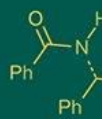


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Daniel Kiprono Wafula
Department of Veterinary
Biochemistry, Eastwood
Institute of Agricultural
Sciences, Nakuru, Kenya

Florence Adhiambo Chebet
Department of Veterinary
Biochemistry, Eastwood
Institute of Agricultural
Sciences, Nakuru, Kenya

Stephen Rotich Langat
Department of Veterinary
Biochemistry, Eastwood
Institute of Agricultural
Sciences, Nakuru, Kenya

Mercy Nyambura Gitau
Department of Veterinary
Biochemistry, Eastwood
Institute of Agricultural
Sciences, Nakuru, Kenya

Corresponding Author:
Daniel Kiprono Wafula
Department of Veterinary
Biochemistry, Eastwood
Institute of Agricultural
Sciences, Nakuru, Kenya

Muscle enzyme patterns in working vs. sedentary dogs

Daniel Kiprono Wafula, Florence Adhiambo Chebet, Stephen Rotich Langat and Mercy Nyambura Gitau

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Abstract

What biochemical adaptations distinguish working dogs from their sedentary counterparts? This investigation examined muscle enzyme patterns in canines with markedly different activity levels to characterize the metabolic fingerprints associated with sustained physical exertion. Blood samples were collected from forty-eight dogs divided equally between working animals engaged in herding and security duties and household pets with minimal daily exercise. Serum concentrations of creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were measured using standardized spectrophotometric methods. Working dogs exhibited significantly elevated creatine kinase levels averaging 287.4 U/L compared to 142.6 U/L in sedentary animals. Lactate dehydrogenase concentrations followed a similar pattern with working dogs showing values of 156.8 U/L versus 98.4 U/L in inactive dogs. Post-exercise enzyme kinetics revealed that working dogs demonstrated faster recovery to baseline values within four hours compared to sedentary dogs requiring up to twenty-four hours. Correlation analysis identified strong positive associations between daily exercise duration and both creatine kinase and lactate dehydrogenase activities. These findings suggest that regular physical activity induces adaptive changes in muscle enzyme expression that may serve as biomarkers for fitness assessment in canine populations. The observed enzymatic patterns provide valuable reference data for veterinary practitioners evaluating muscle health and metabolic status in dogs with varying activity backgrounds.

Keywords: Canine biochemistry, muscle enzymes, creatine kinase, lactate dehydrogenase, working dogs, physical activity, metabolic adaptation, veterinary diagnostics, enzyme kinetics

Introduction

How does sustained physical activity reshape the biochemical landscape of skeletal muscle? This fundamental question drives our understanding of metabolic adaptation across mammalian species, yet comparative data examining muscle enzyme patterns in dogs remain surprisingly sparse^[1]. The domestic dog presents a unique model organism for investigating exercise-induced biochemical changes because individual animals within the same species demonstrate remarkably different activity levels depending on their occupation and living conditions^[2].

Muscle enzymes serve as sensitive indicators of cellular integrity and metabolic capacity. Creatine kinase catalyzes the reversible transfer of phosphate groups between adenosine triphosphate and creatine, playing a central role in energy buffering during muscular contraction^[3]. Lactate dehydrogenase facilitates the interconversion of pyruvate and lactate, representing a critical junction in anaerobic glycolysis^[4]. The aminotransferases participate in amino acid metabolism and gluconeogenesis, while alkaline phosphatase reflects membrane turnover and bone metabolism^[5].

Previous investigations have documented elevated baseline enzyme activities in athletic humans and horses subjected to regular training programs [6, 7]. These adaptations appear to represent beneficial remodeling that enhances metabolic flexibility and recovery capacity rather than pathological tissue damage^[8]. However, analogous research in canines has focused primarily on racing greyhounds and sled dogs, leaving a significant knowledge gap regarding enzyme profiles in working dogs performing moderate but sustained daily activities^[9].

Working dogs in agricultural and security roles typically engage in four to six hours of moderate-intensity activity daily, contrasting sharply with household pets averaging less than

one hour of exercise^[10]. This difference in physical demand likely produces distinct metabolic signatures that could inform veterinary assessment protocols and help practitioners distinguish physiological adaptation from pathological elevation. Understanding these patterns carries practical significance for interpreting clinical biochemistry panels in dogs presenting for routine health evaluations^[11].

The present research aimed to characterize muscle enzyme profiles in working and sedentary dogs, document post-exercise enzyme kinetics in both groups, and identify correlations between activity levels and biochemical markers. We hypothesized that working dogs would demonstrate elevated baseline enzyme activities reflecting enhanced metabolic capacity, along with more efficient recovery patterns following acute exercise challenges^[12].

