






Electrocardiographic changes and changes in cardiac lactate and troponin I levels associated with search and rescue physical activity in military dogs

Alterações eletrocardiográficas e nos níveis de lactato e troponina I cardíaca associadas à atividade física de busca e salvamento em cães militares

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ABSTRACT

Rescue and recovery dogs intercalate the activity intensity developed, which also triggers significant metabolic changes in cardiac physiology. Thus, we evaluated the changes that search simulation causes in glucose, lactate, and cardiac troponin I level (cTnI) and the electrocardiographic and heart rate during the activity and recovery phase to predict the physiological adaptation to the exercise. Five healthy adult dogs from the Rescue and Recovery Service of Military Firefighters Corps were submitted to 60 minutes search operation simulation in the woods. They covered a forest area of approximately 50,000 m². The dogs were loose and accompanied by their driver, and they could perform any physical activity. Were evaluated serum biochemical analysis of glucose, lactate, cardiac troponin I, electrocardiographic, and heart rate (rest, exercise phase, and recovery time). No changes in glucose levels, heart rate, and cardiac rhythm were detected. In comparison to baseline values, there is an increase: in lactate at the end of the exercise phase [EXER] (60'EXER), and in the recovery phase [RCT] at 30'RCT and 60'RCT, and cTnI at 60'RCT, 120'RCT, and 4hRCT. P wave duration was significantly higher at 60'EXER, 15'RCT, and 30'RCT, with no alterations in wave amplitude. QRS interval duration significantly increased at 30'RCT, and the ST segment presented a significant difference at 60'EXER, 15'RCT, and 60'RCT compared to the rest moment. The moderate alterations in lactate and cTnI and few alterations in the electrocardiographic and heart rate maintenance suggest the adaptation of rescue and recovery dogs to the type, intensity, and duration of search operation simulation performed.

Keywords: Cardiac electrophysiology. Dog. Physiology. Metabolism.

RESUMO

Cães de busca e resgate intercalam a intensidade da atividade desenvolvida que desencadeia alterações metabólicas significativas, bem como na fisiologia cardíaca. Assim, foram avaliadas as alterações que a simulação de busca produz nos níveis de glicose, lactato, troponina I cardíaca (cTnI), bem como na frequência cardíaca e atividade eletrocardiográfica durante a fase de atividade e recuperação, a fim de prever a adaptação fisiológica ao exercício. Cinco cães adultos saudáveis do Serviço de Resgate e Salvamento do Corpo de Bombeiros Militares foram submetidos à simulação de operação de busca de 60 minutos na mata e cobriram uma área florestal de aproximadamente 50.000 m². Os cães estavam soltos, acompanhados pelo condutor e estavam livres para realizar qualquer tipo de atividade física. Foram avaliados os níveis séricos de glicose, lactato e troponina I cardíaca, atividade eletrocardiográfica e frequência cardíaca em repouso, na fase de exercício e no tempo de recuperação. Não foram detectadas alterações nos níveis de glicose, frequência cardíaca e ritmo cardíaco. Em comparação com os valores basais houve aumento de lactato ao final da fase de exercício [EXER] (60'EXER) e na fase de recuperação [RCT] aos 30'RCT e 60'RCT; e cTnI aos 60'RCT, 120'RCT e 4hRCT. Duração da onda P foi significativamente maior em 60'EXER, 15'RCT e 30'RCT, sem alterações na amplitude da onda. Duração do intervalo QRS teve aumento significativo em 30'RCT e o segmento ST apresentou diferença significativa em 60'EXER, 15'RCT e 60'RCT quando comparado ao basal. As alterações moderadas nos níveis de lactato e cTnI, bem como a pouca alteração na atividade eletrocardiográfica e manutenção da frequência cardíaca sugerem boa adaptação dos cães de busca e resgate ao tipo, intensidade e duração da operação de busca simulada realizada.

Palavras-chave: Eletrofisiologia cardíaca. Cão. Fisiologia. Metabolismo.

