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Circadian variability of bioelectric muscle tone in static and dynamic anaerobic exercises in men

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Purpose: Physiological processes in the body are characterized by a 24-hour circadian rhythm. The circadian variability of physiological processes affects exercise capacity. The aim of the study was to determine the circadian variability of bioelectric muscle tone in static and dynamic exercises with the use of surface electromyography (sEMG), which allows for the assessment of neuromuscular activity and muscle function during physical activity. *Methods*: The research sample consisted of 16 men aged 21.6 ± 0.62 years, with intermediate chronotypes, who were not professional athletes. The tests were conducted at 2:00, 10:00, 18:00 and 22:00 and included measurements of bioelectric tension (sEMG) of the vastus lateralis muscle of the right and left limbs during static exercise with maximum voluntary isometric contraction (MVC) and during dynamic exercise, such as the Jumping test – vertical jump with arm swing (CMJ) and without arm swing (ACMJ), as well as during a 10-second cycle ergometer test. The tests were repeated after 24 hours. *Results*: The circadian periodization of biopotential has a varied course with a noticeable decline in values at night time. The level of bioelectric tension recorded during static exercise did not show significant circadian variability. However, in dynamic exercises, significant variability ($p < 0.05$) in bioelectric tension (sEMG) of the vastus lateralis muscle of the left limb during the Jumping test with arm swing (CMJ) was observed between 22:00 and 18:00 in the first series of tests. A tendency to achieve higher average amplitude values was observed at 10:00 in both limbs, with the lowest values observed at 2:00. After 24 hours, the highest bioelectric activity was observed in both limbs at 10:00 during the jump with arm swing (CMJ), while the lowest values in the left limb were observed at 2:00. During the jump without arm swing (ACMJ), the lowest level of bioelectric activity of the tested muscle in the right limb was observed at 2:00. In the 10-second anaerobic cycle ergometer test, significant variability ($p < 0.05$) in bioelectric tension of the muscle in the left limb was shown between 18:00 (highest result) and 2:00 (lowest result). After 24 hours, the measurement values were generally lower compared to the results of identical measurements from the first series of tests. *Conclusions*: the level of bioelectric tension in the studied muscles during anaerobic exercises at different times of the day varies depending on the time and type of workout. The highest sEMG biopotential amplitude values were recorded in the evening and afternoon, while the lowest values were recorded at night time, at 2:00.

Key words: *circadian variability, sEMG, electromyography, static and dynamic exercises, men*

1. Introduction

In the human body, many vital processes occur rhythmically. Among the various biological rhythms, the best known are circadian rhythms (circa – about, dies – day), generated by endogenous biological clocks. The circadian rhythm is an innate mechanism, which regulates many physiological processes in the human body. Numerous physiological parameters oscillate within this cycle, such as body temperature, hormone secretion, changes in the cardiovascular system and tonic activity of the autonomic nervous system: the activity of the sympathetic part, which activates the body, increases during the day and decreases at night, while the parasympathetic part, which has an inhibitory effect, is more

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active during sleep. The primary synchronizer of the circadian rhythm and its simultaneous stimulus is the "day-night" cycle, which dictates the phases of activity and rest (wake-sleep). The specific structures of the central nervous system are the regulators of circadian rhythms in physiological, biochemical and behavioral processes: suprachiasmatic nuclei (SCN) located bilaterally in the anterior part of the hypothalamus, above the optic chiasm, on both sides of the third ventricle of the brain. The primary biochemical coordinator of the biological rhythm is melatonin, synthesized in a circadian rhythm dependent on the lighting conditions and produced by the endogenous biological clock. Melatonin production is the highest during night time. The circadian rhythm is characterized with the 24-hour sleep–wake rhythm, heart rate, blood pressure, body temperature changes (fluctuations of up to 0.5 °C) and variations in blood hormone concentrations such as ACTH, adrenaline, cortisol, growth hormone, prolactin, melatonin, testosterone, thyroid-stimulating hormone, luteinizing hormone, aldosterone, insulin, renin and triiodothyronine. It also affects the digestive system (hydrochloric acid secretion, liver function), the cardiovascular system (morphological blood components such as leukocyte count, hemoglobin concentration, clotting), the immune system (neutralizing bacterial toxins), drug metabolism, DNA synthesis, psychomotor efficiency and autonomic nervous system activity. A noticeable increase in body activity is observed between 10.00 and 12.00, and 16.00 and 18.00 hours. Body temperature also undergoes circadian changes, rising at around 15.00 and between 21.00 and 23.00 hours, and reaching the lowest values between 5.00 and 6.00, with an increase occurring about three hours before waking up. The rhythm of internal organ function depends on the activity and rest phases and changes accordingly [3], [17].