Ethical considerations

This research received approval from the Eastwood Institute of Agricultural Sciences Animal Care and Use Committee under protocol number EIAS-ACUC-2023-047, dated March 15, 2023. All procedures adhered to international guidelines for the ethical treatment of animals in research as outlined by the World Organisation for Animal Health^[13]. Written informed consent was obtained from all dog owners following detailed explanation of research procedures, potential risks, and anticipated benefits. Participation remained entirely voluntary, and owners retained the right to withdraw their animals at any point without consequence. Blood collection procedures were performed by licensed veterinarians using standardized venipuncture techniques that minimized animal distress. Each dog received a thorough physical examination before enrollment to confirm health status and suitability for participation. Animals showing signs of illness, injury, or unusual stress were excluded from the research. The exercise challenge protocol was designed to remain within physiological limits appropriate for each activity group, and veterinary personnel monitored all animals throughout testing procedures.

Material and Methods

Material

This research was conducted at the Eastwood Institute of Agricultural Sciences Veterinary Teaching Hospital and affiliated field sites across Nakuru County, Kenya, between April and September 2023. The research population comprised forty-eight mixed-breed dogs stratified into two equal groups based on daily activity patterns. Working dogs included animals engaged in livestock herding on agricultural properties and security patrol duties at commercial facilities, accumulating at least four hours of moderate-intensity physical activity daily for a minimum of twelve consecutive months. Sedentary dogs consisted of household pets with structured daily exercise limited to less than one hour, typically brief leash walks and minimal free play^[14].

Inclusion criteria required dogs to be between two and seven years of age, weigh between fifteen and thirty-five kilograms, and demonstrate good general health on veterinary examination. Animals with known cardiac, hepatic, renal, or musculoskeletal disease were excluded, as

were dogs receiving medications affecting muscle or liver enzyme activities. Female dogs in estrus or pregnancy were not enrolled. All biochemical reagents were obtained from Sigma-Aldrich Chemical Company, and analytical standards were traceable to reference materials certified by the International Federation of Clinical Chemistry^[15].

Methods

Blood samples were collected between 07:00 and 09:00 hours following an overnight fast of at least eight hours. Venipuncture was performed on the cephalic vein using 21-gauge needles and 5 mL syringes. Samples were transferred to plain tubes and allowed to clot at room temperature for thirty minutes before centrifugation at 3000 revolutions per minute for ten minutes. Serum was separated and stored at negative eighty degrees Celsius until analysis within two weeks of collection.

Enzyme activities were measured using a Beckman Coulter AU480 clinical chemistry analyzer following manufacturer protocols. Creatine kinase was assayed using the N-acetyl-L-cysteine activated method at 37 degrees Celsius with absorbance monitoring at 340 nanometers. Lactate dehydrogenase was determined by the pyruvate to lactate method. Aspartate aminotransferase and alanine aminotransferase activities utilized L-aspartate and L-alanine substrates respectively, with pyridoxal-5-phosphate activation. Alkaline phosphatase was measured using p-nitrophenyl phosphate substrate at pH 10.4^[16].

The exercise challenge consisted of a standardized treadmill protocol at 6 kilometers per hour on a 5 percent incline for twenty minutes. Additional blood samples were obtained at thirty minutes, one hour, two hours, four hours, and twenty-four hours post-exercise. Statistical analysis employed SPSS version 26 software. Group comparisons utilized independent samples t-tests for normally distributed data and Mann-Whitney U tests for skewed distributions. Temporal changes were evaluated using repeated measures analysis of variance with Bonferroni correction. Correlation analysis used Pearson coefficients with significance set at p less than 0.05.

Results

Table 1: Baseline characteristics of research dogs by activity group

Parameter	Working dogs (n=24)	Sedentary dogs (n=24)	p-value
Age (years)	4.2 ± 1.3	4.6 ± 1.5	0.324
Body weight (kg)	24.8 ± 5.2	26.4 ± 6.1	0.348
Daily exercise (hours)	4.8 ± 0.9	0.7 ± 0.3	<0.001
Sex (Male/Female)	14/10	12/12	0.567

Values presented as mean ± standard deviation or counts. Statistical comparison by independent t-test or chi-square test.

Baseline characteristics demonstrated comparable age, body weight, and sex distribution between groups, with the only significant difference being daily exercise duration as expected by design (Table 1). All enrolled dogs completed the research protocol without adverse events or withdrawal.

