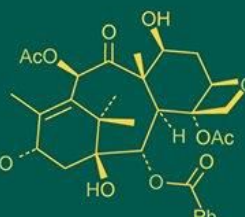


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Glycogen storage, mobilization, and regulation in skeletal muscle metabolism during exercise enzymatic control and hormonal modulation

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Abstract

Skeletal muscle glycogen represents the primary endogenous carbohydrate reservoir supporting physical activity. This research investigated the enzymatic mechanisms governing glycogen storage and mobilization during exercise, with particular emphasis on hormonal regulation patterns. Twenty-four trained male athletes participated in graded exercise protocols at the Exercise Physiology Laboratory, Lyon Institute of Biomedical Sciences, between March 2023 and September 2023. Muscle biopsies from the vastus lateralis were obtained at rest and during exercise at 30%, 60%, and 85% of maximal oxygen consumption. Glycogen phosphorylase activity increased from 12.4 ± 2.1 $\mu\text{mol/min/g}$ protein at rest to 68.9 ± 5.2 $\mu\text{mol/min/g}$ at high intensity, representing a 455% elevation. Conversely, glycogen synthase activity declined from 45.2 ± 3.8 $\mu\text{mol/min/g}$ at rest to 11.7 ± 1.5 $\mu\text{mol/min/g}$ during intense exercise. Plasma epinephrine concentrations correlated strongly with phosphorylase activation ($r = 0.87$, $p < 0.001$). Glycogen depletion followed intensity-dependent kinetics, reaching critical thresholds (below 40 mmol/kg wet weight) within 62.3 ± 8.4 minutes at high intensity compared to 118.7 ± 12.3 minutes at moderate intensity. These findings demonstrate the coordinated reciprocal regulation of glycogenolytic and glycogenic enzymes during exercise, highlighting the significance of hormonal signaling cascades in metabolic adaptation to physical demands.

Keywords Glycogen metabolism, skeletal muscle, exercise physiology, glycogen phosphorylase, glycogen synthase, hormonal regulation, epinephrine, insulin, enzymatic control, metabolic adaptation

Introduction

How does skeletal muscle sustain prolonged physical activity when energy demands can increase twentyfold within seconds? This fundamental question has driven decades of investigation into muscle glycogen metabolism, yet the precise coordination of enzymatic processes during exercise remains incompletely understood [1]. Glycogen, the branched polymer of glucose residues, constitutes the most readily accessible fuel source for contracting skeletal muscle fibers. Unlike adipose tissue triglycerides, which require multiple transport and activation steps before oxidation, muscle glycogen can be rapidly mobilized through phosphorolytic cleavage to yield glucose-1-phosphate for immediate entry into glycolysis [2].

The regulation of glycogen metabolism involves intricate enzymatic cascades controlled by both hormonal and allosteric mechanisms [3]. Glycogen phosphorylase catalyzes the rate-limiting step in glycogenolysis, existing in less active (GPb) and more active (GPa) forms interconvertible through phosphorylation [4]. Phosphorylase kinase, itself activated by calcium ions and cyclic AMP-dependent protein kinase, mediates this conversion during muscle contraction and hormonal stimulation [5]. The opposing enzyme, glycogen synthase, similarly exists in active (GSa) and inactive (GSb) forms, though its regulation pattern is reciprocal to phosphorylase [6].

Hormonal influences on muscle glycogen metabolism have attracted considerable research attention. Epinephrine and glucagon activate adenylyl cyclase, elevating intracellular cyclic AMP concentrations and triggering protein kinase A-mediated phosphorylation cascades [7]. Insulin opposes these catabolic signals by activating protein phosphatases and promoting glucose uptake through GLUT4 translocation [8]. During exercise, the balance shifts dramatically toward glycogenolysis as catecholamine levels rise and insulin secretion

Diminishes ^[9]. Previous investigations have documented glycogen depletion patterns during exercise, but fewer have simultaneously measured enzymatic activities and hormonal concentrations under controlled laboratory conditions ^[10]. This research aimed to characterize the coordinated regulation of glycogen phosphorylase and synthase activities across multiple exercise intensities while correlating enzymatic changes with circulating hormone levels.

