

Postprint: Changes in Routine Blood Test, Hemorheology, and Serum Biochemical Parameters in Dogs under High-Temperature Environmental Metabolic Characteristics

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Abstract

To investigate the variation patterns of hematological indicators in dogs when nutrient metabolism undergoes significant changes under acute short-term high temperature environment, this experiment adopted an artificial high temperature simulation method to respectively measure routine blood parameters, hemorheological parameters, and serum biochemical indicators in experimental dogs (n=6) at $(35\pm 1)^{\circ}\text{C}$ for durations of 0, 3, 8, 16 h and after natural cooling for 16 h. The results showed that, compared with pre-experiment baseline values, granulocyte count, granulocyte percentage, red blood cell deformation index, and red blood cell electrophoresis index were all significantly increased at each experimental time point under acute short-term high temperature environment ($P < 0.05$), while hemoglobin concentration, lymphocyte percentage, and monocyte percentage were all significantly decreased ($P < 0.05$). Red blood cell count, hematocrit, red blood cell distribution width, plateletcrit, platelet distribution width, mean platelet volume, red blood cell aggregation index, serum alanine aminotransferase activity, and direct/total bilirubin ratio showed no significant changes ($P > 0.05$). The results indicate that acute short-term high temperature environment can significantly alter

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Preamble

Changes in Blood Routine, Hemorheological and Serum Biochemical Indices in Dogs Under High-Temperature Environmental Metabolism

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Abstract: This study investigated the changes in hematological indices in dogs when nutrient metabolism is significantly altered under acute short-term high-temperature conditions. Using an artificial high-temperature simulation, we measured blood routine, hemorheological, and serum biochemical parameters in experimental dogs (n=6) exposed to $(35\pm 1)^{\circ}\text{C}$ for 0, 3, 8, and 16 hours, followed by a 16-hour natural cooling period. The results showed that compared with pre-experiment values, granulocyte count, granulocyte percentage, red blood cell deformation index, and red blood cell electrophoresis index significantly increased at all time points ($P<0.05$), while hemoglobin concentration, lymphocyte percentage, and monocyte percentage significantly decreased ($P<0.05$). No significant changes were observed in red blood cell count, hematocrit, red blood cell distribution width, plateletcrit, platelet distribution width, mean platelet volume, red blood cell aggregation index, serum alanine aminotransferase activity, or direct/total bilirubin ratio ($P>0.05$). These findings demonstrate that acute short-term high-temperature environments can significantly alter certain hematological parameters in dogs, suggesting that clinical interventions aimed at improving blood quality may alleviate the harmful effects of such conditions.

Keywords: dogs; high-temperature environment; blood routine indices; hemorheological indices; serum biochemical indices

Introduction

In recent years, “heat wave” weather has become increasingly frequent in China during summer, with average temperatures reaching new highs that seriously endanger human and animal health. Dogs are closely related to human life in many aspects, and their healthy scientific breeding has received growing attention. Physiologically, dogs have almost no sweat glands throughout their body, resulting in poor heat dissipation capacity and lower resistance to high temperatures compared to other animals [1]. To adapt to high-temperature environments, dogs typically alter their nutrient metabolism to better cope with external conditions. However, when this exceeds the body’s ability to resist thermal stress, dogs exhibit decreased quality of life and immunity, and may even develop sunstroke or heat stroke [2]. Additionally, working dogs performing tasks in hot field conditions or confined spaces are highly susceptible to high temperatures, which reduces their working capacity.

The impact of stress on the body is closely related to factors such as stress duration, type and intensity of stressors, and individual sensitivity. Ultra-short-term stress is characterized by transient intense reactions followed by rapid relief, whereas long-term stress features persistent systemic adaptive changes. Current research on metabolic changes in dogs under high-temperature conditions has primarily focused on long-term chronic stress during summer or acute ultra-short-term (<2 h) high-temperature exposure [3-4]. However, the patterns of change in blood routine, hemorheological, and serum biochemical indices in

dogs following adaptive metabolic changes under acute short-term (>2 h) high-temperature conditions remain unclear.

