86.7 (45.1; 108.7)	**91.8 (73.7; 114.3)**

Note: Mo – mode, AMo – amplitude of mode, RRNN – average duration RR-intervals, SDNN – standard deviation of values of normal RR-intervals, CV – coefficient of variation, HRV-index – heart rate variability index, HF – absolute indexes of high-frequency oscillation, LF – absolute indexes of low-frequency component of the spectrum, VLF – absolute indexes of very low-frequency component of the spectrum, LF/HF – indicator of vegetative balance between the activity of sympathetic and parasympathetic departments of the autonomic nervous system, TP – total spectrum power, HF% – relative indexes of high-frequency oscillation, LF% – relative indexes of low-frequency component of the spectrum, VLF% – relative indexes of very low-frequency component of the spectrum, VEI – vegetative equilibrium index, VRR – vegetative rate of rhythm, WI – workload index

Indicators that had the biggest differences between the groups are given in bold.

* differences between groups at $p \leq 0.05$ (Kruskal–Wallis criterion)

link of regulation – 2.2 (1.25; 3.02) un. [22]. WI, at the same time, was 59.5 (39.6; 155.2) un. and pointed to the absence of intension of the organism's regulatory systems and predominance of the activity of the autonomous regulation contour [20]. Other indicators of HRV (the lowest – AMo [25.32 (22.28; 40.18), ms, $p \leq 0.05$], the highest HRV-index [17 (14; 19), un., $p \leq 0.05$] showed a high level of organism functioning under the influence of educational intension [14].

Indicators of HRV of students from group III were within the norm [20]. The value of the spectral indicator of LF/HF [0.88 (0.63; 2.39], un., $p \leq 0.05$] gives grounds for claiming the high activity of the autonomous regulation contour [22]. It can be assumed that the training intension corresponded to the high psychophysiological abilities of the persons in this group (Table 1) and had no stress influence at the organisms. That was displayed in a satisfactory functional state of central hemodynamics (Table 2) and heart rhythm formation.

In students from group IV values of most indicators of HRV met the standards [22]. The indicator of vegetative balance of LF/HF [2.05 (0.66; 3.16), un.] indicated the predominance of the activity of the sympathetic regulatory link. They were characterized by the lowest values of VLF% [33 (24; 47), %; $p \leq 0.05$] among the examined groups.

Factor analysis of the data was conducted for each examined group. Among the highlighted factors, the largest contributors to the overall dispersion were the first factors. That is why only the indicators, which were parts of the first factors of each group, were included in further analyses [8]. When comparing the data of each group with the optimal ones, a chart was presented (Figure 1).

As we can see in Figure 1, the largest deviations were found among the students of group I: spectral indicators LF/HF and VLF showed deviations of 80-100%; 20--40% – for VS, HRV, CV, HF%, VRR, WI; indicators of central hemodynamics, LF, LF%, HF, NN50, IARP – to 20%; the smallest deviations (to 10%) were found for AP, DR, Mo, RRNN, and RMSSD. A significant deviation of LF/HF (78%) was recorded [3, 11, 22].

In group II, factor I was formed by HRV indicators. The HRV index showed the highest deviations – 42%. VLF, WI and IARP were lower at 20-40%, NN50 and VEI – 10-20%, other indicators – to 10%, or were at the optimal level. Students of this group were characterized by high functional capacity of the organism, which was manifested in the negative deviations of indicators WI, IARP, VEI and significant positive deviations in indicators VS, HRV.

Factor I of persons from group III included psychophysiological indicators (memory for syllables

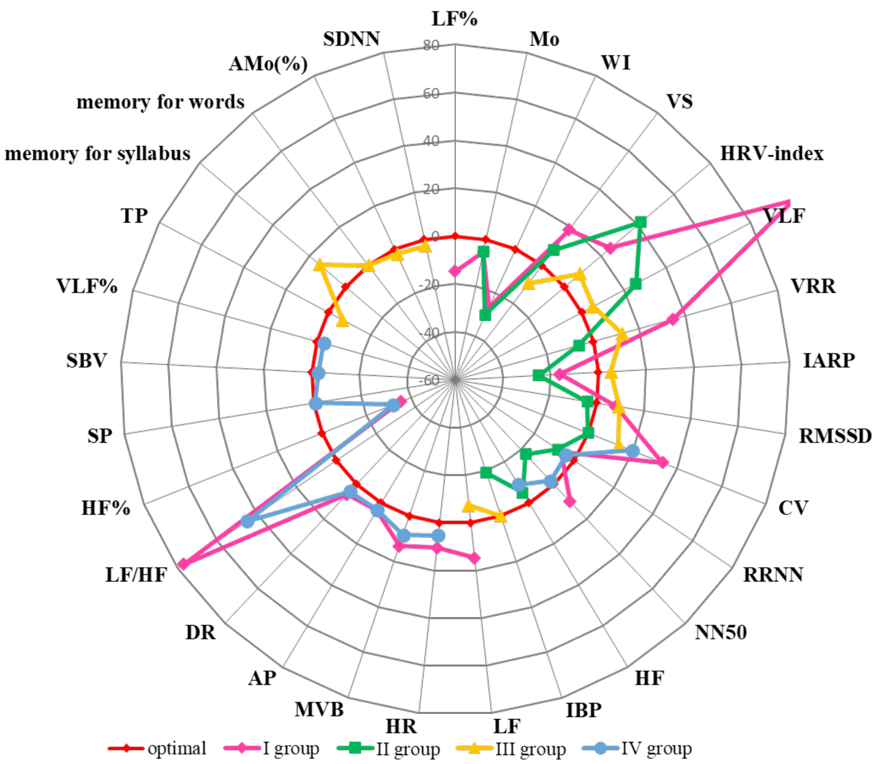

Figure 1. Deviation in % of indicators I factor of each group from the optimal

and words) and vegetative regulation. The values of these indicators were within ±14%. It can be stated that the regulatory activity of the heart rhythm occurred at an autonomous level without involving regulatory mechanisms of central hemodynamics.

Group IV. Spectral indexes of vegetative balance showed the largest deviations (LF/HF) – 45% and HF% – 32%, index CV – 20%, with the other indicators (Figure 1) showing deviations of 10%. Regulatory mechanisms of central hemodynamics were realized by strengthening the heart work. The deviation of the relative value of the respiratory component of the spectrum (HF%) against the background of negligible RRNN, NN50, HF and a significantly higher CV indicated decreasing in the activity of the autonomous regulation contour [24].

Discussion

Mechanisms of mental intension, which provide resultative human activity, in spite of their complexity and diversity, they eventually lead to the same result – changes in the functions of organs and systems of the organism [2, 3, 7, 14, 15, 18]. Stress appears as a result of an interaction between the individual and the environment, where the individual considers requirements of a situation as excess of functional resources [1].

Evaluating the psychophysiological support of the mental capacity of a person uses indicators of heart rate variability as a reliable marker of the functioning of the organism in general [15]. If psychophysiological functions and vegetative reactions of the individual are low and not able to rebuild in new functional creations, then they become a prerequisite for the development of maladaptation, greater severity of changes in the functioning of the cardiovascular system [4, 13, 14]. Markers of maladaptation include deviation of heart rhythm indicators, insufficient vegetative support, disturbance of the daily profile of blood pressure and heart rate, hypertensive response to professionally directed intension, disturbance of cerebral venous blood flow [21]. The development of autonomic reactions is a summary and a non-specific result that characterizes the influence of information processes. Stress perception activates physiological responses. Any changes that appear due to changes in the wave structure of the signal of the central nervous system will be reflected in the change in the rhythmic activity of the heart [27].

In group I the result of imbalance of regulatory influences on the heart rhythm upon activation of central hemodynamics is found as a decompensatory mechanism for the formation of adaptive reactions. Adaptation for training intensions was accompanied by high activity of cerebral ergotropic influences. Regulatory influences were manifested in increased blood flow due to HR, which affected the systolic heart function.

Insignificant deviations from the optimal values of cardio intervals and their statistical derivatives can testify to the influence of age peculiarities related to maturation of organism structures, which is manifested in the imperfect regulation of the heart rhythm [13]. The increased regulatory effects of central hemodynamics on the background of vagal activity corresponded to the compensatory mechanism of regulation by type A in group II.

Students of group III were characterized by indicators, which had no significant differences from the optimal ones, and indicated high parasympathetic activity of ANS. The presence of memory indicators in factor I confirms the data obtained by researchers that individuals with more memory capacity less often exhibit physiological signs of stress [6]. The optimal mechanism for the development of adaptive reactions was a specific characteristic for the studied group of students.

Adaptive reactions were formed with the activation of the sympathetic part, the enhancement of the activity of the hemocirculatory apparatus was regulated by cardiac output of the activity of the hemocirculatory apparatus and was regulated by cardiac output. The compensatory mechanism for the development of type B adaptive reactions was specific to students of group IV.

The described mechanisms of adaptive changes have been explained by models of visceral integration, which examines the physiological mechanisms of adaptation through the formation of the integration system of the brain that provides flexible management and coordination of the peripheral link of regulation [23], and double-circuit regulation of heart rate [3].

Conclusions

Differences of mental functions in students of different groups were established. Group I – the highest indicators of memory for words, syllables ($p \leq 0.05$), group III – high speed of switching attention, memory for numbers and figures, group IV – productivity, volume and distribution of attention, group II – the lowest indicators of psychophysiological functions. Such results may be due to the specifics of professional workloads, which differ in content and volume. High levels of MVB [6.0 (5.4; 6.6) l/min], HR [83 (74; 89) beats/min], SDNN [63.3 (55.0; 70.1), ms] and LF/HF [2.43 (1.18; 3.64), un.] in students from group I indicated an increase in the activity of the hemocirculatory system and the predominance of sympathicotonic effects on heart rhythm. In group II the highest SBV [75.6 (70.1; 83.0), ml/min] and lowest HR [77 (65; 82), beats/min, $p \leq 0.05$] with high LF/HF [2.2 (1.25; 3.02), un., $p \leq 0.05$] indicated the optimal level of regulation of central hemodynamics in the activation of the sympathetic link of regulation. Students from group III had the lowest values of LF/HF [0.88 (0.63; 2.39), un., $p \leq 0.05$] and hemodynamic parameters that corresponded to the norm. Students from group IV had the lowest VLF and VLF% [854 (612; 1616), ms^2 and 33 (24; 47), %; $p \leq 0.05$], the highest LF% (39 (29; 52), %, $p \leq 0.05$] and VEI [122.7 (91.4; 157.0), un., $p \leq 0.05$]. This may be the result of reduced ergotropic resources under stress and increased sympathicotonia.

According to the results of factor analysis, the important indicators were identified. Students from group I were characterized by a decompensatory mechanism of reactions to the workload, group II – a compensatory mechanism of regulation type A, group III – the optimal one, IV – a compensatory mechanism of regulation of type B.

However, there are some limitations. The expanding literature on HRV norms requires careful interpretation. Due to the lack of standardization of short-term measurement protocols, concurrent validity criteria, normative values for healthy non-athlete (in our case – students of pedagogical universities), optimal performance, and clinical populations, it is not recommended to use short-term interchangeably with 5-minute values. At the same time, recordings of heart rate variability in populations may be affected by duration, intensity, type of physical activity, food and drink consumption or testing environment. In our research, the potential environmental influences, such as testing participants in a similar environment at the same time of day, were not accounted for and controlled. These could contribute to inaccurate analyses and interpretations of received results.

Disclosure and recognition

Indicators of psychophysiological functions, central hemodynamics and heart rate of students with different types of initial load were analyzed. The mechanisms of adaptive reaction development in an organism were defined and characterized.

Conflict of Interest

The authors declare no conflict of interest.

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Serum irisin concentration and its association with muscle and fat mass in aerobic and anaerobic endurance athlete men and women

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Abstract

Introduction. **Irisin is released in response to exercise, but the regulatory effect of exercise on serum irisin is controversial. Evidence linking irisin with muscle mass or fat mass is limited. Little is known about the connection of irisin with the type and intensity of exercise in athletes.** Aim of Study. **This study sought to determine serum irisin concentration (SIC) in athletes and non-athletes and assess its association with anthropometric indices, including body weight (BW), body mass index (BMI), waist-to-height ratio (WHtR), mid-upper arm circumference (MUAC), lean body mass (LBM), and fat mass (FM).** Material and Methods. **We conducted a case-control study on 72 athletes and non-athletes comprising three age-and sex--matched groups with a 1 : 1 sex ratio: 24 footballers (aerobic endurance exercise), 24 bodybuilders (anaerobic strength exercise), and 24 non-exercised controls. Standard protocols for measuring anthropometric indices and quantifying SIC were followed.** Results. **Whole athletes had higher SIC than controls, with footballer men and women having higher values than bodybuilders and controls. Athletic men and women exhibited higher SIC than control men. SIC showed no sex differences within each experimental and control group. SIC negatively correlated with BW, BMI, LBM, MUAC, and WHtR in athlete women, BMI and MUAC in bodybuilders, FM in whole footballers, and BW in total control, but positively correlated with overall bodybuilders.** Conclusions. **The findings indicate that irisin is exercise-dependent, as it is enhanced in aerobic endurance more than in anaerobic strength exercise but is gender-independent. The results also support the relationship between irisin and body composition, as it generally correlates negatively with BW, BMI, FM, and WHtR.**

KEYWORDS: irisin, soccer players, bodybuilders, muscle mass, fat mass.