Numerous scientific papers discuss the impact of the circadian rhythm on human exercise capacity, which has led to a significant interest in this subject among researchers worldwide. Studying circadian rhythms is particularly important in case of athletes since the time of day when exercise is performed affects various components of physical performance. Identifying the time of day when the body's functional efficiency is at its peak is vital for sports physiology. This knowledge allows for optimal training schedules to maximize an athlete's exercise capacity and effectively influence their performance outcomes. Additionally, coachers and athletes' knowledge and understanding of how to modify the biological rhythm enable better preparation for exercise under unfavorable conditions, such as changes in sleep patterns due to long-distance travel and time zone shifts. The proper training, which is planned well in advance and aims at modifying the circadian rhythm, can prepare the body for new time zone, minimize the negative effects of jet lag, and, therefore, positively impact an athlete's performance. The influence of the circadian rhythm on the functioning of human body has been of interest to researchers globally for many years, with ongoing scientific literature continually reporting new findings. There are available studies on bioelectric muscle activity measurements during static or dynamic efforts, often conducted only during the day, either in the morning or afternoon. However, there are only few studies that describe such measurements being taken at several times throughout the day or night time. Also, no studies have been found that simultaneously address the variability of bioelectric muscle tension during static and dynamic efforts at different times of the day (day and night) and after a 24-hour recovery period. Many current studies focus on circadian changes in muscle strength and power, core body temperature, or hormonal responses to physical exertion in athletes [11], [12], [17]–[19], [25], [36]. Some researchers, such as Abedelmalek et al. [1], in their studies on circadian variations in core body temperature and muscle strength and power, also highlight daily differences in the measured muscle strength among individuals of different races. It is justified to thoroughly investigate the circadian rhythm and its influence on functional efficiency and the body's exercise capacity. Among various methods applied in the measurement of functional efficiency and physical performance, the non-invasive method of surface electromyography (sEMG) is gaining increasing interest. sEMG involves the detection, recording and interpreting of the electric activity of muscles resulting from the physiological changes taking place in the sarcolemma. As a needle-free technique, it allows for non-invasive and painless detection, recording, and analysis of myoelectric signals from the skin's surface. It allows for the objective, qualitative assessment of neuromuscular activity during postural changes, functional movements, dynamic and static exercise, as well as for the evaluation of muscle efficiency during exertion. The electromyogram is characterized by amplitude and frequency. It is believed that the electromyogram amplitude, measured in microvolts [μV], depends on the number of muscle fibers engaged in muscle action. The discharge frequency [Hz] measures the synchronization of motor unit excitation [4], [8]. The scientific literature documents the use of electromyography to study bioelectric muscle tension and motor unit recruitment changes in muscles. Electromyography has been used to develop innovative methods for determining the anaerobic threshold, the topic that has been investigated since the 1980s. The issue of determining the anaerobic threshold using electromyography remains relevant among researchers who have confirmed the effectiveness and non-invasiveness of the method [5], [23], [40], [41].

Studying changes in muscle bioelectrical tension during static and dynamic exercises constitutes an interesting aspect of using surface electromyography (sEMG) in sports physiology. Studies on the daily variability of muscle bioelectrical tension with the use of electromyography were usually performed at two chosen hours, typically at 6:00 and 18:00. They showed that the highest values of strength and muscle power were achieved in the afternoon [2], [27], [28]. Castaingts et al. [6] demonstrated the variability of bioelectrical tension of the triceps surae muscle during static and dynamic exercises at two times of the day (6:00 and 18:00) under various experimental conditions (electrically induced contractions, contraction during natural movement, submaximal voluntary isometric contraction, jump). In turn, Sedliak et al. [33] did not show significant variability in the biopotential recordings when they measured the strength and bioelectrical tension of the quadriceps muscle during maximum isometric contraction (MVC) with the knee extension among two study groups – the morning (7:00–9:00) and the afternoon ones (17:00–19:00). In another experiment, they analyzed these parameters during maximum (MVC) and submaximal (MVC40) isometric contraction at four times of the day: 7:00–8:00, 12:00–13:00, 17:00–18:00, 20:30–21:30 [34]. The results published by Pereira et al. [28] concerned the muscle strength along with the recordings of muscle bioelectrical tension during maximum isometric contraction (MVC) of the vastus lateralis and rectus femoris muscles performed at three times of the day: 7:30–9:30, 13:30–15:30 and 19:30–21:30. Their studies did not show circadian periodicity in the recording of muscle bioelectrical activity, although they observed significant differences in muscle strength and internal body temperature values, with the highest values achieved at 19:30 and the lowest at 7:30. Tamm et al. [39] studied the circadian variability of motor unit activity of the triceps surae muscle among individuals with morning and evening chronotypes. Biopotential measurements during maximum isometric contraction (MVC) were performed at 9:00, 13:00, 17:00 and 21:00, and their results suggest the influence of the subjects' chronotype on the daily changes in the analyzed activities. Chtourou et al. [9] did not observe significant changes in the bioelectrical activity of the vastus lateralis and vastus medialis muscles between 7:00 and 17:00 during the 30-second Wingate test. In the light of various research findings, it can be concluded that there is still a deficit of scientific information on the shaping of skeletal muscle bioelectrical tension during intense workout at different times of the day. Therefore, an attempt was made to assess changes in muscle bioelectrical tension (sEMG) in static conditions and during short-term dynamic workouts at different times of the day (also at night) and after a 24-hour restitution phase.

