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Acute Effects of Aerobic Endurance Training with Different Glycogen Levels on Some Biochemical Parameters in Football Players

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ABSTRACT

This study aims to determine the acute effects of aerobic endurance exercises performed at different body glycogen levels on biochemical parameters related to energy metabolism. The study included 14 male amateur football players with an average age of 20.38 ± 2 years. Aerobic endurance exercises were performed under conditions of low liver glycogen after a 10-12 hour fasted (FST) state and under a postprandial (PPD) state, as well as under conditions of full and low body glycogen (partially reduced by the first exercise). These exercises consisted of two 60-minute sessions on a cycle ergometer with a 60-minute rest interval in between. Blood samples were collected from participants before and after all exercises. Statistical analyses were performed using SPSS 28.0 software, utilizing the paired simple t-test, Wilcoxon test, one-way ANOVA, Friedman analysis, and post-hoc tests with a significance level of $p < 0.05$. In PPD with low glycogen, glucose levels decreased during exercise, whereas in FST with low glycogen, insulin levels decreased in both exercises. Cortisol levels increased in the FST low glycogen exercise. Triglycerides also increased in the FST low glycogen exercise. Albumin levels increased in the FST, and the PPD and low glycogen exercise; similarly, levels increased in the PPD low glycogen exercise ($p < 0.05$). In conclusion, glucose levels were maintained during the FST and PPD low glycogen exercises, while the highest triglyceride breakdown occurred during the FST low glycogen exercise.

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INTRODUCTION

The human body is designed for movement (Şerare et al., 2024). Physical activity plays a crucial role in maintaining overall health, while training is essential for enhancing physical performance (Potteiger, 2018). Coaches increasingly rely on evidence-based medicine to design training programs and off-field strategies that optimize performance (Bhandari & Giannoudis, 2006). Among these off-field methods, experimenting with different combinations of training and nutrition plans to indirectly influence an athlete's energy metabolism is of primary importance. However, the literature review highlights the complexity and incomplete understanding of carbohydrate and fat utilization during aerobic endurance exercises. (Paşaoğlu et al., 2019).

Moreover, there is growing evidence that starting exercise with reduced glycogen reserves activates lipolysis to a greater extent, resulting in less dependence on glycogen (Earnest et al., 2019) and affecting biochemical parameters (Andrade-Souza et al., 2019; Lundberg et al., 2014). These studies generally involve pathological examinations through muscle biopsies, yielding findings that support muscle adaptation (Hansen et al., 2005). Additionally, increased research suggests that altering dietary intake can modify the metabolic responses associated with exercise (Earnest et al., 2019). Various methods have been employed to enhance metabolic efficiency, such as increasing carbohydrate consumption before and during exercise or performing exercise in a FST state (10-12 hours of fasting; Jeukendrup, 2017). A 12-hour FST period is known not to alter muscle glycogen stores; however, evidence has increasingly indicated that training under conditions of low liver glycogen reserves (Iwayama et al., 2021) may potentially influence metabolic efficiency (Gonzalez et al., 2015). Furthermore, carbohydrate and lipid metabolism changes during during aerobic exercise are recognized as significant factors affecting exercise performance (Fernández-Verdejo et al., 2018; Maunder et al., 2018). In addition to these studies, there is research investigating the impact of fasting on the performance of football players during Ramadan. These studies focus on the effects of exercise performed in a FST state on aerobic capacity, endurance (Meckel et al., 2008), speed, power, and ball dribbling skills (Kirkendall et al., 2008; Zerguini et al., 2007). The literature indicates that many exercises conducted with low glycogen stores are typically performed at 70% VO_2 peak, with durations ranging from 60 to 105 minutes, predominantly among cyclists and triathletes (Rosa et al., 2019; Webster et al., 2016; Yeo et al., 2008). Although football is considered one of the most popular sports worldwide, the application of such

endurance exercises lasting 120 minutes or more, often consisting of two 45-minute halves with potential extra time, is relatively rare among football players.

It is known that aerobic exercise does not increase cortisol hormone (COR) levels (Setiakarnawijaya et al., 2022; Torres et al., 2021), may reduce plasma glucose (GLU), insulin (INS), and insulin resistance index (Sabzikar et al., 2018), lead to significant reductions in serum triglyceride (TG) concentrations (Santiago et al., 2020), and cause no significant changes in serum albumin (ALB) levels immediately after exercise (Zhang et al., 2023).

This study aims to evaluate the acute effects of aerobic endurance exercises performed under FST and PPD conditions, and with full and partially reduced (low) body glycogen reserve on some biochemical parameters indicative of energy metabolism in male amateur soccer players. It was hypothesized that aerobic endurance exercises in soccer players may have different effects on biochemical parameters indicative of energy metabolism depending on different body glycogen levels.

METHODS

Participants

The research group consisted of 14 healthy adult amateur male football players with a mean age of 20.38±2, who had participated in football-specific training for at least 8-weeks during the season and were actively competing in local amateur leagues (n = 14; Table 1). After the potential risks were explained in detail, participants signed the informed consent form and were included in the study. Although the study began with 14 participants, it was completed with 13 participants due to one individual being unable to finish the exercise trial.