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Introduction

Dogs have been used more frequently by men as a work tool (Gordon, 2018; Lima et al., 2022), and one of the activities in which they are widely used is the search, rescue, and recovery service of the military organs (Corpo de Bombeiros Militar de Goiás, 2020; Moraes et al., 2017). This activity triggers significant metabolic changes (Diverio et al., 2016; Rovira et al., 2008) that begin as a consequence of the physical training routine that occurs from the first year of life of the animal and lasts throughout its useful life in the military service (Corpo de Bombeiros Militar de Goiás, 2020).

It is known that different mechanisms of energy production for muscle contraction—phosphate system, aerobic glycolysis, and anaerobic glycolysis—co-occur. However, the type and intensity of the activity being performed determine the pathway that will be prevalent (Caputo et al., 2009). During prolonged submaximal exercise in dogs, a strong hormonal stimulus for glycogenolysis/gluconeogenesis occurs for sparing and replenishment of muscle glycogen that remained increased during later rest periods and for several days after the conclusion of the exercise, showing the importance of the hepatic glucose output to support prolonged submaximal exercise (Davis et al., 2020). Thus, lactate is a metabolite of the anaerobic glycolytic pathway and a muscle fatigue indicator in dogs (Rovira et al., 2007).

Cardiac adaptations secondary to a physical activity routine also trigger cardiac remodeling in dogs (Civelek et al., 2012; Santos et al., 2018), and electrocardiography (ECG) changes consistent with this condition can be observed, such as an increase in QRS duration, QT

interval, and S wave amplitude (Bavegems et al., 2009; Constable et al. 2000). In time, significant alterations to the electrocardiographic exam were observed in military service dogs after a six-week physical training process, where the electrocardiogram revealed physiological adaptations at a cardiovascular level triggered by the practice of physical activity (Moraes et al., 2017). Moreover, the “athlete’s heart” syndrome was described in search, rescue, and recovery dogs due to the physical activity routine, but without electrocardiographic changes (Santos et al., 2018). A study using electrocardiography in several breeds of dogs identified that German Shepherds presented arrhythmias the most (Noszczyk-Nowak et al., 2017); also, such breed is widely used by military services (Ferworn, 2009; Lima et al., 2016).

Additionally, healthy dogs had increased cardiac troponin I level (cTnI) after six-stage submaximal exercise test (ET) on a motorized treadmill (Wall et al., 2018), and sled dogs that performed resistance activities for several days showed increased levels of cardiac troponin I (cTnI) after physical activity, and this was not indicative of myocardial injury, but instead of cardiovascular adaptation to the exercise (Spratt et al., 2005).

As the primary determinant of cardiac output and oxygen consumption, the heart rate (HR) is used as a parameter to evaluate the effort exerted by the cardiovascular system during exercises (Muñoz et al., 1999; Rovira et al., 2008), and its degree of variation as a function of physical activity reveals the physical conditioning of the animal (Rovira et al., 2008). However, the sport practiced must be considered since the type, duration, and intensity of the physical exercise also interfere with the magnitude of the HR variation (Piccione et al., 2012).

Nevertheless, the knowledge and interpretation of physical fitness indicator variables for the different categories of activity performed by the animals must consider the requirements of the modality since the degree of adaptation varies according to it. In addition, it would be possible to differentiate a physiological response to exercise from a pathological response, considering that some physiological and biochemical parameters may differ from normal values when the patient is an athlete animal (Rovira et al., 2007).

Thus, the objective of this study was to evaluate the changes that search simulation of 60 minutes causes in glucose, lactate, and cTnI levels, as well as in the ECG and HR during the activity and recovery phase, to predict the physiological adaptation to the exercise presented by rescue and recovery dogs.