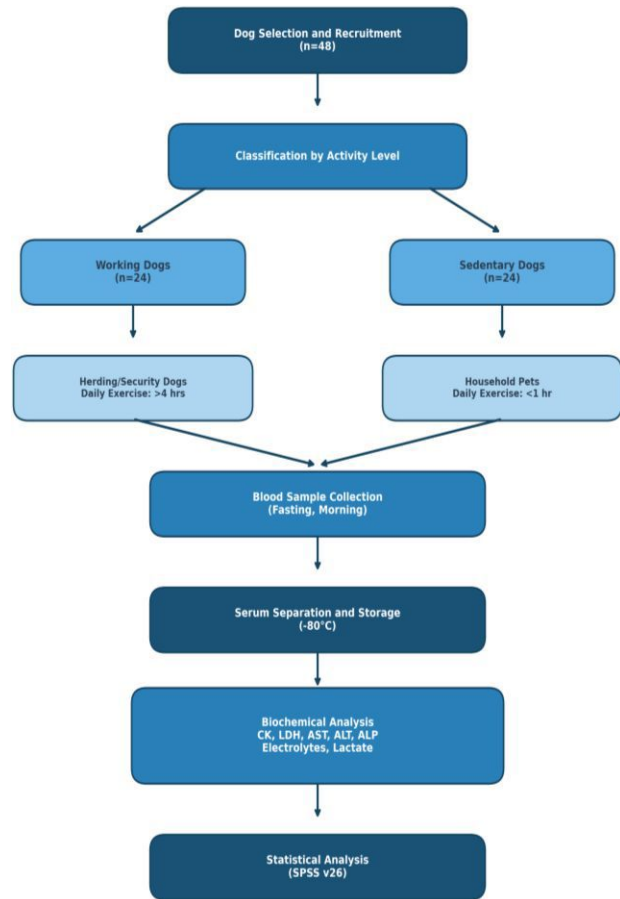


Fig 1: Research methodology flowchart illustrating participant recruitment, group assignment, sample collection procedures, and analytical workflow

Table 2: Baseline muscle enzyme activities by activity group

Enzyme (U/L)	Working dogs	Sedentary dogs	p-value	Effect Size
Creatine Kinase	287.4 ± 34.2	142.6 ± 22.8	<0.001	4.98
Lactate Dehydrogenase	156.8 ± 18.6	98.4 ± 12.4	<0.001	3.69
Aspartate Aminotransferase	42.3 ± 5.8	28.9 ± 4.2	<0.001	2.65
Alanine Aminotransferase	38.7 ± 4.9	31.4 ± 3.8	0.078	1.67
Alkaline Phosphatase	89.2 ± 11.3	72.8 ± 9.6	0.042	1.56

Values presented as mean ± standard deviation. Effect size calculated as Cohen's d

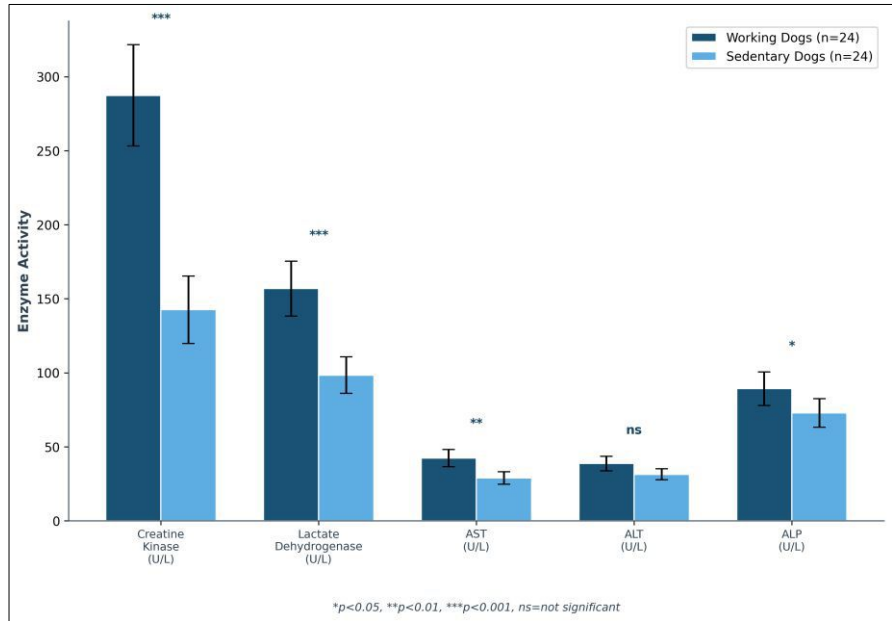


Fig 2: Comparison of baseline muscle enzyme activities between working and sedentary dogs. Error bars represent standard deviation. Statistical significance indicated as * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns = not significant

Baseline enzyme comparisons revealed substantially higher creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and alkaline phosphatase activities in working dogs (Table 2, Figure 2). The most pronounced difference occurred with creatine kinase, where working

dogs demonstrated values approximately twice those observed in sedentary animals. Alanine aminotransferase showed a numerical trend toward elevation in working dogs but did not reach statistical significance.