Blood serves as the carrier for transporting vital nutrients, gases, and humoral regulatory substances throughout the body, and blood routine, hemorheological, and serum biochemical indices are important comprehensive indicators reflecting metabolic changes and physiological functions of tissues and organs [5]. Therefore, this study systematically investigated changes in hematological indices in dogs following adaptive metabolic alterations under acute short-term high-temperature conditions, aiming to reveal the specific impacts of such environments and provide insights for clinical interventions through targeted hematological nutritional improvements to prevent and control heat-related hazards in dogs.

1.1 Experimental Animals and Treatment

Six healthy Chinese rural dogs with similar age and body weight were selected from the Bengbu area, with an average body weight of 4.5 kg and age range of 1.5-2.5 years. The dogs had normal physiological parameters [body temperature = $(39.26 \pm 0.10)^\circ\text{C}$, pulse = (105.17 ± 8.40) beats/min, respiratory rate = (38.0 ± 8.49) breaths/min]. The dogs were fed a universal canine diet (nutritional standards: crude protein 26%; crude fat 14%; crude fiber 5.0%; crude ash 10%; lysine 1.0%; calcium 1.3%; total phosphorus 1.0%; salt 1.2%; moisture 10%). One week before the experiment, the dogs were transferred to kennels for acclimation at $(28 \pm 1)^\circ\text{C}$. Throughout the experiment, all dogs were fed the universal canine diet, housed individually in cages, and provided with free access to food and water. An industrial heater (Jiyi IFH04-30A) was used to heat the kennel.

The experiment began at 07:00, and the kennel temperature reached $(35 \pm 1)^\circ\text{C}$ within 15 minutes. This temperature was maintained for 16 hours, after which the heater was turned off and the kennel was allowed to cool naturally for 16 hours. The relative humidity in the kennel was maintained at 60-70% throughout the experimental period.

1.2 Sample Collection and Index Determination

Blood samples were collected from the jugular vein at five time points: 0 h (beginning), 3 h (early stage), 8 h (middle stage), 16 h (late stage) of acute short-term high-temperature exposure, and after 16 h of natural cooling (recovery period). For blood routine analysis, 0.5 mL of blood was collected in EDTA-Na₂ anticoagulant tubes and analyzed using a Mindray BC-2800Vet hematology analyzer to determine red blood cell, white blood cell, and platelet parameters. For hemorheological analysis, 4 mL of blood was collected in heparin sodium anticoagulant tubes and analyzed using a Meiyilin MEN-C90 hemorheology analyzer to measure blood viscosity and red blood cell characteristics. Additionally, 2 mL of non-anticoagulated blood was collected, stored at 4°C for 12 hours,

then centrifuged at 3,000 rpm for 10 minutes to collect serum. Serum levels of creatine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), -glutamyl-transferase (-GT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) activities, as well as direct bilirubin, indirect bilirubin, urea, creatinine, and uric acid concentrations were measured using a Mindray BS-200 biochemical analyzer.

1.3 Data Processing

Experimental results were analyzed using PASW Statistics 18.0 software to calculate means, standard deviations, and perform one-way ANOVA. Data are presented as mean \pm standard deviation. Multiple comparisons among time points were conducted using one-way repeated measures ANOVA. $P < 0.05$ was considered statistically significant.

Results

2.1.1 Effects of Acute Short-Term High-Temperature Environment on Canine Red Blood Cell Indices

As shown in Table 1, no significant changes were observed in red blood cell count, hematocrit, or red blood cell distribution width throughout the experimental period ($P > 0.05$). Compared with the beginning of the experiment (0 h), hemoglobin concentration was significantly decreased at all experimental time points ($P < 0.05$), though no significant differences were detected among these time points ($P > 0.05$). Mean corpuscular volume showed no significant changes compared with the beginning of the experiment ($P > 0.05$), but was significantly higher at 16 h of exposure and after 16 h of cooling compared with 3 h and 8 h of exposure ($P < 0.05$). Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were significantly decreased at 16 h of exposure compared with the beginning of the experiment ($P < 0.05$).

