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Effect of Bruce and Wingate Protocols on PGC-1α in Well-Trained Endurance Athletes

İyi Antrenmanlı Dayanıklılık Sporcularında Bruce ve Wingate Protokollerinin PGC-1α Üzerine Etkisi

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Abstract: PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha) is a transcriptional coactivator that plays a key role in exercise-induced metabolic adaptations. The purpose of this study is to compare serum PGC-1α levels before and after aerobic and anaerobic power tests in well-trained endurance athletes. Twenty-five healthy male cross-country skiers with an average age of 20.56 ± 2.27 years, who had at least three years of endurance training experience and trained regularly at least 5 days a week, were included in the study using cluster sampling. Two different test protocols were applied to the participants 48 hours apart: the Wingate anaerobic power test and the Bruce treadmill protocol. The Wingate test was used to assess anaerobic capacity, while the Bruce protocol was used to evaluate maximal aerobic capacity. Blood samples were taken at three time points: before each test (in the morning in a fasting state), 2 hours after, and 48 hours after, and serum PGC-1α levels were analyzed using the ELISA method. A mixed design ANOVA was used to examine the time-dependent changes within each protocol, while a one-way ANOVA was used for inter-protocol comparisons. Both test protocols produced significant time-dependent increases in serum PGC-1α levels (Bruce: $p < .001$, $\eta^2 = .456$; Wingate: $p = .020$, $\eta^2 = .953$). Statistically significant differences were found between the pre- and post-tests in both protocols ($p < .05$). In inter-protocol comparison, significant differences were only observed in pre-test values ($p = .05$). The average PGC-1α levels (ng/mL) were as follows: Bruce – pre-test: 4.949 ± 0.807, 2 hours: 5.739 ± 1.050, 48 hours: 6.343 ± 1.001; Wingate – pre-test: 3.984 ± 1.037, 2 hours: 4.622 ± 0.782, 48 hours: 5.254 ± 0.747.
Both aerobic and anaerobic exercise protocols lead to significant increases in serum PGC-1α levels. However, the Bruce treadmill protocol appears to elicit this increase more strongly. This suggests that aerobic exercise may be more effective in enhancing transcriptional activity related to mitochondrial biogenesis.

Keywords: PGC-1α, aerobic power, anaerobic power, mitochondrial biogenesis, endurance athletes.

Özet: PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha), egzersize bağlı metabolik adaptasyonlarda kilit rol oynayan bir transkripsiyonel koaktivördür. Bu çalışmanın amacı, iyi antrene edilmiş dayanıklılık sporcularında aerobik ve anaerobik güç testleri öncesi ve sonrası serum PGC-1α düzeylerini karşılaştırmaktır.
Yöntem: En az 3 yıllık dayanıklılık antrenman geçmişine sahip ve haftada en az 5 gün düzenli olarak antrenman yapan, yaş ortalaması 20.56 ± 2.27 yıl olan 25 sağlıklı erkek kayak kros sporcusu, küme örnekleme yöntemiyle çalışmaya dahil edilmiştir. Katılımcılara 48 saat arayla iki farklı test protokolü uygulanmıştır: Wingate anaerobik güç testi ve Bruce treadmill protokolü. Wingate testi anaerobik kapasiteyi, Bruce protokolü ise maksimal aerobik kapasiteyi değerlendirmek amacıyla uygulanmıştır. Her test öncesi (sabah açlık durumunda), 2 saat sonrası ve 48 saat sonrası olmak üzere üç zaman noktasında kan örnekleri alınarak serum PGC-1α düzeyleri ELISA yöntemiyle analiz edilmiştir.
İstatistiksel Analiz: Her bir protokolde zamana bağlı değişimi incelemek amacıyla tekrarlı ölçümlerde karma desen ANOVA analizi, protokoller arası karşılaştırmalar için ise tek yönlü ANOVA uygulanmıştır.
Bulgular: Her iki test protokolü de serum PGC-1α düzeylerinde zamana bağlı anlamlı artışlar oluşturmuştur (Bruce: $p< .001$, $\eta^2 = .456$; Wingate: $p = .020$, $\eta^2 = .953$). Her iki protokolde de ön-ara ve ön-son testler arasında istatistiksel olarak anlamlı fark tespit edilmiştir ($p< .05$). Protokoller arası karşılaştırmada sadece test öncesi değerlerde anlamlı farklılık saptanmıştır ($p = .05$). Ortalama PGC-1α düzeyleri (ng/mL) şu şekildedir: Bruce – ön test: 4.949 ± 0.807, 2. saat: 5.739 ± 1.050, 48. saat: 6.343 ± 1.001; Wingate – ön test: 3.984 ± 1.037, 2. saat: 4.622 ± 0.782, 48. saat: 5.254 ± 0.747.
Sonuç: Hem aerobik hem de anaerobik egzersiz protokolleri serum PGC-1α düzeylerinde anlamlı artışa yol açmaktadır. Ancak Bruce treadmill protokolünün bu artışı daha güçlü şekilde tetiklediği görülmektedir. Bu durum, aerobik egzersizin mitokondriyal biyogenezle ilişkili transkripsiyonel aktiviteyi artırmada daha etkili olabileceğini göstermektedir.

Anahtar Kelimeler: *PGC-1α, aerobik güç, anaerobik güç, mitokondriyal biyogenez, dayanıklılık sporcuları.*

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INTRODUCTION

Physical exercise is considered a stimulus that brings about multilayered and complex effects on many physiological systems of the organism. Exercise is known, especially at the level of skeletal muscle, to trigger adaptations at the metabolic, biochemical, and genetic levels. These adaptations not only enhance the athlete's performance but also provide an important biological infrastructure for the prevention and management of clinical issues such as aging, metabolic diseases, and chronic inflammatory conditions (Egan & Zierath, 2013; Joyner & Coyle, 2008). Among these exercise-specific cellular responses are mitochondrial biogenesis, reprogramming of energy metabolism, and the enhancement of oxidative capacity. One of the molecular keys to these processes is PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha), which plays a critical role in the regulation of energy homeostasis and muscle adaptations (Handschin & Spiegelman, 2006; Kupr & Handschin, 2015).

PGC-1α, as a transcriptional coactivator, does not bind directly to DNA but coordinates the expression of mitochondrial genes by interacting with many transcription factors (Puigserver & Spiegelman, 2003). Located on chromosome 4 in the human genome, this gene is highly expressed in tissues rich in mitochondria, such as skeletal muscle, heart tissue, brain, and brown adipose tissue (Mootha et al., 2003; Rowe et al., 2013). The main biological functions of PGC-1α include mitochondrial biogenesis, activation of oxidative metabolism, muscle fiber type transformation (switching from fast-twitch to slow-twitch fibers), enhancement of antioxidant defense, and maintenance of energy balance (Handschin & Spiegelman, 2008; Scarpulla, 2011). This molecule also plays a role in the regulation of cellular redox status and inflammation, thereby providing protective functions against metabolic and neurodegenerative diseases (Agudelo et al., 2019; Cartoni et al., 2005).