An attempt was made to determine the circadian variability of muscle bioelectrical tension during anaerobic static and dynamic exercises performed at 2:00, 10:00, 18:00, 22:00, and after a 24-hour restitution period.

2. Materials and methods

Study group

Out of a group of 50 volunteers, 16 non-training men aged 21.6 ± 0.62 years, with similar weekly physical activity, were qualified for the study. The chronotype of the subjects was determined based on the Polish version of the Morningness-Eveningness Questionnaire (MEQ) by Horne and Ostberg. The selected group carried out a self-assessment of their chronotype by answering 19 questions in the questionnaire, including preferences for rest time, wake up time, morning mood, and the preferred time to start work. Men with an intermediate chronotype "between night owl and early bird" were selected to participate in the exercise tests so as to eliminate the influence of extreme types on the achieved results. The selection of 16 individuals was based on preliminary studies, including measurements of biometric body indicators BH [cm] and BM [kg] and structural nutritional status indicators (PF, LBM, BMI). The selected men had balanced levels of morphological body structure indicators, i.e., BH: 180.2 cm ± 6.20 cm, BM: 75.3 kg ± 6.42 kg, PF: 9.45% ± 4.23, LBM: 68.9 ± 4.02 kg, BMI: 20.86 ± 2.16. The qualification procedure also required them to obtain appropriate results from their medical examinations, such as blood pressure measurement (BP), heart rate (HR), and ECG in order to participate in exercise tests. The selected group conducted a qualitative-quantitative assessment of their diet using the "Dietary records" method. For seven days, the men kept their "food diaries" in which they recorded all consumed products, dishes, and liquids in household measures. The "Album with photographs of selected products and dishes" by Szponar et al., 2000 was used to describe the consumed products. Using the tables containing data on the nutritional value of basic products and typical dishes by Kunachowicz et al., 2019, the energy value of the daily food ration (2590 ± 198.02 kcal) and the contribution of macronutrients: proteins (15.5%), fats (35.5%), and carbohydrates (49%) to the daily energy value were calculated using computer software. The candidates' nutrition, non-use of pharmacological agents; drugs or supplements, adherence to a similar diet, and non-smoking were considered. Participants were informed about the research program and consented to participate in the study. The research project was approved by the Bioethics Committee at the District Medical Chamber in Kraków. A test assessing the level of aerobic fitness of the body (graded test to refusal on a bicycle ergometer) was conducted. The initial load was set at 90 W, and the pedaling frequency at 70 revolutions per minute. Every 3 minutes, the load was increased by another 30 W, and the exercise continued until the subject could no longer maintain the tempo set by the metronome. During the exertion, respiratory exchange ratios were recorded, i.e., minute oxygen uptake (VO_2), minute carbon dioxide output (VCO_2), percentage oxygen content in exhaled air (FEO_2), percentage carbon dioxide content in exhaled air ($FECO_2$), minute lung ventilation (VE), respiratory quotient (RQ), respiratory equivalent for CO_2 ($VE \cdot VCO_2^{-1}$), respiratory equivalent for O_2 ($VE \cdot VO_2^{-1}$), breathing frequency (FR) and tidal volume (TV). The heart rate (HR) was recorded, and blood pressure (BP) was measured. The oxygen fitness index VO_2max was 3.58 ± 0.41 [$l \cdot min^{-1}$]. The selected group participated in the tests, which were preceded by a warm-up to adapt to the conditions under which the physiological testing and determination of physical fitness level were carried out. The people taking part in the experiment had a daily sleep duration of 7–9 hours and during the research they participated in the same number of hours of physical activity resulting from the study plan. The subjects did not undertake any additional physical activity individually during the experiment.

The study began at designated times according to a pre-established schedule: 2:00, 10:00, 18:00, and 22:00. The research group was randomly divided into four teams (Team A, Team B, Team C, Team D), with each team starting the first series of tests at a different time. The exercise tests began with a 5-minute warm-up on a bicycle ergometer at an intensity of 50% VO_2max with a set frequency of 60 revolutions per minute and maximum acceleration at the 2nd, 4th, and 5th minutes. Two minutes after completing the warm-up, the subjects proceeded to physiological tests in the following order:

1. Static Test on a standardized biomechanical test-bench. During the workouts, bioelectrical muscle tension (sEMG) of the vastus lateralis muscle of the right and left thigh was measured. The measurements were taken during the contraction in the knee joint under isometric conditions. To determine the circadian changes in muscle bioelectrical tension, the amplitude values of the recorded action potentials (sEMG) of the knee extensor muscle vastus lateralis of the right and left limbs during maximal voluntary contraction (MVC) were calculated.
2. After a 5-minute break, the subjects proceeded to the jumping test with the recording of muscle bioelectrical tension (sEMG) – the normalized EMG signal within a 0.2 s time interval during the rebound phase from the right and left limbs. Each subject performed two types of vertical jumps from a standing position twice, trying to fully engage the lower limb muscles during the rebound phase and achieve the maximum jump height. The jumping test included the following jumps: Counter Movement Jump (CMJ) – a squat and jump upwards from a standing position with an arm swing. Akimbo Counter Movement Jump (ACMJ) – a jump upwards from a standing position without an arm swing (the subject holds a stick on their shoulders).
3. The third stage involved performing a 10s-time anaerobic workout on a cycloergometer with the recording of the bioelectrical tension of the studied muscle of the right and left limbs (normalized EMG signal within a 10s time interval during the test). Each subject aimed to reach the maximum pedaling rate in the shortest possible time and maintain it for as long as possible. The flywheel resistance force was set at 8.3% of body mass (BM).
4. Repetition of the tests after 24 hours (second series of tests). The subjects performed the same tests again at the same time as before, in the same order (as in the first series of tests).

