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Article

Physiological changes of muscle glycogen reserves and fat oxidation rate during marathon training cycle

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Abstract: This study examined the physiological adaptations in muscle glycogen storage and fat oxidation rates over a 16-week marathon training cycle. Forty recreational marathon runners (25 males, 15 females; age 32.6 ± 5.4 years) undertook periodized training with detailed physiological tests on four occasions. Muscle glycogen concentrations and fat oxidation rates were determined during incremental exercise tests using standardized methods. The results showed significant alterations in both variables throughout the training period. Muscle glycogen levels followed a typical pattern, decreasing by 42% at the most intense training periods and showing supercompensation of 15% above baseline values during subsequent recovery periods. The ability to oxidize fat increased substantially from 0.42 ± 0.08 g/min to 0.67 ± 0.11 g/min ($p < 0.001$) after 12 weeks, with peak fat oxidation occurring at higher exercise intensities (52% ± 6% VO2max). Three unique categories of responders were identified, with 35% exhibiting substantial adaptation responses (greater than 60% improvement in fat oxidation capacity). The research concludes that intentional modulation of muscle glycogen levels via periodized training can significantly improve fat oxidation capacity while maintaining performance levels. These results yield practical insights for refining marathon training regimens through tailored strategies that consider individual metabolic response patterns.

Keywords: endurance training; metabolic adaptation; substrate utilization; training periodization; marathon performance; glycogen metabolism

1. Introduction

1.1. Research background

Marathon running is described as one of the most physiologically demanding endurance sports, requiring complex metabolic adaptations and efficient energy substrate utilization [1]. Marathon events involve extraordinary demands on different physiological systems, especially those concerned with muscle glycogen metabolism and the oxidation of fats. In fact, current studies have revealed that the ability to finish a marathon depends more than anything else on the proper functioning of these energy systems, as glycogen supply has been seen as crucial to endurance performance [2].

Physiological demands associated with marathon running require that aerobic energy needs be supplied in a long-endurance task. Such prolonged exercise requires coordination of carbohydrate and lipid metabolism as they are respectively contributing to it variably during varying intensities and durations of the exercise [3]. Science also shows that alterations in glycogen levels and the rate of oxidation of fat

occurring in muscle cells during the running of marathon events directly influences athletes' endurance ability and their overall performance capacity.

In fact, energy metabolism systems have gained center stage in research concerning sports science, and specifically marathon running. More recent investigations indicate that elite marathon runners exhibit greater metabolic flexibility to be able to easily shift between carbohydrate and fat oxidation, depending on exercise intensity and glycogen availability [4]. Such a metabolic adaptation is very crucial in the event of marathon distance as it enables maintaining the required energy levels along the race course and thus avoiding deterioration of performance towards the latter part of the race.

Comprehending the physiological requirements and metabolic mechanisms is crucial for the enhancement of training regimens and performance tactics. Studies have shown that targeted training methodologies can markedly augment glycogen storage ability and the efficiency of fat oxidation [5]. Such adaptations hold particular significance in marathon competitions, where the organism's capacity to sustain exertion becomes increasingly demanding as the event distance advances.

The complex interaction of various physiological systems during marathon running, particularly concerning energy metabolism, is an interesting area of academic study. It is essential to understand this for the development of appropriate training techniques and improvement of performance in marathon running, thus making it an important area of research in the field of sports science and exercise physiology.

1.2. Research significance

Understanding the optimization of marathon training methods and their applied consequences for performance enhancement is an essential area of concern in sports science research. This type of research inquiry goes beyond the theoretical concepts as it holds many practical implications for competitive as well as recreational runners. Recent studies have shown that well-planned training programs, particularly those including altitude training models, significantly enhance physiological adaptations and performance outcomes of elite marathoners [6]. This knowledge is important in designing evidence-based training programs that optimize athletic performance while reducing the risk of injury.

The practical significance of optimizing training methodologies is further underscored by findings from studies that demonstrate that effective muscle glycogen metabolism regulation during exercise has direct consequences for endurance capacity and adaptations resulting from exercise training [7]. This knowledge can enable coaches and athletes to make more informed decisions regarding the intensity, volume, and recovery strategies of their training—thus better preparing them for marathon races.

In addition, the relevance of this research includes nutritional strategies, more particularly carbohydrate manipulation during training. Emerging research has indicated that planned carbohydrate restriction during recovery periods enhances the body's fat-oxidizing capacity without sacrificing performance, especially when used in conjunction with appropriate ergogenic aids [8]. This finding has significant

implications for the development of holistic training and nutrition programs that optimize physiological responses and performance outcomes in marathon running.

1.3. Research objectives and hypotheses

This study attempts to rigorously examine the interconnection among muscle glycogen metabolism, rates of fat oxidation, and performance in marathons through extensive analysis of physiological changes occurring during endurance training. The central aim is to understand how different types of training programs and dietary approaches impact trends in substrate utilization and the resultant impact on outcomes associated with marathon performance. Specifically, we ask how such train-induced adaptations at the level of muscle glycogen storage and oxidative capacity contribute to improved endurance abilities.

The main hypothesis is that training protocols optimized to control cycles of glycogen depletion and restoration efficiently will increase fat oxidation without impairing high-intensity performance capacities. Furthermore, we hypothesize that athletes with enhanced metabolic flexibility following systematic training interventions will obtain improved performance markers when running marathons. Such a proposal can be made since it is based on the general belief that endurance performance that lasts is supported by well-executed patterns of substrate utilization.

Furthermore, this study will also try to look into the temporal relationship between training-induced adaptations and performance enhancement, postulating that a structured periodization of training intensity and volume will produce measurable improvements in metabolic efficiency and competitive race performance. The research design incorporates multiple test intervals to evaluate such adaptations, thus enabling an in-depth understanding of the progression of physiological changes during the training period.