Table 1
Anthropometric Characteristics and Years of Education of Participants Evaluated Once

Anthropometric Characteristics	Mean±SD	Min	Max
Age (years)	20.38±2.06	18.00	25.00
Training Years (years)	9.07±3.06	5.00	16.00
Height (cm)	175.61±6.15	167.00	184.00
Body Weight (kg)	66.06±7.05	53.50	78.20
BMI (kg/m²)	21.03±1.52	17.90	23.80
Body Fat Percentage (%)	7.27±3.65	2.80	13.95
Body Muscle Mass (kg)	57.95±7.41	45.40	71.40

Note. SD: Standard Deviation, Min: Minimum, Max: Maximum, BMI: Body Mass Index, kg: Kilogram, %: Percentage, n = 13

Procedures

The experimental research model derived from the doctoral thesis was supported by the Gazi University "Scientific Research Projects Coordination Unit" (Project Code: 2022-8055). The research was conducted at the Performance Laboratory and the Cardiopulmonary Rehabilitation Unit of Gazi University. This study, conducted in accordance with the procedures outlined in the Helsinki Declaration and derived from a doctoral dissertation, was approved by the "Ethics Committee for Non-Invasive Clinical Research" of Gazi University (Date: July 25, 2022, Decision No: 586).

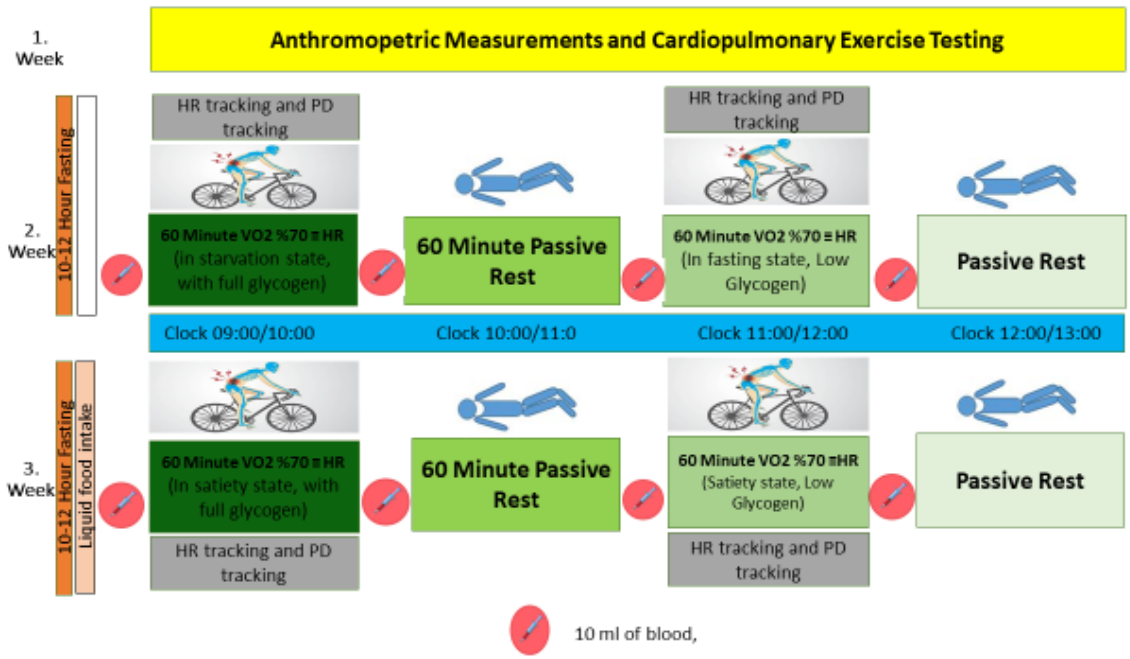
The participants visited our laboratory and unit a total of three times: once for anthropometric measurements and cardiopulmonary exercise tests, with a one-week interval between visits, and twice for experimental exercise sessions. Participants were instructed to arrive between 08:00 and 08:30, and all measurements, tests, and experimental exercises were conducted at the same time of day (±1 hour) between 09:00 and 13:00.

In the first week, anthropometric measurements were taken, body composition was assessed, and cardiopulmonary exercise tests were conducted once. In the second and third weeks, two 60-minute aerobic endurance exercises were conducted under different liver and body glycogen storage conditions, as detailed below (see Figure 1).

Second Week: The first 60-minute exercise (FE) was performed with low liver glycogen reserves (10-12 hours fasting) and full body glycogen reserves, followed by 60 minutes of passive rest. The second 60-minute exercise (SE) was conducted with both liver glycogen reserves (10-12 hours fasting) and body glycogen reserves at a low level (partially reduced from the first exercise).

Third Week: The first 60-minute exercise was performed with full liver glycogen reserves (liquid food intake) and full body glycogen reserves. After 60 minutes of passive rest, the second 60-minute exercise was conducted with full liver glycogen reserves (liquid food intake) and low body glycogen reserves (partially reduced from the first exercise). 10-12 hours of fasting is known to deplete liver glycogen stores (Learsi et al., 2019). The conditions of low liver and body glycogen have been validated by recent studies conducted with a population of elite male cyclists (Bulut & Turnagöl, 2018; Hulston et al., 2010). The acute effects of aerobic endurance exercises performed in both fasting and fed states, and full and partially reduced body glycogen reserves on biochemical parameters indicative of energy metabolism were assessed through statistical analyses of numerical data obtained from blood samples.

Figure 1
Study Protocol



Note. PD: Perceived Difficulty, HR: Heart Rate, VO_2max (ml kg-1 min-1): Maximum Amount of Oxygen (milliliters) Used Per Minute by 1 Kilogram of Muscle, VO_2max 70% ≡ HR: Heart Rate Corresponding to 70% of Maximum Oxygen Utilization, HR ≡ Power (watts) Corresponding to Heart Rate, ml: Mililitre.