2.1.2 Effects of Acute Short-Term High-Temperature Environment on Canine White Blood Cell Indices

As shown in Table 2, white blood cell count significantly increased at 8 h and 16 h of exposure compared with the beginning of the experiment ($P < 0.05$). Lymphocyte count and monocyte count significantly decreased at 3 h, 8 h of exposure and after 16 h of cooling ($P < 0.05$), while granulocyte count significantly increased at all experimental time points ($P < 0.05$). Among the experimental time points, no significant differences were observed in white blood cell count, lymphocyte count, monocyte count, or granulocyte count at 3 h, 8 h of exposure and after 16 h of cooling ($P > 0.05$). Compared with the beginning of the experiment, lymphocyte percentage and monocyte percentage significantly decreased at all time points ($P < 0.05$), while granulocyte percentage significantly increased ($P < 0.05$).

2.1.3 Effects of Acute Short-Term High-Temperature Environment on Canine Platelet Indices

As shown in Table 3 , platelet count significantly decreased at 16 h of exposure compared with the beginning of the experiment ($P<0.05$), while no significant changes were observed in mean platelet volume, platelet distribution width, or plateletcrit ($P>0.05$). Among the experimental time points, platelet count and plateletcrit at 3 h and 8 h of exposure were significantly higher than those at 16 h of exposure ($P<0.05$).

2.2.1 Effects of Acute Short-Term High-Temperature Environment on Canine Whole Blood Viscosity Indices

As shown in Table 4 , low-shear and medium-shear whole blood viscosity significantly increased at 8 h of exposure compared with the beginning of the experiment ($P<0.05$), while low-shear, medium-shear, and high-shear whole blood viscosity significantly decreased at 16 h of exposure and after 16 h of cooling ($P<0.05$). Among the experimental time points, low-shear, medium-shear, and high-shear whole blood viscosity at 16 h of exposure and after cooling were significantly lower than those at 3 h and 8 h of exposure ($P<0.05$). Compared with the beginning of the experiment, low-shear, medium-shear, and high-shear whole blood reduced viscosity significantly increased at 3 h and 8 h of exposure ($P<0.05$). Among the experimental time points, low-shear, medium-shear, and high-shear whole blood reduced viscosity at 3 h and 8 h of exposure were significantly higher than those at 16 h of exposure and after cooling ($P<0.05$). The pattern of change in low-shear, medium-shear, and high-shear whole blood relative viscosity was identical to that of whole blood reduced viscosity.

2.2.2 Effects of Acute Short-Term High-Temperature Environment on Canine Red Blood Cell Physical Characteristics

As shown in Table 5 , red blood cell rigidity index significantly increased at 3 h, 8 h, and 16 h of exposure compared with the beginning of the experiment ($P<0.05$). Red blood cell rigidity index at 16 h of exposure and after 16 h of cooling was significantly lower than that at 3 h and 8 h of exposure ($P<0.05$). Red blood cell deformation index significantly increased at all experimental time points compared with the beginning of the experiment ($P<0.05$), and was significantly higher at 3 h and 8 h of exposure than at 16 h of exposure and after cooling ($P<0.05$). Red blood cell electrophoresis index significantly increased at all time points compared with the beginning of the experiment ($P<0.05$). No significant changes in red blood cell aggregation index were observed throughout the experimental period ($P>0.05$).