After completing the full first cycle, which consisted of tests conducted in two series at the same time, a two-week break followed. After this break, each subject began the second cycle, starting at the next designated test time. After another two-week rest, the third cycle began (starting from the next chosen hour), and after a two-week break again, the final fourth cycle of tests was conducted (tests at the last chosen measurement time). The research scheme is presented in Fig. 1.

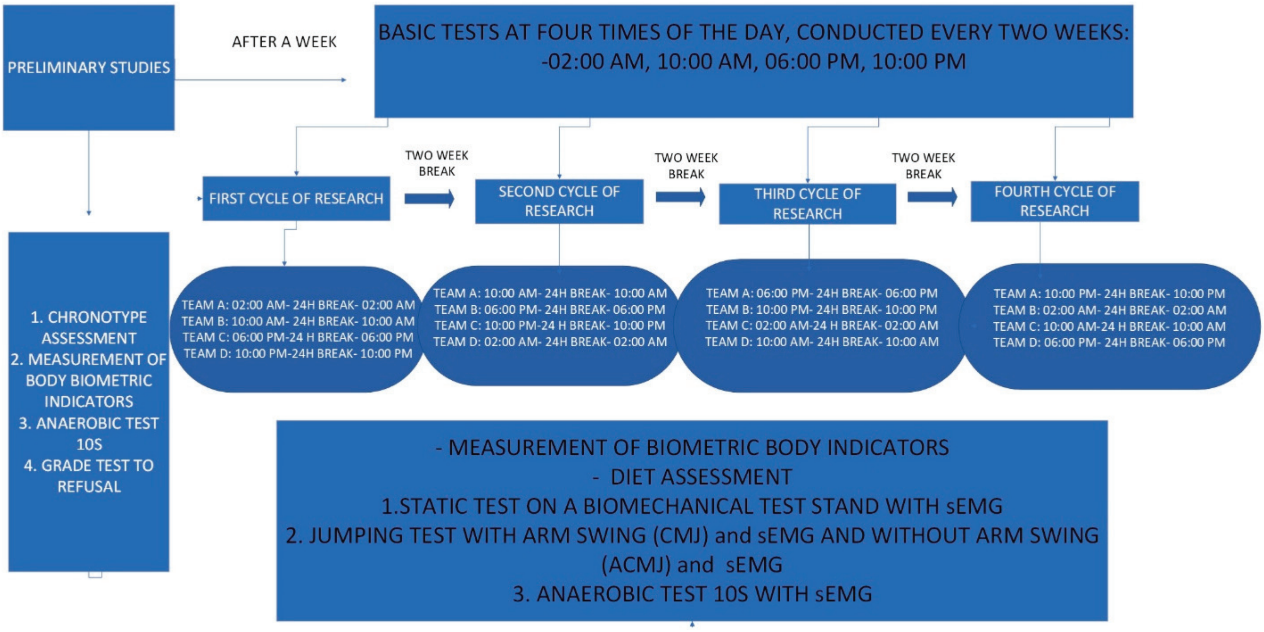

Fig. 1. Research scheme

The preparation for the measurement of the sEMG action potential involved marking the location for attaching the surface electrode over the vastus lateralis muscle belly of the thigh to ensure accurate measurement and repeatability of electrode placement in subsequent tests. During all the tests, bioelectrical activity of the studied muscle (sEMG) was recorded with the use of an 8-channel portable electromyograph from Mega Electronics, with Lead-LOK surface electrodes, size 43 mm, type R-LFO-510, attached to the skin over the muscle belly and MegaWin computer software. The measurements were conducted according to the SENIAM recommendations (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles). The assessment of recorded changes in electrical activity of the vastus lateralis during static workout was based on calculating the average amplitude value [μV] of the normalized EMG signal within a 0.5 s time frame from reaching the maximum muscle tension. The amplitude was defined as the maximum deviation of the potential from the "0" level. After a 5-minute break, the subjects proceeded to perform the Jumping test with the recording of muscle bio electrical tension – normalized EMG signal within a 0.2 s time frame during the rebound phase. Precisely recorded take-off and landing times allowed the determination of the flight duration and its height. The OptoJump optical data acquisition system consists of two instruments in the form of crossbars – 100 cm × 3 cm × 4 cm, which are laid on the ground and responsible for data acquisition, control, and their electronic transmission during the jump. The third stage of the study involved a 10-second anaerobic exercise on a Monark 876E mechanical cycloergometer, along with the recording of bioelectrical tension of the studied muscle of the right and left limbs (normalized EMG signal within a 10 s time frame during the test). The tests at each of the four times of the day were repeated in the same order after 24 hours.