Data Collection Tools

Anthropometric Measurements and Assessment of Body Composition

The participants' height measurements were taken once using a TEM EKO (Made in Turkey) brand electronic scale and stadiometer with a precision of one millimeter. Body compositions were determined using the TANITA MC 580 S (Tokyo, Japan) Bioelectrical Impedance Analysis (BIA) method. BIA estimates parameters such as body weight (kg), fat mass (%), muscle mass (kg), and body mass index (kg/m²) based on previously entered personal information and the body's electrical conductivity (Maliqi et al., 2022). (Table1). The participants' estimated maximum heart rate (HRmax) was calculated using the Karvonen formula (She et al., 2015).

Cardiopulmonary Exercise Test (CPET)

The Astrand Bicycle Ergometer Test protocol was applied once to determine the participants' aerobic capacities. The test used a Monark LC 6 model bicycle ergometer (Monark Exercise AB, Vansbro, Sweden; Stavrinou et al., 2019). During the test, oxygen consumption was recorded using a Cosmed Quark CPET device (Rome, Italy), which was calibrated according to the manufacturer's instructions before each test for cardiopulmonary indices.

Heart rate was measured using a REF: D41480 ANT+ chest strap, part of the Cosmed Quark CPET system, with accuracy reported similarly to electrocardiography (±1 beat/minute). A Cosmed Quark CPET gas analyzer was utilized throughout the data collection process (Price et al., 2022). In the CPET, measurements of key variables such as heart rate, oxygen consumption, respiratory rate, pulmonary ventilation (PVE), oxygen pulse, respiratory exchange ratio (RER), ventilation equivalents for oxygen (VE/VO_2), and ventilation equivalents for carbon dioxide (VE/VCO_2) were obtained.

In the Astrand protocol, participants began with a 5-minute warm-up at 50 Watts and a cadence of 60 revolutions per minute. Subsequently, a workload of 100 Watts was applied, and the intensity was increased by 50 Watts every two minutes until each participant reached voluntary exhaustion. A metabolic cart continuously recorded expired breath (COSMED, Quark CPET, Italy). The peak VO_2 value was defined as the highest oxygen uptake achieved during the final 30 seconds of the test. To assess maximum aerobic fitness levels, participants were verbally encouraged to exert themselves to their fullest potential (Naharudin & Yusof, 2018). The exercise was terminated by the operator when participants felt they could no longer maintain their effort, indicated by a failure of VO_2 or heart rate to increase with rising speed/power, a respiratory rate exceeding 45 breaths per minute, and a perceived exertion on the Borg scale above 18 (Price et al., 2022; Table 2).

Table 2
The Training Status and Aerobic Capacities of The Participants were Evaluated Once (n = 13)

Variables	Mean±SD	Min	Max
Resting HR (beats/min)	69.23±5.38	58.00	76.00
Max HR (beats/min)	186.00±8.66	165.00	197.00
VO_2max (ml kg-1 min-1)	44.78±4.02	37.00	51.70
Max Power (watts)	265.38±37.55	200.00	350.00
VO_2max 70% (ml kg-1 min-1)	32.33±4.17	25.90	42.70
VO_2max 70% ≡ HR (beats/min ±10)	153.76±5.34	141.00	159.00
70% HR ≡ Power (watts)	118.46±23.75	75.00	170.00
Perceived Difficulty (PD)	19.23±.72	18.00	20.00
70% of Perceived Difficulty	13.46±.50	12.60	14.00

Note. SD: Standard Deviation, Min: Minimum, Max: Maksimum, HR (beats/min): Heart Rate Per Minute, Max HR: Maximum Heart Rate, VO_2max (ml kg-1 min-1): Maximum Amount of Oxygen (milliliters) Used Per Minute by 1 Kilogram of Muscle, VO_2max 70% ≡ HR: Heart Rate Corresponding to 70% of maximum Oxygen Utilization, HR ≡ Power (watts): Power (watts) Corresponding to Heart Rate, PD: Perceived Difficulty

Determination of Exercise Intensity

At the end of the Astrand protocol applied to determine aerobic capacity, the heart rate range corresponding to 70% of VO_2max was identified in the CPET table and was designated as the intensity to be maintained in all aerobic exercises (Cabral et al., 2020; Fang et al., 2021; Rogers et al., 2021; Table 2).

Dietary Control Before and During Exercise

To maintain the participants' body glycogen levels and ensure energy balance, they were instructed to cease strenuous training 48 hours before the testing days, minimize caffeine intake especially in the last 24 hours and abstain from alcohol consumption (Ramos et al., 2021). Participants were also asked to avoid significant dietary changes during this period and consume the same food types the day before each exercise day (Hulston et al., 2010).

A specialist in nutrition and dietetics calculated the evening meals, pre-exercise PPD, and interim fluid nutrient intakes. The final evening meals before both exercise days were standardized and calculated to provide an average of 1013.48 ± 64.39 kcal (Fink & Mikesky, 2015). This meal was designed to consist of 55-65% carbohydrates, 30% fats, and 12-15% proteins (Nikolaidis & Theodoropoulou, 2014). The caloric values of the participants' last evening meals were calculated based on their Basal Metabolic Rates (BMR), daily energy requirements, and 25% of the calculated daily caloric needs. For male football players aged 18-30, BMR was calculated using the formula: BMR = (15.3 x Body Weight) + 679 = kcal. Daily energy requirements were determined using the formula: BMR x activity factor = BMR x (1.6 or 2.4) = kcal/day (Eskici, 2015). The recommended 25% of the calculated daily caloric needs was used to determine the last evening meal caloric intake (Şakar, 2009; Table 3).