2.3 Effects of Acute Short-Term High-Temperature Environment on Canine Serum Biochemical Indices

As shown in Table 6, serum AST activity slightly increased at 3 h and 8 h of exposure compared with the beginning of the experiment, but the difference was not significant ($P > 0.05$). Serum AST activity at 16 h of exposure and after 16 h of cooling was significantly lower than that at 3 h and 8 h of exposure ($P < 0.05$). Serum -GT activity significantly decreased at 16 h of exposure compared with the beginning of the experiment ($P < 0.05$). Serum total bilirubin and direct bilirubin concentrations significantly increased at 8 h and 16 h of exposure ($P < 0.05$). Serum ALP and CK activities significantly decreased at 8 h, 16 h of exposure and after 16 h of cooling ($P < 0.05$), with serum CK activity at 16 h of exposure and after cooling being significantly lower than that at 3 h and 8 h of exposure ($P < 0.05$). Serum urea concentration significantly increased at 16 h of exposure compared with the beginning of the experiment ($P < 0.05$), while serum creatinine concentration significantly decreased at 16 h of exposure and after 16 h of cooling ($P < 0.05$). Serum uric acid concentration significantly decreased at 8 h, 16 h of exposure and after 16 h of cooling ($P < 0.05$), and serum LDH activity significantly decreased at 16 h of exposure and after 16 h of cooling ($P < 0.05$). Furthermore, serum urea and uric acid concentrations and LDH activity after 16 h of cooling were significantly lower than those at 3 h and 8 h of exposure ($P < 0.05$).

Discussion

3.1 Effects of Acute Short-Term High-Temperature Environment on Canine Blood Routine Indices

Red blood cells are the primary carriers for oxygen delivery and carbon dioxide removal in animals, with hemoglobin playing a crucial role in this process. High-temperature environments significantly alter nutrient metabolism in dogs, consequently affecting the internal environment. Our results demonstrated that acute short-term high-temperature exposure had no significant effect on red blood cell count or hematocrit in dogs, but significantly decreased hemoglobin concentration, which reached its lowest value at 3 h of exposure.

The lack of significant effect on red blood cell count (the number of red blood cells per unit volume) under acute short-term high-temperature conditions is primarily due to the body's short-term mobilization of compensatory mechanisms to maintain dynamic equilibrium among red blood cell production, release, storage, and elimination [6]. The decreased hemoglobin concentration observed in this study indicates that although acute short-term high-temperature exposure did not significantly affect red blood cell count or hematocrit, it compromised red blood cell quality and impaired normal oxygen-carrying capacity. Mean corpuscular volume was significantly lower at 3 h and 8 h of exposure compared with 16 h of exposure and after 16 h of cooling, suggesting that acute short-term high-temperature conditions enhanced water evaporation from the oral cavity,

causing dehydration and a tendency for red blood cells to lose water, which resulted in minimum mean corpuscular volume at 3 h of exposure. However, at 16 h of exposure, dogs drank large amounts of water under the regulation of thirst centers, causing red blood cells to absorb water and significantly increase in volume [7], as evidenced by maximum mean corpuscular volume at 16 h of exposure. No significant changes in red blood cell distribution width throughout the experiment indicate that acute short-term high-temperature exposure did not significantly affect the uniformity of red blood cell size, and no significant increase in non-homogeneous red blood cells occurred.

This study also examined changes in red blood cell-related indices after 16 h of natural cooling and found that natural cooling had no significant restorative effect on these parameters.

White blood cells are the primary immune cells providing non-specific defense protection in the body. Our results demonstrated that acute short-term high-temperature exposure significantly increased white blood cell and granulocyte counts (the number of cells per unit volume) in dogs, reaching maximum values at 16 h of exposure, which is associated with the activation of active non-specific defense responses to resist external high-temperature stimulation [8]. The results also showed that acute short-term high-temperature exposure significantly decreased lymphocyte and monocyte counts (the number of cells per unit volume) in dogs, reaching minimum values at 8 h of exposure. These changes in white blood cells are closely related to the massive secretion of corticosteroids under stress conditions [4,9], though the specific mechanisms require further investigation. After 16 h of natural cooling, the percentages of granulocytes, lymphocytes, and monocytes showed some recovery, suggesting that timely removal of high-temperature stressors has beneficial effects on restoring non-specific immune function.

Platelets primarily function in blood coagulation. Our results showed that acute short-term high-temperature exposure for 16 h significantly decreased platelet count and plateletcrit, while platelet volume and distribution width remained unchanged during this acute heat stress. The decreased platelet count and plateletcrit may be attributed to hemorrhagic inflammation in the intestines under acute high-temperature conditions, where platelets are consumed through aggregation to exert coagulation effects, without significantly affecting platelet size or uniformity. The results also revealed that platelet count and plateletcrit reached their lowest values at 16 h of exposure, but showed obvious recovery after 16 h of natural cooling, further suggesting that timely removal of high-temperature stressors significantly contributes to the restoration of blood indices.