PGC-1α expression during exercise significantly increases, particularly due to the activation of intracellular signaling cascades resulting from muscle contractions. The increased AMP/ATP ratio, Ca^{2+} levels, and reactive oxygen species (ROS) during muscle contractions lead to the activation of various signaling pathways, primarily AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (p38 MAPK), and calcium/calmodulin-dependent protein kinase (CaMKII) (Jäger et al., 2007; Akimoto et al., 2005). These signaling pathways stimulate transcription factors located in the promoter region of the PGC-1α gene, increasing mRNA synthesis and subsequently triggering

mitochondrial biogenesis (Little et al., 2011; Scarpulla et al., 2012). As a result, muscle cells enhance their oxidative capacity to meet the increased energy demand, thereby improving endurance performance (Baar, 2006). Moreover, the increase of PGC-1α as an adaptive response to exercise is critically important in correcting the energy imbalance involved in the pathophysiology of metabolic diseases and in modulating inflammatory processes (Fernandez-Marcos & Auwerx, 2011; Handschin, 2016).

The effect of exercise on PGC-1α varies depending on the type, intensity, duration, and frequency of the exercise. Long-duration low-to moderate-intensity aerobic exercises significantly increase mitochondrial biogenesis and PGC-1α expression (Granata et al., 2016; Lanza & Nair, 2009). At the same time, high-intensity interval training (HIIT) protocols, which are short but intense, can also activate PGC-1α by triggering alternative signaling pathways (Gibala et al., 2012; Perry et al., 2010). High-intensity tests that measure anaerobic power and capacity, such as the Wingate test, activate glycolytic metabolism in muscles, leading to the stimulation of different molecular mechanisms (MacInnis & Gibala, 2017). These differences are important for understanding how the type of exercise shapes PGC-1α-mediated metabolic adaptations.

Studies examining the temporal dynamics of PGC-1α expression show significant increases in PGC-1α mRNA levels within 3-6 hours post-exercise, but the rise in protein levels peaks at a later time, around 24-48 hours (Camera et al., 2010; Granata et al., 2016). Therefore, monitoring short- and long-term molecular responses to exercise is critically important for a comprehensive assessment of muscle adaptations. Additionally, this data provides a basis for developing practical recommendations regarding the timing of post-exercise recovery and nutritional strategies.

In the exercise science literature, examining PGC-1α responses using test protocols targeting different energy systems holds significant importance. The Bruce treadmill test is a commonly used protocol for measuring maximal aerobic capacity (VO_2max) and is an exercise type where oxidative metabolism and mitochondrial function are predominantly engaged (Bruce et al., 1973). On the other hand, the Wingate anaerobic test measures anaerobic power and capacity through a 30-second maximum intensity sprint exercise (Bar-Or, 1987). Comparing the effects of both tests on PGC-1α can provide important information for understanding molecular adaptations of energy systems and can shed light on the individualization of training programs (Little et al., 2010; Roca-Rivada et al., 2013).

In recent years, findings are increasing that PGC-1α plays a role not only in muscle metabolism but also in systemic inflammation, neurodegeneration, immune response, and microbiota homeostasis. Agudelo et al. (2019) noted that the activation of PGC-1α is a central molecule in exercise-induced neuroprotective effects. This makes PGC-1α a critical target not only in sports physiology but also in the context of holistic health and disease management.

Looking at the theoretical framework regarding the variation of PGC-1α according to exercise types and their reasons; PGC-1α is a co-active protein that plays a central role in cellular energy metabolism, particularly regulating mitochondrial biogenesis and oxidative metabolism (Puigserver & Wende, 2004). Different types of exercise influence the expression of this gene in various ways, leading to metabolic adaptations. In aerobic exercises, the expression of PGC-1α is elevated to increase oxidative phosphorylation and mitochondrial density. Long-duration exercises increase energy demand and accelerate the transcription of PGC-1α to enhance energy production capacity in cells (Egan & Zierath, 2013). This promotes mitochondrial biogenesis, enhances endurance, and provides metabolic flexibility. In anaerobic or resistance exercises, there may also be an increase in PGC-1α levels to optimize muscle adaptation and glucose metabolism. However, this increase may be more limited or exhibit a different regularity compared to aerobic exercises (Laefer et al., 2012). The increased energy demand during exercise triggers the activation of kinases such as AMP-activated protein kinase (AMPK) and mitogen-activated protein kinases (MAPK). These kinases increase the transcription and activation of PGC-1α (Russell et al., 2003). In aerobic exercises, especially with the foreground focus on the AMPK pathway, PGC-1α expression increases significantly. The type of exercise activates different signaling pathways and affects muscle fiber types (type I and II) and metabolic pathways, leading to different regulations of PGC-1α (Holloszy & Booth, 1996). Factors such as warm-up and mechanical stress also facilitate the activation of p53 and other transcription factors, guiding the expression of PGC-1α, particularly in resistance exercises (Gleyzer & Ardilouze, 2014). In summary, while aerobic exercises generally trigger an increase in PGC-1α, anaerobic exercises can also modulate these molecular adaptations but through different mechanisms and signaling pathways. The fundamental reason for these differences lies in the energy demands of exercise, muscle type, and metabolic effects. Therefore, regulation of PGC-1α is an evolutionary adaptation to meet cellular energy demand, dependent on the type and intensity of exercise.

This study aims to compare the changes in serum PGC-1α levels before and after two different exercise protocols with varying metabolic demands; the Bruce maximal aerobic capacity test and the Wingate anaerobic power test, in well-trained elite male endurance athletes (2 hours and 48 hours post-exercise). The hypothesis of the study is that the Bruce protocol, targeting maximal aerobic capacity, will result in a greater and sustained increase in serum PGC-1α levels compared to the short-term anaerobic loading-based Wingate test. This assumption is based on the more comprehensive and lasting effects of aerobic exercises on mitochondrial biogenesis and energy metabolism (Little et al., 2011; Egan & Zierath, 2013).

METHODS

Publication Ethics: This study was approved by the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University on March 20, 2020, with approval number 03/02. Additionally, necessary permissions were obtained from the Turkish Ski Federation Presidency on December 28, 2020, with number 50715. Prior to the study, the purpose of the research, the exercise protocols to be implemented, the blood sampling processes, and safety precautions were verbally communicated to the participants in detail. It was emphasized that participation in the study would be voluntary, and after the information process was completed, participants signed the "Informed Consent Form." Participants' medical histories were inquired in detail, and it was confirmed that they had no chronic diseases, regular medication use, smoking habits, cardiovascular disorders, or hormone treatments (Mostafidi et al., 2016). Throughout the study, the rights and freedoms related to the processing, storage, and protection of participants' personal data under the Personal Data Protection Law (KVKK) were explained to them, and the related information text was read aloud and approved by the participants. Furthermore, in accordance with the Helsinki Declaration, adopted in 1964 and updated in 2024, the

ethical principles of medical research conducted on human subjects were explained to the participants, and they signed the relevant consent form.