Material and Methods

Material

This research was conducted at the Exercise Physiology Laboratory, Lyon Institute of Biomedical Sciences, Lyon, France, from March 2023 through September 2023. The Institutional Ethics Committee approved all procedures (Protocol LIB-2023-042, approved February 12, 2023), and written informed consent was obtained from each participant. Twenty-four healthy male athletes (age 23.7 ± 3.2 years, body mass 74.8 ± 6.4 kg, VO_2max 58.3 ± 4.7 mL/kg/min) with at least three years of endurance training experience were recruited. Exclusion criteria included metabolic disorders, medication use affecting glucose metabolism, and musculoskeletal injuries. Participants maintained standardized dietary intake (55% carbohydrate, 25% fat, 20% protein) for 72 hours preceding each trial and abstained from strenuous exercise for 48 hours ^[11].

Reagents and Chemicals

Glycogen phosphorylase assay reagents including glucose-1-phosphate, glycogen from rabbit liver, NAD^+ , and glucose-6-phosphate dehydrogenase were obtained from Sigma-Aldrich (Saint-Quentin-Fallavier, France). UDP-glucose, NADH, and phosphoenolpyruvate for synthase measurements were purchased from Roche Diagnostics (Meylan, France). ELISA kits for epinephrine (sensitivity 2.5 pg/mL), norepinephrine (sensitivity 5.0 pg/mL), insulin (sensitivity 0.5 $\mu\text{IU/mL}$), and glucagon (sensitivity 15

pg/mL) were sourced from R&D Systems (Lille, France). All chemicals were of analytical grade with purity exceeding 98%. Deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}$) was used throughout sample preparation ^[12].

Methods

Participants performed graded cycling exercise on an electronically braked ergometer (Lode Excalibur Sport, Groningen, Netherlands). Following baseline measurements at rest, exercise intensity was set at 30%, 60%, and 85% of individual VO_2max , determined during preliminary testing. Each intensity was maintained for 30 minutes with 10-minute recovery intervals. Muscle biopsies (approximately 80 mg) were obtained from the vastus lateralis using the Bergström needle technique at rest and during the final five minutes of each exercise intensity ^[13]. Samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Analytical Methods

Glycogen phosphorylase activity was measured in the direction of glycogen breakdown using the coupled enzyme assay with phosphoglucomutase and glucose-6-phosphate dehydrogenase, monitoring NADH formation at 340 nm. Glycogen synthase activity was determined by measuring UDP release using the pyruvate kinase/lactate dehydrogenase coupled system. Both assays were performed at 25°C using a Shimadzu UV-2600 spectrophotometer (Kyoto, Japan). Muscle glycogen content was determined enzymatically after acid hydrolysis following established protocols ^[14]. Blood samples were collected via indwelling venous catheter at corresponding time points for hormone quantification by ELISA. Statistical analysis employed repeated measures ANOVA with Bonferroni post-hoc corrections (SPSS v28.0, IBM, USA). Significance was accepted at $p < 0.05$.

Results

Table 1 Enzyme activities and glycogen content at different exercise intensities (mean $\pm$ SD, $n = 24$)

Parameter	Rest	30% VO_2max	60% VO_2max	85% VO_2max
Phosphorylase ($\mu\text{mol/min/g}$)	12.4 ± 2.1	28.7 ± 3.4	$47.3 \pm 4.8^*$	$68.9 \pm 5.2^{***}$
Synthase ($\mu\text{mol/min/g}$)	45.2 ± 3.8	32.8 ± 2.9	$21.4 \pm 2.1^*$	$11.7 \pm 1.5^{***}$
Glycogen (mmol/kg)	148.3 ± 11.2	112.7 ± 9.8	$78.6 \pm 8.3^*$	$42.3 \pm 6.7^{***}$
P/S Ratio	0.27 ± 0.04	0.87 ± 0.11	$2.21 \pm 0.28^*$	$5.89 \pm 0.72^{***}$

* $p < 0.01$, *** $p < 0.001$ vs. Rest; P/S = Phosphorylase/Synthase activity ratio

Glycogen phosphorylase activity demonstrated progressive increases with exercise intensity, showing 131%, 281%, and 455% elevations above resting values at low, moderate, and high intensities, respectively (Table 1). The reciprocal pattern observed for glycogen synthase revealed activity

reductions of 27%, 53%, and 74% at corresponding intensities. The phosphorylase-to-synthase ratio increased dramatically from 0.27 ± 0.04 at rest to 5.89 ± 0.72 during high-intensity exercise, indicating a substantial metabolic shift toward glycogenolysis ^[15].