The research results were subjected to statistical analysis. The analysis was based on the calculation of standard statistical data (means and standard deviations) and, additionally, standard errors for detailed analyses. Statistical significance was assessed using a three-factor analysis of variance with repeated measures (repeated measures ANOVA) according to the following schedule: Time (4 times) × Measurement (2 measurements) × Limb (2, left and right). The detailed analyses were carried out with the use of contrast analysis (planned comparisons). For general analyses, where each factor was analyzed with the control of other factors, standard deviations were calculated from standard errors. For the factor "Time", the sphericity assumption was checked each time using the Mauchly's test. In case of its disturbance, the Greenhouse-Geisser correction analysis with degrees of freedom was planned.

3. Results

The results of muscle bioelectrical tension, i.e., sEMG amplitude of knee extensors under static conditions during maximal isometric contraction (MVC), showed statistically insignificant variability throughout the day. In the first series of tests, the sEMG measurement at 22:00 was the highest, while at 2:00 it was the lowest in the left limb. In the second series of tests, the lowest results were observed at 2:00 in the right limb. The 22:00 measurement in the second series (after 24 hours) showed a significantly lower sEMG amplitude in the right limb compared to the measurement at this time in the first series (Table 1).

The results of muscle bioelectrical tension (sEMG) under dynamic conditions in the Jumping Test with arm swing (CMJ) and without arm swing (ACMJ). Significant variability ($p < 0.05$) was observed in the amplitude of the sEMG action potential [μV] from the left limb (L) in the jump with arm swing (CMJ) between 18:00 and 22:00, where the result at 18:00 (149.9 ± 59.37 μV) was the lowest, and at 22:00 (185.7 ± 65.08 μV) it was the highest (Table 2). Other results were not significantly different, but in many cases, higher amplitude values were observed at 10:00. In the second series, the mean amplitude values in the left limb at 22:00 in the CMJ jump were significantly lower compared to the measurement at this time in the first series (Table 2).

Table 1. sEMG amplitude values [μV] of the right (R) and left (L) limbs and their sum (R + L) at the studied times of day (Mean, SD) under static conditions during maximal isometric contraction (MVC)

	Mean and standard deviation ($\bar{x}$, SD)			
	Hour	R sEMG [μV]	L sEMG [μV]	Sum R + L sEMG [μV]
First series of tests	2:00	1122.7 ± 170.64	1091.4 ± 199.59	1107.0 ± 157.33
	10:00	1146.9 ± 146.11	1122.9 ± 126.01	1134.9 ± 118.59
	18:00	1089.4 ± 153.71	1121.4 ± 153.85	1105.4 ± 143.04
	22:00	1179.4 ± 185.16	1138.9 ± 143.76	1159.2 ± 99.62
Second series of tests	2:00	1065.2 ± 133.87	1078.0 ± 170.13	1071.6 ± 130.10
	10:00	1096.8 ± 170.19	1074.7 ± 138.57	1085.7 ± 136.25
	18:00	1095.9 ± 185.78	1118.5 ± 213.11	1107.3 ± 193.08
	22:00	1095.3 ± 161.46	1094.0 ± 113.32	1094.6 ± 98.24
The first and second series of tests combined	2:00	1093.9 ± 146.60	1093.9 ± 179.89	1089.3 ± 138.90
	10:00	1121.8 ± 149.95	1121.8 ± 120.27	1110.3 ± 118.27
	18:00	1092.7 ± 164.83	1092.7 ± 172.16	1106.3 ± 160.94
	22:00	1137.3 ± 171.11	1137.3 ± 101.94	1126.9 ± 90.09

Table 2. Amplitude values of sEMG [μV] of the right (R) and left (L) limbs and their sum (R + L) under dynamic conditions in the jumping test with arm swing (CMJ) at different times of day (Mean, SD)

	Mean and standard deviation ($\bar{x}$, SD)			
	Hour	R sEMG [μV]	L sEMG [μV]	Sum R + L sEMG [μV]
First series of tests	2:00	164.4 ± 45.76	180.2 ± 59.28	172.3 ± 51.18
	10:00	183.7 ± 36.71	176.7 ± 50.85	180.2 ± 28.96
	18:00	164.0 ± 36.62	149.9* ± 59.37	156.9 ± 44.41
	22:00	152.4 ± 43.21	185.7* ± 65.08	169.0 ± 49.31
Second series of tests	2:00	151.3 ± 45.36	155.3 ± 52.90	153.3 ± 38.92
	10:00	178.5 ± 40.32	176.2 ± 49.58	177.3 ± 31.89
	18:00	165.4 ± 48.17	170.1 ± 57.62	167.7 ± 50.96
	22:00	143.8 ± 61.36	164.2 ± 54.01	154.0 ± 52.65
The first and second series of tests combined	2:00	157.9 ± 42.78	167.7 ± 53.07	162.8 ± 44.34
	10:00	181.1 ± 33.41	176.5 ± 46.92	178.8 ± 27.20
	18:00	164.6 ± 37.56	160.0 ± 55.80	162.3 ± 45.45
	22:00	148.0 ± 49.75	174.9 ± 58.87	161.5 ± 50.11

* – Statistically significant differences at the level ($p < 0.05$) between the values of the indicator at four different times of the day.