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Introduction

Irisin is a novel hormone identified in 2012 [5]. It is released in response to exercise after stimulation of peroxisome proliferator activator γ coactivator-1 alpha (PGC1α), which in turn stimulates the expression of the fibronectin type III domain containing 5 (FNDC5) and the proteolysis of this gene produces irisin. Irisin is released from skeletal muscle in mice and humans into circulation targeted toward white adipose tissue as a chemical messenger [5]. Irisin is also considered an adipokine released from adipose tissue [23]. The hormone increases the expression of uncoupling protein 1, resulting in increased thermogenesis, enhanced systemic metabolism, and boosted energy expenditure [17]. Thus, irisin seems promising in controlling chronic disorders such as cardiometabolic disease, obesity, and diabetes [14]. In th on the net energy balance than the exercise's direct energy cost; thus, exercise may boost the resting metabolic rate [18, 24]. Browning the white adipose tissue by irisin is one of the suggested mechanisms [5]. Sports are classified according to the type and intensity of exercise, into dynamic and static, and based on muscle metabolism, into aerobic and anaerobic [19]. Most high-intensity static exercises, such as muscle building, are performed anaerobically; while high--intensity, dynamic exercises lasting for more than several minutes, like football, are performed aerobically [19]. Although the connection between irisin and exercise has been suggested, the evidence is inconsistent. Several debatable studies that relate irisin to exercise in normal subjects are available [6, 7, 10, 13, 20, 22, 28]. Circulating irisin levels did not rise after aerobic endurance training, or with strength endurance training [10, 20, 22]. Cooke et al. [6] and Daskalopoulou et al. [7] reported that different protocols of exercise raised irisin levels, while Tsuchiya et al. [28] suggested that different exercise intensities affected irisin secretion. Kraemer et al. [13] indicated that prolonged aerobic exercise produces a transient increase in irisin concentrations during the first hour for both genders. The different experimental protocols, gender, age, body composition, and genetics are among the reasons for the present controversy.

Muscle mass is responsible for about 80% of energy expenditure, and irisin is proposed to increase energy expenditure, suggesting an interchangeable relationship between muscle mass and irisin [26]. Muscle mass is also affected by the type of exercise, and high-intensity anaerobic exercise is responsible for the anabolic process that leads to muscle hypertrophy, a critical adaptation in muscles for optimal performance [27]. Circulating irisin levels decrease as the body mass index (BMI) decreases in normal and obese individuals [25]. However, the connection between irisin and indices of body composition, including body weight (BW), BMI, waist circumference (WC), hip circumference (HC), waist-hip ratio (WHR), waist-to-height ratio (WHtR), mid-upper arm circumference (MUAC), lean body mass (LBM), and fat mass (FM) among athletes and non--athletes is generally lacking. Therefore, the objective of the present study was to determine serum irisin concentration in non-athletes and athletes who regularly engaged in aerobic and anaerobic sports and assess its association with anthropometric indices, including BW, BMI, WC, HC, WHR, WHtR, MUAC, LBM, and FM.

Materials and Methods

Participants and study design

A case-control study was undertaken and included three groups of healthy Jordanian men and women aged 20-35, performing aerobic endurance exercise, strength endurance exercise, and not performing any exercise. The study sample (72 participants, 36 men, and 36 women) included three age-and sex-matched groups with varying levels of physical activity. The first group was 24 participants (12 men and 12 women) who followed their habitual lifestyle and physical activity and were not performing any regular exercise regimens (unexercised status: 1 hour a week of regular activity for at least one year), as described elsewhere [10]. The participants were recruited from office workers who usually came to their job by car. The second and the third groups, each of which were also 24 participants (12 men and 12 women) and for at least one year were regularly performing either dynamic aerobic endurance exercise, i.e., aerobic high intensity and long-duration exercise, or strength endurance exercise, i.e., static anaerobic high--intensity exercise [1]. The second group comprised football players who were regularly training for 90 minutes every day, three times a week (Jordan Football Association, personal communication, 2022) and were recruited from among the professional players of the Jordan Football Association, Amman-Jordan. The third group included bodybuilders who were regularly training for 90 minutes every day, six days a week (Jordan Bodybuilding Federation, personal communication, 2022), and were recruited from among the professional players of the Jordan Bodybuilding Federation, Amman-Jordan.

Inclusion criteria included: age 20 to 35 years, non--smokers, non-pregnant and non-lactating women, taking no medications or medicinal herbs, and having no diseases or abnormalities that would interfere with exercise or require regular medication, including heart, kidney, thyroid, and respiratory problems, diabetes mellitus, and iron deficiency anemia [10]. Any participant who did not fit the inclusion criteria was excluded. The study was approved by the Institutional Ethics Committee. All participants provided written informed consent before their involvement in the study. The investigator interviewed each participant to obtain information regarding their personal, social, and health history to affirm the inclusion and exclusion criteria. The investigator was also authorized to refer to each player's medical records to check their health condition to see if it did fit the inclusion criteria.

Data collection

The investigator interviewed each participant for data collection, including personal information and anthropometric measurements. The BW, height, BMI, WC, HC, MUAC, LBM, and FM were evaluated following standard methods of anthropometry [15]. The weight was measured with light clothing and without shoes to the nearest 0.1 kg using a measuring scale, and height was recorded to the nearest 0.5 cm using a stadiometer. The WC was measured standing to the nearest 5 mm at a level midway between the lower rib margin and the iliac crest during the normal end-expiratory phase. The HC was measured at the level of the greater trochanters. The MUAC was measured on a straight left arm, mid-way between the tip of the shoulder and the tip of the elbow. The BMI was calculated as BW (kg) divided by height (m^2), WHtR was obtained by dividing the WC by height, and WHR was computed as the WC divided by HC. A Harpenden skinfold caliper (British Indicators Ltd., England) was used to measure the skinfold thickness of two body sites, from which body fat and LBM were calculated according to the regression equations of Durnin and Womersley, 1974 [8]. Systolic (SBP) and diastolic (DBP) blood pressure were measured twice using a standardized mercury sphygmomanometer after seating the subject for at least 15 minutes, and then the average blood pressure was recorded [15].

Serum irisin determination

Blood samples (5 ml) were collected from each participant following an overnight fast (10-12 h) and 24 hours of not performing any exercise by a licensed phlebotomist in a sitting position according to a standard protocol. Serum was obtained using a serum separator tube. Samples were allowed to clot at room temperature for 30 minutes before centrifugation for 20 minutes and stored frozen at –20°C until analysis. Serum from each participant was processed in one batch for irisin concentrations using standard biochemical kits and the ELISA (catalog # K4761-100, 100assays; Biovision, S. Milpitas Blvd., Milpitas, CA 95035 USA) assay method.

Statistical analysis

Data analysis was performed using statistical analysis software (SPSS Inc., version 19.0.1, Chicago, USA) and processed using ANOVA followed by a Tukey *post hoc* test. Results were presented as means ± standard error of the mean (SEM). Significance was set at $p < 0.05$. Pearson correlations were used to test the relationship between anthropometric measures and serum irisin concentrations.

Results

Table 1 shows the anthropometric measures and blood pressure of athletic and control men and women. Men of both athletics and control groups had the highest height and were significantly different ($p \leq 0.05$) from women of both groups, but were non-significantly different

Table 1. Group-gender anthropometric measures and blood pressure in the study sample

Variable	Control		Footballers		Bodybuilders	
	Men n = 12	Women n = 12	Men n = 12	Women n = 12	Men n = 12	Women n = 12
Height (cm)	174.6 ± 1.9^{a}	160.9 ± 1.6^{b}	178.3 ± 1.9^{a}	162.3 ± 1.9^{b}	176.5 ± 1.8^{a}	160.90 ± 1.4^{b}
Weight (kg)	67.9 ± 2.5^{bc}	56.2 ± 1.6^{de}	74.2 ± 2.3^{b}	54.5 ± 1.9^{e}	91.1 ± 3.6^{a}	64.4 ± 1.0^{cd}
BMI (kg/m²)	22.2 ± 0.6^{cd}	21.7 ± 0.5^{cd}	23.3 ± 0.4^{bc}	20.7 ± 0.4^{d}	29.2 ± 1.0^{a}	24.9 ± 0.2^{b}
MUAC (cm)	$28.4 \pm 1.0b^{cd}$	26.4 ± 0.8^{cd}	29.3 ± 0.6^{bc}	24.8 ± 0.8^{d}	41.3 ± 1.2^{a}	31.7 ± 0.7^{b}
FM (kg)	9.90 ± 0.6^{b}	13.5 ± 0.7^{a}	10.5 ± 0.8^{b}	11.0 ± 0.7^{ab}	11.8 ± 0.8^{ab}	9.3 ± 0.7^{b}
LBM (kg)	57.9 ± 2.0^{bc}	42.7 ± 1.1^{d}	63.7 ± 1.9^{b}	43.4 ± 1.2^{d}	79.4 ± 3.1^{a}	55.1 ± 1.3^{c}
WC (cm)	82.4 ± 2.2^{ab}	72.3 ± 2.6^{c}	82.2 ± 1.5^{b}	75.1 ± 1.9^{bc}	90.9 ± 2.4^{a}	79.3 ± 1.7^{bc}
HC (cm)	95.2 ± 1.6^{b}	95.3 ± 1.4^{b}	98.8 ± 1.4^{b}	93.7 ± 1.5^{b}	106.0 ± 2.3^{a}	96.4 ± 1.8^{b}
WHR	0.87 ± 0.02^{a}	0.76 ± 0.02^{b}	0.83 ± 0.01^{a}	0.80 ± 0.01^{ab}	0.86 ± 0.01^{a}	0.82 ± 0.02^{ab}
WHtR	0.47 ± 0.01^{abc}	0.45 ± 0.02^{c}	0.46 ± 0.01^{bc}	0.46 ± 0.01^{bc}	0.51 ± 0.01^{a}	0.49 ± 0.01^{ab}
SBP (mmHg)	124.3 ± 3.0^{a}	107.9 ± 2.7^{b}	127.1 ± 2.9^{a}	116.4 ± 2.2^{ab}	127.0 ± 5.9^{a}	113.3 ± 2.6^{ab}
DBP (mmHg)	83.8 ± 3.0^{ab}	74.3 ± 3.2^{b}	78.5 ± 2.2^{ab}	81.5 ± 3.0^{ab}	75.7 ± 3.6^{b}	91.3 ± 4.0^{a}

Note: BMI – body mass index, MUAC – mid-upper arm circumference, FM – fat mass, LBM – lean body mass, WC – waist circumference, HC – hip circumference, WHR – waist-to-hip ratio, WHtR – waist-to-height ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure

Data are given as means ± SEM.

Means in rows with different superscripts are significantly different ($p \leq 0.05$).

($p > 0.05$) for the entire group. Athletic men had the highest BW, BMI, MUAC, LBM, and HC and were significantly different ($p \leq 0.05$) from the other study groups. These variables of total athletics were ($p \leq 0.05$) higher than those of the overall control. The highest FM was that of the control women and significantly different ($p \leq 0.05$) from the other study groups. On the other hand, FM was non-significantly different ($p > 0.05$) when comparing total control to total athletics. Control men had the highest WHR and were non-significantly different ($p > 0.05$) from athletic men and women, but significantly different ($p \leq 0.05$) from control women. No significant difference ($p > 0.05$) in WHR existed between total control and total athletics. Athlete men had the highest WHtR and were non-significantly different ($p > 0.05$) from other study groups except for control women. Whole athletics had significantly ($p \leq 0.05$) higher WHtR than total control. Athlete men had higher ($p \leq 0.05$) SBP than overall athletics and control women. The highest DBP was that of athlete women and it non-significantly differed ($p > 0.05$) from that of control men. The DBP and SBP were non-significantly different ($p > 0.05$) between total athletics and total control.

