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CHANGES IN ALDOLASE, LDH, CPK AND MYOGLOBIN BLOOD LEVELS IN MIDDLE-AGED MEN AFTER ONE HOUR BRISK TREADMILL WALKING OVER A PERIOD OF FOUR MONTHS

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Abstract: Regular physical activity induces adaptive biochemical responses in skeletal muscle. Enzymes including aldolase, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), along with oxygen-binding protein myoglobin, serve as established indicators of muscle metabolism, membrane integrity, and exercise-induced stress. However, longitudinal behavior of these biomarkers during sustained moderate-intensity exercise remains incompletely characterized. To evaluate temporal changes in serum aldolase, LDH, CPK, and myoglobin levels in middle-aged men following a structured four-month treadmill-based brisk walking program. Prospective interventional study was conducted on 40 sedentary men aged 40–55 years. Participants performed supervised treadmill walking for one hour daily, five days per week, over four months. Blood samples were obtained at baseline and at monthly intervals. Biochemical parameters were analyzed using standardized enzymatic and immunoassay techniques. Significant elevation in CPK, LDH, and myoglobin was observed after one month ($p < 0.05$), followed by progressive decline and stabilization, with values approaching or falling below baseline by month four. Aldolase demonstrated minimal variation throughout study duration. Moderate-intensity aerobic exercise induces an initial phase of muscle stress followed by adaptive stabilization, reflected by normalization of biochemical markers. Findings support safety and physiological benefits of structured walking programs in middle-aged men.

Key words: Brisk walking, CPK, LDH, Aldolase, Myoglobin, Exercise adaptation, Middle-aged men

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INTRODUCTION

Regular physical activity represents a cornerstone in prevention and management of metabolic and cardiovascular disorders, particularly in middle-aged populations where risk of sarcopenia, insulin resistance, and cardiovascular disease progressively increases. Among various exercise modalities, moderate-intensity aerobic activity such as brisk walking remains widely advocated due to practicality, safety profile, and consistent evidence demonstrating improvements in cardiovascular function, metabolic regulation, and overall longevity (Warburton *et al*., 2006; ACSM, 2018; World Health Organization, 2023).

At cellular level, exercise imposes mechanical and metabolic demands on skeletal muscle, initiating a cascade of biochemical responses that reflect both acute stress and long-term adaptation. Circulating enzymes such as creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and aldolase serve as indirect markers of muscle membrane permeability and metabolic flux. Elevations in these enzymes typically indicate disruption of sarcolemma integrity or increased enzymatic turnover associated with heightened muscular activity (Brancaccio *et al*., 2007). Myoglobin, a cytoplasmic heme protein involved in intracellular oxygen transport, is rapidly released into circulation following muscle fiber perturbation and is regarded as a sensitive early indicator of muscle injury (Clarkson & Hubal, 2002).

Recent investigations have refined understanding of these biomarkers by emphasizing influence of exercise intensity,

duration, and training status. Longitudinal data suggest that sustained moderate exercise not only attenuates baseline enzyme levels but also enhances mitochondrial efficiency and structural resilience of muscle fibers. Adaptation is further supported by evidence demonstrating reduced oxidative stress and improved sarcolemmal stability with repeated aerobic activity.

Emerging research also highlights interaction between systemic inflammation, redox balance, and muscle enzyme kinetics. Structured aerobic programs have been shown to reduce inflammatory mediators alongside normalization of muscle-derived biomarkers, suggesting integrated systemic and muscular adaptation (Khan, *et al*., 2022). Furthermore, attenuation of myoglobin release with continued training reflects improved intracellular oxygen handling and reduced mechanical disruption of muscle fibers (So-ichiro *et al*., 2020). Despite growing body of evidence, longitudinal studies focusing specifically on non-athletic, middle-aged individuals engaged in moderate-intensity exercise remain limited. Much of existing literature is derived from athletic populations or high-intensity protocols, restricting applicability to general population.