On the FST exercise day, participants performed 60 minutes of aerobic endurance exercises after a 10-12 hour FST period. During the one-hour rest interval, they could consume water up to 0.5 milliliters (Hulston et al., 2010). On the PPD exercise day, participants consumed a liquid meal two hours before exercise, consisting of 55% carbohydrates, 30% fats, and 15% proteins, calculated at kg/10 kcal based on their body weight. After 60 minutes of aerobic endurance exercise and one-hour rest interval, they ingested a liquid meal containing 44% carbohydrates, 24% fats, and 28% proteins, calculated at kg/2 kcal (Bulut & Turnagöl, 2018). The liquid food beverages were sourced from Nestlé Turkey Food Inc. (Table 3).

Aerobic Endurance Exercise

Aerobic endurance exercises were conducted on an electronic-braked bicycle ergometer (Monark 928 E, Sweden; Jones et al., 2021). The exercise intensity was maintained at a pedaling rate of 70 RPM, corresponding to 70% of VO_2max, using heart rate (HR) data (Fang et al., 2021; Rogers et al., 2021). During the exercise, HR was monitored using a Polar H1 chest strap (Dennis et al., 2021) and a Polar FT80 watch (Polar Electro Oy, Kempele, Finland; Manjunath et al., 2019). In the first week of the experimental exercises, participants performed 60 minutes of aerobic endurance exercises at a pedaling rate of 70 RPM, aiming to maintain the HR corresponding to 70% VO_2max after a 10-12 hour fast. This was repeated twice with a 60-minute rest interval. In the second week of the experimental exercises, the same protocol was followed under fed conditions (liquid food intake; Bulut & Turnagöl, 2018). The aim was to reduce body glycogen reserves with the first 60-minute exercise and to begin the second exercise with partially reduced glycogen reserves (Bulut, 2014; Yeo et al., 2010).

Table 3
Dietary Calories and FST - PPD Blood Glucose Levels for Before - After FST and PPD Status Exercises (n = 13)

Variables	Mean±SD	Min	Max
Basal Metabolic Rate (BMR; kcal)	1689.15±107	1497.55	1875.46
Daily Calorie Needs (kcal)	4053.92±257	3594.12	4501.10
Last Evening Diet (kcal)	1013.48±64.39	898.75	1125.27
Week 2 PPD blood GLU (mg/dL)	118.69±11.41	99.00	137.00
FST blood GLK (mg/dL) after 10-12 hours	92.69±6.66	83.00	101.00
Week 3 PPD blood GLU (mg/dL)	121.38±15.34	93.00	146.00
FST blood GLU (mg/dL) after 10-12 hours	92.92±9.85	80.00	111.00
PPD PRE-FE diet (kcal)	653.07±68.25	535.00	732.00
PPD POST-FE diet (kcal)	132.89±16.96	107.00	167.40

Note. SD: Standard Deviation, Min: Minimum, Max: Maximum, GLU: Glucose, kcal: Kilocalories, mg/dL: Milligrams per Deciliter, PPD: Postprandial, FST: Fasting, PRE-FE: Pre-First Exercise, POST-FE: Post-First Exercise.

Biochemical Sample Collection and Measurement

Venous blood samples were collected from participants a total of four times, both before and after each exercise session, by cardiology specialists. For hormone and metabolite measurements, 5 mL gel tubes (serum) were used, with particular attention to measuring the levels of hormones such as cortisol (COR) and insulin (INS), as well as metabolites including albumin (ALB), glucose (GLU), and triglycerides (TG). The venous blood samples collected

from participants were transported to the biochemistry laboratory, where they were centrifuged at 3500 RPM at -4 °C to separate plasma and serum. Different devices were used to determine the levels of each serum component. Hormone and metabolite levels were measured using a fully automated clinical biochemistry analyzer (Cobas 6000, Roche Hitachi, Mannheim, Germany). Blood samples taken for gas analysis were measured using a blood gas analyzer (ABL800™, Radiometer, Copenhagen, Denmark).

Data Analysis

The determination of the number of participants was conducted by a biostatistics expert, with the PRE-FE glucose parameter (75.7 ± 15.1) under the PPD condition in Bulut's (2014) study primarily referenced (Bulut, 2014). A power analysis conducted using *GPower 3.1 with $\alpha = 0.05$, $\beta = 0.10$, and $1-\beta = 0.90$ determined that 14 volunteer participants should be included in the study, with the test power calculated as $p = 0.90431$. Data analysis was performed using SPSS 28.0 statistical software. Normality assumptions were tested using the Shapiro-Wilk test. Equality of variances among all relevant group combinations was determined using Levene's test. When parametric assumptions were met, measurements obtained from the same individuals under different conditions were compared using the paired sample t-test. For comparisons involving more than two measurements, one-way ANOVA, Bonferroni test, and post-hoc tests were employed. When parametric assumptions were not met, the Wilcoxon test was used to compare measurements from the same individuals under different conditions, and the Friedman test was applied to compare measurements involving more than two measurements. The significance level was set at $p < 0.05$.