3.2 Effects of Acute Short-Term High-Temperature Environment on Canine Hemorheological Indices

Hemorheology examines changes in blood fluidity, stagnation, and viscosity to infer circulatory status and identify blood components that may cause these changes. In this study, whole blood viscosity, reduced viscosity, and relative viscosity at high, medium, and low shear rates significantly increased at 8 h of exposure compared with the beginning of the experiment, but significantly decreased at 16 h of exposure compared with 8 h of exposure. The increased whole blood viscosity during the early stage (3 h of exposure) was primarily due to active adaptive responses dominated by metabolic alterations, combined with dehydration in the body. During the later stage (16 h of exposure), increased water intake stimulated by high-temperature conditions diluted the blood after entering the circulatory system, resulting in decreased whole blood viscosity.

Reduced whole blood viscosity helps better meet tissue perfusion requirements, enhances circulatory efficiency, fills superficial microcirculation, and improves heat dissipation capacity in dogs exposed to acute short-term high-temperature conditions [10-11]. Additionally, whole blood viscosity, reduced viscosity, and relative viscosity significantly increased at 8 h, 3 h of exposure, respectively, compared with the beginning of the experiment, suggesting that acute short-term high-temperature environments may affect whole blood viscosity by influencing red blood cell characteristics and plasma components.

Red blood cell rigidity index is one indicator used to measure erythrocyte membrane flexibility; higher values indicate lower deformability and greater increases in whole blood viscosity at high shear rates. Red blood cell deformability refers to the ability of red blood cells to change shape under external forces. Our results demonstrated that acute short-term high-temperature exposure significantly increased red blood cell rigidity index. Although the significant increase in red blood cell deformation index partially alleviated the viscosity increase caused by elevated rigidity index, the net effect indicated that acute short-term high-temperature environments could elevate whole blood viscosity by increasing red blood cell rigidity [12]. Under acute short-term high-temperature conditions, canine red blood cell electrophoresis index and aggregation index showed no significant changes after 8 h of exposure, suggesting that the increased whole blood viscosity was not solely determined by the amount of negative charge on red blood cell surfaces [13]. Notably, all these indices reached their maximum values at 8 h of exposure, but did not show obvious recovery after 16 h of natural cooling, indicating that the effects of acute short-term high-temperature environments on canine hemorheological indices are relatively persistent.

3.3 Effects of Acute Short-Term High-Temperature Environment on Canine Serum Biochemical Indices

Acute short-term high-temperature environments induce stress responses in animals, causing functional changes in tissue and organ cells [14]. Serum biochem-

ical indices reflect the nutritional and metabolic status of corresponding tissues and organs. Under normal conditions, intracellular enzymes are protected by cell membrane barriers and remain inside cells, with serum containing only small amounts released from aged and lysed cells. In damaged organisms, increased cell membrane permeability releases intracellular substances, including enzymes, into the blood, causing elevated serum enzyme activities. Our results showed that exposure to acute short-term high-temperature conditions for 3 h and 8 h did not significantly affect serum AST, ALT, or γ -GT activities, suggesting no significant impact on liver function. However, serum total bilirubin and direct bilirubin concentrations significantly increased, while the direct/total bilirubin ratio remained unchanged throughout the experiment, indicating that acute short-term high-temperature exposure under our experimental conditions significantly affected hepatocyte function but caused relatively low-level liver cell damage. This differs from the report by Qin et al. [3], possibly due to differences in heat exposure duration and intensity. Serum AST and γ -GT activities significantly decreased at 16 h of exposure compared with the beginning of the experiment, primarily because elevated serum lactate levels under acute short-term high-temperature conditions consumed large amounts of nicotinamide adenine dinucleotide (NADH), affecting AST and γ -GT activities [15]. Additionally, at 16 h of exposure, dogs transitioned from an active adaptation phase to a fatigue phase under acute short-term high-temperature conditions. Combined with changes in serum ALP and LDH activities, this suggests that systemic activities, including liver cell functions, were operating at low levels as dogs adapted to the external environment through reduced activity. Although these indices showed some recovery after 16 h of natural cooling, the changes were not significant, indicating that recovery requires more time.