Participants: In this study, the sample group was determined using cluster sampling. Since the sample size and characteristics were fixed according to the specific requirements of the research, power analysis (G-Power) was not applied. The study included 25 male athletes who had been participating in endurance training for at least three years and trained at least five days a week as part of the Turkish cross-country ski national team. To ensure homogeneity in performance and environmental factors, participants remained in an intensive training camp in Erzurum for the duration of the study, where they followed the same nutrition plan and training protocols. This approach aimed to minimize variability in the study conditions, thereby increasing the reliability of the biochemical and physiological data obtained. The sample group comprises elite-level athletes, including those with experience in Olympic and international championships. Due to the insufficient number of female cross-country skiers, only male athletes were included in the study to eliminate the impact of gender as a factor in the analysis. The health status of the participants was evaluated in detail prior to the study and assessed according to exclusion criteria. These criteria included acute infectious diseases, regular medication use, smoking, sleep disorders, irregular dietary habits, existing injury status, voluntary withdrawal from the study, chronic or acute health issues, and any physical risk factors. Athletes who did not meet these criteria were excluded from the study, and it was confirmed that all participants were fit to complete the protocol from a health perspective.

Participants' anthropometric and physiological characteristics were measured using standard methods. Age information was obtained directly from participants, height was measured with a stadiometer, and body weight was precisely determined using a digital scale. Maximum heart rate (HRmax) was calculated using the Karvonen method, and maximal oxygen consumption (VO_2max) was determined in a laboratory setting using the Bruce treadmill test protocol. The obtained average values are as follows: age 20.56 ± 2.27 years, height 172.56 ± 10.11 cm, body mass 64.37 ± 10.62 kg, HRmax 145.82 ± 9.16 bpm, and VO_2max 68.72 ± 8.18 mL/kg/min. These values indicate that the participants are elite athletes with a high level of endurance capacity. This comprehensive and homogeneous sample selection has provided the opportunity to examine exercise-induced biochemical adaptations in a healthy and reliable manner, enhancing the internal validity of the results.

Table 1. Standard nutrition program during the camp

	Details
Nutrition Program	
- Daily Caloric Intake	3000–3500 kcal
- Macronutrient Distribution	Carbohydrates: 55-60% Protein: 15-20% Fat: 20-25%
- Number of Meals	5 meals (3 main meals + 2 snacks)
- Fluid Intake	Minimum of 3 liters of water daily
- Special Nutritional Supplements	Vitamin D, Omega-3, Magnesium (under the supervision of a sports dietitian)
- Nutritional Timing	Light carbohydrate-rich meal 2 hours before training; rapid protein intake after training
- Prohibited Foods	Processed foods, high-sugar and fatty snacks, excessive caffeine and alcohol

Table 2. Detailed weekly training schedule for the camp

Day	Training Type	Content and Duration	Sets x Reps/Duration	Intensity/Goal	Purpose
Monday	Endurance Run	90 minutes, low-moderate intensity (70-75% HRmax) long-distance run	Duration-based	%70-75 HRmax	Increase aerobic capacity, fat burning
Monday	Technical Training	Cross-country skiing technical practice (turns, balance, sliding)	3 sets x 15 minutes	Moderate intensity	Technical development, movement quality
Tuesday	Strength Training	Weight training (squats, deadlifts, core exercises)	4 sets x 8-12 repetitions (per exercise)	Moderate-high intensity	Developing muscle strength and endurance
Tuesday	Flexibility and Mobility	Dynamic stretching and mobility exercises	3 sets x 30-45 seconds (per area)	Low intensity	Muscle elasticity, injury prevention
Wednesday	Interval Endurance Run	10 x 3-minute tempo runs, with 2 minutes of active rest in between	10 sets	%85-90 HRmax	VO2max gelişimi, yüksek yoğunluk dayanıklılığı
Wednesday	Technical & Speed Training	Short sprints and speed-focused technical training	6 sets x 30 seconds sprint + 5 minutes rest	High intensity	Anaerobic power, fast movement coordination
Thursday	Active Rest	Light swimming or cycling	45 minutes	%50-60 HRmax	Recovery, low-intensity aerobic
Thursday	Regeneration and Massage	Professional massage and stretching	30 minutes	Passive	Muscle recovery, reducing injury risk
Friday	Endurance Running	Tempo running	60 minutes	%75-80 HRmax	Improving lactate threshold
Friday	Strength Endurance	Bodyweight exercises (push-ups, planks, burpees)	3 sets x 20 repetitions (per exercise)	Moderate intensity	Increasing muscle endurance
Saturday	Long-term Endurance	Low-intensity running or skiing	120 minutes	%65-70 HRmax	Enhancing aerobic base endurance
Saturday	Technical Training	Incline and balance exercises	3 sets x 10 minutes	Moderate intensity	Technical coordination, balance control
Sunday	Complete Rest	-	-	-	Physical and mental recovery

Experimental Procedure and Test Implementation Process: Athletes participating in the study were asked to avoid the consumption of caffeine, dietary supplements, and other ergogenic aids, as well as to refrain from intense physical activities for at least 48 hours before the tests began. This practice aims to prevent external influences on biochemical and molecular parameters post-exercise and to enhance the reliability of the measurements (Amaro-Gahete et al., 2019; Thompson et al., 2021). During this process, participants were thoroughly informed about compliance with dietary and activity restrictions, and pre-test inquiries were conducted to identify any instances of non-compliance. Throughout the study, participants engaged in two different exercise tests with a minimum 48-hour interval between tests. By randomizing the order of the tests, any potential order effects (carry-over effects) were minimized, thereby increasing the validity of the measurements (Smith & Jones, 2020). This approach allowed for an objective assessment of the independent effects of exercise protocols on the participants.

On test days, participants were ensured to have restorative sleep for 6-8 hours overnight, and fasting basal blood samples were collected at 09:00. After blood sampling, participants were provided with a standard breakfast, and to ensure the standardization of pre-test hydration, they consumed 150-200 mL of fluid and voided their bladder (Kovacs et al., 2022). These preparatory steps are crucial for minimizing variations in biochemical measurements and standardizing the physiological condition.

In the first test session, anaerobic performance was evaluated using a cycle ergometer (Monark 894E). Participants performed a controlled warm-up lasting 5 minutes at 60 rpm under a fixed load of 75 g/kg. Following the warm-up, they underwent sprint pedaling tests in three sets, each lasting 30 seconds, to maximize anaerobic power capacity. A 4-minute rest period was provided between sets to allow for partial recovery of the muscles and maintenance of performance. This protocol is a widely accepted anaerobic power assessment method with high test-retest reliability (Akgül et al., 2016; Barbosa et al., 2021). Throughout the tests, experienced staff continuously monitored the participants for motivation and the standardization of technical execution.

In the second test session, the Bruce treadmill test was applied to determine maximal aerobic capacity. The test began at a speed of 2.7 km/h with a 10% incline, progressively increasing both speed and incline every 3 minutes. This incremental increase is designed to assess the limits of oxygen consumption capacity. Participants continued the test until they reached their endurance limits (Bruce et al., 1973). During the test, parameters such as heart rate and subjective fatigue were monitored. The total test duration was converted to VO_2max using the formula developed by Foster et al. (1984):

$$VO_2max = 14.76 - (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)$$ (T: test duration, in minutes).