RESULTS

As shown in Table 4, in all exercise trials conducted in a fasting state (FST), there were significant differences in repeated measurements: INS levels decreased between POST-FE and the second exercise post (POST-SE), TG levels decreased between POST-FE and PRE-SE, TG measurements increased between PRE-SE and POST-SE, COR measurements increased between PRE-SE and POST-SE, ALB measurements increased between PRE-FE and POST-FE, decreased between POST-FE and PRE-SE, and increased between PRE-SE and POST-SE ($p < 0.05$). Table 5 presents a comparison of blood parameters collected from participants during exercise trials under the PPD condition.

Table 4
The Statistical Comparison of Blood Levels Obtained From All Exercise Trials in the FST Condition (n=13).

FST		Mean±SD	p	Friedman Test		p
GLU (mg/dL)	PRE-FE	88.53±9.76	0.071			
	POST-FE	86.30±9.66				
	PRE-SE	85.00±8.80				
	POST-SE	82.00±13.65				
INS (µIU/mL)	PRE-FE	6.32±2.97	**0.0001***	PRE-FE	POST-FE	0.055
	POST-FE	4.37±2.70		POST-FE	PRE-SE	0.420
	PRE-SE	3.54±2.32		POST-FE>	PRE-SE	**0.0001***
	POST-SE	2.39±2.38		PRE-SE	POST-SE	0.055
COR (micg/dl)	PRE-FE	14.94±2.72	**0.0001***	PRE-FE	POST-FE	0.664
	POST-FE	13.53±4.01		POST-FE	PRE-SE	0.082
	PRE-SE	11.90±2.84		POST-FE	POST-SE	0.172
	POST-SE	16.43±5.17		PRE-SE<	POST-SE	**0.0001***
TG (micg/dl)	PRE-FE	67.23±26.24	**0.0001***	PRE-FEB	POST-FE	0.710
	POST-FE	71.61±23.89		POST-FE>	PRE-SE	**0.0001***
	PRE-SE	60.07±18.78		POST-FE	POST-SE	0.094
	POST-SE	78.38±17.88		PRE-SE<	POST-SE	**0.0001***
				Bonferroni Test		
ALB (g/dL)	PRE-FE	50.23±2.56	**0.0001***	PRE-FE<	POST-FE	**0.0001***
	POST-FE	52.37±0.98		POST-FE>	PRE-SE	**0.0001***
	PRE-SE	48.98±2.18		POST-FE	POST-SE	1.000
	POST-SE	52.54±1.22		PRE-SE<	POST-SE	**0.0001***

Note. GLU: Glucose, INS: Insulin, COR: Cortisol, TG: Triglyceride, ALB: Albumin, Mean ± SD: Mean ± Standard Deviation, mg/dl: Milligram/Deciliter, µIU/mL: Micro-International Units Per Millilite, micg/dl: Micrograms/Decilitre, g/DL: Gram/Decilitre <: Less than, >: Greater than, PRE-FE: Pre-first exercise, POST-FE: Post-first exercise, PRE-SE: Pre-second exercise, POST-SE: Post-second exercise, p*: p value between tests. Significance level: $p < 0.05$.

Table 5
Statistical Comparison of Blood Levels Obtained From All Exercise Trials in the PPD Condition Among Participants (n = 13)

FST	PDD	Mean±SD	p	Pairwise		p
GLU (mg/dL)	PRE-FE	93.92±15.45	**0.0001***	PRE-FEB	POST-FE	0.969
	POST-FE	93.84±6.41		POST-FE	PRE-SE	0.059
	PRE-SE	86.38±12.39		POST-FE>	POST-SE	**0.0001***
	POST-SE	84.92±5.78		PRE-SE	POST-SE	0.944
INS (µIU/mL)	PRE-FE	38.07±23.93	**0.0001***	PRE-FE>	POST-FE	**0.0001***
	POST-FE	15.61±17.21		POST-FE	PRE-SE	0.055
	PRE-SE	21.95±18.10		POST-FE	POST-SE	0.239
	POST-SE	7.63±14.37		PRE-SE>	POST-SE	**0.0001***
COR (micg/dl)	PRE-FE	13.43±4.01	0.107			
	POST-FE	11.04±2.42				
	PRE-SE	10.93±2.64				
	POST-SE	12.77±3.66				
				Bonferroni		
TG (mg/dL)	PRE-FE	114.75±62.44	0.489			
	POST-FE	127.46±52.13				
	PRE-SE	121.07±50.34				
	POST-SE	125.23±44.74				
ALB (g/dL)	PRE-FE	49.91±3.50	**0.0001***	PRE-FE	POST-FE	0.348
	POST-FE	51.60±2.80		POST-FE>	PRE-SE	**0.0001***
	PRE-SE	48.68±3.15		POST-FE	POST-SE	1.000
	POST-SE	51.70±2.87		PRE-SE<	POST-SE	**0.0001***

As shown in Table 6, when comparing the blood parameters measured in the PRE-FE, FST, and PPD conditions, there is a difference between the FST and PPD conditions regarding INS and TG levels. Similarly, when comparing the blood parameters measured in the POST-FE, FST, and PPD conditions, there are differences between the FST and PPD conditions regarding GLU, COR, and TG levels ($p<0.05$).