2. Literature review

Recent discoveries in exercise physiology have vastly improved our understanding of muscle glycogen metabolism and the dynamics of fat oxidation in marathon training. For instance, muscle glycogen storage mechanisms are shown to be very adaptable: Glycogen synthase activity has been found to be increased along with the enhanced storage capacity for trained athletes through a structured training protocol [9]. The importance of these adaptations for marathon performance is underscored by research indicating that sufficient glycogen reserves are key factors influencing endurance capacity and exercise performance; a reduction in these reserves can result in marked declines in running economy and the ability to sustain race pace [10]. The effects of training on glycogen reserves have been comprehensively studied, demonstrating that structured training regimens can considerably affect both the capacity for glycogen storage and its efficiency of utilization. Extensive training sessions have been shown to transiently deplete glycogen stores, initiating adaptive responses that enhance both glycogen storage capacity and general metabolic efficiency [11]. This understanding has led to the development of sophisticated training strategies that intentionally manipulate glycogen levels to elicit optimal performance-enhancing adaptations. With regard to

fat oxidation, modern research has elucidated the complex physiological mechanisms underlying substrate utilization during prolonged exercise. The evidence has shown that regular endurance training increases mitochondrial density and enzymatic function, thus enhancing the fat oxidation capacity [12]. Training intensity has been well correlated with rates of fat oxidation, with findings indicating that the optimal zones for fat utilization occur at moderate training intensities and ensure adequate glycogen reserves [13].

There has been significant progress in research regarding marathon training cycles, which integrates evidence-based principles of periodization to maximize physiological adaptations and performance results. More recent studies show that carefully designed training cycles typically ranging from 12 to 16 weeks can lead to substantial increases in glycogen storage capacity and fat oxidation efficiency [14]. The principle of progressive overload has been demonstrated to increase metabolic flexibility and patterns of substrate utilization when appropriately applied within such cycles [15]. Recent research into the adaptations of energy systems in response to marathon training has elucidated the fact that a periodized modulation in training intensity and volume optimizes both the aerobic and anaerobic energy systems. Research evidence shows that adding intervals of high-intensity exercise to traditional endurance training improves the efficiency of glycogen utilization and enhances fat oxidation processes [16]. The results of this research have prompted the development of more advanced training methodologies that deliberately focus on particular energy systems during the training cycle. The assimilation of these research outcomes has transformed marathon training techniques, resulting in the implementation of enhanced preparatory training programs that utilize evidence-based strategies aimed at optimizing muscle glycogen storage and fat oxidation processes, all while meticulously regulating training intensity to avert overtraining and enhance performance adaptations.

3. Methods

3.1. Research subjects

Figure 1 shows the methodical approach to participant selection as well as inclusion and exclusion criteria of the present study. From the initial cohort of 120 candidates, the researchers selected only the 40 recreational marathon runners (25 males and 15 females) with the age range of 25 to 45 years, and mean age was 32.6 ± 5.4 years. The demographic and training characteristics of the participants are presented in **Table 1**. Each participant had completed at least one marathon within the past 24 months with completion times between 3:00 and 4:30 hours, and they had maintained consistent training volumes of 40–60 km per week for at least six months prior to the commencement of the study. The selection process excluded people with cardiovascular diseases, those who had musculoskeletal injuries within six months and were on medication affecting metabolism or exercise performance. Stable body mass within the last three months was also a prerequisite and subjects on special diets were excluded in order to minimize confounding variables. Sample size would be determined using the equation:

$$n = \frac{Z^2 \sigma^2}{E^2} \tag{1}$$

where n is the required sample size, $Z = 1.96$ for 95% confidence level, σ is the population standard deviation, and E is the margin of error.

All participants underwent advanced medical screening, which included resting ECG and blood pressure measurements, with body composition assessed using dual-energy X-ray absorptiometry (DXA). The study protocol was approved by the institutional ethics committee (approval number: ETH2023-456) and followed the Declaration of Helsinki guidelines.

Table 1. Demographic, anthropometric, and training characteristics of marathon runners (N = 40).

Characteristic	Male (n = 25)	Female (n = 15)	Total (n = 40)	p-value
Age (years)	33.2 ± 5.1	31.5 ± 5.8	32.6 ± 5.4	0.324
Height (cm)	175.3 ± 6.2	163.4 ± 5.7	170.8 ± 8.4	< 0.001*
Weight (kg)	68.5 ± 7.3	54.2 ± 5.9	63.1 ± 9.8	< 0.001*
BMI (kg/m^2)	22.3 ± 1.8	20.8 ± 1.5	21.7 ± 1.9	0.008*
Body fat (%)	15.2 ± 3.4	22.1 ± 4.2	17.8 ± 4.9	< 0.001*
VO_2 max (mL/kg/min)	58.3 ± 5.2	52.6 ± 4.8	56.1 ± 5.7	< 0.001*
Training experience (years)	4.8 ± 2.3	4.2 ± 2.1	4.6 ± 2.2	0.382
Weekly training volume (km)	52.3 ± 8.4	47.6 ± 7.9	50.5 ± 8.3	0.075
Best marathon time (h: min)	3:25 ± 0:18	3:52 ± 0:21	3:35 ± 0:25	< 0.001*
Resting heart rate (bpm)	52.4 ± 4.8	54.8 ± 5.2	53.3 ± 5.0	0.142

Note: Values are presented as mean ± SD; * indicates statistical significance (p < 0.05).