Present study was therefore designed to evaluate temporal changes in aldolase, LDH, CPK, and myoglobin over four months in middle-aged men undergoing structured brisk treadmill walking, with emphasis on adaptive biochemical responses.

MATERIALS AND METHODS

Study Design

Prospective interventional design was employed over a four-month period to investigate biochemical responses of skeletal muscle to sustained moderate-intensity exercise. Study population comprised forty sedentary but otherwise healthy male participants aged 40-55 years. Rigorous screening ensured exclusion of individuals with cardiovascular disease, musculoskeletal disorders, metabolic abnormalities, or recent history of structured physical training. Participants receiving medications known to influence muscle metabolism were also excluded. Ethical compliance was maintained throughout study, and written informed consent was obtained from all participants.

Exercise intervention

Exercise intervention consisted of a standardized, supervised treadmill walking protocol. Participants performed brisk walking for one hour per day, five days per week. Exercise intensity was maintained at 60–70% of maximum heart rate, ensuring consistency within moderate aerobic range as recommended by American College of Sports Medicine guidelines (ACSM, 2018). Continuous heart rate monitoring was utilized to ensure adherence, while supervised sessions minimized inter-individual variability.

Blood collection and biochemical analysis:

Venous blood samples were collected at baseline and subsequently at end of each month for four months. To minimize acute exercise-induced fluctuations, sampling was performed 24 hours after last exercise session. This approach allowed assessment of stable biochemical adaptations rather than transient post-exercise changes.

Biochemical analysis was conducted using standardized laboratory methods. Serum aldolase, Creatinine Kinase, Lactate dehydrogenase and myoglobin has been estimated as per standard method referred in Table 1.

Table 1: Methods of analysis and references of serum enzymes and myoglobin in Middle-Aged Men Performing One Hour of Brisk Treadmill Walking Over a Period of Four Months.

Marker	Roche Instrument	Method Type	Principle	Reference
Aldolase	cobas c503 / c501	Enzymatic UV assay	Cleavage of F-1,6-bisphosphate → G3P + DHAP	Siekmann Clinical Enzymology. EJIFCC. 2001
Creatine Kinase (CK)	cobas c503 / c501	UV kinetic assay	Creatine + ATP → Phosphocreatine + ADP (coupled NADH detection)	Schumann *et al.* 2002
Lactate Dehydrogenase (LDH)	cobas c503 / c501	UV assay (IFCC)	Lactate + NAD^+ → Pyruvate + NADH	Schumann *et al.*, 2002
Myoglobin	cobas e801	Immunoassay (ECL)	Sandwich immunoassay with biotinylated and ruthenium-labeled antibodies	Roche Diagnostics. Myoglobin assay on cobas e 801

Statistical analysis

Statistical analysis was performed using repeated measures analysis of variance (ANOVA) to evaluate temporal changes within study group. Data were expressed as mean ± standard deviation. Statistical significance was defined as $p < 0.05$

RESULTS AND DISCUSSION

Observed biochemical trends demonstrate a distinct biphasic response characterized by early muscle stress followed by progressive adaptation. Baseline values for all parameters were within physiological limits, indicating absence of underlying muscle pathology.

At one month, significant elevations were noted in CPK, LDH, and myoglobin ($p < 0.05$), reflecting acute muscular response to initiation of exercise. CPK increased from 120 ± 15 U/L to 180 ± 20 U/L, LDH from 190 ± 22 U/L to 240 ± 25 U/L, and myoglobin from 45 ± 10 ng/mL to 80 ± 12 ng/mL (Table 2). These changes are indicative of increased sarcolemmal permeability and metabolic demand associated with unaccustomed physical activity. Aldolase exhibited only marginal, non-significant variation.

Subsequent months demonstrated progressive decline in enzyme levels. By second month, reductions in CPK, LDH, and myoglobin suggested early adaptation, likely mediated by improved muscle fiber stability and repair mechanisms. This trend continued through third month, with values approaching baseline.