Table 2 shows group-gender serum irisin concentrations of the study sample. Footballer women had higher ($p \leq 0.05$) serum irisin (0.290 ± 0.010 mcg/ml) than bodybuilder women (0.210 ± 0.009 mcg/ml) and control men (0.200 ± 0.008 mcg/ml) and women (0.220 ± 0.009 mcg/ml), but non-significantly different ($p > 0.05$) from both footballer men (0.260 ± 0.014 mcg/ml) and bodybuilder men (0.250 ± 0.011 mcg/ml). Serum irisin concentrations did not show gender differences within each experimental and control group.

Table 3 presents serum irisin concentrations of athletic and non-athletic subjects of the study. Serum irisin did not differ ($p > 0.05$) between athlete men, athlete women, and control women or between control men and women. Serum irisin of athlete men and women was significantly higher ($p \leq 0.05$) than those of control men. The highest mean value of serum irisin was that of athlete men (0.260 ± 0.009 mcg/ml). The respective mean values of control men, control women, and athlete women were 0.200 ± 0.008, 0.220 ± 0.009, and 0.250 ± 0.010 mcg/ml. The overall athletics had significantly ($p = 0.000$) higher serum irisin (0.250 ± 0.007 mcg/ml) than the total control (0.210 ± 0.006 mcg/ml).

Serum irisin concentrations in the overall control, football players, and bodybuilders are shown in Table 4. Footballers had the highest serum irisin and were significantly different ($p \leq 0.05$) from both bodybuilders and the control. The respective serum irisin mean values for the footballer, bodybuilder, and control groups were 0.280 ± 0.009, 0.230 ± 0.008, and 0.210 ± 0.006 mcg/ml.

Table 5 shows the Pearson correlations between serum irisin anthropometric indices and blood pressure in the study sample. Serum irisin positively correlated with

Table 2. Group-gender serum irisin levels of the study sample

Variable	Control		Footballers		Bodybuilders	
	Men n = 12	Women n = 12	Men n = 12	Women n = 12	Men n = 12	Women n = 12
Serum irisin (mcg/ml)	0.200 ± 0.008^{c}	0.220 ± 0.009^{bc}	0.260 ± 0.014^{a}	0.290 ± 0.010^{a}	0.250 ± 0.011^{ab}	0.210 ± 0.009^{bc}

Data are given as means ± SEM.
Means in rows with different superscripts are significantly different.

Table 3. Serum irisin levels of athletic and non-athletic subjects of the study

Variable	Control		Athletics		Total control n = 24	Total athletics n = 48	*p-value
	Men n = 12	Women n = 12	Men n = 24	Women n = 24			
Serum irisin (mcg/ml)	0.200 ± 0.008^{b}	0.220 ± 0.009^{ab}	0.260 ± 0.009^{a}	0.250 ± 0.010^{a}	0.210 ± 0.006	0.250 ± 0.007*	0.000

Data are given as means ± SEM.
Means in rows with different superscripts are significantly different.
*p-value significant at 0.05 levels donates a significant difference between total athletics and total control.

Table 4. Serum irisin concentrations of the overall control, footballers, and bodybuilders

Variable	Total control n = 24	Total footballers n = 24	Total bodybuilders n = 24
Serum irisin (mcg/ml)	0.210 ± 0.006[b]	0.280 ± 0.009[a]	0.230 ± 0.008[b]

Data are given as means ± SEM.
Means in rows with different superscripts are significantly different.

height in the whole bodybuilder group ($r = 0.498$, $p \leq 0.05$) and negatively correlated in the entire control group ($r = –0.44$, $p \leq 0.05$). A significant negative correlation existed between serum irisin and BW in athlete women ($r = –0.545$, $p \leq 0.01$) and total control ($r = –0.432$, $p \leq 0.05$). The BMI of athlete women negatively correlated with serum irisin ($r = –0.698$, $p \leq 0.01$). The MUAC negatively correlated with serum irisin in bodybuilder women ($r = –0.826$, $p \leq 0.05$) and athlete women ($r = –0.431$, $p \leq 0.05$), whereas positively correlated with this variable in total bodybuilders ($r = 0.494$, $p \leq 0.05$). Serum irisin negatively correlated with body FM in overall footballers ($r = 0.433$, $p \leq 0.05$). Furthermore, serum irisin negatively correlated with LBM and WHtR in athlete women ($r = –0.636$, $p \leq 0.01$; $r = –0.422$, $p \leq 0.01$, respectively). The DBP negatively correlated with serum irisin in footballer women, athlete women, and overall athletes ($r = –0.676$, $p \leq 0.05$; $r = –0.322$, $p \leq 0.05$ and $r = 0.432$, $p \leq 0.05$, respectively). No correlations were found between serum irisin and other studied variables.

Discussion

Serum irisin concentrations between footballer men and women and bodybuilder men did not differ significantly, while footballer women had the highest value. Non-significant differences were also seen between bodybuilder men and women and control women and between bodybuilder women, control women, and control men. In this study, gender did not affect irisin concentrations, which is consistent with other studies that encountered no significant main or interaction effect of gender on the irisin [3, 11]. It was also found that the circulating irisin of men and women are almost similar [25, 30]. However, after adjusting for LBM, Anastasilakis et al. [2] found that males had lower

Table 5. Pearson correlations between serum irisin and study variables

Variables	Footballers		Body builders		Control		Athletics		Overall athletics n = 48	Overall footballers n = 24	Overall bodybuilders n = 24	Overall control n = 24
	Men n = 12	Women n = 12	Men n = 12	Women n = 12	Men n = 12	Women n = 12	Men n = 24	Women n = 24				
Height (cm)	0.109	0.571	0.418	–0.149	–0.473	–0.317	0.254	0.145	0.169	–0.133	0.498*	–0.440*
Weight (kg)	0.181	0.500	0.051	–0.406	–0.325	–0.444	–0.020	–0.545**	–0.090	–0.135	0.387	–0.432*
BMI (kg/m²)	0.206	0.148	–0.196	–0.441	–0.085	–0.309	–0.144	–0.698**	–0.284	–0.090	0.197	–0.208
MUAC (cm)	0.311	0.438	–0.07	–0.826*	–0.111	–0.366	–0.098	–0.431*	–0.155	0.017	0.494*	–0.284
FM (kg)	0.407	0.533	0.161	–0.267	–0.212	–0.431	0.256	0.290	0.278	–0.433*	0.202	–0.086
LBM (kg)	0.039	0.423	0.019	–0.184	–0.343	–0.389	–0.083	–0.636**	–0.146	–0.231	0.391	–0.396
WC (cm)	0.266	0.241	–0.019	–0.302	0.074	–0.489	–0.003	–0.324	–0.091	–0.019	0.231	–0.329
HC (cm)	0.2	0.487	0.137	–0.193	–0.216	–0.385	0.056	–0.188	–0.018	0.044	0.284	–0.282
WHR	0.159	–0.061	–0.196	–0.121	0.242	–0.410	–0.069	–0.234	–0.139	–0.075	0.017	–0.233
WHtR	0.239	–0.002	–0.209	–0.254	0.33	–0.457	–0.108	–0.422*	–0.250	0.105	–0.045	–0.199
SBP (mmHg)	0.565	–0.288	0.071	–0.324	–0.541	0.05	0.231	–0.036	0.138	0.063	0.173	–0.353
DBP (mmHg)	0.257	–0.676*	–0.493	0.042	–0.16	–0.161	–0.131	–0.457*	–0.322*	–0.119	–0.432*	–0.248

Note: BMI – body mass index, MUAC – mid-upper arm circumference, FM – fat mass, LBM – lean body mass, WC – waist circumference, HC – hip circumference, WHR – waist-to-hip ratio, WHtR – waist-to-height ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure
* correlation is significant at the 0.05 level; ** correlation is significant at the 0.01 level

irisin levels than females. Huh et al. [12], on the other hand, found that male adolescents had a higher increase in irisin levels following acute swimming than female adolescents. The discrepancy in these results could be attributed to the individuals' varying ages, sample sizes, and experimental protocols. The participants in this study were between the ages of 20 and 35. Huh et al. [11] used middle-aged women, while Stengel et al. [25] omitted those under the age of 18, and Huh et al. [12] included adolescents as participants. Moreover, a small sample size of elite taekwondo competitors (7 males and 6 females) and college students (8 males and 6 females) between the ages of 16 and 20 were the participants in a recent study [3].

Irisin concentrations were substantially higher in the entire athletes than in total controls. This study is possibly the first case-control study to compare serum irisin in professional athletes and regular active people. Arıkan et al. [3], using a small sample size, reported that irisin levels are exercise independent. On the other hand, other studies examined the levels of irisin in the athlete population and revealed a connection between irisin levels and exercise and considered irisin to be one putative mediator of the positive effects of exercise on the metabolic profile [30]. The results of the numerous studies in the literature presented in the debate frequently lack a suitable control group sample size, necessitating an interpretation and partial downsizing of their findings.

Almost all previous study designs were intervention training programs, with the subjects' pre-exercise intervention serving as the control group. The current research, which included professional Jordanian athletes in two different sports, reveals that irisin may be an exercise-related hormone. Our findings are consistent with those of Bostrom et al. [5], who found that exercise can promote irisin expression in human muscle and blood. As a result, irisin has been proposed as a potential treatment drug for metabolic disorders [5]. Another study also suggested that children and adults both have an acute and brief rise in blood irisin levels after short bursts of intense exercise, but not after sustained increases in physical activity [16].

There are significant inconsistencies in the results of several studies looking at irisin and its association with exercise. Type, intensity, and duration of exercise, age, gender, and sample size, along with other lifestyle factors, such as energy intake, diet quantity and quality, nutritional status, and body composition, as well as different experimental protocols and the genetic factor, are among the many potential confounders that may contribute to this discrepancy. Some investigations have agreed with the findings of the current study. Daskalopoulou et al. [7] looked at the post-to-pre--exercise variations in irisin levels after a maximal relative and absolute workload exercises and found that serum irisin increased after three workloads. Cooke et al. [6] also discovered comparable results. Irisin levels increased at the end of interventional exercise [2]. Huh et al. [11] found that circulating irisin levels rise in response to exercise. Norheim et al. [20] found that in pre-diabetic participants compared to controls, plasma irisin levels increased initially 45 minutes after exercise and then dropped after 2 hours of rest in an intervention study. Huh et al. [12] investigated the influence of exercise intensity on serum irisin levels in high- and moderate--intensity swimming in teenage boys and girls. Circulating irisin levels were shown to peak immediately after high--intensity interval exercise and then fall 1 hour later [12]. Irisin levels were also higher in men and women during the first hour following exercise [13].

On the other hand, other research yielded mixed results. Compared to previously untrained women, no changes in serum irisin were seen following 12 weeks of intense strength training [9]. Serum irisin was measured after 1 hour of low-intensity aerobic exercise, heavy-intensity resistance exercise, 21 weeks of endurance exercise, and a combination of endurance and resistance exercise [22]. A majority of the participants were men of various ages and BMIs. There were no substantial changes in serum irisin after any procedure [22]. Irisin levels were also not enhanced following training [10]. In this study, Jordanian athlete participants competed in two sports: football and bodybuilding. Footballers and bodybuilders were compared to one another and to a control group. Footballers had the highest serum irisin levels and were considerably higher than bodybuilders and controls. This result indicated that aerobic endurance exercise, but not anaerobic resistance strength exercise, could change circulating irisin levels, which go in line with the findings reported by Bostrom et al. [5]. Serum irisin increased 2-fold following ten weeks of aerobic endurance training exercise compared to the non-exercised state [5]. These findings are consistent with Huh et al. [11], who stated that plasma irisin decreased after eight weeks of anaerobic intermittent sprint training. Endurance-trained athletes were found to have higher concentrations of circulating irisin than sedentary controls [4]. Furthermore, Ellefsen et al. [9] reported similar results.

The result of the present study is inconsistent with those of [22], who observed no changes in irisin levels after 21 weeks of combined endurance aerobic exercise and resistance exercise. Norheim et al. [20] found that plasma irisin levels acutely increased 45 minutes after aerobic exercise of ergometer cycling and then decreased after 2 hours of rest in pre-diabetes subjects vs controls, but declined after 12 weeks of training. On the other hand, Huh et al. [12] reported that serum irisin increased after high-intensity interval exercise in an adolescent swimmer who depended on an anaerobic system, and it did not increase after moderate-intensity aerobic exercise. In the same study, eight weeks of sprint training significantly induced irisin biomarkers FNDC5 and PGC1_ mRNA levels. Tsuchiya et al. [28] suggested that irisin is affected by the intensity of exercise as it increased after high-intensity anaerobic exercise, but not after low-intensity aerobic exercise. Several reports indicated that circulating irisin was also influenced by the method of analysis used [5, 11, 20], a matter that could be responsible for the dissimilarity in the results of different studies. The muscle phenotype could also be another confounding factor. Ellefsen et al. [9] observed that FNDC5 expression is related closely to aerobic muscle fibers, the myosin heavy chain 2X isoform (MyHC2X), but not with 2A ioform (MyHC2A), which increased in response to strength training. Participants in the present study were all healthy with normal BMIs. Participants who were overweight BMIs were bodybuilders with high lean body mass and not high--fat mass. However, subjects enrolled in the Huh et al. [11] study; their MBIs were all above 37 kg/m^2, while those of Stengel et al. [25] had a wide range of BMIs. Nevertheless, irisin, a new myokine, has recently been linked to human exercise-induced changes in oxidative stress and antioxidant defense, and its concentrations were unaffected by increased training volume or intensity [29].