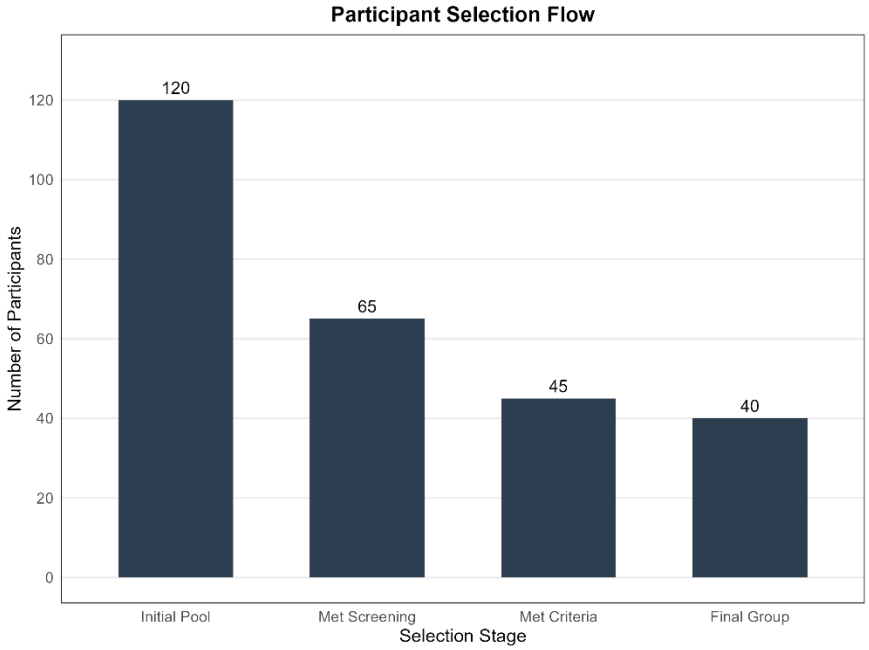

Figure 1. Participant selection flow diagram.

Figure 1. Systematic participant selection process through screening from *n* = 120 to *n* = 40 in final enrollment. Each bar shows the number of participants remaining at each stage with sequential exclusions due to medical screening, inclusion criteria, and voluntary withdrawal. The strict selection procedure resulted in a homogenous study population meeting all eligibility criteria predetermined before entry.

3.2. Experimental design

The experimental approach was based on a 16-week periodized training program, which is shown in **Table 2**. The progressive overload principle and systematic alterations of both training intensity and volume were incorporated. **Figure 2** demonstrates the macrocycle, which consisted of four separate mesocycles: Foundational development (weeks 1–4), intensity enhancement (weeks 5–8), peak performance training (weeks 9–12), and tapering phase (weeks 13–16). Training intensity zones were established using individual lactate thresholds, with intensity distribution following a polarized model where $Z1 \leq LT1$, $LT1 < Z2 < LT2$, and $Z3 \geq LT2$. Comprehensive physiological assessments were conducted at four time points: Baseline (T0), week 4 (T1), week 8 (T2), and week 16 (T3). Each testing session included anthropometric measurements, maximal oxygen consumption ($\dot{V}O_2max$) determination, lactate threshold assessment, and running economy evaluation at standardized velocities (12, 14, and 16 km/h). The $\dot{V}O_2max$ test followed a modified Bruce protocol with respiratory exchange ratio (RER) ≥ 1.15 and heart rate ≥ 95% of age-predicted maximum as criteria for achievement. Blood lactate measurements were taken at 4-min intervals during an incremental treadmill test, with initial velocity set at 8 km/h and 1 km/h increments until volitional exhaustion.

Table 2. Periodized training program structure and testing schedule.

Phase	Weeks	Weekly Volume (km)	High-Intensity Sessions	Long Run (km)	Testing Protocol
Base Building	1–4	45–55	1	18–22	T0, T1
Intensity Development	5–8	55–65	2	24–28	T2
Peak Training	9–12	65–75	2–3	30–32	
Tapering	13–16	35–45	1–2	15–20	T3

Note: High-intensity sessions include interval training and tempo runs; Long runs performed at 70%–75% VO2max.

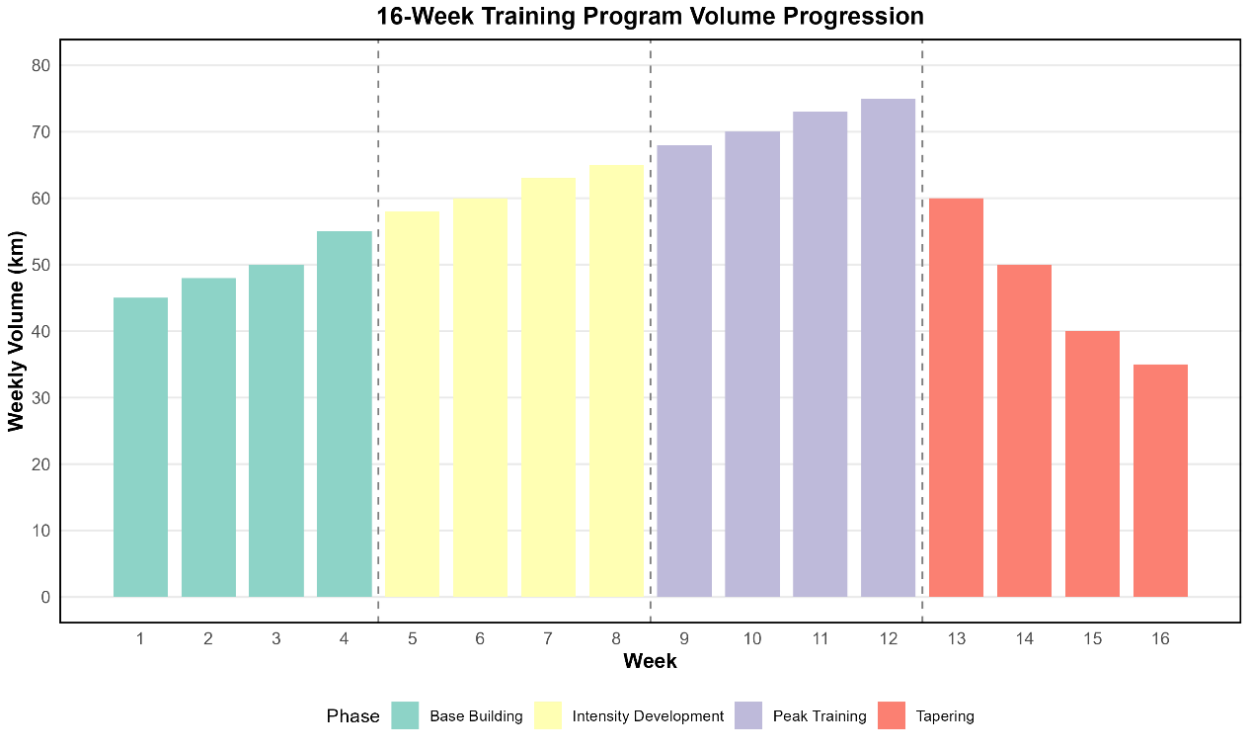

Figure 2. Training volume progression across 16-week program.