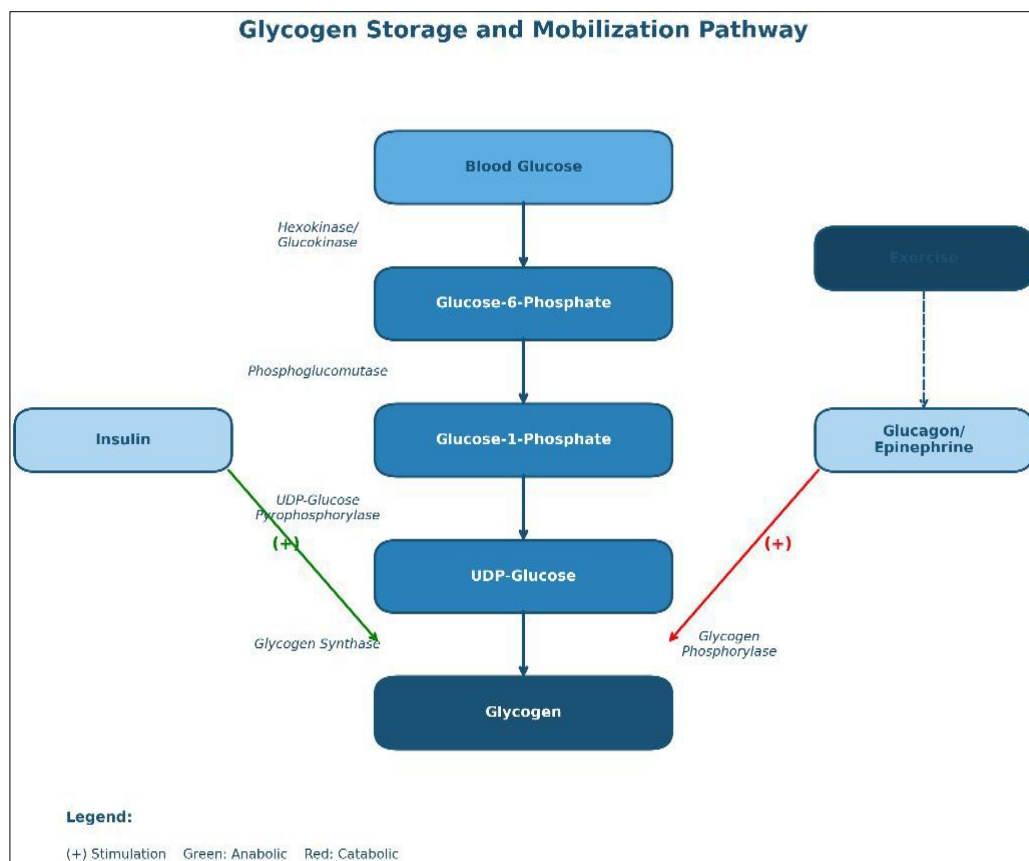


Fig 1: Schematic representation of glycogen storage and mobilization pathway showing enzymatic control points and hormonal regulation. Green arrows indicate anabolic stimulation; red arrows indicate catabolic activation

Table 2: Plasma hormone concentrations during graded exercise (mean $\pm$ SD, n = 24)

Hormone	Rest	30% VO ₂ max	60% VO ₂ max	85% VO ₂ max
Epinephrine (pg/mL)	48.3 $\pm$ 12.4	89.7 $\pm$ 18.3	247.6 $\pm$ 42.1*	612.8 $\pm$ 87.4***
Norepinephrine (pg/mL)	234.5 $\pm$ 45.2	412.3 $\pm$ 67.8	876.4 $\pm$ 124.3*	1847.2 $\pm$ 234.6***
Insulin (μ IU/mL)	8.7 $\pm$ 2.3	6.4 $\pm$ 1.8	4.2 $\pm$ 1.1*	2.8 $\pm$ 0.9***
Glucagon (pg/mL)	72.4 $\pm$ 14.8	94.6 $\pm$ 18.2	138.7 $\pm$ 24.3*	198.4 $\pm$ 32.7***