The present study also investigated the correlations between serum irisin levels and several anthropometric indices. Irisin positively correlated with height in the bodybuilders' group and negatively in the control group. Serum irisin levels showed a strong negative correlation with body weight and BMI in athlete women and a negative correlation with body weight in the control group. Conversely, much research showed that irisin positively correlated with body weight and BMI [9, 11, 21, 25]. This controversy could be due to the differences between subjects enrolled in different studies. While the participants in the present study were all healthy with normal BMI, participants in previous studies were obese [11, 25]. Obese subjects could have insulin resistance. Stengel et al. [25] reported a positive correlation of serum irisin with insulin. The increase in irisin levels in the obese subject could indicate a physiological function to improve glucose intolerance and insulin sensitivity, which is often impaired in obese subjects [25].

The MUAC is an indicator for muscle mass, and irisin correlated negatively with MUAC in bodybuilders and athlete women, while it was positively correlated in total bodybuilders. These results merit further investigations. The positive correlation for irisin with MUAC in total bodybuilders agrees with that reported by Huh et al. [11]. In this study, irisin negatively correlated with MUAC and LBM in athlete women. Similarly, Ellefsen et al. [9] reported a positive correlation between LBM and irisin in untrained women, while this correlation seemed to disappear in trained women after 12 weeks of strength training. Ellefsen et al. [9] explained this result that strength training could affect the regulation of irisin secretion in skeletal muscle and is likely to be linked to the complex biological induction imposed on muscle cells by training. In turn, Stengel et al. [25] and Ellefsen et al. [9] reported positive correlations between body fat mass and serum irisin, while the present study showed a negative correlation between irisin and body fat mass in footballers. These differences could be due to different exercise habits, as irisin may interact with fat cells resulting in its removal from the bloodstream [9].

In the present study, a negative correlation was observed between serum irisin and WHtR, which is consistent with that reported elsewhere [21]. No correlation was obtained between serum irisin and WHR, which accords with the finding of Stengel et al. [25].

One limitation of the present study is the small sample size, and the sample was a convenient one. The timing of sampling could affect the results. Anastasilakis et al. [2] reported that irisin levels followed a day-night rhythm with a peak at 9:00 pm. In this study, all samples were collected before training. The prime feature of this study is that it is possibly the first case-control investigation to compare serum irisin concentration in professional athletes and regular active people.

Conclusions

Taken together, the current findings point to regular exercise training as a means of inducing irisin in the general population. Irisin appears to be a hormone linked to physical activity, and gender did not affect it. The data confirm the theory that the type of exercise can impact circulating irisin, as footballers had the highest levels of irisin. Strength training or static sports may enhance serum irisin levels more than aerobic endurance exercise or dynamic sports. The control group and the participants of the static sport were not statistically different. The complex pattern of inter-group variation in correlations between irisin and anthropometric indices suggests a complex relationship between body composition and regulation of serum irisin and the complexity of understanding irisin biology.

Conflict of Interest

The authors declare no conflict of interest.

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The influence of bodyweight high-intensity interval training on critical velocity and sprinting abilities in well-trained soccer players

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Abstract
Introduction. The need for efficient training programs is critical for high-performance in competitive soccer. High--intensity interval training is an effective exercise modality that can improve both the aerobic and anaerobic energy systems, but its efficacy to enhance performance-related tests in well--trained soccer players is unknown. Aim of Study. The purpose of this study was to evaluate a bodyweight high-intensity interval training program and its effects on critical velocity via a 50-meter 3-minute all-out test and maximal sprint speed by a 40-yard dash in well-trained soccer players during the competitive season. Material and Methods. The experimental subjects performed a progressive series of high-intensity exercises and conditioning, twice per week for 4 consecutive weeks, in addition to their regular practice and match schedules. A 2 × 2 ANOVA was used for both performance tests, along with the smallest worthwhile change and smallest real difference to measure practical significance compared to the athletes who did not perform the additional training protocol. Results. No statistically significant differences were found between time or groups for the 40-yard dash ($p = 0.079$; $p = 0.161$) or 50-m 3-minute all-out tests ($p = 0.052$; $p = 0.351$), however, significant small ($p = 0.024$) and trivial ($p = 0.04$) interactions were found, respectively. For practical significance, considerable differences between the experimental and control groups were found for both performance tests. Conclusions. The results can be a practical option for strength and conditioning coaches to improve game-impacting sprinting and critical velocity in well-trained soccer players within a 4-week in-season training period with minimal additional training investments.

KEYWORDS: soccer, critical velocity, high-intensity interval training, performance, maximal sprint speed.

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Introduction

Soccer emphasizes cardiovascular and neuromuscular systems throughout matches which requires participants to jog, run, sprint, jump, kick, and slide tackle, both with and without the possession of the ball [2, 23]. As the competition levels increase, the physiological demands and the quality of specific skills and tactics required to be successful on an individual and team basis require more attention [2, 23]. Systematically planned training programs and assessments in soccer require an emphasis on the aerobic and anaerobic energy systems while also addressing the need for frequent changes in speed, direction, power, strength/endurance, and intensities [2, 20]. Athletes expressing the highest levels of these performance traits during matches will typically improve their chances of success during match play [2, 20]. However, during the competitive season, tactical/technical training sessions generally are prioritized, which emphasize specific skill sets needed to execute team gameplay, limiting the opportunities for training specific cardiovascular or neuromuscular needs for performance enhancement leading up to a match [2, 20].

As physiological and psychological demands increase throughout a match, soccer players will likely encounter various levels of fatigue that may negatively impact performance [2, 17]. These fatigue levels could result from muscle acidification, neuromuscular inefficiencies, depleted muscle glycogen stores, or a combination [20]. The high variability of fatigue depends considerably on the athletes' playing position, the severity of acute and chronic stressors, and their ability to recover between intense performance periods, all of which reflect the efficiency of an athlete's physiological systems [17, 20]. The capabilities of these high-performing athletes can be practically evaluated using numerous running field tests such as the 40-yard dash (40-YD) to assess maximal sprint speed (MSS) and the 3-minute all-out test (3AOT) to determine the efficiency of the aerobic and anaerobic systems [15, 17]. Improving fatigue resistance is possible, but these improvements are mainly unknown in well-trained athletes, specifically, soccer players who already have high aerobic capacities and fatigue resistance [2].

High-Intensity Interval Training (HIIT) is an exercise modality centered on altering sets and recovery periods that vary between high and low intensities/volumes for specific times [7]. HIIT programs have improved aerobic and anaerobic capabilities in various populations, with additional neuromuscular benefits when incorporating resistance training [7, 17, 27]. In a landmark study by Tabata et al. [28], 2 groups (n = 14) were evenly divided to perform an aerobic (70% of $\dot{V}O_{2max}$, 60 minutes per day) or anaerobic (7 to 8 sets of 20 seconds of work at 170% of $\dot{V}O_{2max}$, followed by 10 seconds of rest) cycling program 5 days per week for 6 consecutive weeks. The aerobic group significantly ($p < 0.01$) improved their $\dot{V}O_{2max}$ (53 ± 5 to 58 ± 3 ml·kg^{-1}·min^{-1}), but did not significantly ($p > 0.10$) improve anaerobic capacity, while the anaerobic group significantly improved ($p < 0.01$) both $\dot{V}O_{2max}$ and anaerobic capacity by 7 ± ± 1 ml·kg^{-1}·min^{-1} and 28%, respectively, when compared to pre-training values [28]. Furthermore, HIIT can improve aerobic and anaerobic conditioning in athletes, usually in less time than traditional cardiovascular training [7, 17, 18, 23, 27]. For instance, McRae et al. [18] evaluated aerobic running (~85% maximal heart rate for 30 minutes; n = 7) and a HIIT (20 seconds of as many repetitions as possible of whole-body exercises, followed by 10 seconds of rest for 8 rounds) program against non-exercise controls, 4 days per week for 4 weeks. Collectively, all subjects significantly ($p < 0.05$) improved their $\dot{V}O_{2peak}$ (aerobic: 45 ± 5.0 to 48 ± ± 3.0 ml·kg^{-1}·min^{-1}; HIIT: 43 ± 7.6 to 46 ± 7.8 ml· ·kg^{-1}·min^{-1}), but only the HIIT group saw significant ($p < 0.05$) improvements in leg extension, chest press, sit up, push-up, and back extension capabilities [18]. The performance upgrades with the HIIT group were due to the subjects invoking an average of 80% or more of their HRmax per session while creating additional local and systemic muscular endurance adaptations via metabolic stress [18, 22]. HIIT programs provide opportunities to train at different intensities and volumes, reflecting an athlete's current physical and psychological abilities to work in high-stress environments while invoking numerous positive adaptations [7, 8, 17].

HIIT programs have also demonstrated numerous improvements among soccer players, such as improving fatigue resistance, recovery capabilities, and aerobic power, all of which can positively impact performance during match-play [2, 7, 23, 27]. For example, Rowan et al. [23] divided $\dot{V}O_{2max}$ matched collegiate soccer players into a HIIT and aerobic training group where they performed their conditioning sessions twice per week over 5weeks during the season. The HIIT group performed 5 repetitions of 30-second sprints as fast as possible, with 3.5 to 4.5 minutes of active recovery, and the aerobic-only group performed a 40-minute run at 80% of their $\dot{V}O_{2max}$ [23]. Both HIIT and aerobic groups significantly improved mean $\dot{V}O_{2max}$ (50.68 to 53.04 ml·kg^{-1}·min^{-1}, 4.73% increase; 50.64 to 52.31 ml· ·kg^{-1}·min^{-1}, 3.42% increase, $p = 0.002$) and average distance covered during the Yo-Yo Intermittent Recovery test (1857 ± 423 to 2131 ± 436 m, $p = 0.001$; 1473 ± 494 to 1613 ± 510 m, $p = 0.042$) with no significant differences between groups [23]. The application of HIIT created just as substantial aerobic and anaerobic performance values as traditional endurance training, but with considerably less time [23]. Moreover, a meta-analysis by Kunz et al. [16] further supports the use of HIIT in soccer and found that small-sided games (intense ball-handling games performed on smaller fields) and running-based HIIT programs produced similar positive performance outcomes in young soccer players, including moderate-to-large positive effects on $\dot{V}O_{2max}$, $\dot{V}O_{2peak}$, running economy, and change-of-direction performance. Despite the documented effectiveness of HIIT in soccer athletes, the use of bodyweight high-intensity interval training (BW HIIT) programs in well-trained soccer players has not been established, and it is unknown if these athletes would benefit from this type of training, especially in the middle of the season, to improve or better prepare for the most crucial times of competition.

Aim of Study

The purpose of the research study was to determine the potential impact a bodyweight high-intensity interval training (BW HIIT) program has on a well-trained soccer player's critical velocity (CV) via a 50-meter 3AOT and MSS by a 40-YD [2, 19]. The study's results can provide options to improve athletic performance during the competitive season without using equipment.

Material and Methods

Subjects

Twenty highly aerobically trained soccer players (mean age = 24 $\pm$ 6 years) representing Livonia City Football Club, a semi-professional team representing the Midwest Premier League in the United States, were included in this study. All participating subjects had at least 4 years of competitive experience at the high school or college levels and were either current members of a collegiate soccer program working with the team during their off-season training or graduated from a collegiate program and were working with the team solely. Subjects were not divided into position-specific groups due to individuals playing multiple positions based on team needs and strategies; however, subjects who play goalie were excluded since goalkeepers traditionally have the lowest aerobic capacities compared to other positions due to their unique position-specific needs [2, 13]. Subjects were instructed to avoid caffeine/stimulant consumption at least 12 hours before testing, along with limiting a large consumption of food/mixed macronutrient meals at least 90 minutes beforehand to minimize ergogenic or ergolytic interferences [8, 14]. After obtaining informed consent, subjects were randomized into experimental and control groups and then participated in baseline performance testing for the 40-YD and 3OAT.