Here, T represents the total time spent on the treadmill (in minutes). The Bruce protocol is considered the gold standard for measuring maximal aerobic capacity and allows for reliable assessment of cardiorespiratory performance (Myers et al., 2017). Both test protocols are reliable and valid methods that specifically evaluate the different components of energy systems and the physical capacities of participants. Furthermore, the appropriateness of these tests for monitoring the temporal changes of post-exercise biochemical parameters provides a strong experimental basis for analyzing molecular adaptations within the scope of the study (Egan & Zierath, 2013; Gibala et al., 2012).

Wingate Anaerobic Power Test Procedure: The study employed the Wingate Anaerobic Power Test to assess anaerobic capacity. This test is designed as a short-duration, high-intensity cycle ergometer test to measure the maximum anaerobic power of participants (Bar-Or, 1987; Inbar et al., 1996). Before the test, participants conducted a 5-minute low-intensity warm-up followed by a 3-minute rest period. A Monark 894E cycle ergometer was used during the test, and the resistance was set at 7.5% of the participant's body weight (0.075 kg/kg) (Dotan & Bar-Or, 1983). Participants aimed to maintain the highest possible pedaling speed for 30 seconds, targeting maximum power output. During the test, pedal revolutions per minute (rpm) and power output (Watts) were recorded automatically. A 4-minute active rest was provided between test repetitions. Maximum power, average power, and fatigue index were calculated from the obtained data to assess anaerobic power and endurance (Beneke, 2011; Zajac et al., 2015). The Wingate test was preferred due to its reliable and valid measurement of high-intensity anaerobic performance in athletes (Pyne et al., 2018). The results of the test were used to objectively determine the anaerobic power capacities of participants and compare their biochemical responses against different exercise protocols.

Bruce Treadmill Test Protocol: To evaluate aerobic capacity, participants underwent the Bruce treadmill test, a progressively increasing speed and incline exercise test widely used for estimating maximal oxygen consumption capacity (VO_2max) (Bruce et al., 1973). The test began at a speed of 2.7 km/h with a 10% incline and continued with incremental increases every 3 minutes. This progressive increase raises the oxygen demand of the muscles, allowing participants to reach their maximal exercise tolerance. The test continues until terminated by participant request or medical indication (Myers et al., 2017).

In the Bruce protocol, the total test duration is used for VO_2max estimation, calculated using the following formula for men (Foster et al., 1984):

$$VO_2max = 14.76 - (1.379 \times T) + (0.451 \times T^2) - (0.012 \times T^3)$$ (T: test duration, in minutes).

Here, T represents the total duration on the treadmill (in minutes). Throughout the test, heart rate, subjective fatigue level (Borg scale), and other vital parameters were monitored. The Bruce test demonstrates high reliability and validity for assessing endurance performance, as well as cardiovascular health (Balady et al., 2010; Howley et al., 1995).

Sample Collection and Biochemical Analysis Protocol: In this study, a standardized and meticulous sample collection

and biochemical analysis protocol was implemented to determine PGC-1α levels before and after aerobic and anaerobic power tests. Participants were instructed to avoid stimulants, supplements, ergogenic aids, and excessive physical activity at least 48 hours prior to the tests. This precaution aligns with the gold standard approaches recommended in the literature aimed at minimizing external influences on metabolic and hormonal parameters to enhance the validity of biochemical measurements (Amaro-Gahete et al., 2019; Gibala et al., 2012). Blood samples were collected at 09:00 in a fasting state from the antecubital vein of participants in a seated position by experienced and certified healthcare personnel using aseptic techniques. Stabilizing posture is critical for controlling hemodynamic changes, thereby reducing biological variability related to sampling (Kushner et al., 2018). At 11:00, corresponding to the start time of the tests, participants began to implement the designated exercise protocols. To monitor the acute and delayed effects of exercise, follow-up blood samples were taken 2 hours and 48 hours post-exercise, considering the individual test completion times of participants. These time intervals were found suitable for revealing the temporal profile of expression dynamics of proteins associated with mitochondrial biogenesis and energy metabolism, such as PGC-1α (Peake et al., 2017; Granata et al., 2016). Early transcriptional activation is expected immediately post-exercise, while increases at the protein level are anticipated within the subsequent 48 hours (Egan & Zierath, 2013). The blood samples were transported under cold chain conditions (4°C) over a distance of 165-177 km from the test site to the medical faculty biochemistry laboratory, adhering to appropriate temperature and transport standards. Upon arrival at the laboratory, the samples were centrifuged without delay to separate serum and plasma fractions, which were stored at +4°C. The quality of the samples was checked with a hemolysis test, and hemolyzed samples were excluded from the study, as this practice is among the standard procedures to prevent pre-analytical errors in biochemical analyses (Chen et al., 2007; McArdle et al., 2001). The ELISA (Enzyme-Linked Immunosorbent Assay) method was chosen for the quantitative measurement of PGC-1α protein levels, providing high specificity and sensitivity. The study used the Elabscience brand, human PPARγC1α specific ELISA kit (E-EL-H1359). The kit is manufactured in the USA, with production taking place in the People's Republic of China. Measurements were performed according to the protocols specified by the manufacturer, involving two dilutions and three technical repetitions. Epoch Biotek ELISA readers and Biotek ELx50TM automatic strip washer devices were used for the analyses, enhancing measurement accuracy through precise optical measurements and standardized washing processes (Lee et al., 2021; Zhang et al., 2022). The technical specifications of the kit report a detection range of 0.156 to 10 ng/mL and an analytical sensitivity of <0.057 ng/mL. A high correlation was observed with $R^2 \geq 0.99$ within the linear working range, and intra-assay and inter-assay variation coefficients remained within the ideal biochemical analytical reliability limits at 9.07% and 9.99%, respectively (Lee et al., 2021). These values indicate that the study achieved high repeatability and consistency in biomarker measurements. The sampling and analysis protocol applied is conducive to revealing the temporal changes in post-exercise PGC-1α levels with high precision, establishing a reliable basis for monitoring molecular-level exercise adaptations (Agudelo et al., 2019; Handschin & Spiegelman, 2006).



Figure 1. Microplate ELISA equipment

Data Analysis: The data were analyzed using the IBM SPSS (Statistical Package for the Social Sciences) version 30.0. The Shapiro-Wilk test was performed to determine the distribution of the data, and descriptive statistics were used to identify the means of the variables. Repeated Measures Mixed-Design ANOVA was conducted to compare serum PGC-1α levels across pre-, mid-, and post-tests for the Bruce and Wingate protocols, allowing for the assessment of temporal effects (this analysis was chosen because more than two tests were conducted within a single group). The Bonferroni correction was applied for Type I error control. For the three repeated tests, a significance level of $p < 0.016$ was accepted. To monitor changes in PGC-1α levels, a one-way ANOVA (Post-hoc = Tukey) analysis was used to compare the Bruce and Wingate protocols (this analysis was chosen because more than two tests were conducted between the two groups/procedures), with a significance level of $p < 0.05$. To determine the effectiveness of these two protocols, which require both aerobic and anaerobic muscle strength, in stimulating and increasing PGC-1α, and which plays a greater role, a one-way ANOVA (Post-hoc = Tukey) analysis was used for the comparison between the Bruce and Wingate protocols (this analysis was chosen because more than two tests were performed between the two groups/procedures), and the significance level was set at $p < 0.05$.