Table 3. Amplitude values of sEMG [μV] of the right (R) and left (L) limbs and their sum (R + L) under dynamic conditions in the jumping test without arm swing (ACMJ) at different times of day (Mean, SD)

	Mean and standard deviation ($\bar{x}$, SD)			
	Hour	R sEMG [μV]	L sEMG [μV]	Sum R+L sEMG [μV]
First series of tests	2:00	163.7 ± 49.29	209.9 ± 64.36	186.8 ± 48.06
	10:00	200.9 ± 56.12	201.2 ± 33.58	201.1 ± 30.27
	18:00	186.8 ± 40.86	199.1 ± 70.60	192.9 ± 54.75
	22:00	189.5 ± 57.14	209.5 ± 83.31	199.5 ± 66.21
Second series of tests	2:00	168.0 ± 65.38	203.2 ± 65.31	185.6 ± 45.62
	10:00	182.5 ± 42.83	204.5 ± 48.40	193.5 ± 38.44
	18:00	180.2 ± 49.18	193.2 ± 83.24	186.7 ± 64.85
	22:00	177.3 ± 59.18	200.1 ± 76.59	188.7 ± 63.73
The first and second series of tests combined	2:00	165.8 ± 56.09	206.5 ± 58.33	186.2 ± 44.58
	10:00	191.6 ± 47.43	202.8 ± 37.95	197.2 ± 32.38
	18:00	183.5 ± 39.40	196.1 ± 74.38	189.8 ± 56.21
	22:00	183.4 ± 51.91	204.8 ± 73.72	194.1 ± 60.52

Table 4. Amplitude values of sEMG [μV] of the right (R) and left (L) limbs and their sum (R + L) in the 10-second anaerobic test at different times of dDay (Mean, SD)

	Mean and standard deviation ($\bar{x}$, SD)			
	Hour	R sEMG [μV]	L sEMG [μV]	Sum R + L sEMG [μV]
First series of tests	2:00	3995.7 ± 559.90	4201.7 ± 1060.81	4098.7 ± 580.37
	10:00	4130.0 ± 865.69	4280.0 ± 603.39	4205.0 ± 552.46
	18:00	4519.7 ± 853.80	4220.4 ± 648.39	4370.1 ± 631.70
	22:00	3817.4 ± 1015.27	3820.1 ± 1334.99	3818.7 ± 1063.37
Second series of tests	2:00	3979.1 ± 965.56	3606.2* ± 823.77	3792.6 ± 851.87
	10:00	4254.3 ± 885.66	4330.8 ± 867.39	4292.6 ± 527.40
	18:00	4169.2 ± 762.96	4425.2* ± 797.50	4297.2 ± 714.33
	22:00	3655.2 ± 613.98	4134.1 ± 1182.55	3894.6 ± 853.58
The first and second series of tests combined	2:00	3987.4 ± 676.42	3904.0 ± 850.19	3945.7 ± 681.81
	10:00	4192.1 ± 850.29	4305.4 ± 644.38	4248.8 ± 484.99
	18:00	4344.5 ± 640.17	4322.8 ± 645.51	4333.6 ± 612.54
	22:00	3736.3 ± 781.95	3977.1 ± 1187.44	3856.7 ± 913.63

* – Statistically significant differences at the level ($p < 0.05$) between the values of the indicator at four different times of the day.

The measurements of the bioelectrical muscle tension during the jump without arm swing (ACMJ) did not show significant daily variation (Table 3).

The results of muscle bioelectrical activity (sEMG) in a short anaerobic test on a cycle ergometer. The sEMG results during the 10-second workout on the cycle ergometer indicated statistically significant variability in the second series of tests for the left limb between 2:00 (lowest result) and 18:00 (highest result). The sEMG activity measurements in the first series of tests did not show statistically significant differences, but the highest values were observed at 18:00 (right limb). The measurement results are presented in Table 4.

4. Discussion

The daily variability of the body's various responses to physical exercise is a topic of interest for sports physiologists. The biological clock, circadian rhythms and their impact on physical exercise has particular applications in sports sciences and exercise physiology, which is reflected in the new concept of chrono-exercise – a discipline that aims to maintain and improve people's health by paying attention to "when" people exercise during the day [24]. Current research examines two important aspects: the effect of exercise on the circadian rhythm and the relationship between exercise and circadian disruption and related diseases. Long-term circadian disorders not only have a negative impact on the body's adaptation to physical exercise, but are closely related to the onset and progression of various mental and physical diseases, including cardiovascular diseases, metabolic syndrome, neurodegenerative diseases and cancer [36]. New studies often focus on the effects of morning versus evening resistance training on adaptations in skeletal muscle hypertrophy and muscle strength [15]. Nevertheless, many aspects regarding the influence of circadian rhythms on exercise capacity are not yet sufficiently known.