Test selection

Soccer players perform a series of low-, medium-, and high-intensity actions and movements throughout a soccer match, and the amount/capacity of high-speed running, along with the ability to recover between bouts, is a significant distinguishing factor between high and low competition levels [2, 21, 22] Therefore, consistently evaluating high-intensity exercise outcomes is necessary for soccer as a general and position-specific measurement of athletic performance [2, 17, 21, 22]. A high aerobic capacity, marked by relative $\dot{V}O_{2max}$, is associated with greater performance outcomes across many sports and activities [2, 12, 17]. Yet, due to the varying demands and actions within the sport of soccer, additional performance measurements, such as lactate threshold (LT), maximal lactate steady state (MLSS), MSS, and CV, are also evaluated to provide a more accurate performance assessment both in laboratories and the field [2, 15, 18, 20]. Even though laboratory tests typically provide more data sets in controlled settings, field tests are more likely to stimulate performance demands encountered during matches since they occur in similar environments encountered during practices and matches [2, 15, 17, 20].

Speed tests evaluate an individual's ATP-PCr and anaerobic glycolytic energy systems and typically last 20 seconds or less to determine acceleration and MSS capabilities [17]. The 40-YD is a popular performance test assessing linear speed, with high correlation values ($r = 0.89$-0.97) and reliabilities (ICC = 0.985; $p < 0.001$) when assessing multiple attempts and can also be manipulated based on individual or sport-specific needs [17]. It is a practical field-based test evaluating an individuals ability to accelerate (0-20 yards) linearly and achieve/maintain MSS (20-40 yards), both of which are primary attributes expressed repeatedly during soccer competitions for most playing positions [2, 17, 20, 22].

CV describes the efficiency between the energy systems and the capability to operate at various intensities/distances and recovery intervals while providing activity-specific assessments and performance predictions, especially at high-intensity capacities, making it a valuable tool in assessing most soccer positions [2, 15, 21]. The 3AOT is a valid and reliable performance test when evaluating CV [1, 17, 20]. For example, research by Alves de Aguiar et al. [1] had 7 male runners complete time trials of 800, 1600, and 2400 meters (m), along with two 3AOTs for CV comparisons. Both 3AOTs displayed excellent reliability scores for CV (trail 1: 3.90 $\pm$ 0.41 m/s^{-1}, trail 2: 3.89 $\pm$ 0.48 m/s^{-1}, ICC = 0.95, coefficient of variation = 2.97%) and D' (trial 1: 176 $\pm$ 42 m, trial 2: 183 $\pm$ 35 m, ICC = 0.93, coefficient of variation = = 5.12%) in comparison to prediction models of the 3 time trials [1]. In addition, Saari et al. [24] demonstrated that shuttle run 3AOT (2.94 $\pm$ 0.39 m/s^{-1}) displayed similar ($p = 0.71$, coefficient of variation = 7.7%) CV outcomes compared to continuous (3.00 $\pm$ 0.36 m/s^{-1}) 3AOT in 12 active subjects, further validating either method for CV predictions. Both the 40-YD and 3AOT are excellent field options to evaluate numerous sprinting and CV characteristics that soccer players frequently emphasize during training and matches [1, 15, 17, 20, 22, 24].

Testing protocols

The 40-YD and the 3AOT pre- and post-performance assessments were conducted 1 hour before a regularly scheduled evening practice at an outdoor soccer practice facility. A tape measure and cones outlined the 40-YD, marking both the start and finish lines. When ready and after individual warm-ups, subjects approached the starting line going into a split stance, placing one foot of their choosing as close to the start line as possible, with the option of placing one or both hands on the ground representing a 3 or 4-point stance. Timing began as soon as the subject initiated movement and stopped once they crossed the finish line. Subjects rested for approximately minutes and then performed a second trial, with their best running time achieved between both attempts counted as their official score.

After all subjects performed both 40-YD trials and completed a 5-minute recovery period, they performed one test of the 3AOT. The primary investigator outlined end lines 50-m apart, with cones marking each 10-m increment. After the subjects recovered and understood expectations/completed a walk-through, they approached the starting line similarly to the 40-YD trials. Subjects ran as fast as possible to the other end line, touching the line with one foot, and then returned to the starting line, repeating this process for 3 minutes. Timing started when the subject initiated movement and tracked how many yards were covered during the trial. Once 3 minutes had elapsed, the primary investigator marked where the subjects finished and noted the total distance covered during the test. Once all data sets were collected and recorded on paper, each subject's assigned number, position, and scores for each test were recorded on an electronic spreadsheet and used for scoring comparisons during the post-tests.

After completing the tests, all athletes continued their regularly scheduled training, practice, and match schedules during the competitive season, which typically contained 2 to 3 practices and 1 to 2 matches per week. The athletes were not required to perform any additional training sessions together outside of their practices, and if they were performed, they were completed on an individual basis and not tracked/ recorded for this study. The subjects were assigned to either the experimental (n = 10) or control (n = 10) groups based on their ability to commit to the exercise program and desire to participate in additional training sessions. The experimental group began their additional workouts one week after completing the first round of testing and performed 2 BW HIIT workouts weekly (8 total) for 4 consecutive weeks (Appendix A). Subjects used a Rating of Perceived Exertion (RPE) scale of 6 (no exertion at all) to 20 (maximal exertion) to execute their runs and recovery periods appropriately and according to the directions outlined (Appendix A), noting all sensations and feelings of physical stress and fatigue [9]. Subjects were instructed on how to rate their level of exertion using the RPE scale (both verbally and within the informed consent), why the scale/level of effort matters during each set/ repetition, and how to record their effort levels using the sheets provided to meet their goals (Appendix B). In addition to RPE, subjects also recorded the number of repetitions performed during each bout of exercise using a data tracking sheet to ensure high levels of effort were maintained throughout the program, along with aiming for a 10 percent increase in total repetitions during weeks 3 and 4 of training.

The primary investigator provided verbal encouragement to the subjects to help ensure proper form and execution of each exercise round as hard as possible. After individual warm-ups, the exercise program took approximately 20 minutes for each session, considering the 1-minute rest periods between each exercise protocol. When the exercise program finished, subjects joined their team for the rest of their scheduled practice or could do what they wanted if the program took place on a non-practice day. All subjects in the experimental group were expected to complete 8 exercise sessions during the 4 weeks, with attendance/compliance measured electronically by the primary investigator before beginning each session. If subjects did not make a session, they arranged a make-up day within the same week with the primary investigator. If 2 of the 8 regularly scheduled exercise sessions were missed, the subjects were dropped from the experiment and not included in the final data collection. Once the experimental group completed 8 exercise sessions over 4 weeks, all subjects retested their 40-YD and 3AOT for comparison to their initial values.

Data analysis

The data from each group's performance for both pre- and post-tests are outlined in Table 1. Two, 2 × 2 mixed factor ANOVAs [(time: pre, post) × (group: exp, control)] were conducted to investigate the exercise training program's impact on 40-YD and 3OAT scores between each group, respectively. Tukey HSD post hoc tests were administered to probe any significant main effects. An alpha level of 0.05 was used for all inferential analyses. For effect size, partial eta squared was set to <0.25, 0.25-

-0.50, 0.50-1.0, and >1.0 as trivial, small, moderate, and large effect size, respectively [26]. Practical significance was measured using the smallest worthwhile change (0.2 × between-subject SD) and smallest real difference (1.96 × √2 × standard error of measurement) determine performance differences and potential effectiveness of the training program on both field tests [2].

Table 1. Descriptive statistics for the experimental and control groups

Test	Timing group	M (SD)	Range
40-YD (sec)	EXP pre	5.07 (0.17)	4.68-5.28
	EXP post	4.99 (0.16)	4.65-5.21
	CON pre	4.90 (0.22)	4.5-5.25
	CON post	4.91 (0.23)	4.46-5.22
3AOT (yd)	EXP pre	753 (39.24)	680-830
	EXP post	775.3 (38.36)	734-848
	CON pre	750.6 (28.59)	710-790
	CON post	749.9 (30.47)	700-796

Note: EXP – experimental group, CON – control group, 40-YD – 40-yard dash, 3AOT – 3-minute all-out test, pre – pre-test, post – post-test

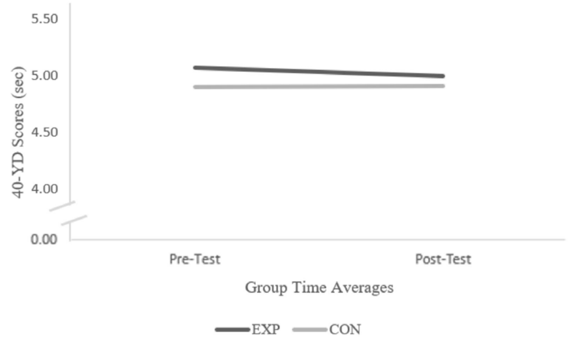

Figure 1. Interaction effects of avarage 40-yard dash (40-YD) pre-test and post-test times between the experimental (EXP) and control (CON) groups

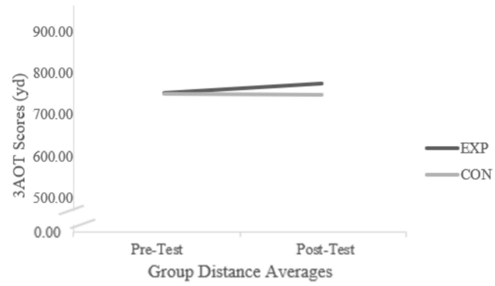

Figure 2. Interaction effects of avarage 3-minutes all-out test (3AOT) pre-test and post-test distances (yd) between the experimental (EXP) and control (CON) groups

Results

There was not a significant main effect for time [F(1, 18) = 3.47, p = 0.079, η_p^2 = 0.162] or groups [F(1, 18) = 2.14, p = 0.161, η_p^2 = 0.11] involving 40-YD dash performance (Figure 1). There was a significant interaction between groups and time [F(1, 18) = 6.12, p = 0.024, η_p^2 = 0.25], however, Tukey's HSD post hoc tests assessed the differences between each pre and post-test scores of the experimental and control groups, confirming no significant differences (p > 0.05) were achieved in 40-YD performances. Another 2 × 2 mixed factor ANOVA [(time:pre,post) × (group:exp,control)] was conducted to investigate the exercise training program's impact on 3AOT scores between each group (Figure 2). There was not a significant main effect for time [F(1, 18) = 4.32, p = 0.052, η_p^2 = 0.19] or group [F(1, 18) = 0.92, p = 0.351, η_p^2 = 0.05] regarding 3AOT scores. There was a significant interaction between time and group [F(1, 18) = 4.90, p = 0.04, η_p^2 = 0.21], however, Tukey's HSD post hoc tests assessed the differences between each pre and post-test scores of the experimental and control groups, confirming no significant differences (p > 0.05) were achieved in 3AOT performances.

The smallest worthwhile change and smallest real difference were also used to measure practical significance for the 40-YD and 3AOT pre- and post--test performances. Eight out of 10 subjects from the experimental group achieved the smallest worthwhile change of at least 0.03 seconds for the 40-YD, while the control group had only subjects achieve at least a 0.04 second improvement. The experimental group also produced the smallest real difference of 0.11 second improvement, while the control group reached only 0.01 seconds between the pre- and post- 40-YD performances. For the 3AOT, the experimental and control groups had 6 out of 10 subjects obtain the smallest worthwhile change of 7.85 and 4.52 yards between the pre- and post-tests, respectively. The experimental group also displayed the smallest real difference of 30.91 yards, while the control group collectively achieved 0.98 yards between initial and final tests.

Discussion

The main findings failed to demonstrate significant differences between the experimental and control groups for BW HIIT on the 40-YD and 3AOTs. However, the significant interaction effects for the 40-YD and

3AOTs, along with the smallest worthwhile changes and smallest real differences, showcase that the experiment provided performance-impacting improvements in the experimental group's sprinting and CV abilities after 4 weeks. These findings provide the foundations for future research to determine the usefulness of the BW HIIT training program and its impact on sprinting and CV abilities across a longer training period [2].