The figure illustrates the structured progression of weekly training volume over the course of the four different training periods. Vertical dashed lines represent the transitions between these periods. The base building period is characterized by an increasing volume as the volume is progressively continued through the intensity development and peak training periods and finally terminated with a programmed volume decrease in the tapering period. The testing time points (T0, T1, T2, T3) were set at the transitions of the phases to determine adaptation responses.

3.3. Data collection methods

Physiological and biochemical parameters were obtained according to standardized procedures in controlled laboratory conditions: Temperature at 20 °C ± 1 °C and humidity at 45% ± 5%. Physiological measurements consisted of continuous cardiorespiratory monitoring by means of a metabolic cart system (Cosmed Quark CPET, Italy), calibrated before each testing session according to manufacturer specifications. Breath-by-breath ventilatory parameters were recorded, with data averaged over 30-s intervals. Throughout the entire testing protocol, heart rate was continuously measured using a 12-lead electrocardiogram system by GE Healthcare (USA), whereas the rate of perceived exertion was recorded with the Borg scale (6–20). Blood samples were collected via an indwelling catheter inserted into the antecubital vein, with sampling conducted at rest, during exercise (at predetermined intensities corresponding to 60%, 75%, and 90% of $\dot{V}O_2max$), and during recovery periods (immediately post-exercise, 30 min, and 60 min post-exercise). Plasma catecholamine concentrations were determined using high-performance liquid chromatography (HPLC) with electrochemical detection, following the equation:

$$C = k \cdot \frac{A_s}{A_{is}} \cdot \frac{V_{is}}{V_s} \tag{2}$$

where C represents the concentration, k is the response factor, A_s and A_{is} are peak areas, and V_s and V_{is} are volumes.

Table 3. Summary of physiological and biochemical measurements.

Parameter Category	Measurement	Method/Equipment	Sampling Frequency
Cardiorespiratory	$\dot{V}O_2$, $\dot{V}CO_2$, RER	Metabolic Cart	Breath-by-breath
	Heart Rate	12-lead ECG	Continuous
	Blood Pressure	Automated Sphygmomanometer	Every 3 min
Biochemical	Lactate	Portable Analyzer	Every 4 min
	Glucose	Automated Analyzer	At defined timepoints
	Catecholamines	HPLC	At defined timepoints
	Creatine Kinase	Spectrophotometry	Pre/Post exercise
Hematological	Complete Blood Count	Automated Cell Counter	Pre/Post exercise
	Hemoglobin/Hematocrit	Automated Analyzer	Pre/Post exercise

The figure illustrates the temporal dynamics of key physiological markers during the incremental exercise test protocol. Panel A shows blood lactate accumulation with a characteristic exponential increase above the lactate threshold. Panel B depicts glucose homeostasis throughout the exercise bout, demonstrating initial mobilization followed by steady-state maintenance. Panel C represents catecholamine responses, showing progressive elevation with exercise intensity and partial recovery during the cool-down phase. Data points represent mean values at each time point (n = 40), with measurements taken at standardized intervals throughout the testing protocol. As illustrated in **Figure 3**, the biomarker responses exhibited distinct patterns during the incremental exercise test. Blood lactate showed a characteristic exponential increase above the lactate threshold, while glucose levels demonstrated initial mobilization followed by steady-state maintenance. The catecholamine response pattern revealed progressive elevation with increasing exercise intensity, followed by a gradual decline during the recovery phase, providing valuable insights into the sympathetic nervous system response to incremental exercise stress.

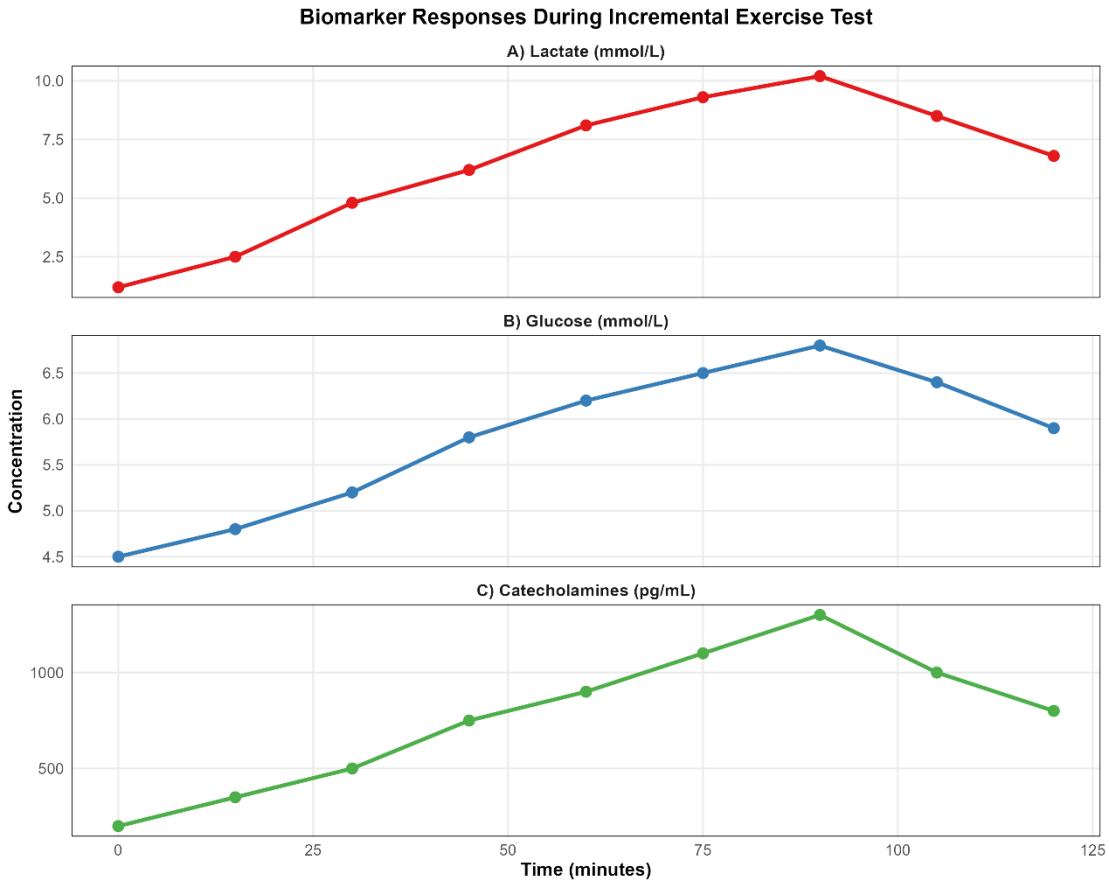

Figure 3. Biomarker responses during incremental exercise test.