Table 6
Statistical Comparison of the Blood Parameters Before and After the First Exercise in the FST and PPD Conditions Among Participants (N=13)

Variables	FST (Mean±SD)	PPD(Mean±SD)	t	Z	P
Pre-First Exercise (PRE-FE)					
GLU(mg/dL)	88.53±9.76	93.92±15.45	-.978		0.348
INS(µIU/mL)	6.32±2.97	38.07±23.93	-5.113		**0.0001***
COR(micg/dl)	14.94±2.72	13.43±4.01	1.118		0.286
TG(mg/dL)	67.23±26.24	114.75±62.44		-2.132	**0.0001***
ALB(g/dL)	50.23±2.56	49.91±3.50	.293		0.774
Post-First Exercise (POST-FE)					
GLU(mg/dL)	86.30±9.66	93.84±6.41	-3.458		**0.0001***
INS(µIU/mL)	4.37±2.70	15.61±17.21		-1.818	0.069
COR(micg/dl)	13.52±4.01	11.04±2.42	3.004		**0.0001***
TG(mg/dL)	71.61±23.89	127.46±52.13	-3.950		**0.0001***
ALB(g/dL)	52.37±0.98	51.60±2.80	1.043		0.317

Mean ± SD: Mean ± Standard Deviation, Glucose, INS: Insulin hormone, COR: Cortisol hormone, ALB: Albumin enzyme, TG: Triglycerides, p*: p value between tests. Significance level: $p < 0.05$

As shown in Table 7, when comparing the blood parameters measured in the PRE-SE, FST, and PPD conditions, there is a difference between the FST and PPD conditions regarding INS and TG levels. Similarly, when comparing the blood parameters measured in the POST-SE, there are differences between the FST and PPD conditions regarding COR and TG levels ($p<0.05$).

Table 7
Statistical Comparison of the Blood Parameters Before and After the Second Exercise in the FST and PPD Conditions Among Participants (N = 13)

Variables	FST (Mean±SD)	PPD (Mean±SD)	t	Z	P
Pre-Second Exercise (PRE-SE)					
GLU(mg/dL)	85.00±8.80	86.38±12.39		-.035	0.972
INS(µIU/mL)	3.54±2.32	21.95±18.10	-3.723		**0.0001***
COR(micg/dl)	11.90±2.84	10.93±2.64	1.123		0.283
TG(mg/dL)	60.07±18.78	121.07±50.34	-4.789		**0.0001***
ALB(g/dL)	48.98±2.18	48.68±3.15	.352		0.731
Post-Second Exercise (POST-SE)					
GLU(mg/dL)	82.00±13.65	84.92±5.78	-.895		0.389
INS(µIU/mL)	2.39±2.38	7.63±14.37		-1.782	0.075
COR(micg/dl)	16.43±5.17	12.77±3.66	2.599		**0.0001***
TG(mg/dL)	78.38±17.88	125.23±44.74	-3.938		**0.0001***
ALB(g/dL)	52.54±1.22	51.70±2.87	1.331		0.208

DISCUSSION

Although it has been demonstrated that diet and exercise intensity can significantly alter skeletal muscle glycogen content, which in turn can affect exercise capacity (Ramonas et al., 2023; Guest et al., 2021), many questions remain unanswered regarding how the full and depleted states of liver and body glycogen reserves affect blood parameters related to energy metabolism during exercise. Therefore, this study investigated the acute effects of 60 minutes of aerobic endurance exercise at four different glycogen reserve levels: low liver glycogen FST, filled liver glycogen PPD, and body glycogen reserves that were either filled or low (partially reduced). The most significant findings related to energy metabolism are that GLU levels decreased following exercise with low body glycogen under the PPD condition and remained low after all other exercise trials. Additionally, TG levels increased following exercise with low body glycogen under the FST condition, whereas no significant increase in TG was observed in any exercise trials under the PPD condition.

It is known that as the duration of low to moderate intensity exercise increases, carbohydrate reserves decrease, leading to a reduction in carbohydrate oxidation (Potteiger, 2011). In our study, while the GLU level in the resting state for the filled liver glycogen PPD condition was not significantly higher than that in the state FST, it was observed to be higher nonetheless. Indeed, Turan (2010) reported higher GLU levels in the resting state for PPD compared to FST levels (Turan, 2010). After aerobic endurance exercise performed with filled body glycogen reserves, the GLU levels in the PPD condition were found to be significantly higher than in the FST condition ($p<0.05$; Table 6). Supporting our findings, de Lima et al. (2015) conducted a study on physically active individuals. They found that during moderate intensity aerobic exercise lasting over 30 minutes at 65% of VO_2max, GLU levels were maintained in the FST condition, while GLU concentration increased in the PPD condition (de Lima et al., 2015). In our study, a significant decrease in GLU levels was observed in the post-exercise state with low body glycogen (POST-SE) for the PPD condition ($p<0.05$; Table 5).

Bulut & Turnagöl (2018) conducted a study involving nine male triathletes with a mean age of 21.5±2.06 years, performing 60 minutes of aerobic endurance exercise under filled and low muscle glycogen reserves in FST and PPD conditions. They found that total carbohydrate oxidation significantly decreased after the second PPD exercise compared to the first PPD exercise (Bulut & Turnagöl, 2018). Although our participants were football players, the findings of Bulut & Turnagöl (2018) support our results.

According to Haub et al. (2003), maximal effort exercise in trained cyclists did not significantly differ in pre- and post-exercise blood GLU levels (Haub et al. 2003). In our study, under the FST condition, a non-significant decrease in blood GLU levels was observed following exercise performed with full and low body glycogen stores, indicating that GLU levels were maintained. Numerous studies conducted under FST conditions have reported that the exercise trials increase fat utilization without causing a significant change in GLU utilization (Van Proeyen et al., 2011). In the FST condition, where liver glycogen reserves are low, gluconeogenesis likely remains active, thereby preserving the already low blood GLU levels, particularly for glucose-dependent tissues such as brain tissue (Quintard et al., 2016).