Various factors influence athletic performance, including genetics, environment, injury status/ history, and physical preparation [8]. While some of these aspects cannot be manipulated, power, technique, and aerobic/ anaerobic capabilities are all traits that continue to be emphasized to maximize an individual's performance in soccer [2, 20, 22]. The higher performing an individual or team is compared to their opponent, the greater the likelihood of positively influencing key outcomes, plays, and winning matches [2, 20, 22]. For instance, in an analysis of professional soccer players, the top division performed 25 to 33% longer sprints ($p < 0.001$) during match play compared to the third division despite similar distances covered between playing positions, showcasing higher workload capabilities of top performing players [12]. In addition, elite players displayed 41% higher distances covered in progressive running tests (965 $\pm$ 251 vs 685 $\pm$ 217 m; $p < 0.05$) in comparison to the sub-elite players, further demonstrating their abilities to perform under severe-intensity conditions [12]. In another analysis during the 2016-2017 season, the top 4 finishing teams in the elite Italian professional circuit demonstrated lower percentages of running (65.98 $\pm$ 1.51 vs 66.84 $\pm$ $\pm$ 2.18; $p < 0.001$) and higher percentages of jogging (25.61 $\pm$ 1.71 vs 25.30 + 1.97; $p = 0.037$) and sprinting (8.41 $\pm$ 1.04 vs 7.86 $\pm$ 0.82; $p < 0.001$) in comparison to the rest of the teams in the league [28]. The total goals scored ($r = 0.906$; $p < 0.001$) and final position ranking ($r = 0.850$; $p < 0.01$) were also strongly correlated, indicating that the best teams displayed high levels of jogging and sprinting during match-play, which positively influenced outcomes throughout the season [28]. Improving athletic performance, however, becomes increasingly difficult as individuals become more trained and get closer to reaching their maximal potential, which is a considerable factor in these well--trained subjects [25].

There are notable shortcomings that may have contributed to the lack of statistical significance in addition to the ones identified before the experiment was conducted. The most significant limitation that occurred was the sample size. For the a priori levels to be set to 0.05, a minimum sample size of 52 subjects from both the experimental and control groups would be required to avoid a type II error [3, 5, 6]. However, the small sample size of 20 total subjects underpowered the results, which is a significant factor in failing to reject the null hypothesis [3, 5, 6]. Due to the small sample size, the collective improvements of the subjects needed to drastically improve beyond their pre-test values to obtain statistical significance [3]. Since there were at least 50 subjects to choose from between both teams, the primary investigator aimed to have at least 20 subjects for the experimental group and 20 for the control group to empower the sample size and better understand the results obtained. Unfortunately, the lack of subjects completing the study was attributed to injuries sustained during the 6-week timeframe, the inability to commit to the additional conditioning sessions, or current collegiate players transitioning from the club to their university teams. Despite the primary investigator's desire to investigate if significant performance improvements can improve within the season, these factors reduced subject participation and limited the results.

In addition to the small sample size, the training stimuli/ volume may not have been enough or too much to improve sprinting or CV abilities significantly due to the offsetting demands of regular practices/conditioning sessions and normal competition schedules, all of which may impact recovery capabilities and positive adaptations [2, 10]. The modalities of backward sprinting, HIIT, and high-speed interval running have previously been documented to significantly improve athletic performance variables in soccer players by enhancing sprinting capabilities, muscular endurance, and fatigue resistance, respectively [5, 10, 17, 22, 23, 30]. The in-season training period may have limited performance adaptations due to the physical preparation that took place in previous training periods and current demands of the competitive season, further necessitating additional testing of the BW HIIT intervention during other cycles of the season, such as the off-season or pre--season [5, 9, 22, 23]. Previous studies that used similar training methods took place during the off-season training periods, which provides a greater opportunity for extensive adaptations to occur [10, 23, 30]. The exercise sessions in these previous studies were also the primary training modality, with subjects performing 2 to 5 weekly bouts lasting between 5 to 10 weeks [10, 20, 30]. The current study had subjects perform the training program twice per week for 4 weeks in addition to practices 2 to 3 times per week and regular competitions, speculating that competing demands and fatigue could have been primary factors in not achieving significant performance improvements, along with the program's duration [9, 10, 17, 21, 22, 23, 30].

The age and skill levels of the subjects may have also played a vital role in the lack of statistically significant performance improvements. For instance, the typical high school age range is between 14 to 18 years of age, and the subjects recruited for the current study ranged between 18 to 30 years [9]. Peak performance for world-class track and field sprinting times usually peaks between 25 to 27 years, with longer distance events observing higher peak performance ages [9]. Since physical maturation has likely not peaked in the adolescent or high-school athletes observed in previous studies [11, 30], their potential training adaptations may be more extensive than subjects within the next age group decade [9, 25]. The subjects of the current research study were all current or former colligate athletes, indicating they had more training years and are likely closer to their genetic ceiling for performance capabilities compared to the younger counterparts in the other studies [9, 10, 11, 25, 30]. In addition, all subjects of the current research study were part of a semi-professional team, demonstrating their high skill and performance levels before the experiment [2, 11, 25]. Despite the lesser likelihood of achieving substantial performance improvements in the current study's subjects versus other sub-elite populations, the need to research performance improvements in these athletes remains high for influencing outcomes for competitions [2, 25].

A final primary limitation is that the novel exercise program used by the experimental group is based on training programs derived from previous studies [5, 18, 27, 30], and while each program found significant performance improvements, the training protocols utilized by the experimental group in this study used only parts of these programs for additional conditioning purposes. Even though the subjects were part of a semi-professional team, the team's programs lacked specific periodized training modalities and exercise tools that other clubs may have regular access to, providing them with potential performance training disadvantages, which is why the goal was to determine if these attributes can improve within minimal investments in time and equipment. Regardless of a team's circumstances, there are specific periods when training programs are designed to achieve peak performance levels during the competitive season, usually in preparation for the most important competitions, further highlighting the study's importance [10].

Current academic studies and publications rely heavily on finding innovative research that produces statistically significant results, which prompts researchers to make determinations based on absolute terms and may create biases or manipulation of data/analyses without a true effect [3, 6, 29]. The findings in sports science research can vary greatly due to numerous dependent/independent variables and sample sizes, which all play a role in determining significance [3, 6]. If findings are excluded by not achieving these standards, the progress of understanding and progressing human performance may be limited [3, 6]. Relying solely on published statistically significant data could also impact follow-up or meta-analysis research, and it may not consider the entirety of findings regarding specific athletic performance tests and training modalities [3]. Therefore, the data in sport science research should be presented clearly for readers to translate the findings instead of relying on the author's interpretations, which can be accomplished through alternative statistical methods that highlight practical significance, feasibility, and create opportunities for future research/applications, especially when statistical significance is not found [3, 4, 6]. For instance, top level professional starting soccer players that play the majority of the match displayed greater total distance covered and high-intensity running vs nonstarters, and starters also displayed higher instances of countermovement jump height and peak power [9]. Despite not achieving statistical significance, exercise training strategies will likely need to differentiate between starters and nonstarters to best prepare for competition and performance capabilities [9]. Furthermore, the top 100 sprinters in the world from 2002 to 2016 achieved mean annual improvements between 0.1-0.2% [9]. The top ten athletes in all track and field categories except throwers also had small, yet the greatest improvements as they reached their peak age compared to athletes ranked 11 to 100 (1.8 + 1.1 to 1 + 0.9%) during this timeframe, further emphasizing the need for athletic performance to improve in elite athletics, even marginally, to create significant impacts in competition [3, 9].

Other soccer performance variables/assessments could also test the program's efficacy, such as vertical or horizontal jumps for lower body power and other aerobic capacity tests such as the Yo-Yo Intermittent Recovery test [4, 23]. In addition, testing different age groups/genders and applying the protocols to other field sports can all offer greater insight into understanding the usefulness of the exercise program and addresses the gaps found [3, 4, 7, 23, 29]. If similar results are obtained in the replication studies, or additional variables/relationships are identified, it increases the confidence of the original analysis while also providing converging evaluations [3, 6]. When key variables, specific problems, and potential outcomes are identified, additional follow-up research designs are needed to determine further if a cause-and-effect relationship can be established, along with highlighting the exercise program's efficacy and practicality in practice for athletes and coaches [3, 4, 6, 29].

The data from the present study provides an exploratory hypothesis in that the training program did produce performance-related, practical improvements in both the 40-YD and 3AOT, but these results were not similarly supported by traditional inferential analyses [3, 4, 6]. Future trials with large sample sizes or longer durations for training would likely reveal additional, useful information regarding the practicality of including a program such as this into a competitive season schedule [3, 4, 6].

Conclusions

In soccer, the athleticism required for competitive success is dependent on the level of competition, playing position, and tactics, which is why administering and analyzing performance tests are crucial for understanding individual and team capabilities/goals. The variety of exercise programs available can improve many traits required, but executing a training program during the competitive season that complements the demands of practices and games is less clear. This study aimed to understand if sprinting and CV abilities can improve within well-trained soccer players during the competitive season. Despite the lack of statistical significance, the training program produced practically significant results for both performance attributes after a 4-week mesocycle, which coaches can use to prepare their athletes for the most important competitions. The results provide the framework for future investigations and replication studies to determine the impact of a BW HIIT on well-trained soccer players and potentially other field sports.

Conflict of Interest

The authors declare no conlifct of interest.

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Appendix A

Exercise Program

	Backward Running (RPE)	Tabata Split Jumps (Reps each set)	Tabata Alternate 1-Leg Plyometrics + Modified Burpees (Reps each set)	Sprint + Walk (RPE)
Week 1	15 yards 2 sets @ Mod 6 sets @ Sev*	20 sec AMRAP 10 sec of rest 8 rounds*	20 sec AMRAP 10 sec of rest 8 rounds*	10 sec @ Sev 20 sec @ Low For 6 mins
Week 2	15 yards 2 sets @ Mod 8 sets @ Sev*	20 sec AMRAP 10 sec of rest 8 rounds*	20 sec AMRAP 10 sec of rest 8 rounds*	10 sec @ Sev 20 sec @ Low For 6 mins
Week 3	20 yards 2 sets @ Mod 6 sets @ Sev*	20 sec AMRAP 10 sec of rest 8 rounds*	20 sec AMRAP 10 sec of rest 8 rounds*	10 sec @ Sev 20 sec @ Low For 7 mins
Week 4	20 yards 2 sets @ Mod 8 sets @ Sev*	20 sec AMRAP 10 sec of rest 8 rounds*	20 sec AMRAP 10 sec of rest 8 rounds*	10 sec @ Sev 20 sec @ Low For 8 mins
Goals	Increase volume or distance each week while maintaining RPE intensities	Improve total repetitions per 8 rounds by 10% during weeks 3 + 4	Improve total repetitions per 8 rounds by 10% during weeks 3 + 4	Maintain RPE intensity levels throughout

Note: Low – low intensity (RPE ≤ 8), Mod – moderate intensity (RPE 12-14), Sev – severe intensity (RPE 18+), AMRAP – as many repetitions as possible
* Subjects rest 1 minute after all sets/rounds are completed for each category before moving on to the next series

Description of Each Exercise Series

Backward Running: After the subject's warm-up using their own methods, they will perform sets at moderate intensities (RPE 12-14) and 6-8 sets at severe intensities (RPE 18+) within the distance outlined each week. Sets will be performed with one subject at a time, and once a set is completed, the subject walks back to the starting line as a form of active recovery. During the active recovery time, the following subject up begins their backward running protocol. This process is repeated until all sets for the day are completed for each subject. RPE is recorded at the end of the end of all sets to ensure appropriate intensities are utilized.

Split Jumps: Starting in the split stance with one foot completely flat/forward and the other foot behind the torso on their toes, individuals then dip their body down by bending their legs. Subjects immediately jump upward and reposition their legs to land softly in opposite directions, and the sequence is repeated until their time is reached. Subjects record their repetitions for each set for tracking purposes.

1-Leg Plyometrics + Modified Burpees: Subjects alternate between both exercises during each round, performing a total of 4 sets of 1-leg plyometrics (2 each leg) and 4 sets of modified burpees. The focus of the 1-leg plyometrics is to perform as many jumps either side-to-side or front-to-back on one leg as possible during the round, focusing on both explosive power and speed. For the modified burpees, the subjects stand upright, then bend over and squat down while placing their hands on the ground slightly wider than shoulder width apart. While bracing the upper body, subjects kick both of their legs up simultaneously until their legs are fully extended, and they land on their toes. Subjects then reverse the process by bringing both legs inward and then jumping upwards as high as possible while extending their body, followed by a quiet landing where the lower body absorbs the impact. Repetitions are recorded for each set for tracking purposes.

Sprint + Walk: The final session consists of all subjects performing the protocol at the same time. Subjects line up at the starting line and then sprint as fast as possible for 10 seconds, followed by 20 seconds of walking as a form of active recovery. The process is repeated for 6 total minutes or 12 total repetitions. Auditory timing cues will be announced by the principal investigator, and subjects will sprint/walk back-and-forth within a 50-meter start and end line. RPE is recorded at the end of all sets to ensure appropriate intensities are utilized.