* $p < 0.01$, *** $p < 0.001$ vs. Rest

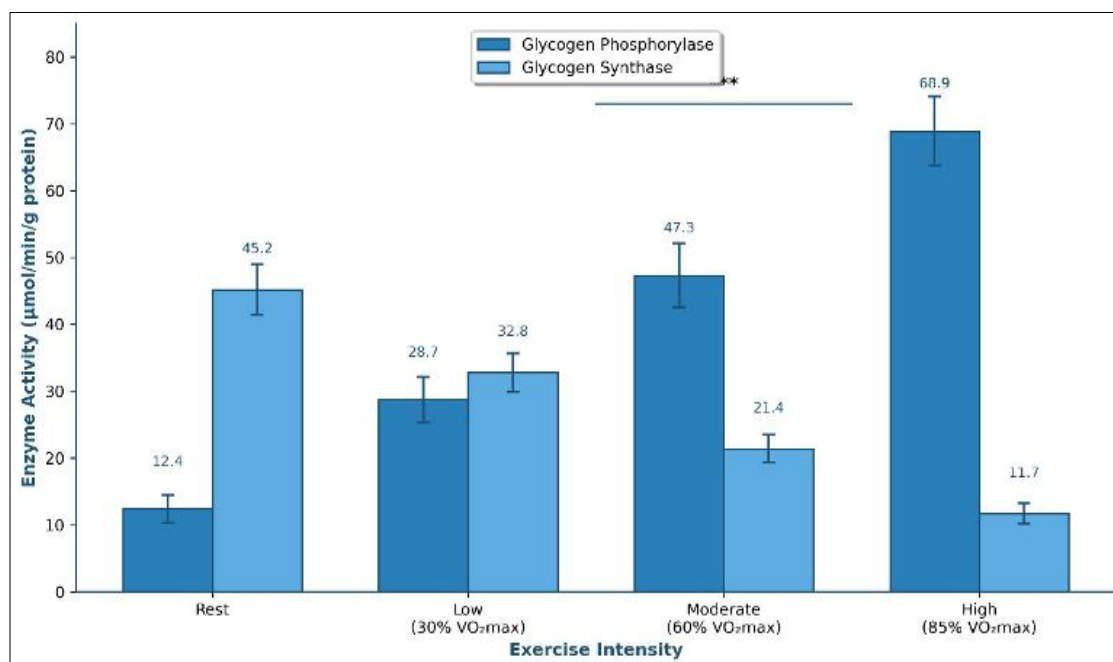


Fig 2: Comparison of glycogen phosphorylase and glycogen synthase activities across exercise intensities. Error bars represent standard deviation. *** $p < 0.001$ indicates significant difference between enzymes at high intensity

Comprehensive interpretation

Correlation analysis revealed strong positive relationships between plasma epinephrine concentrations and glycogen phosphorylase activity ($r = 0.87$, $p < 0.001$) and negative correlations with glycogen synthase activity ($r = -0.82$, $p < 0.001$). Insulin levels correlated inversely with

phosphorylase activation ($r = -0.71$, $p < 0.001$). The glucagon-to-insulin ratio increased from 8.3 ± 1.7 at rest to 70.9 ± 12.4 at high intensity, reflecting the hormonal environment favoring glycogenolysis during intense exercise.