Prolonged fasting leads to the inability of liver reserves to meet metabolic demands. (Hall & Hall, 2020). It has been reported that after an overnight fast, the blood GLU levels decrease, accompanied by a reduction in INS levels when no food is consumed (Atkinson et al., 2020). In our study, the significantly lower INS levels in the FST condition compared to the PPD condition, observed prior to exercises conducted with both filled and low body glycogen, align with the literature ($p<0.05$; Tables 6-7). In the FST condition, where INS levels were much lower, a linear decrease in blood INS was found, indicating that the reduction in POST-SE after exercise with low glycogen was greater compared to that with filled body glycogen ($p<0.05$) (Table 4). Conversely, in the PPD condition, which had higher INS levels, significant decreases were observed post-exercise compared to the aerobic endurance exercise trials conducted with both filled and low body glycogen reserves ($p<0.05$; Table 5). In a study by Chycki et al. (2019), involving 18 individuals (6 obese, 6 athletic, and 6 with endurance training), participants performed treadmill exercises for 20 minutes at 30% VO_2max, 10 minutes at 50% VO_2max, and 5 minutes at 70% VO_2max. They found that blood INS levels decreased across all groups ($p<0.05$; Chycki et al., 2019). Although the exercise protocols employed were significantly shorter than those employed in our study, the findings of Chycki and colleagues strongly support our results.

INS secretion inhibits COR, thereby enhancing carbohydrate entry into cells (Lin et al., 2012). On the other hand, during aerobic endurance exercise, free fatty acids (FFA) are stimulated by catecholamines such as epinephrine, norepinephrine (Jaworski et al., 2007), glucagon, and cortisol, which are derived from adipose tissue triglycerides (Birbrair et al., 2013; Lafontan & Langin, 2009). The opposing effects of the anabolic hormone INS and the catabolic hormone COR significantly impact glycogen reserves (Ferlazzo et al., 2020; Robyn et al., 2017). In the FST condition, the COR levels were found to be higher after aerobic endurance

exercises conducted with both filled and low body glycogen reserves compared to the PPD condition ($p<0.05$; Tables 6-7). It has been noted that there are no significant changes in COR levels during short-duration low-intensity exercises; however, a significant increase occurs following exercises performed at or above 60% of VO_2 max (Civan et al., 2018). In our study, while there were no significant changes in COR levels across all exercise trials in the PPD condition, a significant increase in COR was observed post-exercise in the FST condition compared to the PRE-SE with low body glycogen reserves ($p<0.05$; Table 4). Moreover, Terink et al., (2021) reported that exercising with reduced muscle glycogen reserves elevated FFA and COR levels between the 90th and 120th of exercise (Terink et al., 2021). Our findings, particularly the significant increase in POST-SE COR levels in the FST condition, align with the results of Terink et al., (2021). The data indicate that aerobic endurance exercises performed under FST conditions and with low body glycogen reserves increase COR levels due to metabolic stress. The rise in COR levels suggests the activation of gluconeogenesis to maintain blood GLU levels.

Ruíz-Moreno et al. (2020) stated that exercise intensity significantly affects substrate utilization, with intramuscular triglycerides (IMTG) contributing more to fat oxidation as exercise intensity increases. Their findings indicate that the contribution of IMTG to fat oxidation is particularly significant during moderate-intensity exercise, when fat oxidation rates peak (Ruíz-Moreno et al., 2020). Howard and Margolis, (2020) demonstrated that muscle triglycerides (TG) are significantly utilized during prolonged submaximal exercise (Howard & Margolis, 2020). In our study, the TG levels that converted to free FFA after lipolysis were found to be significantly higher in the PPD condition compared to the FST condition in all measurements taken before and after exercise ($p<0.05$; Tables 6-7). Turan (2010) also reported that resting TG levels were higher in the PPD condition than FST, supporting our findings of resting TG levels in both FST and PPD before exercise (Turan, 2010). Rothschild et al. (2021) found that fat oxidation rates remained significant even in a glycogen-depleted state, suggesting that intramuscular triglycerides (IMTG) are a crucial energy substrate during prolonged exercise. Their study further supported the notion that IMTGs are readily utilized as an energy source, as fat oxidation levels during moderate-intensity cycling were similar between fasting and protein-fed states (Rothschild et al., 2021). De Lima et al. (2015) found that in physically active individuals, plasma TG levels increased significantly more after FST exercise compared to PPD exercise during moderate-intensity aerobic trials performed at 65% of VO_2max for over 30 minutes ($p<0.05$; de Lima et al., 2015). Our study determined that

aerobic endurance exercises performed in the PPD condition, whether with filled or low body glycogen reserves, did not alter TG levels. However, the second aerobic endurance exercise performed in the FST condition with low body glycogen resulted in increased blood TG levels ($p<0.05$; Tables 4-5). Furthermore, Hulston et al. (2010) conducted a study with trained cyclists divided into low and high glycogen groups. The low group performed 90 minutes of aerobic endurance exercise at 70% VO_2max every other day, followed by eight bouts of five-minute high-intensity interval training (HIIT) one hour later. The high group performed aerobic endurance exercises on one day and HIIT on the next for three weeks. They found that during aerobic endurance exercise, the low muscle glycogen group showed a higher rate of FFA utilization in parallel with increased usage of muscle IMTG (Hulston et al., 2010). Although our participants were amateur football players, the significant increase in TG levels observed post-exercise in both the FST condition and the partially reduced body glycogen reserves aligns with the findings of Hulston et al. (2010), which noted a corresponding increase in lipolysis of muscle triglycerides in the low muscle glycogen group. Bulut & Turnagöl (2018) studied trained male triathletes and found that during 60 minutes of aerobic endurance exercises in the PPD and FST conditions, the FFA levels were higher in the PPD condition compared to those performed post-exercise with low glycogen reserves ($p<0.05$; Bulut & Turnagöl, 2018).