The influence of circadian rhythms on the formation of muscle bioelectrical activity during anaerobic exercises has not been thoroughly studied yet. Scientific literature reports daily fluctuations in muscle electrical activity, which were analyzed with the use of surface electromyography (sEMG), but the results of various studies were inconclusive or even contradictory [2], [7], [10], [12]–[14], [16], [21]–[23], [26], [27], [30], [31], [33], [34]. The application of surface electromyography in physiological research is justified due to the non-invasiveness of the method (electrodes are attached to the skin) compared to invasive needle methods. Moreover, surface electromyography is considered more reliable for recording muscle functional changes over a longer period of time[22]. Surface electromyography is a reliable and frequently used technique for studying muscle function in kinesiology, exercise physiology, and biomechanics [2], [5], [6], [8], [9], [13], [14], [21], [22], [27], [31], [32], [37], [38], [42]–[44]. There have been studies in which conducted static sEMG recordings, examining the efficiency of wrist extensor muscle units depending on the time of day and the gender of the subjects. The highest amplitude values were observed between 11:00 and 13:00, and it was noted that the variability in muscle activity correlates with changes in body temperature (surface temperature measured above the muscle) during the studied time intervals [22]. Similar circadian fluctuations were observed in both women and men, with slightly higher values in men [22]. However, the results of some authors suggest that muscle bioelectrical activity is not dependent on the time of day and does not undergo circadian changes. Pereira et al. [28] did not confirm the daily periodicity in the recording of motor unit activity of the vastus lateralis and rectus femoris muscles, despite observing significant differences in strength and internal body temperature values, with the highest values achieved at 19:30 and the lowest at 7:30 [28]. Sedliak et al. [33] did not observe significant variations in muscle bioelectrical activity recordings at different times of the day during maximum voluntary isometric contraction (MVC) with the knee joint extension. The results of the study did not show significant variability in the biopotential recordings at different times of the day, but statistically significant differences were observed in the maximum strength and power [33]. Tamm et al. [39] suggest that the influence of circadian rhythms on muscle performance depends on the type of chronotype represented by the research group. They observed daily differences in motor unit activity of the triceps surae muscle among individuals with morning and evening chronotypes, with sEMG measurements showing daily variations in amplitude only in the evening chronotype group. In this study, a group of men with an intermediate chronotype was selected for research to eliminate this factor that could influence the results achieved at chosen times of the day. Our own observations regarding static exertion performed in the first and second series of tests did not show statistically significant circadian differences in the level of electromyographic activity of the vastus lateralis muscle. These observations are consistent with the results of the studies by Pereira et al. [28] and Sedliak et al. [33], who did not observe daily fluctuations in the amplitude level of bioelectrical potential.

However, it is worth noting that our measurement results indicated a tendency to achieve the lowest amplitude values in the left limb at night at 2:00, which is confirmed by other studies showing that the lowest muscle bioelectrical activity occurs at night or early in the morning. The measurements of muscle bioelectrical activity during maximum voluntary isometric contraction (MVC) did not show statistically significant differences, but higher activity was observed at 22:00, and the lowest at night, i.e., 2:00. The highest values in this test were recorded at 22:00 (left limb, first series of tests), and the lowest values at 2:00 (right limb, second series of tests). This suggests that changes in muscle bioelectrical activity may exhibit circadian variability. The comparison of both series of our own tests showed that after 24 hours, statistically significant ($p < 0.05$) lower values of muscle bioelectrical potential amplitude were recorded at 22:00 in the right limb compared to measurements in the first series of the tests.

Latest study Hirono et al. [21] aimed to investigate the time-of-day effects on neural excitability and muscle contractile properties by assessing the firing properties of tracked motor units and electrically evoked twitch muscle contraction. Neuromuscular function was measured in the morning (10:00), at noon (13:30), in the evening (17:00), and at night (20:30). The measurements consisted of maximal voluntary contraction (MVC) strength of knee extension, recording of high-density surface electromyography (HDsEMG) from the vastus lateralis during ramp-up contraction to 50% of MVC, evoked twitch torque of knee extensors by electrical stimulation. Findings suggest that neural excitability may be affected by the time of day, but it did not accompany changes in peripheral contractile properties in a diurnal manner. In another study Croskery et al. [12], during strength training, with the use of electromyography, evaluated the reliability and exercise relationship of SEMG amplitudes of the biceps brachii (BB) during the concentration, dumbbell, hammer, and incline resistance arm curl exercises. SEMG amplitude shows high heterogeneity between individuals, reliability for resistance training exercise generally demonstrates favourable results. It is important to note that the synergistic muscles involved contribute significantly to movement and can influence the activation of target muscles. Therefore, the influence of SEMG amplitude on training adaptation should be investigated.