RESULTS

Table 3. Descriptive statistics - mean values

n=25	Protocol-Test	Min.	Max.	$\bar{X}$±SD
"Bruce Treadmill Test HR (beats/min)"	0	83	127	104.0±11.97
	3.min	105	140	117.5±9.89
	6. min	129	155	137.9±7.27
	9. min	135	155	144.7±5.97
	12. min	138	160	147.8±6.56
	15. min	146	168	155.4±6.60
	18. min	158	179	164.9±7.17
PGC-1α (ng/mL) Bruce	Pre-test	4.015	6.813	4.949±.807
	Mid-test	4.231	8.564	5.739±1.050
	Post-test	4.404	8.263	6.343±1.001
PGC-1α (ng/mL) Wingate	Pre-test	2.741	5.377	3.984±1.037
	Mid-test	3.913	5.725	4.622±.782
	Post-test	4.728	6.110	5.254±.747
Wingate leg (W)	PPO	261.50	2131.0	820.752±614.595
	APO	181.20	1799.0	655.705±523.482
	MPO	101.20	1272.0	439.594±371.777

Minimum = minimum observation value, Maximum = maximum observation value, $\bar{X}$±SD = mean and standard deviation, HR = heart rate, W = watts, PPO = peak power output, AP = average power output, MP = minimum power output.

Table 3 presents the average maximum oxygen consumption (VO_2max) per minute, the heart rate (HR) measured every 3 minutes throughout the Bruce test, the average PGC-1α levels reached in the Bruce-Wingate tests, and the average power outputs from the Wingate leg power test.

It was determined that the VO_2max capacity is at a high/good level (68.72±8.18 mL/kg/min). The highest HR during the Bruce test was observed at the 18th minute (164.9±7.17 beats/min). Both protocols reached the highest average PGC-1α levels in the post-test (Bruce: 6.343±1.001 ng/mL, Wingate: 5.254±0.747 ng/mL). In the Wingate leg power test, it was found that the maximum anaerobic power produced (PPO = 820.752±614.595W), average power/maximum anaerobic capacity (APO = 655.705±523.482W), and minimum mechanical power (MPO = 439.594±371.777W) averages were at high limits.

The high standard deviation values in Wingate power outputs may suggest significant variance and heterogeneity within the sample. However, the deviations obtained from both the Bruce and Wingate protocols minimize this perception by demonstrating homogeneity in the data from the same sample group.

Table 4. Results of mixed-design Anova for repeated measures (Differences Between Tests)

Protocol n=25	PGC-1α (ng/mL)		SD	MD	p
Bruce test η^2=.703	Pre-test	Mid-test	.242	-.791*	.016
		Post-test	.259	-1.395*	.000
	Mid-test	Pre-test	.242	.791*	.016
		Post-test	.329	-.604	.258
	Post-test	Pre-test	.259	1.395*	.000
		Mid-test	.329	.604	.258
Wingate test η^2=.703	Pre-test	Mid-test	.121	-1,075*	.037
		Post-test	.211	-2,074*	.031
	Mid-test	Pre-test	.121	1,075*	.037
		Post-test	.314	-,999	.259
	Post-test	Pre-test	.211	2,074*	.031
		Mid-test	.314	,999	.259

η^2 = effect size, SD = standard deviation, MD = mean difference, p = significance level. Bonferroni correction was applied for Type I error control, and a significance level of $p < 0.016$ was accepted for three repeated tests.

In Table 4, the comparison of serum PGC-1α levels from samples taken before, 2 hours after, and 48 hours after the Bruce and Wingate tests is presented separately. Significant differences in PGC-1α levels were found between the pre-mid and pre-post tests in both protocols (η^2 = .703, $p < 0.05$). Based on this result, it is suggested that PGC-1α gradually increases after exercise, with the significant increase occurring in the phases after 48 hours.

Table 5. Results of mixed-design Anova for repeated measures (Time Effect)

Protocol n=25	Source of Variance	KT	Sd	KO	F	p	η^2
Bruce test	PGC-1α	15.654	1.733	9.031	12.568	.000	.456
	Error	18.684	26.000	.719			
Wingate test	PGC-1α	6.454	1.066	6.056	40.895	.020	.953
	Error	.316	2.132	.148			

KT = sum of squares, sd = degrees of freedom, KO = mean square, η^2 = effect size. The source of variance for PGC-1α indicates whether there are differences between the tests over time. Bonferroni correction was applied for Type I error control, and a significance level of $p < 0.016$ was accepted for three repeated tests.

In Table 5, it has been established that there are significant differences in PGC-1α over time for both protocols (Bruce η^2 = .456, Wingate η^2 = .953, p 0.05). In the analysis of differences over time, which supports and predicts the results in Table 4, a gradual and sustained increase in the later hours after exercise was observed and confirmed.

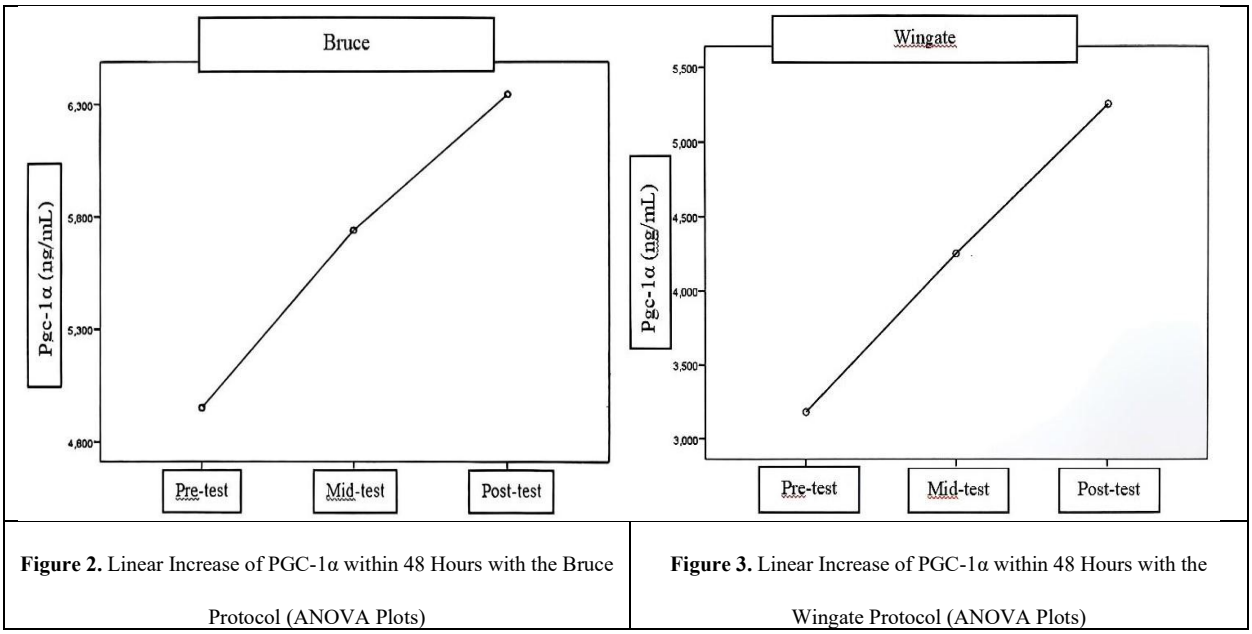


Figure 2. Linear Increase of PGC-1α within 48 Hours with the Bruce Protocol (ANOVA Plots)

Figure 3. Linear Increase of PGC-1α within 48 Hours with the Wingate Protocol (ANOVA Plots)

In Figures 2 and 3, the time effect from the mixed-design ANOVA results is presented through curves plotted based on the means. The graphs serve as a visual representation of the findings in Tables 2 and 3. The vertical x-axis represents the PGC-1α levels, while the horizontal y-axis denotes the time of the tests (pre-test: before, mid-test: 2 hours after, post-test: 48 hours after). It has been determined that PGC-1α exhibits significant changes over time for both protocols.