According to the findings, a significant increase in TG utilization was observed when exercise was performed with low glycogen reserves compared to filled glycogen reserves, At the same time, the same result was not found in the PPD condition. We believe the PPD condition positively affects glycogen reserves compared to the FST condition. Indeed, significant decreases in GLU measurements during exercise trials were observed in the PPD condition. In contrast, such decreases were not found in the FST condition, which already had lower GLU levels. This suggests that the PPD condition may help preserve glycogen reserves, albeit with a delay.

During exercise with low glycogen, an increase in circulating catecholamine levels is observed (López-Soldado et al., 2021). Elevated catecholamine levels increase fat metabolism by activating hormone-sensitive lipase (HSL) via protein kinase A. When HSL activity triggers lipolysis in adipose tissue and skeletal muscle, free FFA are released from both adipose and intramuscular tissues (Muscella et al., 2020). Once triglycerides are reduced to FFAs, they bind to plasma albumin and are transported to active tissues for energy use (Günay et al., 2018). There was no significant difference in ALB levels between FST and PPD conditions in all

measurements involving circulating FFA. Bulut & Turnagöl (2018) also found no significant differences between FST and PPD exercises in their study on male triathletes who performed 60 minutes of aerobic endurance exercise with filled and low glycogen (Bulut & Turnagöl, 2018). These findings support our results. It was determined that aerobic endurance exercises performed in both FST conditions with filled and low body glycogen reserves elevated blood ALB levels. In contrast, in the PPD condition, only the second aerobic exercise with low body glycogen reserves increased blood ALB levels ($p<0.05$; Tables 4-5).

The results indicate that in the FST condition, an increase in ALB levels after POST-FE coincided with an increase in the transport of FFAs. In the PPD condition, an increase in FFA transport was observed during POST-SE, parallel to the increase in ALB levels when body glycogen reserves were low.

Limitations

This study's limitations are that the participants were individuals who play amateur football, and the analysis of blood parameters, which are indicators of energy metabolism, was restricted to the current tests conducted at the Biochemistry Laboratory of Sivas Cumhuriyet University Hospital.

CONCLUSIONS

According to the findings obtained from aerobic endurance exercises performed under conditions of low liver glycogen reserves (FST) and filled satiety (PPD), as well as with filled and partially reduced (low) body glycogen reserves, the following conclusions can be drawn:

- *GLU Metabolism:* In the FST condition, where glycogen levels were low, GLU levels were maintained during both filled and low glycogen exercises, while a significant decrease was observed during low glycogen exercise in the PPD condition.
- *Insulin Levels*: Insulin levels decreased in the FST condition during low glycogen exercise. In the PPD condition, significant reductions in insulin levels were observed in both filled and low glycogen exercises.
- *Cortisol Levels:* Cortisol levels increased only during low glycogen exercise in the FST condition, stimulating gluconeogenesis. In the PPD condition, there were no changes in cortisol levels during either filled or low glycogen exercises.

- *Blood TG Levels:* Blood TG levels were higher in the PPD condition compared to the FST condition across all measurements. In the FST condition, low glycogen exercise increased TG levels, whereas no changes in TG levels were observed in both filled and low glycogen exercises during the PPD condition.
- *Albumin Levels:* Blood ALB levels increased in the FST condition during both filled and low glycogen exercises, whereas in the PPD condition, an increase was only noted during low body glycogen exercise.

Therefore, it can be concluded that aerobic endurance exercises performed by male amateur football players under conditions of filled liver glycogen (PPD) and low liver glycogen (FST), as well as with filled and low body glycogen reserves, resulted in the highest fat oxidation, particularly during FST and low glycogen exercises.

PRATICAL IMPLICATIONS

This study reveals the effects of glycogen levels on aerobic endurance exercises in male amateur football players. The findings suggest that coaches and athletes need to pay attention to glycogen status, providing a basis for enhancing athletic performance and reducing the risk of injuries. It is crucial for coaches to regularly monitor athletes' glycogen levels to optimize training. Customized training programs can be adjusted based on the athletes' glycogen status. Aerobic endurance exercises performed under low liver glycogen levels (fasted state) can be integrated to enhance fat utilization. Carbohydrate loading is important prior to critical competitions. Nutrition should aim to optimize glycogen stores. Nutritionists can develop individualized diet plans that consider glycogen levels before training. Future research should focus on evaluating the long-term effects of this training model and nutrition strategies on performance outcomes in football players. Additionally, the applicability of these strategies at different levels of competition should be examined.

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Authors' contributions

The first and second authors designed the manuscript; the first, third, fourth, fifth, and sixth authors were responsible for data collection, while the first author analyzed and interpreted the data. The first author also prepared the draft of the manuscript. All authors, except for the first author, performed a critical review, and all authors read and approved the final version of the manuscript.

Declaration of conflict interest

The authors have no conflicts of interest to disclose.

Ethics Statement

The study was approved by the Gazi University Non-Interventional Clinical Research Ethics Committee (Date: July 25, 2022, Decision No: 586).

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