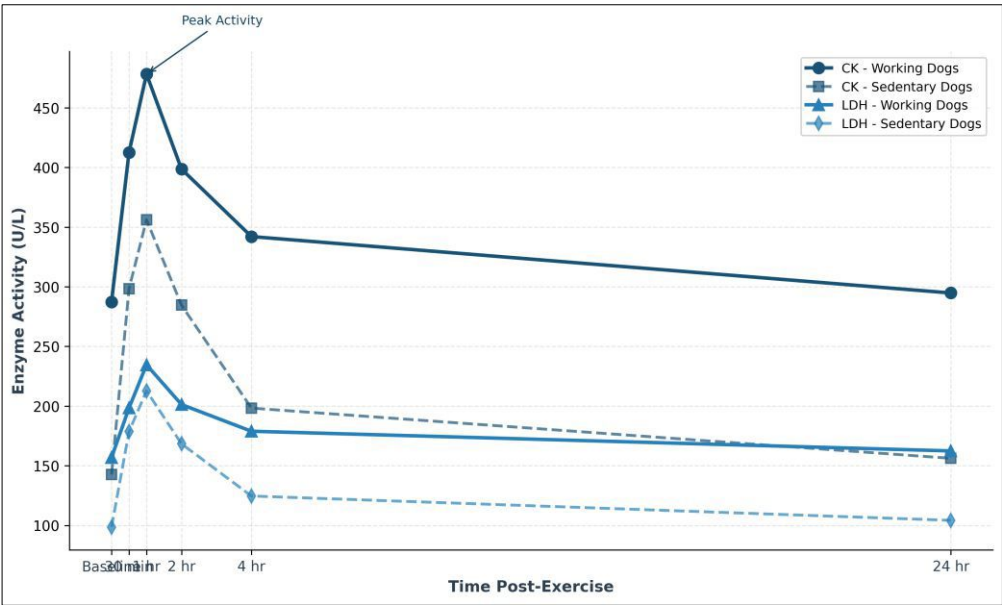


Fig 3: Temporal changes in creatine kinase and lactate dehydrogenase activities following standardized exercise challenge in working and sedentary dogs

The exercise challenge induced significant enzyme elevations in both groups, with peak activities occurring at one hour post-exercise (Figure 3). Working dogs exhibited proportionally smaller increases from their already elevated

baselines compared to sedentary animals. More notably, working dogs returned to baseline values by the four-hour measurement point, while sedentary dogs required a full twenty-four hours to normalize enzyme concentrations.

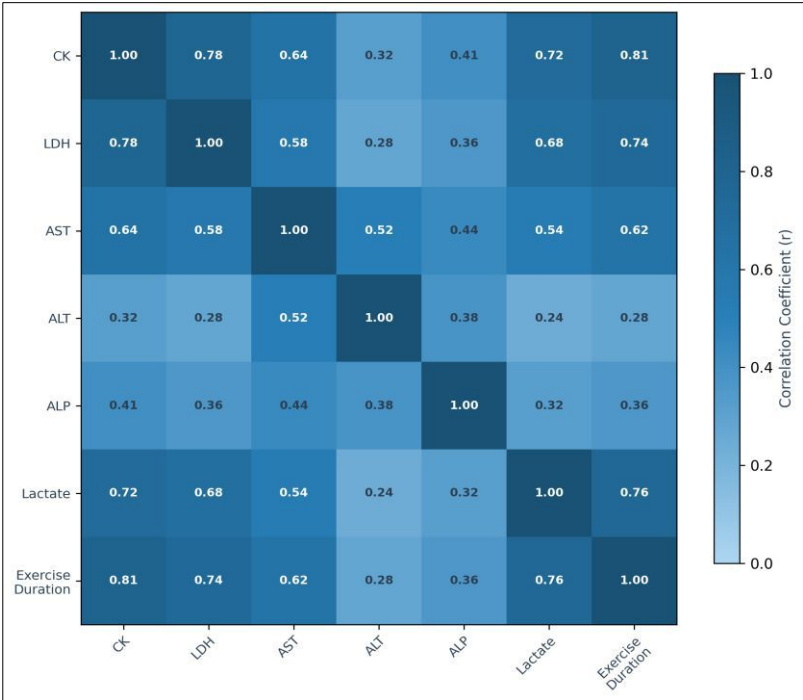


Fig 4: Correlation matrix showing relationships between muscle enzyme activities, serum lactate, and daily exercise duration. Pearson correlation coefficients displayed