Table 6. Comparison of PGC-1α according to bruce and wingate protocols (One-Way ANOVA)

n=25	Protocol	$\bar{X}$±SD	F	p	Levene's
Pre-test	Bruce	4.949±.807	5.862	.025	.196
	Wingate	3.984±1.037			
Mid-test	Bruce	5.739±1.050	3.909	.064	.636
	Wingate	4.622±.782			
Post-test	Bruce	6.343±1.001	3.156	0.94	.612
	Wingate	5.254±.747			

In Table 6, it is observed that there is a statistically significant difference between the protocols in the pre-test PGC-1α levels ($p < 0.05$). However, in the mid-test and post-test results, the mathematical differences between the protocols were not statistically significant (p 0.05). Based on the comparison results and average values, it is suggested that the Bruce aerobic power protocol is more effective than the Wingate anaerobic power protocol. This analysis, conducted to determine the effectiveness of these two protocols—requiring both aerobic and anaerobic muscle power—in stimulating and increasing PGC-1α, and which one plays a greater role, suggests that the difference observed in pre-tests is due to the subjects' existing potential before exercise, while the lack of a difference afterward supports the increase in PGC-1α with both protocols, bringing the situation to a similar level of equality.

DISCUSSION AND CONCLUSION

In this study, the effects of different exercise protocols (aerobic and anaerobic) on the acute changes in PGC-1α levels in human skeletal muscle were investigated, and the obtained results were interpreted in light of current literature. PGC-1α is a transcriptional coactivator that plays a central role in mitochondrial biogenesis, oxidative metabolism, and muscle adaptations (Handschin & Spiegelman, 2006), and it has been shown to be stimulated

by both aerobic and anaerobic exercise types (Egan & Zierath, 2013; Lira et al., 2010).

In our study, a significant increase in PGC-1α levels (%28.15) was observed after the Bruce test, with this increase becoming more pronounced at the 2-hour mark post-exercise and plateauing at 48 hours. This finding aligns with the rapid mRNA increases in PGC-1α expression reported by Popov et al. (2014) following acute aerobic exercise. It is known that the increases in mRNA levels observed at 3 hours post-exercise are reflected in protein levels within 24-48 hours (Granata et al., 2016). Accordingly, the chosen sampling timings in our study were optimized to capture changes at both transcriptional and translational levels.

The data obtained after the Wingate protocol are also noteworthy. A more limited increase in PGC-1α levels was detected after this anaerobic, short-duration, high-intensity test compared to the Bruce protocol (pre-test: 3.984±1.037 ng/mL → post-test: 5.254±0.747 ng/mL; %31.8 increase). The lower effect of anaerobic exercise on PGC-1α is likely associated with the dominance of the glycolytic pathway over mitochondrial oxidative metabolism (Tadaishi et al., 2011). Nonetheless, even in short-duration maximal exercises, the increase in PGC-1α levels supports its role as an important molecular marker for adaptive responses in energy metabolism, regardless of the type of exercise.

Edgett and colleagues (2013) observed increases in PGC-1α mRNA levels ranging from 400% to 800% following HIIT protocols and indicated that exercise intensity influences these responses. In our study, the Wingate protocol (high intensity, short duration) also increased PGC-1α levels, but not as robustly as the Bruce protocol. This finding supports the perspective that while HIIT-like protocols have strong effects at the transcriptional level, the effect may be more limited at the proteomic level (Edgett et al., 2016).

Additionally, high variance (especially large standard deviations for PPO, APO, and MPO) was observed after the Wingate test, indicating that individual capacity differences in anaerobic power production are more pronounced. This variance was also reflected in the PGC-1α protein responses and may reflect inter-individual metabolic adaptation differences (Gibala et al., 2012).

Ahmadi et al. (2021) conducted a study using an animal model in which significant increases in PGC-1α mRNA levels were found in the HIIT group, while no significance was observed in the MICT group. This finding suggests that short but high-intensity protocols trigger a stronger cellular stress response. Along parallel lines, the increase in PGC-1α levels in the Wingate protocol was statistically significant ($p < 0.05$).

In line with the main hypothesis of our study, it was anticipated that both aerobic (Bruce) and anaerobic (Wingate) exercise protocols would increase PGC-1α levels, but the magnitude of this increase would vary depending on the type and duration of exercise. The findings obtained are supportive of this hypothesis. After the aerobic protocol, a significant increase in PGC-1α levels was observed compared to both baseline values ($p < 0.001$) and the anaerobic protocol, indicating more elevated expression levels. This suggests that PGC-1α plays a central role in mitochondrial biogenesis and oxidative phosphorylation processes, becoming more clearly activated under prolonged aerobic stress (Popov et al., 2014; Tadaishi et al., 2011). The Wingate test, due to its short duration and high intensity, primarily targets glycolytic systems but still led to meaningful PGC-1α activation, thereby supporting the claim that the anaerobic protocol is also effective at the molecular level (Edgett et al., 2013; Ahmadi et al., 2021). These findings demonstrate that PGC-1α is sensitive to both aerobic and anaerobic stimuli, but aerobic loading triggers this sensitivity with a more sustainable and comprehensive response.

However, the biomarker levels obtained from the aerobic protocol were statistically stronger ($\eta^2 = 0.703$) and had higher absolute values. This finding supports the hypothesis that long-duration and low-to-moderate intensity exercise more effectively stimulates mitochondrial biogenesis (Bishop et al., 2019; Camera et al., 2010). Furthermore, the VO_2max value calculated based on the Bruce test (68.72±8.18 mL/kg/min) indicates a high aerobic capacity in the elite skiers participating in the study, parallel to the increases in PGC-1α levels. Some studies indicate that these biomolecular increases do not always translate to protein levels. For instance, Edgett et al. (2016) observed increases in PGC-1α mRNA levels following 6 weeks of sprint interval training, yet protein levels remained stable. Such discrepancies can be explained by post-translational regulatory mechanisms and individual variations in protein degradation rates (Egan & Zierath, 2013).