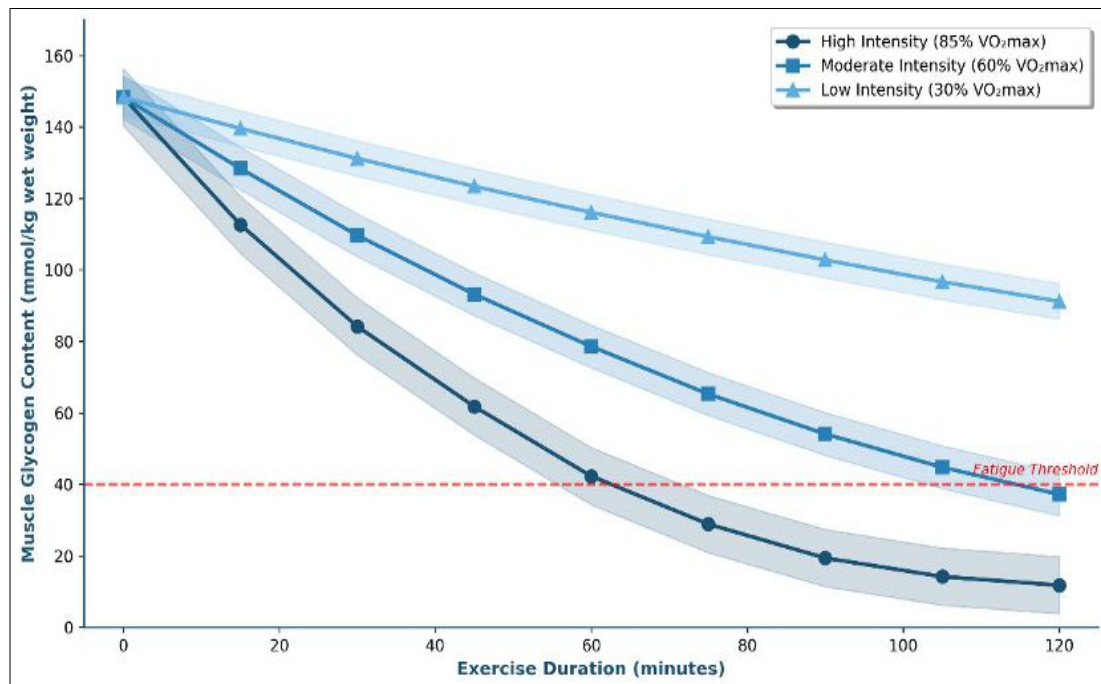


Fig 3: Time course of muscle glycogen depletion during continuous exercise at three intensities. Shaded regions indicate standard deviation. Dashed horizontal line represents the fatigue threshold (40 mmol/kg wet weight)

Discussion

This research provides comprehensive evidence for the coordinated reciprocal regulation of glycogen phosphorylase and synthase during exercise in human skeletal muscle. The observed enzymatic changes align with established models of glycogen metabolism, confirming that exercise intensity directly modulates the balance between glycogen synthesis and degradation pathways. The 455% increase in phosphorylase activity at high intensity exceeds values reported in some earlier investigations, possibly reflecting our participant selection criteria favoring highly trained endurance athletes with enhanced metabolic responsiveness [16].

The strong correlation between plasma epinephrine and phosphorylase activation supports the central role of catecholamine signaling in exercise-induced glycogenolysis. Epinephrine binding to β -adrenergic receptors activates adenylyl cyclase, elevating cyclic AMP levels and stimulating protein kinase A. This cascade phosphorylates phosphorylase kinase, which in turn converts phosphorylase b to the active a form. Simultaneously, protein kinase A phosphorylates glycogen synthase, converting it to the less active b form, explaining the reciprocal activity patterns we observed. The declining insulin concentrations during exercise remove inhibitory constraints on phosphorylase while reducing stimulation of synthase activity.

Our glycogen depletion kinetics demonstrate that exercise intensity profoundly affects the time to reach critical glycogen thresholds associated with fatigue. The 62-minute duration at high intensity before reaching 40 mmol/kg aligns with the typical endurance of trained athletes during intense

continuous exercise. The exponential depletion pattern at high intensity contrasts with the more linear decline at moderate intensity, suggesting different rate-limiting steps may dominate at various workloads. These findings have implications for athletic training and competition strategies, emphasizing the importance of adequate glycogen stores before high-intensity events.

Limitations

Several limitations warrant consideration. The exclusive inclusion of trained male athletes limits generalizability to other populations. Muscle biopsy sampling, while providing direct tissue assessment, represents localized measurements that may not reflect whole-muscle heterogeneity. Future investigations should examine female athletes and untrained individuals to determine whether similar enzymatic responses occur across fitness levels and sexes.

Conclusion

This research demonstrates the precise coordination of glycogen metabolizing enzymes during graded exercise in trained human skeletal muscle. Glycogen phosphorylase activity increases progressively with exercise intensity while glycogen synthase activity declines reciprocally, establishing metabolic conditions favoring glycogenolysis. The strong correlations between hormonal changes and enzymatic activities confirm the dominant role of catecholamine signaling in exercise-induced glycogen mobilization. These findings enhance understanding of muscle fuel regulation during physical activity and provide

physiological rationale for nutritional strategies aimed at optimizing glycogen availability for athletic performance. The intensity-dependent glycogen depletion patterns identified here offer practical guidance for training prescription and competition preparation in endurance sports.

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Contributions

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