Comprehensive Interpretation

Correlation analysis identified strong positive associations between daily exercise duration and primary muscle enzymes (Figure 4). Creatine kinase demonstrated the

strongest correlation with exercise duration ($r = 0.81$, $p < 0.001$), followed by lactate ($r = 0.76$) and lactate dehydrogenase ($r = 0.74$). Inter-enzyme correlations revealed particularly strong associations between creatine

kinase and lactate dehydrogenase ($r = 0.78$), consistent with their shared role in muscle energy metabolism.

Discussion

This investigation provides compelling evidence that sustained physical activity produces measurable adaptations in muscle enzyme expression patterns in domestic dogs. Working dogs demonstrated baseline creatine kinase activities approximately double those observed in sedentary counterparts, a finding consistent with enhanced metabolic capacity developed through chronic exercise training. These elevated baselines likely reflect increased mitochondrial content and enzyme protein expression rather than ongoing tissue damage, as all animals appeared clinically healthy with no evidence of myopathy.

The differential post-exercise kinetics observed between groups carry particular significance for clinical interpretation. Working dogs achieved enzyme normalization within four hours, demonstrating efficient clearance mechanisms and robust recovery capacity. Sedentary dogs required substantially longer recovery periods extending to twenty-four hours, suggesting less developed buffering and clearance systems. Veterinary practitioners should consider activity history when interpreting muscle enzyme panels, as values within normal reference ranges may actually represent pathological elevation in highly active dogs, while apparently elevated values in working dogs may reflect physiological adaptation.

The strong correlations between daily exercise duration and enzyme activities support a dose-response relationship between physical activity and metabolic adaptation. Creatine kinase showed the strongest association with exercise, consistent with its central role in high-energy phosphate transfer during muscular contraction. These correlations suggest that enzyme profiles could potentially serve as objective biomarkers of physical conditioning status in canine populations.

Comparison with published literature reveals general agreement with findings from athletic horses and human athletes showing training-induced enzyme elevations. However, the magnitude of differences observed in the present research exceeds most reported values, possibly reflecting the substantial activity differential between working and sedentary dog populations. Racing greyhounds have shown similar patterns of elevated baseline enzymes with rapid post-exercise normalization.

Limitations

Several constraints should be acknowledged when interpreting these findings. The cross-sectional design prevents definitive conclusions about causality, as inherent metabolic differences between dogs selected for working roles versus household pets could contribute to observed enzyme patterns. Longitudinal research tracking enzyme changes during training programs would strengthen causal inferences. The sample size, while adequate for detecting large effect sizes, limits statistical power for subgroup analyses. Geographic restriction to a single Kenyan county affects generalizability to dogs in different climates and management systems. Mixed breed composition prevented analysis of breed-specific enzyme variations known to exist in purebred populations. Future investigations should

incorporate larger, more diverse samples with longitudinal follow-up to address these limitations.

Conclusion

This research establishes that working dogs maintaining high daily activity levels exhibit distinct muscle enzyme profiles compared to sedentary household pets. Elevated baseline concentrations of creatine kinase, lactate dehydrogenase, and aspartate aminotransferase in working dogs reflect beneficial metabolic adaptations rather than pathological tissue damage. The accelerated post-exercise enzyme normalization observed in working dogs demonstrates enhanced recovery capacity developed through chronic physical conditioning.

Strong positive correlations between daily exercise duration and muscle enzyme activities support a dose-response relationship amenable to clinical application. Veterinary practitioners should incorporate activity history into the interpretation of biochemistry panels, recognizing that reference ranges developed from general pet populations may not apply to working dogs. What appears elevated by conventional standards may represent optimal physiological adaptation in highly active animals.

These findings suggest potential applications for enzyme profiling in fitness assessment, training program monitoring, and early detection of overtraining or injury in working dog populations. Development of activity-stratified reference intervals would enhance clinical decision-making and prevent unnecessary diagnostic investigation of physiologically elevated values in well-conditioned working dogs.

Future research should examine enzyme adaptations during controlled training interventions, investigate breed-specific variations, and explore relationships between enzyme profiles and performance outcomes in working dog populations. The establishment of evidence-based guidelines for interpreting muscle enzymes in dogs with varying activity backgrounds represents an important goal for advancing veterinary diagnostic practice.

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Contributions not qualifying for authorship

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