At fourth month, CPK and LDH levels fell slightly below baseline, while myoglobin returned to near initial levels. Aldolase remained stable throughout study period, suggesting limited sensitivity to moderate exercise-induced stress.

Table 2: Changes in Blood Levels of Aldolase, LDH, CPK, and Myoglobin in Middle-Aged Men Performing One Hour of Brisk Treadmill Walking Over a Period of Four Months

Parameter	Baseline	Month 1	Month 2	Month 3	Month 4
CPK (U/L)	120 ± 15	180±20*	150±18	130±16	110± 14
LDH (U/L)	190 ± 22	240±25*	210±20	195±18	180± 17
Aldolase (U/L)	6.5 ± 0.8	7.0 ± 0.9	6.8±0.7	6.6±0.8	6.4± 0.7
Myoglobin (ng/mL)	45 ± 10	80 ± 12*	60 ± 11	50 ± 9	46 ± 8

*Significant increase compared to baseline ($p < 0.05$)

DISCUSSION

Findings illustrate characteristic transition from acute exercise-induced muscle stress to chronic physiological adaptation. Initial elevation of CPK, LDH, and myoglobin reflects disruption of muscle membrane integrity and increased metabolic turnover, particularly in previously sedentary individuals (Clarkson & Hubal, 2002; Brancaccio *et al*., 2007).

Magnitude of CPK elevation underscores its sensitivity as marker of muscle damage. Exaggerated early response aligns with reduced sarcolemmal stability in untrained individuals, as described in recent longitudinal analyses (Haller *et al*., 2023). Progressive decline observed in subsequent months supports adaptive strengthening of muscle membrane and reduced enzyme leakage.

LDH dynamics further reinforce metabolic adaptation. Initial increase corresponds to enhanced glycolytic activity, while subsequent normalization indicates improved mitochondrial efficiency and shift toward aerobic metabolism (Smith *et al*., 2023).

Transient rise in myoglobin reflects early muscle fiber disruption, with rapid normalization indicating improved structural integrity and oxygen utilization efficiency. Attenuation of myoglobin response with training has been linked to enhanced intracellular oxygen handling (Fakuda *et al.*, 2020).

Aldolase stability throughout study suggests limited responsiveness to moderate-intensity exercise, consistent with its relatively lower sensitivity compared to other muscle enzymes (Khan *et al*., 2022).

Observed pattern strongly supports concept of repeated bout effect, wherein repeated exposure to identical exercise stimulus results in reduced biochemical evidence of muscle damage (McHugh, 2003). Recent mechanistic study attribute this phenomenon to improved antioxidant capacity, reduced inflammatory signaling, and enhanced muscle repair pathways (Baxter *et al*., 2023).

Notably, reduction of enzyme levels below baseline by fourth month suggests improvement in baseline muscle health, potentially reflecting reduced subclinical muscle damage and enhanced cellular integrity (Guo, 2024).

From clinical standpoint, findings emphasize that transient elevation in muscle enzymes during early phases of exercise should be interpreted cautiously and not mistaken for pathological conditions. This has direct relevance in clinical practice, where mildly elevated enzyme levels in physically active individuals may otherwise prompt unnecessary diagnostic interventions.

Limitations include absence of control group, relatively small sample size, and lack of control over dietary intake, hydration, and muscle fiber composition variability. Future research incorporating larger, more diverse populations and molecular markers would further clarify mechanisms underlying exercise-induced adaptation.

CONCLUSION

Sustained moderate-intensity treadmill walking induces predictable biochemical response characterized by early transient elevation of muscle injury markers followed by progressive normalization and stabilization. This adaptive response reflects improved muscle integrity, metabolic efficiency, and overall physiological resilience. Structured walking programs represent safe and effective intervention for promoting musculoskeletal and metabolic health in middle-aged populations.

Conflict of interest

Authors declare no conflict of interest.

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