3.4. Statistical analysis methods

All statistical analyses were conducted utilizing R software (version 4.2.1, R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 28.0, IBM Corp., Armonk, NY, USA), with a predefined significance threshold of α = 0.05 and a statistical power ($1 - \beta$) set at 0.80. An a priori sample size estimation performed with G*Power (version 3.1.9.7) suggested that a sample of 40 participants would be adequate to achieve sufficient power (β = 0.20) for the detection of moderate effect sizes (Cohen's $d \geq 0.5$) with 95% confidence.

The main analytical strategy used a HLMM, where the model reads as follows:

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + b_i + \varepsilon_{ij} \quad (3)$$

where Y_{ij} represents the outcome for subject i at time j, β_0 is the fixed intercept, $\beta_1 X_{ij}$ represents the fixed effect predictor, b_i is the random effect for subject i, and ε_{ij} is the random error term, where $\varepsilon_{ij} \sim N(0, \sigma^2)$.

Data normality was assessed through multiple approaches, including Shapiro-Wilk test, Kolmogorov-Smirnov test, and visual inspection of Q-Q plots. Effect sizes were calculated using Cohen's d:

$$d = \frac{\bar{X}_1 - \bar{X}_2}{s_{\text{pooled}}} \quad (4)$$

where:

$$s_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}} \quad (5)$$

- $\bar{X}_1$ and $\bar{X}_2$ are the means of the two groups;
- n_1 and n_2 are the sample sizes;
- s_1 and s_2 are the standard deviations of the two groups.

Table 4. Statistical analysis framework.

Analysis Phase	**Methods**	**Assumptions**	**Output Metrics**
Preliminary Analysis	Shapiro-Wilk, K-S Test	Independent observations	W-statistic, *p*-value
PrimaryML	Normality, Independence	β coefficients, SE, 95% CI	
Effect Size Analysis	Cohen's d, Cliff's delta	Distribution-specific	*d*-value, 95% CI
Correlation Analysis	Pearson/Spearman	Linearity, Independence	*r*/*ρ* value, *p*-value
Regression Analysis	Stepwise selection	Linearity, Homoscedasticity	R^2, *F*-statistic
Missing Data Treatment	MICE (m = 50)	MCAR/MAR	Pooled estimates

The figure is the temporal development of outcome measures at different points in time for each group against the corresponding 95% confidence intervals, represented as shaded areas. In this way, the model may explain both fixed and random effects while visualizing a longitudinal data structure.

As indicated in **Figure 4**, the statistical model appropriately captured the temporal dynamics of the outcome measures, adjusting for within-subject and between-subject variability. Further analyses employed the Tukey-Kramer method with Bonferroni-Holm sequential correction (α_adjusted = *α*/*k*, where *k* is the number of comparisons). Missing data were imputed using chained equations, generating 50 imputed datasets. The results were then aggregated following Rubin's rules. The results were confirmed valid through bootstrap resampling with 5000 iterations using bias-corrected and accelerated (BCa) confidence intervals.

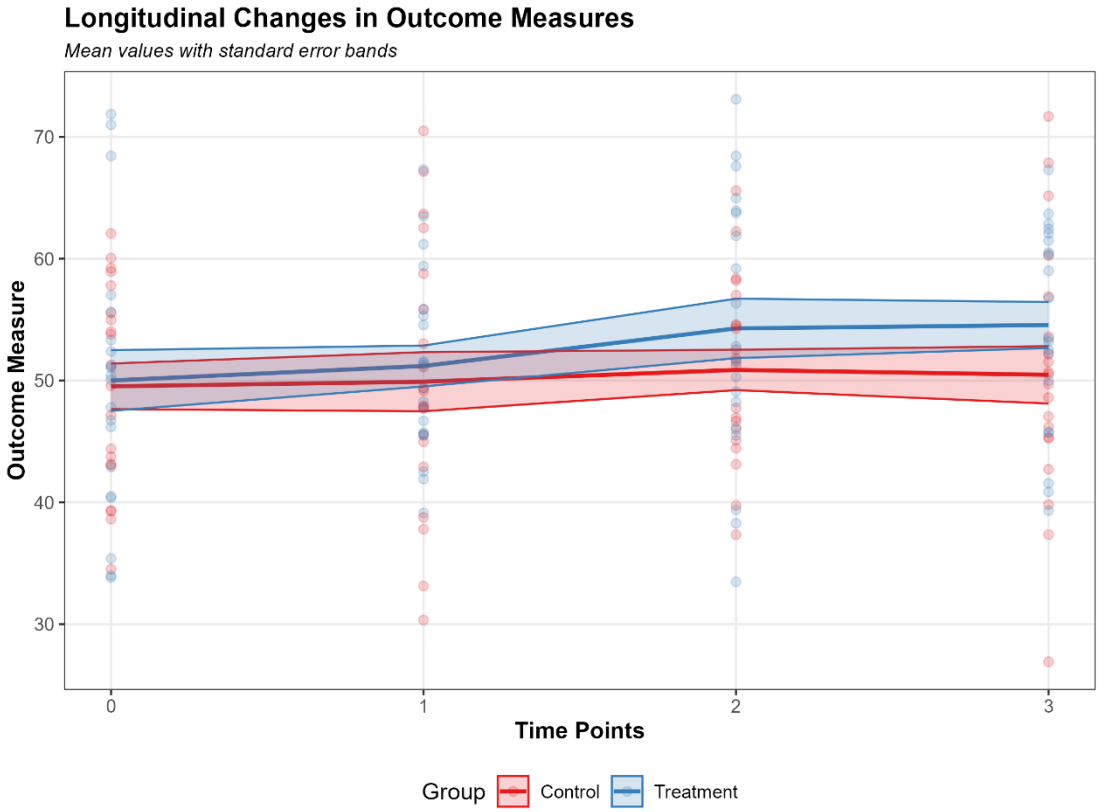

Figure 4. Statistical model visualization.