PGC-1α plays an essential role in differentiation at the molecular level. This coactivator promotes mitochondrial biogenesis and oxidative phosphorylation by activating transcription factors that regulate cellular energy metabolism, thus helping to meet energy demands during differentiation (Puigserver & Madi, 2003). For example, during the differentiation of muscle cells, the expansion of PGC-1α increases oxidative capacity, contributing to the phenotypic transformation of muscle fibers (Handschin & Spiegelman, 2006). Moreover, PGC-1α supports the morphological and functional development of neurons by optimizing mitochondrial functions and energy production during neuronal differentiation (St-Pierre et al., 2006). At the molecular level, PGC-1α has been shown to become active through signaling pathways such as p38 MAPK and AMPK, interacting with various transcription factors during differentiation processes (Wender et al., 2014). These mechanisms enable cells to be directed toward specific phenotypes and regulate differentiation processes. Therefore, PGC-1α plays a central role in the biological mechanisms of cell differentiation through the regulation of energy metabolism.

Finally, the strengths of this study include the randomized application of protocols, the careful determination of sampling times in line with the dynamics of transcriptional biology, and the high analytical accuracy of the biochemical kits used. However, limitations of the study include the sample consisting solely of male athletes, the limited number of samples, and the lack of investigation into other signaling pathways (e.g., AMPK, SIRT1) aside from PGC-1α.

Conclusion

This study compared the PGC-1α responses to aerobic (Bruce) and anaerobic (Wingate) exercise protocols in elite trained endurance athletes (n=25). The participants' VO_2max level (68.72±8.18 mL/kg/min) aligns with values reported for top endurance athletes in the literature (Joyner & Coyle, 2008), confirming their high cardiovascular capacity. Reaching a maximum heart rate at the 18th minute (164.9±7.17 beats/min) during the Bruce test reflects the athletes' high aerobic adaptation level and cardiovascular economy. These values being lower than those observed in sedentary individuals indicate a higher cardiac vagal tone and more efficient heart function in athletes (Aubert et al., 2003).

Significant increases in PGC-1α levels were recorded following both exercise protocols, with the highest values recorded in the final test measurements (Bruce: 6.343±1.001 ng/mL; Wingate: 5.254±0.747 ng/mL). This finding shows that PGC-1α expression is activated following not only aerobic but also high-intensity anaerobic exercises, indicating that mitochondrial biogenesis responds integratively to both energy systems (Egan & Zierath, 2013; Bartlett et al., 2020). However, when considering the statistical significance of the differences and the magnitude of difference between mean values, it can be interpreted that the Bruce protocol provides a stronger molecular stimulus compared to the Wingate protocol. This situation supports the existing literature that suggests PGC-1α is more closely related to oxidative metabolism (Popov et al., 2014; Little et al., 2010).

The high anaerobic power outputs achieved in the Wingate test [PPO=820.752±614.595W; APO=655.705±523.482W; MPO=439.594±371.777W] demonstrate that advanced anaerobic systems contribute to high performance in endurance-based athletes. This suggests that intense aerobic training indirectly supports muscle glycogen stores, phosphocreatine metabolism, and anaerobic glycolytic capacities (Hawley, 2002).

Upon examining the statistical analyses, it was observed that there were significant increases in PGC-1α levels between the pre-test and mid-test and between the pre-test and post-test in both Bruce and Wingate protocols ($p<0.05$), but the differences between the mid-test and post-test were not significant. This situation indicates a biological rhythm where PGC-1α expression peaks in the first few hours post-exercise but reaches a plateau at 48 hours, resulting in a slower rate of increase (Granata et al., 2016). Considering effect sizes (η^2: .456 for Bruce; .953 for Wingate), the high effect size obtained for the Wingate test ($\eta^2 = 0.953$) is particularly noteworthy. It suggests that the differences between the measurements over time are largely attributable to physical or biochemical changes. This value indicates that approximately 95.3% of the total variance originates from differences between tests, a score considered very high in psychophysical or biological research. This high η^2 value also demonstrates that PGC-1α levels change significantly and meaningfully over time and that these changes are strongly associated with the applied protocol and time factors. Thus, the changes in PGC-1α levels observed during the Wingate test underscore the significance of the study within both clinical and research contexts. Literature suggests that high effect sizes enhance the practical implications and reliability of the results. Notably, in biochemical parameters such as these, an η^2 value exceeding 0.14 is regarded as large (Cohen, 1988). A high η^2 indicates that the change in PGC-1α levels is significant and influential over time, serving as a strong finding that reinforces the overall results of the study. This could particularly suggest that the effects of previous probiotic interventions or other interventions on PGC-1α levels have become apparent over time. In other words, the time factor appears to have a significant and clinically relevant influence on this molecular marker. However, the absolute PGC-1α levels attained at the biochemical level were higher in the Bruce test. As such, careful differentiation should be made between statistical significance and physiological significance, with attention not only to p-values but also to the biological importance of mean values (Sterne & Davey Smith, 2001).

The hypothesis of the research was that both aerobic and anaerobic exercises would significantly increase PGC-1α expression. The findings supported this hypothesis, revealing that the Bruce test was more effective at the molecular level. This situation is associated with a more intense transcriptional response in oxidative muscle fibers during aerobic exercise (Handschin & Spiegelman, 2008).

Recommendations

Future studies with groups of different genders, ages, and training histories will enhance the generalizability of the findings. Multimolecular analysis designs measuring other biomarkers associated with mitochondrial biogenesis, such as NRF-1 and TFAM, will provide more comprehensive results. Sample analyses conducted at additional time points, such as 24, 72, and 96 hours post-exercise, could reveal the complete temporal profile of PGC-1α expression. Comparisons of acute and chronic effects through long-term training interventions will more clearly elucidate PGC-1α's role in adaptive regulations (Granata et al., 2016).

Limitations

This study presents significant findings by examining the acute biochemical responses of PGC-1α levels to aerobic and anaerobic exercise protocols in well-trained elite male endurance athletes. However, like scientific studies, this research has certain limitations. Firstly, due to the study group consisting only of male athletes, the results cannot be directly generalized to female athletes. Similarly, the sample group's elite endurance background provides limited information on whether similar results can be obtained in individuals with varying training levels. Additionally, although efforts were made to ensure blood samples were collected in a fasting state and at specific time intervals, controlling for some physiological variables that may arise from individual differences was not entirely feasible. However, individual variation is generally accepted at a specific level in biological studies. Finally, while the biochemical analysis methods used in the study have high reliability, biopsies from muscle tissue were not performed, and PGC-1α levels were assessed via serum. This approach is widely used in the literature and is considered a non-invasive method.

All these limitations do not impact the core findings and validity of the study; however, it is believed that future research with larger sample groups, gender comparisons, and different exercise protocols will contribute to the field.

Ethics Statement: *In the present article, the ethical rules of the journal were followed in the research process in the current article. The responsibility for any violations that may arise regarding the article belongs to the author. The approval of Erzincan Binali Yıldırım University Ethics Committee dated 20.03.2020 and numbered 03/02 was obtained.*

Conflict of Interest: *There is no personal or financial conflict of interest between the authors in the present study.*

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GENİŞLETİLMİŞ ÖZET

Çalışmanın Amacı

Bu çalışmanın amacı, iyi antrene edilmiş dayanıklılık sporcularında aerobik ve anaerobik güç testleri öncesi ve sonrası serum PGC-1α düzeylerini karşılaştırmaktır.