The available literature provides conflicting information regarding the diurnal variability of bioelectrical muscle tension during dynamic efforts. Chtourou et al. [9] investigated the muscle electrical activity, muscle power output, and muscle fatigue of the vastus lateralis and vastus medialis muscles at two different times of the day, namely 7:00 and 17:00, during a 30-second Wingate test. They did not observe significant changes in the bioelectrical muscle activity during the analyzed hours. Similarly, the results of Zarrouk et al. [45] indicated a lack of diurnal variability in the bioelectrical activity of the vastus lateralis, rectus femoris, vastus medialis, and biceps femoris muscles during a cycling test conducted at two selected hours, namely 6:00 and 18:00. On the other hand, studies on changes in the neuromuscular function during the Wingate test in boys [38], showed differences in the parameters of muscle bioelectrical activity in the vastus lateralis, rectus femoris, and vastus medialis muscles at the level of mean power frequency (MPF) and neuromuscular efficiency (NME), where higher values were observed at 17:00 compared to 7:00. No significant differences were observed for the average frequencies (root-mean-square RMS) between the two measurement times [38]. Nicolas et al. [27] attempted to determine the diurnal periodization of bioelectrical tension in the vastus lateralis muscle during dynamic effort. The results indicated significantly higher bioelectrical activity in the muscle during the afternoon tests (18:00) compared to the morning ones (6:00), confirmed the correlations between muscle motor unit activity, increased oxygen consumption, and the onset of the anaerobic threshold during cycling exercise [23]. Tyka et al. [40], [41] observed correlations between the level of bioelectrical activity of the quadriceps femoris muscle, vastus lateralis, and rectus femoris, and the time to reach the anaerobic threshold during incremental exercise both at neutral (23 °C) and elevated (31 °C, 37 °C) ambient temperatures. Renziehausen at al. [32] wanted to determine the effects of time of day on performance during a maximal effort sprinting assessment (30 nmt) and determine potential differences based on chronotype and sex at different times of day: 9:00, 14:00 and 19:00. Research concerned differences in peak/mean power, peak/mean velocity, distance, resting heart rate, temperature, and kcals at each time point. There was a significant main effect for temperature, resting heart rate, and pre-exercise caloric intake throughout the day. No significant main effects for time were found for peak power, mean power, peak velocity, mean velocity, or distance. Significant differences were shown between the peak and nadir of each performance variable. The goal of one recent study Porta at al. [30] was to investigate the relationship between speed and myoelectric activity, measured during an incremental 25 m shuttle running test. Myoelectrical activities of the gluteus, hamstrings, and quadriceps muscles were recorded. The speed of each player during testing was measured using GPS technology, sampling at 50 Hz. This study indicates that GPS and sEMG are valid and consistent in estimating external load and internal load during incremental shuttle running.

The obtained results regarding the level of bioelectrical tension (sEMG) of the vastus lateralis muscle during short-term dynamic anaerobic exercises were not conclusive in determining the time of greatest muscle activity. A varied course of bioelectrical muscle tension was demonstrated depending on the time and type of exercise during which measurements were taken. Personal observations conducted in the first series of Jumping tests showed significant variability ($p < 0.05$) in the amplitude of the action potential of the left vastus lateralis muscle during a countermovement jump (CMJ) between 22:00 and 18:00, where the amplitude value at 22:00 was the highest. No significant diurnal variation was observed during other measurement hours or in the jump without a countermovement (ACMJ). There was a trend of achieving the highest amplitude values during both jumps (CMJ, ACMJ) at 10:00 in the right limb. The results of the second series of tests (after 24 hours) generally showed lower levels compared to the first series, but the diurnal variability in the analyzed hours was similar to the first series of tests. This may have been due to micro-injuries to skeletal muscles that occurred during the efforts in the first series of tests. After rigorous anaerobic exercises for 24 to even 72 hours, increased concentrations of certain muscle damage markers are observed, although it should be noted that markers of muscle damage were not investigated. No significant differences were observed in the level of bioelectrical activity, but it was observed that, on average, the highest values of the characteristic bioelectrical amplitude were at 10:00, and the lowest at 2:00. The greatest changes in bioelectrical activity (sEMG) of the vastus lateralis muscle may indicate a greater involvement of fast-twitch motor neurons IIa and IIx during dynamic effort. The measurement results for the bioelectrical activity of the vastus lateralis muscle during a 10-second anaerobic effort on a cycle ergometer in the first series of tests were not statistically significantly differentiated. However, the highest values were observed at 18:00 in the right limb. However, in the second series of tests, the measurement results showed significant variation between the highest values at 18:00 and the lowest at 2:00 in the left limb.

The presented results have limitations in conclusions resulting from the relatively small sample size (16 people), which was largely determined by the extensive methodology of the experiment. In post-hoc tests, statistically significant differences were noted: between 22:00 and 18:00 in the first series of Jumping tests (CMJ) and between 18:00 and 2:00 in the left limb during anaerobic effort on a cycle ergometer in the second series of tests.

The obtained research results may have applications in planning and implementing sports training, especially for athletes preparing to compete in different time zones.

5. Conclusions

1. The level of bioelectrical muscle tension exhibits variability throughout the day.
2. The level of bioelectrical muscle tension during anaerobic efforts at the analyzed times of day shows a diverse pattern depending on the measurement time and type of exercise.
3. A definitive peak time of electrical activity in the tested muscles was not clearly identified. However, the highest sEMG amplitude values were recorded during the day and evening time, while the lowest values were observed at night, around 2:00.
4. The sEMG results for tests conducted after 24 hours were generally lower compared to the initial series of tests but the circadian variability remained similar to the initial observations.

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