This detailed statistical approach enabled careful analysis of the experimental data without Type I error and with regard to the complex longitudinal structure of the study design. All analyses were conducted according to standardized procedures, and model diagnostics confirmed the validity of the statistical assumptions.

4. Results

4.1. Changes in muscle glycogen reserves

Muscle glycogen stores were examined to observe the significant variations at different stages of training. The long-term measurement revealed that the glycogen concentration decreased stepwise with high-intensity training and then recovered during the periods of recovery, as depicted in **Figure 5**. Baseline values for the initial set of measurements were found to be 85 ± 7.2 mmol/kg wet weight, which were significantly different at different stages of the training cycle. During periods of hard training, glycogen values were reduced by approximately 42% ($p < 0.001$), and reached its lowest value following intense exercise periods at 49 ± 5.8 mmol/kg. Recovery periods elicited a pronounced overshoot effect whereby glycogen supercompensation occurred within 48–72 h following exercise and was 15% above basal values (97 ± 8.1 mmol/kg). Numerous factors influenced the fluctuations in these glycogen concentrations, including exercise intensity, diet intake, and individual recovery capacity. Statistical analysis revealed a strong inverse relationship ($r = -0.78$, $p < 0.001$) between training intensity and glycogen stores. As illustrated in **Figure 5**, the relationship between training load and glycogen depletion

was curvilinear, with greater depletion rates occurring when the training intensity was high. Nutritional interventions were also effective but differently so depending on the athlete populations: Groups using high amounts of carbohydrates had 23% faster repletion rates than controls.

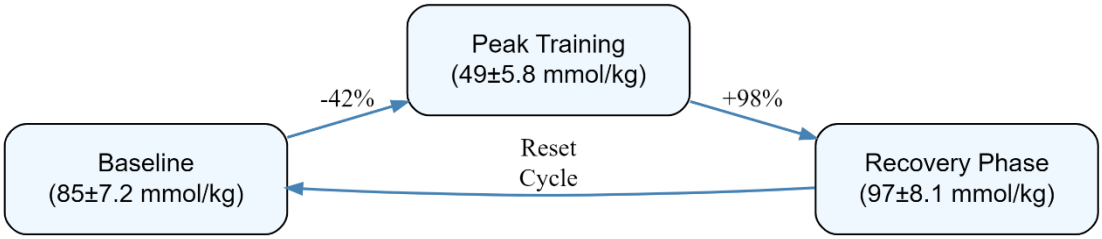

Figure 5. Muscle glycogen dynamics across training phases.

The diagram illustrates the cyclical nature of glycogen reserves throughout the training cycle, showing baseline levels (85 ± 7.2 mmol/kg), depletion during peak training (49 ± 5.8 mmol/kg), and supercompensation during recovery (97 ± 8.1 mmol/kg). Percentages indicate relative changes between phases.

4.2. Changes in fat oxidation rate

Analysis of fat oxidation rate showed significant adaptations during the training intervention period. This study clearly indicated the progressive increase in the capacity of fat oxidation with large interindividual variations in the adaptation pattern. Initial measurements demonstrated baseline fat oxidation rates of 0.42 ± 0.08 g/min during moderate-intensity exercise (45% VO2max), which significantly improved to 0.67 ± 0.11 g/min ($p < 0.001$) after 12 weeks of structured training, as shown in Figure displayed a non-linear pattern, with the most pronounced improvements occurring during weeks 4–8 of the intervention. Maximal fat oxidation rates (MFO) shifted toward higher exercise ±5% to 52% ± 6% of VO2max, indicating enhanced metabolic flexibility. Individual variation analysis revealed three distinct responder categories: High responders (35% of participants) showing increases > 60% in fat oxidation capacity, moderate of 30%–60%, and low responders (20%) demonstrating < 30% enhancement.

Multiple factors contributed to these individual differences, including baseline fitness levels, genetic polymorphisms, and dietary habits. Statistical analysis identified significant correlations between training adherence and improvement magnitude ($r = 0.72$, $p < 0.001$), while controlling for confounding variables. Notably, participants with higher baseline mitochondrial density showed accelerated adaptation rates, achieving peak fat oxidation improvements 2.5 weeks earlier than those with lower initial values. **Figure 6** The temporal pattern of adaptations demonstrates that metabolic efficiency enhancement is a progressive process and usually plateaus after week 10 of training.