Appendix B

Tracking Sheet for Each Participating Subject in the BW HIIT Program

Assigned number: ____________

	Backward Running (Total RPE)	Tabata Split Jumps (Reps each set)	Tabata Plyos/Burpees (Reps each set)	Walk + Sprint (Total RPE)
Session 1				
Session 2				
Session 3				
Session 4				
Session 5				
Session 6				
Session 7				
Session 8				

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Fatigue thresholds as a marker for transition from aerobic-to--anaerobic exercise intensity in intermittent sport players: an electrophysiological study

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Abstract

Introduction. **In recent years, there have been contradictions regarding the fatigue thresholds denoting the boundary between heavy and severe exercise domain as the marker for transition from aerobic-to-anaerobic exercise intensity.** Aim of Study. **This study aims to examine the various fatigue thresholds (critical power, respiratory compensation point and neuromuscular fatigue threshold) as marker for transition from aerobic-to--anaerobic exercise intensity in intermittent sport players based on the VO_2 kinetics, muscle activations and mathematically derived models.** Material and Methods. **Thirteen male intermittent sport players (age = 21.30 ± 2.52 years; height = 164.76 ± 6.2 cm; weight = 57.03 ± 4.93 kg; BMI = 21.01 ± 1.41 kg/m^2) were recruited for this study. The participants performed total 11 sessions, one incremental test for evaluating electromyographic fatigue threshold (EMGFT), four time-to-exhaustion trials for evaluating critical power with mathematical models (CP) and two verification trials, four constant load tests for evaluating critical power based on VO_2 kinetics (CP') and respiratory compensation point (RCP).** Results. **The Bland–Altman analysis revealed that CP was not in agreement with CP' (r^2 = 0.89, $p < 0.00$; t = 9.70, Cohen's f = 0.99). Also, VO_2 corresponding to the work rates of CP was significantly different from the CP' and RCP ($p < 0.001$). However, work rates corresponding to the CP' were not significantly different and in agreement to the work rates corresponding to RCP (t = –1.65, p = 0.062; r^2 = 0.61, p = 0.66, t = –4.18, Cohen's f = 1.26) and EMGFT (t = –0.633, p = 0.269; r^2 = 0.43, p = 0.342, t = –4.18, Cohen's f = 0.99), respectively.** Conclusions. **The CP', EMGFT and RCP corresponds to similar work rates and can depict the boundary between heavy to severe exercise domains which could be used for performance testing and training the intermittent sport players.**

KEYWORDS: VO_{2max}, exercise domains, critical power, respiratory compensation point, EMG fatigue threshold.

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Introduction

According to the principle of specificity of training, adaptations and performance efficiency are based on exercise intensity or domains, in which the sportsperson is trained [15]. The frequently used classification of the exercise domains is moderate, heavy, severe and extreme domains [23]. The transition of exercise from one domain to the other is highly studied as distinct aerobic and anaerobic thresholds. The transition between the heavy--to-severe domain is denoted as the anaerobic threshold or fatigue threshold, which can distinguish between the "fatiguing" and "non-fatiguing" work rates [8]. In recent years, there have been several debates concerning the transition from heavy-to-severe or aerobic-to--anaerobic exercise, as well as the methods of evaluating this transition. This article aims to explore the anaerobic/fatigue thresholds as markers for testing and training in intermittent sport players because these players spend a significant period of time within the severe-intensity domain, therefore, these anaerobic thresholds become very important when determining the intensity of training protocols and monitoring their improvements.

Critical power is considered as the metabolic steady state widely accepted as a boundary between the heavy and severe exercise domain. Metabolically, critical power represents the limit of the sustainable rate of oxidative metabolism. According to the VO_2 kinetics, it is the highest exercise intensity where exercising VO_2 can be stabilized before reaching VO_{2peak} [8, 12, 25] (denoted as CP' for reference of this article). Based on mathematical models, critical power is explained by the relationship between power output and time to exhaustion. This duration follows a hyperbolic function of output power versus time, where the asymptote represents critical power, whereas the curvature constant (W') corresponds to the finite amount of work that can be consumed on critical power [19, 25]. Critical power is usually evaluated using various mathematical models (denoted as CP, for the purpose of this article) by performing three or more time-to-exhaustion trials [7, 25]. However, whether a true maximal metabolic steady state representing critical power can be evaluated by the mathematical models or not, is still controversial [7]. A recent meta-analysis also suggested that between critical power and other indices of physiological function, critical power (if evaluated correctly) represents a unique work rate as it constitutes the maximal metabolic steady-state, which other ventilatory and metabolic thresholds are unlikely to represent [9]. Studies have found a contradiction that exercising VO_2 might not always attain VO_{2peak} even if the work rate exceeded mathematically evaluated critical power (CP) [3].

Electromyographic fatigue threshold (EMGFT) is the exercise intensity an individual can maintain indefinitely without the need to recruit more motor units and hence depicts the neuromuscular fatigue threshold. EMGFT represents an EMG amplitude without trend, slope, or rate of change [18]. EMGFT has also been proposed and utilized as a reliable correlate of anaerobic and critical thresholds [8, 18]. Respiratory compensation point (RCP) is the maximum workload that can be sustained before metabolic acidosis causes hyperventilation [4, 14]. Some authors propose that RCP resembles the same workload as other markers of the aerobic-to-anaerobic transition. Moreover, in a recent series of investigations Broxterman et al. [4] and Ozkaya et al. [21] reported a high degree of intraindividual variability between CP and RCP and the difference between CP and RCP may question interchangeability of these phenomena [16]. To the best of our knowledge, no other study has ever investigated all the fatigue thresholds jointly on the same group of subjects. The authors aim to investigate and appraise the method to estimate critical power and to examine whether critical power, EMGFT and RCP represent the transition from heavy-to-severe or aerobic-to-anaerobic exercise domains and can be markers for testing and training in intermittent sport players. For this study, the authors hypothesized that RCP and EMGFT would not be statistically different from critical power.

Material and Methods

Participants

Thirteen (age = 21.30 ± 2.52 years; height = 164.76 ± ± 6.2 cm; weight = 57.03 ± 4.93kg; BMI = 21.01 ± ± 1.41 kg/m^2) trained national-level male intermittent sport players (football players, n = 13; hockey players, n = 8) participated in this study, determined according to the power analysis conducted with G*Power 3.1.9.2 for a power of 0.95 and the effect size of 0.8 and an α set at a priori of 0.05. The players had at least 4 years of training with an absence of lower limb injury in the last six months. Exclusion criteria specified individuals who were >18 years old, had a history of health-related concerns (e.g., cardiovascular, pulmonary, metabolic, muscular or coronary, etc.), or a positive physical activity readiness questionnaire (PAR-Q).

Compliance with ethical standards

The study was approved by the Institutional Ethics Committee of the university (details provided). The aims, methodology, and potential risks associated with the study were described to the participants. The participants signed an informed consent form that explained their rights as research participants. The research protocols were carried out according to the Declaration of Helsinki, 1964, its later amendments and also in accordance with Harris et al. [11].

Procedure

Before the maximal bout, the subject's body mass (via balance beam scale) and height (via stadiometer) were recorded. Subjects were fitted for their optimal seat height on the inertially braked cycle ergometer by aligning the seat at the level of the participant's iliac crest. Participants in this study completed 11 sessions at the same time assigned to report to the laboratory (to ensure no interactions with the effect of circadian fluctuations) with at least 24 hours in between.

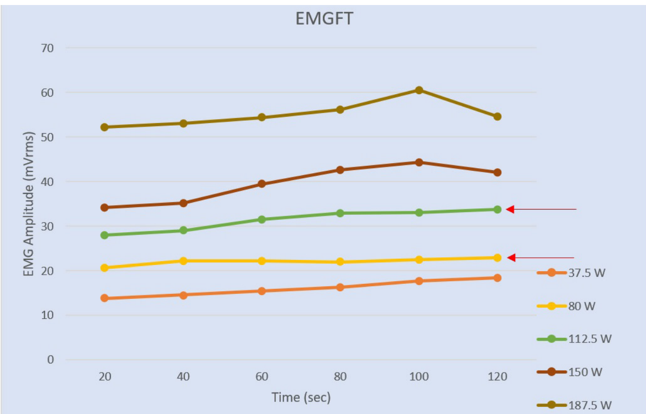
Outcome measures

Incremental tests for RCP and EMGFT

The subjects were instructed to warm up for 15 minutes before the incremental test, and then they were instructed to take a 10-minute break. Pulmonary gas exchange variables [minute ventilation (VE), VO_2, and VCO_2, maximal oxygen consumption (VO_{2max})] were measured breath by breath via an open circuit metabolic system (Powerlab/8M Metabolic System, ADInstruments Pty Ltd, Castle Hill, Australia) using Lab Chart software (version-8, ADInstruments Pty Ltd) sampling at 1000 Hz, and were later analysed as 10-second epochs. Heart rate and rhythm were monitored continuously during all tests via a heart rate monitor (Polar RS800, Polar Electro Oy, Kempele, Finland).

The DelSysTrigno™ Wireless EMG device (Delsys Inc., Boston, USA) was used to detect surface EMG activity through the surface EMG signal during the incremental test of the vastus lateralis muscles of the right lower limb. According to the SENIAM recommendations, Trigno sensors (Trigno Lab System; DelSys Inc., Boston, USA), consisting of two dry bar electrodes spaced 10 mm apart, were positioned in the middle of the muscle belly and aligned in the direction of the muscle fibres. The LabChart software was used to store the EMG signals with a band-pass filter (10–500 Hz), a sampling frequency of 1000 Hz, and also to assess the speed of the cycle ergometer. Each participant's initial power in the incremental test was set at 25 watts (W) with an increment of 25 W every 2 minute until the participant reached volitional exhaustion [10, 14].

Throughout the test, the individual kept a consistent pedal cadence of 70 revolutions per minute (rpm). When the subject failed to sustain a pedalling rate of 60 rpm despite significant verbal encouragement, the incremental test was stopped. The greatest 30-second



Note: mVrms – millivolt root mean square, W – watts, EMGFT – EMG fatigue threshold

Figure 1. Representative results for a participant for EMG fatigue threshold (EMGFT). Linear regression was performed for the EMG amplitude vs time relationship for each power output. The red arrow denotes the first significant result

VO_2 value obtained during the incremental test was considered the VO_{2max}. All the three authors blindly and separately determined RCP by evaluating VE/VCO_2 plotted against VO_2 and located the second breakpoint in the VE to VO_2 relation [12]. The procedure described by Galen et al. [10] was used to analyze recorded EMG signals to determine EMGFT (Figure 1).

Critical power based on mathematical assumptions (CP)

Data from four exhaustive tests lasting 2-10 minutes were applied to calculate CP in widely used equations from the mathematical model using nonlinear total work (Equation 1), linear total work (Equation 2), and linear 1/time (Equation 3) equations. Subjects underwent these four exams on different days in random order. To capture the VO_2 response to the mathematically assessed CP, 2 additional verification sessions (+15 W) were conducted. The termination criteria were identical as for the incremental test.

$$t = W'/(P - CP) \quad \text{(Equation 1)}$$

$$W = W' + (CP \times t) \quad \text{(Equation 2)}$$

$$P = CP + (W' \times \frac{1}{t}) \quad \text{(Equation 3)}$$

Critical power by constant-load tests (CP')

CP' was the physiologically attained work rate corresponding to a slightly lower power output than the lowest work rate giving VO_{2peak}. Four constant-load exercises performed on several days yielded individual power outputs corresponding to CP'. The value of CP' was calculated using the 95% threshold [22], i.e., the lowest work rate, at which the 30-second VO_2 mean data were closer than 95% to VO_{2max}. At +15 W intervals, the individual power outputs associated with CP' were recorded. The termination criteria were the same as for the incremental test.

Statistical analysis

Data are presented as means and standard deviation (SD). To ascertain if the data were normally distributed, the Shapiro–Wilk test was used. One-way repeated measures analysis of variance was performed to evaluate differences across variables and the least significant difference was employed as a post hoc test. Two sample means were compared using a paired-sample t-test. The limits of agreement between CP, CP', RCP, and EMGFT were determined using the Bland–Altman analysis (mean of differences ± 1.96 SD). The one-sample t-test was used to assess bias values of the variables to determine whether they differed substantially from zero ($p < 0.05$). Regression r^2 values were used to analyse the effect size (ES). ES of Cohen's f were categorized as no effect (>0.01), small effect (0.01-0.24), medium effect (0.25--0.39), and large effect (<0.40) [20]. Results with $p < 0.05$ were considered statistically significant. Results were evaluated using SPSS 21.0 (SPSS, Inc., Chicago, IL).