Araştırma Problemleri

İyi düzeyde antrenmanlı dayanıklılık sporcularına uygulanan aerobik (Bruce) ve anaerobik (Wingate) kapasite testleri serum PGC-1α (ng/mL) proteinini arttırır mı?

Serum PGC-1α (ng/mL) proteinini arttırmada aerobik egzersiz mi anaerobik egzersiz mi daha etkilidir?

Literatur Araştırması

Fiziksel egzersiz, organizmanın pek çok fizyolojik sistemi üzerinde çok katmanlı ve kompleks etkiler ortaya koyan bir uyaran olarak kabul edilmektedir. Egzersiz, özellikle iskelet kası düzeyinde metabolik, biyokimyasal ve genetik düzeyde adaptasyonların tetiklenmesini sağlamaktadır. Bu adaptasyonlar, sadece sporcunun performansını artırmakla kalmaz, aynı zamanda yaşlanma, metabolik hastalıklar ve kronik inflamatuar durumlar gibi klinik sorunların önlenmesi ve yönetilmesinde de önemli bir biyolojik altyapı oluşturmaktadır (Egan&Zierath, 2013; Joyner&Coyle, 2008). Egzersize özgü bu hücresel yanıtlar arasında mitokondriyal biyogenez, enerji metabolizmasının yeniden programlanması ve oksidatif kapasitenin artırılması yer alır. Bu süreçlerin moleküler anahtarlarından biri, PGC-1α (Peroxisomeproliferator-activatedreceptor gamma coactivator 1-alpha), enerji homeostazının ve kas adaptasyonlarının düzenlenmesinde kritik bir rol üstlenmektedir (Handschin&Spiegelman, 2006; Kupr&Handschin, 2015).

Yöntem

Çalışmaya, en az üç yıl süreyle dayanıklılık antrenmanı yapan, haftada en az beş gün antrenman gerçekleştiren, Türkiye kayak krosu milli takımında yer alan 25 erkek sporcu dahil edilmiştir. Katılımcılar, performans ve çevresel faktörler açısından homojenlik sağlamak amacıyla, çalışma süresi boyunca Erzurum'da gerçekleştirilen yoğun bir kamp ortamında bulunmuş, kampta aynı beslenme programı ve antrenman protokollerine tabi tutulmuşlardır. Bu yaklaşım, çalışma koşullarındaki değişkenliği minimize ederek, elde edilen biyokimyasal ve fizyolojik verilerin güvenilirliğini artırmayı hedeflemiştir.

Katılımcıların antropometrik ve fizyolojik özellikleri, standart yöntemler kullanılarak ölçülmüştür. Yaş bilgisi doğrudan katılımcılardan alınmış, boy ölçümü stadiometre ile, vücut ağırlığı ise dijital baskül kullanılarak hassas şekilde belirlenmiştir. Maksimal kalp atım hızı (HRmax), Karvonen yöntemiyle hesaplanmış, maksimal oksijen tüketimi (VO_2max) ise Bruce koşu bandı testi protokolü kullanılarak laboratuvar ortamında tespit edilmiştir. Elde edilen ortalama değerler şu şekildedir: yaş 20.56 ± 2.27 yıl, boy 172.56 ± 10.11 cm, vücut kütlesi 64.37 ± 10.62 kg, HRmax 145.82 ± 9.16 atım/dk ve VO_2max 68.72 ± 8.18 mL/kg/dk.

Çalışmaya katılan sporculardan, testlere başlamadan önceki en az 48 saatlik sürede kafein, besin takviyesi ve diğer ergojenik desteklerin tüketiminden kaçınmaları ve yoğun fiziksel aktivitelerden uzak durmaları istenmiştir. Bu uygulama, egzersiz sonrası biyokimyasal ve moleküler parametrelerin dış etkenlerden etkilenmemesini ve ölçümlerin güvenilirliğini artırmayı hedeflemektedir. Bu süreçte katılımcılar, beslenme ve aktivite kısıtlamalarına uyum konusunda ayrıntılı bilgilendirilmiş ve uyumsuzluk durumlarının tespiti için test öncesi sorgulamalar yapılmıştır. Katılımcılar, çalışma boyunca iki farklı egzersiz testine katılmış ve bu testler arasında minimum 48 saat ara verilmiştir. Test sırasının randomize edilmesi ile herhangi bir sıralama etkisinin (carry-overeffect) önüne geçilmiş ve ölçümlerin geçerliliği artırılmıştır. Test günlerinde, katılımcıların gece 6-8 saat arasında dinlendirici uyku almaları sağlanmış ve sabah saat 09:00'da, açlık durumunda bazal kan örnekleri toplanmıştır. Kan örneklemesinin ardından katılımcılara standart bir kahvaltı verilmiş, test öncesi hidrasyonun standardizasyonu amacıyla 150-200 mL sıvı alımı sağlanmış ve idrar boşaltımı gerçekleştirilmiştir.

Her test öncesi (sabah açlık durumunda), 2 saat sonrası ve 48 saat sonrası olmak üzere üç zaman noktasında kan

örnekleri alınarak serum PGC-1α düzeyleri ELISA yöntemiyle analiz edilmiştir.

Sonuç ve Değerlendirme

Her iki test protokolü de serum PGC-1α düzeylerinde zamana bağlı anlamlı artışlar oluşturmuştur (Bruce: $p < .001$, $\eta^2 = .456$; Wingate: $p = .020$, $\eta^2 = .953$). Her iki protokolde de ön-ara ve ön-son testler arasında istatistiksel olarak anlamlı fark tespit edilmiştir ($p < .05$). Protokoller arası karşılaştırmada sadece test öncesi değerlerde anlamlı farklılık saptanmıştır ($p = .05$). Ortalama PGC-1α düzeyleri (ng/mL) şu şekildedir: Bruce – ön test: 4.949 ± 0.807, 2. saat: 5.739 ± 1.050, 48. saat: 6.343 ± 1.001; Wingate – ön test: 3.984 ± 1.037, 2. saat: 4.622 ± 0.782, 48. saat: 5.254 ± 0.747.

Hem aerobik hem de anaerobik egzersiz protokolleri serum PGC-1α düzeylerinde anlamlı artışa yol açmaktadır. Ancak Bruce treadmill protokolünün bu artışı daha güçlü şekilde tetiklediği görülmektedir. Bu durum, aerobik egzersizin mitokondriyal biyogenezle ilişkili transkripsiyonel aktiviteyi artırmada daha etkili olabileceğini göstermektedir.

Araştırmanın hipotezi, hem aerobik hem de anaerobik egzersizlerin PGC-1α ekspresyonunu anlamlı düzeyde artıracağı yönündeydi. Bulgular bu hipotezi desteklemiş, ancak Bruce testinin moleküler düzeyde daha etkili olduğu ortaya koyulmuştur. Bu durum, aerobik egzersizin oksidatif kas liflerinde daha yoğun bir transkripsiyonel yanıt oluşturmasıyla ilişkilidir.