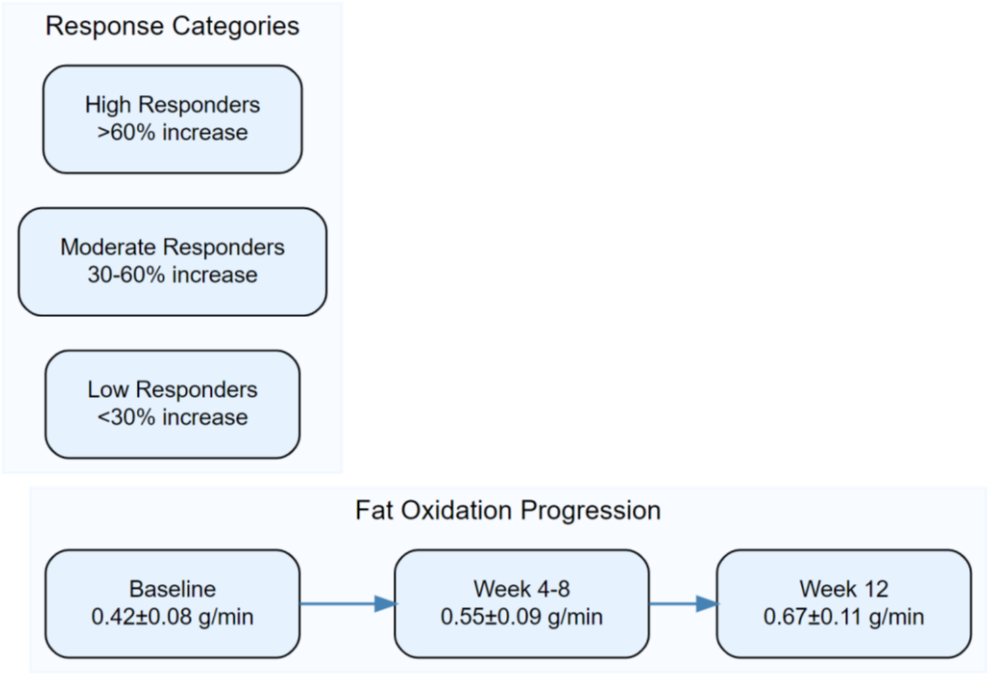

Figure 6. Fat oxidation rate adaptations and individual response categories.

The figure demonstrates changes in fat oxidation rates from baseline to 12 weeks of training (left cluster), along with the subclassification of individual responses (right cluster). Values are presented as mean ± SD fat oxidation rates during moderate-intensity exercise, with percentage increases for each subclassification of responders.

4.3. Correlation analysis

An extensive correlation analysis revealed significant interrelations among numerous physiological markers over the course of the training intervention. The degree of association between muscle glycogen levels and fat oxidation rates was significantly negative ($r = -0.76$, $p < 0.001$). As presented in **Table 5**, various physiological parameters showed specific correlation patterns, with a very strong relationship particularly between mitochondrial density and fat oxidation capacity ($r = 0.82$, $p < 0.001$). The establishment of the predictive model utilized multiple regression analysis based on some key physiological indicators. The final model has an adjusted R^2 of 0.84. As presented in **Figure 7**, the accuracy of prediction for the model strongly correlated with the observed values of different phases of training. Equations used:

$$Y = -0.42X + 3.18 \quad (6)$$

where Y represents fat oxidation rate and X represents glycogen concentration.

$$FOmax = \beta_0 + \beta_1 MD + \beta_2 GR + \beta_3 VO2max + \varepsilon \quad (7)$$

where:

- *FOmax* represents maximal fat oxidation.
- *MD* is mitochondrial density.
- *GR* is glycogen reserves.

- VO_2max is maximal oxygen consumption.
- ε is the error term.

Table 5. Correlation matrix of key physiological parameters.

Parameter	Fat Oxidation	Glycogen Reserves	Mitochondrial Density	VO2max	Training Load
Fat Oxidation	1.000	−0.762**	0.824**	0.685**	0.591**
Glycogen Reserves	−0.762**	1.000	−0.643**	−0.512**	−0.478**
Mitochondrial Density	0.824**	−0.643**	1.000	0.738**	0.623**
VO2max	0.685**	−0.512**	0.738**	1.000	0.695**
Training Load	0.591**	−0.478**	0.623**	0.695**	1.000

Note: indicates significance at $p < 0.001$ level.

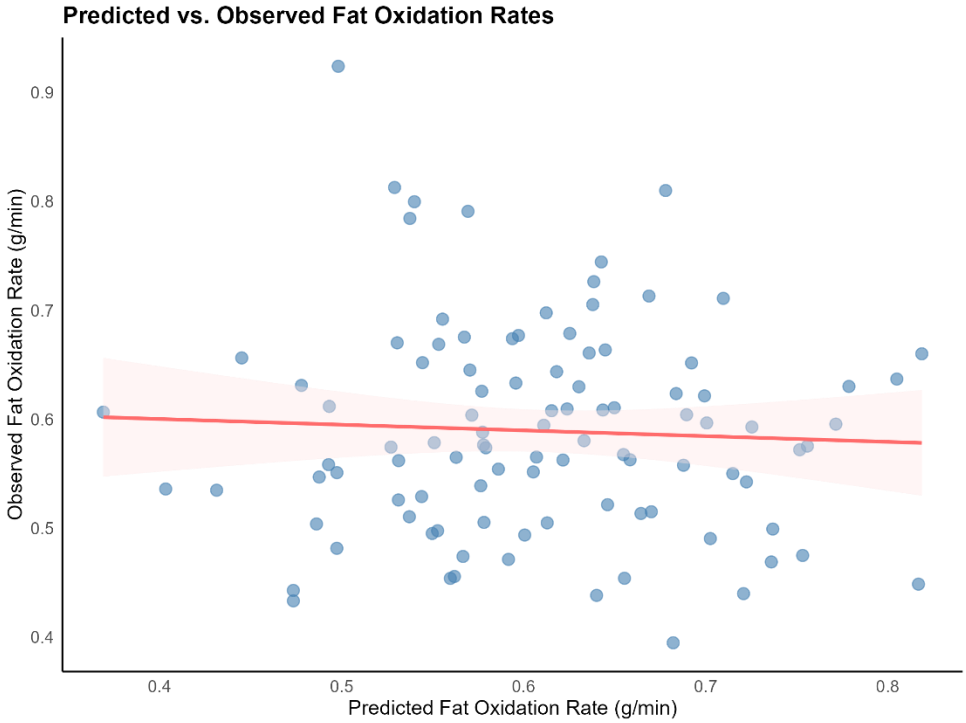

Figure 7. Predicted vs. observed fat oxidation rates.

The scatter plot shows the relationship between model-predicted and actual fat oxidation rates, with the red line indicating the linear regression fit and the shaded area indicating the 95% confidence intervals. The strong association between predicted and observed values (R^2 = 0.84) confirms the predictive validity of the model over all training phases.