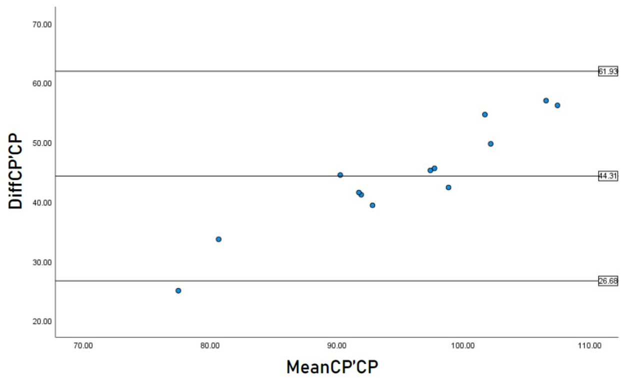
Results

The baseline data of the subjects was found to be normally distributed (Table 1). As a result of repeated measure analysis of variance, the work rates were found to be significantly different from one another ($p < 0.001$). Meanwhile, the interpretation of the paired t-test showed that the work rates corresponding to CP were significantly different from the work rates corresponding to CP' ($t = 17.77$; $p < 0.001$), RCP ($t = –17.113$; $p < 0.001$) and EMGFT ($t = –18.61$; $p < 0.001$). However, work rates corresponding to CP' were not different from the work rates corresponding to RCP ($t = –1.65$; $p = 0.062$) and EMGFT ($t = –0.633$; $p = 0.269$). Also, VO_2 corresponding to the work rates of CP was significantly different from CP' and RCP ($p < 0.001$). Following the Bland–Altman analysis, CP work rates (72.95 ± 4.94 W) were found to be significantly lower than the CP' work rates (117.26 ± ± 13.32 W). Results also indicated that there was low agreement between the work rates corresponding to CP and CP' ($r^2 = 0.89$, $p < 0.001$, $t = 9.70$, Cohen's f = 0.99), RCP (115.63 ± 13.32W; $r^2 = 0.88$, $p < 0.001$, $t = 8.7$, Cohen's f = 2.7) or EMGFT (118.28 ± 12.58W; $r^2 = 0.56$, $p = 0.037$, $t = –1.151$, Cohen's f = 1.12) (Bland–Altman plots are illustrated in Figures 2, 3 and 4, respectively), whereas there was a high agreement between CP' and RCP ($r^2 = 0.61$, $p = 0.66$, $t = –4.18$, Cohen's f = 1.26) and EMGFT ($r^2 = 0.43$, $p = 0.342$, $t = –4.18$, Cohen's f = 0.99) and also between RCP and EMGFT ($r^2 = 0.47$, $p = 0.735$, $t = 9.6$, Cohen's f = 0.86). Limits of agreement and bias values for exercise intensities in watts and results of one sample t-test between CP and the other performance indices are presented in Table 2.

Table 1. Descriptive and baseline data of the athletes

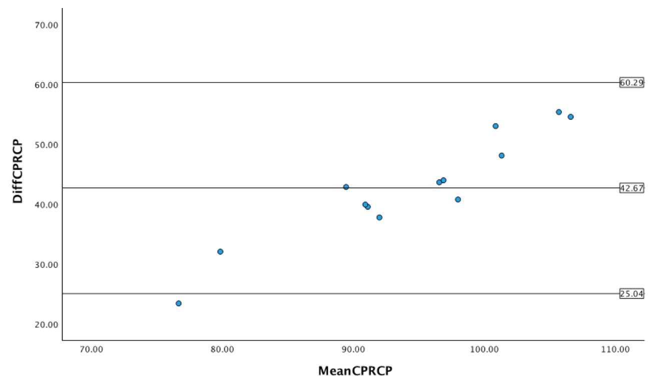
Variable	Mean	± SD
Age (years)	21.30	2.52
Height (cm)	164.76	6.2
Weight (kg)	57.03	4.93
BMI (kg/m)	21.01	1.41
VO_2 resting (ml/kg/min)	3.61	0.23
VO_{2max} (l/min)	3.66	0.27
CP' (W)	117.26	13.32
VO_2 at CP' (l/min)	3.37	0.27
CP (W)	72.95	4.9
VO_2 at CP (l/min)	2.97	0.27
RCP (W)	115.63	13.32
VO_2 at RCP (l/min)	3.31	0.29
EMGFT (W)	118.28	12.58

Note: BMI – body mass index, VO_2 – oxygen consumption, VO_{2max} – maximal oxygen consumption, CP' – critical power based on VO_2 kinetics, CP – critical power based on mathematical models, RCP – respiratory compensation point, EMGFT – EMG fatigue threshold, SD – standard deviation
Data are presented as means and SD.



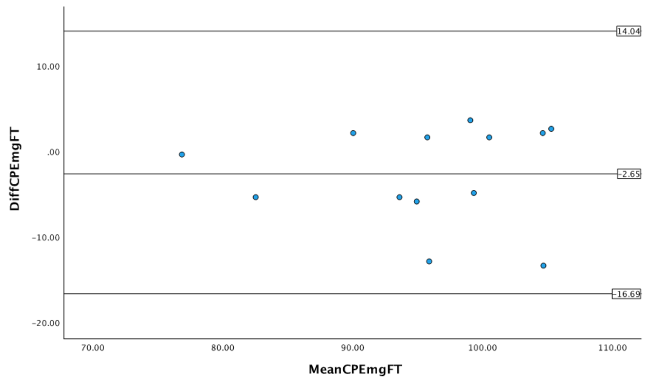
Note: CP – critical power by mathematical models, CP' – critical power by constant load tests, X axis – mean of CP and CP', Y axis – mean difference between CP and CP'

Figure 2. Limits of agreement between CP and CP' (±1.96 SD). The middle solid line represents the mean bias



Note: CP – critical power, RCP – respiratory compensation point, X axis – mean of CP and RCP', Y axis – mean difference between CP and RCP

Figure 3. Limits of agreement between CP and RCP (±1.96 SD). The middle solid line represents the mean bias



Note: CP – critical power, EMGFT – EMG fatigue threshold, X axis – mean of CP and EMGFT, Y axis – mean difference between CP and EMGFT

Figure 4. Limits of agreement between CP and EMGFT (±1.96 SD). The middle solid line represents the mean bias

Table 2. Results of one sample t-test, mean values, mean differences, and SDs of power output values between CP and other performance indices (CP', RCP, EMGFT)

Variable	Mean	±SD	Mean difference	
			Mean	±SD
CP' (W)	95.11	8.99	44.31	8.99
RCP (W)	94.29	8.98	42.67	8.98
EMGFT (W)	95.62	8.49	–2.65	5.80

Note: CP' – critical power based on VO_2 kinetics, CP – critical power based on mathematical models, RCP – respiratory compensation point, EMGFT – EMG fatigue threshold, SD – standard deviation
Data are presented as means, SD and mean differences.

Discussion

This study was an attempt to investigate the fatigue threshold as a marker for transition from aerobic-to--anaerobic exercise intensity in intermittent sport players. Moreover, the authors also aimed to investigate the agreement between various fatigue thresholds (CP, CP', RCP and EMGFT). The crucial point emerging from this study was that CP based on mathematical models underestimates the boundary between heavy and severe exercise domain, in comparison to its physiologically evaluated substitute CP', which could be an answer to the query summarized in a review on critical power by Dotan [7].

Also, this is among the first few studies to directly demonstrate a commonality between these fatigue thresholds in intermittent sport players. Previous studies debated whether or not exercise conducted at or slightly over CP reached VO_{2peak} or not [8, 12]. The results of this study showed that VO_2 could be kept constant during exercises performed above CP (+15 W), but when CP' was exceeded by 15 W, the exercising VO_2 responses immediately increased and reached VO_{2peak} for each subject. Therefore, above CP', metabolic (phosphocreatine, pH) and systemic (VO_2, blood lactate) homeostasis decreased, validating the notion of a "critical metabolic rate" (above which intramuscular phosphocreatine, phosphate ions and pH could not be stabilized) [25, 26].

RCP and CP' were shown to be in accord with one another in our investigation, but not with CP, similarly to the result reported by Ozkaya et al. [21]. The reason behind these changes can be the rapid compensatory reflex increase in ventilation (i.e., RCP) brought on by intensities above the critical metabolic rate, which overwhelms this regulatory system, leading to a near--immediate accumulation of hydrogen ions, an uncoupling of ventilation from carbon dioxide (CO_2) production, and a systematic decrease in end-tidal partial pressure of CO_2. By evacuating CO_2 from the lungs at a speed comparable to its synthesis, the hydrogen ion concentration is related to both metabolic and non-metabolic CO_2 production [24]. Importantly, RCP must happen after the critical metabolic rate, hence in this instance CP' applies. Thus, CP' and RCP may be a superior approach to determine the lower boundary of the severe exercise domain [21]. In the heavy intensity domain, however, there are increases in EMG amplitude. Furthermore, the fatigue-induced increase in muscle activation suggests that the increase in EMG amplitude, which is used to determine EMGFT, is due primarily to increases in motor unit recruitment [5, 18]. Also, RCP and EMGFT corresponded to the similar work rates. Similar to our study, Zuniga et al. [28] and Bergstrom et al. [2] indicated that the work rate corresponding to EMGFT was equal to RCP based on the disassociation of VE from VCO_2. According to Darabi et al. [6] and Bergstrom et al. [2], "the disassociation of VE from VCO_2 may be more closely related to the stimulation of peripheral catecholamine by increased arterial potassium". Indeed, it was previously observed that the change in ventilation associated with RCP was produced by increases in the circulatory concentration of potassium released from the recruitment muscle fibers [17]. It can also be postulated that if the subject can reach an intensity of RCP, then EMGFT should occur, which is also supported by a study done by Mäestu et al. [17]. CP' was also in accord with EMGFT, which was a novel finding to this study, moreover, this can be a response to the increasing rate of hyperkalemia [21]. EMGFT reflects the recruitment of additional motor units, increased firing rates, and/or synchronization [1]. The result of this study supports the previous finding that EMGFT can be an alternative to open-circuit spirometry to detect an aerobic to anaerobic transition in athletes on a cycle ergometer [13]. Even if some scientists theorize that CP best depicts the upper limit boundary of heavy exercise domain exercise, the result of this study can support the findings of Ozkaya et al. [21] that there is a significant gap between heavy and severe exercise domains, which was referred to as the “grey zone”.

The limitation of this study was being a sex-biased study. Future studies can focus on evaluating these trends in female athletes. Moreover, in this study the sample was taken from the intermittent sports players only, therefore the results will only be applicable to these players, hence lacking generalizability to all the sports players. Future studies should analyse estimation of various fatigue thresholds in different populations.

Conclusions

Critical power can truly be estimated physiologically via VO_2 kinetics (CP’), rather than being mathematically derived (CP). Therefore, CP’ can help in evaluating performance efficiency and also helps monitor the effect of training programs especially for interval training protocols and team sports such as association football, rugby and hockey. As RCP and EMGFT agree with CP’, therefore CP’, RCP, and EMGFT can be considered as the boundary between aerobic and anaerobic work rates or heavy and severe exercise domains. CP’ can be used as a surrogate to EMGFT and RCP and vice versa, therefore, evaluating either one of them can give an overview of exercise tolerance and fatigue resistance of the intermittent sport players. CP’ should be evaluated individually of all athletes to have the best-fit prescription for the high or severe intensity exercise in athletes. However, if critical power is evaluated using mathematical models, then the possibility of the grey zone between heavy and severe domains should be considered during testing and training.

Conflict of Interest

The authors declare no conflict of interest.

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1. Up to six authors: Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002 Jul 25;347(4):284-287.
2. More than six authors – list the first six authors followed by et al.: Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-46.
3. Article published electronically ahead of the print version: Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002 Nov 15;100(10):3828-3831. Epub 2002 Jul 5.

Book citation

References to books should include surname(s) of the author(s) with first name initials (list the first six authors followed by et al.), title, number of an edition (not required for the first edition), city and name of the Publishing House and year of publication.

1. Gardiner PF. Advanced neuromuscular exercise physiology. Champaign: Human Kinetics; 2011.

Book chapter

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1. Renson R. Sport Historiography in Belgium. Status and Perspectives. In: Renson R, Lämmer M, Riordan J, editors. Practising Sport History. Sankt Augustin: Akademia Verlag Richarz; 1987. pp. 1-18.

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Abbreviations and symbols

Use only standard abbreviations and symbols. The expansion of an abbreviation should precede its first use

Table 1. Descriptive statistics and comparative analysis of maximal oxygen uptake (VO_2max in ml/kg·min^{-1}) between genotypes of the I/D *UCP2* gene polymorphism

UCP2	DD					ID					II				
Sex	*N*	$\bar{x}$	*SD*	*Min*	*Max*	*N*	$\bar{x}$	*SD*	*Min*	*Max*	*N*	$\bar{x}$	*SD*	*Min*	*Max*
F	42	45.65	*6.14*	32.30	59.00	36	45.66	*7.18*	30.60	59.80	7	45.07	*7.60*	35.00	54.80
M	72	54.01[a]	*6.20*	40.30	79.00	70	55.60	*7.32*	42.30	76.80	12	59.07[a]	*9.04*	49.70	74.90

in the text and be repeated in the legend under a figure or a table in which the abbreviation is used.
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