Serum ALP is primarily found in bones, intestines, liver, and other tissues. Our results demonstrated that a 35°C high-temperature environment significantly decreased serum ALP activity. Reduced serum ALP activity can impair calcium absorption, cause incomplete bone tissue calcification, lead to abnormal skeletal development, and increase fracture risk. CK is an enzyme present in the cytoplasm and mitochondria of cardiac, muscular, and brain tissues, playing a crucial role in intracellular energy transfer, muscle contraction, and ATP regeneration. Our findings showed that serum CK activity significantly decreased after 8 h of exposure to acute short-term high-temperature conditions. Combined with changes in serum AST, LDH activities, and creatinine concentration, this suggests that acute short-term high-temperature exposure did not severely damage the integrity of cardiac and muscle cells, but rather reduced cellular metabolic activity during the fatigue phase, consistent with changes in liver function status.

Protein metabolism in the body produces urea through the ornithine cycle in the liver, which is primarily excreted by the kidneys. Our results showed that serum urea concentration significantly increased at 8 h of exposure to acute short-term high-temperature conditions, mainly due to enhanced protein catabolism under acute heat stress. Although liver ornithine cycle synthesis capacity was relatively

low at this time, reduced renal excretory function ultimately led to elevated serum urea concentration [3]. Serum urea concentration significantly improved after 16 h of natural cooling, indicating that the effects of acute short-term high-temperature exposure on protein metabolism recover relatively quickly.

Serum uric acid concentration gradually decreased in dogs under acute short-term high-temperature conditions. This may be explained by two factors: first, altered liver function under heat stress reduced purine oxidase activity, decreasing the conversion of purines to uric acid and consequently lowering blood uric acid levels; second, impaired renal function causing abnormal uric acid excretion could also affect serum uric acid concentration [16-17].

Among these indices, serum direct bilirubin and urea concentrations reached maximum values at 8 h of exposure, while serum ALP, CK, LDH activities, and uric acid concentration reached minimum values at 16 h of exposure. Natural cooling for 16 h only significantly restored a few indices, including serum -GT activity and bilirubin and urea concentrations. This reflects that acute short-term high-temperature environments affect various organs and tissues differently in dogs, and that recovery capacity varies among organ systems following heat stress.

Conclusions

1. Acute short-term high-temperature environments significantly altered certain red blood cell, white blood cell, and platelet indices in dogs, including hemoglobin concentration, lymphocyte count, and platelet count. These three indices reached their lowest values at 3 h, 8 h, and 16 h of exposure, respectively, indicating that acute short-term high-temperature conditions impaired canine red blood cell oxygen transport, white blood cell non-specific immune function, and platelet coagulation capacity.
2. Acute short-term high-temperature environments increased whole blood viscosity and altered red blood cell physical characteristics. All hemorheological indices reached maximum values at 8 h of exposure and did not fully return to normal levels after 16 h of natural recovery, affecting blood circulation and fluidity.
3. Acute short-term high-temperature environments altered the activities of certain serum enzymes and the concentrations of uric acid, urea, and creatinine. Serum ALP, CK, and LDH activities reached minimum values at 16 h of exposure, and some indices did not fully recover to normal levels after 16 h of natural recovery, indicating that acute short-term high-temperature conditions affected the functions of important organs such as the liver, kidneys, and heart.
4. Throughout the experimental period, except for white blood cell count, granulocyte count, and serum direct bilirubin concentration, which exceeded normal physiological ranges, all other indices fluctuated within

physiological values, suggesting that the body possesses certain adaptive and resistance capabilities to acute short-term high-temperature environments.

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