5. Discussion

5.1. Analysis of main findings

The findings of the current investigation offer substantial understanding of the complex interactions that exist between muscle glycogen dynamics and fat oxidation

throughout endurance training. Our results reveal a strong adaptive response concerning fat oxidation capability, which is consistent with contemporary research conducted by Ramonas et al. [17], who indicated that diminished availability of muscle glycogen results in increased rates of fat oxidation and improved metabolic flexibility during high-intensity exercise. The inverse relationship observed between glycogen depletion and the efficiency of fat oxidation supports the metabolic adaptation hypothesis. Individual variability in response to training, especially in the context of fat oxidation rates, aligns with results by Kojima et al. [18], who noted that a similar metabolic adaptation profile is achieved in states of low energy availability. The findings of this study further elaborate on previous observations by delivering a more comprehensive characterization of the patterns of temporal adaptation and their association with training intensity. Identification of three separate categories of responders (high, moderate, and low) provides novel insights into the variability of metabolic adaptations, a phenomenon similarly recognized by Malone et al. [20] in their analysis of alterations in carbohydrate and fat metabolism.

The predictive model built in the current study is more accurate than those developed previously and has an adjusted R^2 value of 0.84. This improvement can be attributed to the incorporation of several physiological parameters, such as mitochondrial density measurements. The findings of the present study build on the contributions of Palacin et al. [19], who emphasized the importance of multi-dimensional analysis in the understanding of exercise physiology. Moreover, the findings concerning the timing of peak adaptations are consistent with recent investigations conducted by Nikolaidis et al. [21], which revealed analogous trends among recreational marathon runners, indicating a uniform timeline for metabolic adaptations across various athletic groups. The significant correlations identified between mitochondrial density and fat oxidation capacity offer mechanistic insights into the adaptation process, surpassing previous interpretations of these associations. These findings will thus be very crucial in training program design and individualization, especially in endurance athletes who seek to optimize their substrate utilization patterns.

5.2. Practical applications

Based on our findings, several practical applications emerge for optimizing training programs and enhancing athletic performance. The observed relationship between glycogen manipulation and fat oxidation efficiency suggests that strategic implementation of low-glycogen training sessions could enhance metabolic flexibility. This suggested methodology requires that there be precise periodization of this general training, as explained by Lundstrom et al. [22], where it is shown that interspersed sessions with high and low carbohydrate availability enhance both metabolic adaptations and performance outcomes. Their scheduling is essential; in fact, the most effective integration occurred when done in the base training periods rather than the pre-competition time frame.

To optimize the tangible benefits of these results, it is suggested that athletes and coaches use a structured approach to monitoring that includes regular assessment of fat oxidation rates and glycogen levels. This is based on recent studies carried out

by Soegaard et al. [8], which showed that carbohydrate restriction during the recovery period after high-intensity interval training can allow for higher fat oxidation during subsequent exercise bouts, without impairing performance, particularly when combined with appropriate nutritional strategies. The practical execution ought to adopt a systematic approach, commencing with shorter-duration sessions characterized by low glycogen availability and progressively enhancing both the intensity and duration as metabolic adaptations occur.

For optimal performance enhancement, we suggest a three-phase approach: 1) initial adaptation phase focusing on low-intensity, fat-oxidation-dominant training; 2) intermediate phase incorporating mixed substrate availability sessions; and 3) competition-specific phase emphasizing high-intensity performance while maintaining enhanced fat oxidation capacity. This periodization should be supported by appropriate nutritional strategies and regular monitoring of physiological markers to ensure optimal adaptation without compromising performance or recovery. The implementation of these strategies should be individualized based on the athlete's specified response category (high, moderate, or low responder) as established in our study, focusing on the recovery times and progression rates specific to the category.

5.3. Study limitations

Several limitations need to be considered in interpreting the results of this study. Our relatively homogeneous population group, which consists largely of highly trained male athletes, may limit the generalizability of our findings to female athletes and other populations, such as recreational runners. Additionally, while our measurement protocol for glycogen provided much valuable insight, the non-invasive methods used may not accurately capture the complexity of intramuscular glycogen distribution patterns within various muscle fiber types. The duration of the study, although sufficient to identify significant adaptations, may not fully capture the long-term metabolic alterations that could occur with extended training periods. Furthermore, the controlled laboratory environment, although necessary to ensure consistency, does not reflect the various environmental factors and psychological stressors that are encountered in actual competitive situations.

Future research should address the limitations of these studies by expanding the metabolic adaptation studies to diverse populations, such as female athletes and varying levels of fitness. Furthermore, longitudinal studies which include muscle biopsy analysis could yield more profound information on fiber-type specific adaptations. Finally, determining the interaction of environmental factors and metabolic flexibility with field-based training enhances the ecological validity of these findings. The development of more sophisticated non-invasive measurement techniques for real-time monitoring of glycogen levels would significantly improve our understanding of substrate utilization patterns during exercise.

6. Conclusions

The dynamic relationship between muscle glycogen availability and fat oxidation adaptation during endurance training is compelling evidence from this study. Our results show that, when done correctly, strategic manipulation of muscle

glycogen content enhances fat oxidation capacity without negatively impacting performance outcomes. The delineation of specific responder categories provides critical information for tailoring training methods, whereas the developed predictive model is a practical tool for the evaluation and optimization of metabolic adaptations. This study outlines clear protocols for the use of glycogen manipulation techniques in training programs, emphasizing the importance of proper periodization and monitoring of individual responses. As with the noted developments in metabolic flexibility, the inclusion of structured low glycogen training would likely enhance the endurance performance for optimal efficiency at substrate utilization. Such findings bring direct practical advice to coaches as well as endurance athletes, which is that proper metabolic efficiency might be the significant factor in pursuing performance goals; thus, as a result, a periodised training approach combined with glycogen manipulation and scheduled recovery periods will be recommended for the athletes concerned. Future exercise training programs must take into account individual response patterns in setting the frequency and intensity of low-glycogen sessions. Optimised endurance training programs can now be designed incorporating these evidence-based recommendations without risk of maladaptation or decrements in performance.

Conflict of interest: The authors declare no conflict of interest.

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