Materials and Methods

Animals

Five dogs from the Rescue and Recovery Service of Military Firefighters Corps of Espírito Santo, Brazil, between 3 and 5 years old, not neutered (mean \pm SD body weight, 30.24 ± 2.70 kg), and on a training routine of more than one year were used. The studied group consisted of three female German Shepherds, one male Belgian Malinois, and one male mixed breed of German Shepherd and Belgian Malinois. For inclusion criteria, dogs were judged to be healthy by physical examination, ECG and echocardiographic (ECHO) exams, complete blood count (CBC), and serum biochemical analysis (urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum albumin, and total protein). To ensure that previous physical activities would not influence the baseline values, the dogs did not participate in any search operation or moderate to intense effort training one week before the beginning of the study. The exclusion criteria were dogs that presented any abnormalities in physical exam, ECG and ECHO, hemogram, or biochemical analysis and participated in any search operation.

Procedures

The dogs were submitted to a 60-min search operation simulation in the woods and covered a forest area of approximately 5 ha ($20^{\circ}13'23.0''$ S $40^{\circ}15'29''$ W) (Figure 1).

To ensure that all animals worked throughout the proposed time, the victim was included in the operation

45 minutes after the beginning of the activity. Dogs did not access water during the exercise phase.

The dogs were loose and were accompanied by their driver. They could perform any physical activity, including walking, jogging, running, and jumping over obstacles. In addition, the conductors encouraged the animals to work whenever necessary. The mean \pm SD temperature and relative humidity were $25 \pm 2.5^{\circ}\text{C}$ and $70.5 \pm 7.5\%$, respectively.

Blood samples of the dogs were evaluated in two phases of the study, exercise, and recovery time. Before the exercise (rest moment), all dogs were subjected to serum biochemical analysis of glucose, lactate, and cardiac troponin I (cTnI). Then, during the search operation simulation (exercise phase - EXER), samples were taken every 15 min. After simulation (recovery time - RCT), samples were collected at 15, 30, and 60 min (glucose and lactate) and 2, 4, 12, and 24 h (cTnI). Blood samples for determining the glucose, lactate, and cTnI levels were taken from the cephalic or lateral saphenous vein. Glucose (Accu-Chek® Performa, Roche Diabetes Care Brasil LTDA, São Paulo, Brazil) and lactate (Accutrend® Plus (Roche) Roche Diagnóstica Brasil LTDA, São Paulo, Brazil) measurements were performed using portable devices. Serum samples for determining cTnI levels were conditioned at $2-8^{\circ}\text{C}$ until arrival at the laboratory and stored at -20°C for not more than 20 days, and the analysis was performed using chemiluminescence immunoassay (RTI Turbo Troponin I for IMMULITE® 1000 benchmark - Siemens Healthcare Diagnostics S.A.), in a duplicate analysis using a sensitivity of 0.01 ng/mL.



Figure 1 – Delimitation of the study area of approximately 5 ha of Atlantic Forest. Source: Google Earth.

In addition, ECG (Veterinary PV ECG –TEB – Brazilian Electronic Technology[®]) was performed in rest moment, immediately after the search operation simulation and at 15, 30, and 60 minutes of recovery time. Electrocardiographic tracings were analyzed in bipolar leads (I, II, and III) and unipolar limb leads (aVR, aVL, and aVF). Wave measurements were obtained in triplicate, in random regions of the electrocardiographic tracing for each evaluation moment, by the same observer, with P, PR, QRS, and QT durations measured in seconds, while P, Q, R, S, and T amplitudes measured in millivolts; the ST segment deviation was calculated from the baseline.

Heart rate was measured in rest moments every 15 min during the simulation (15, 30, 45, and 60 min of exercise phase) and 15, 30, 60, and 120 min at recovery time. The heart rate was measured by palpating the femoral pulse since tachypnea made auscultation difficult, especially in the exercise phase.

Statistics

The normal distribution of variables was tested and confirmed by the Shapiro-Wilk normality test. To assess changes in glucose, lactate, cTnI, ECG variables, and HR in the exercise and recovery phases, baseline measurements (rest moment) were used for comparison with each moment in the study using Paired Samples *t*-test. Statistically significant results were indicated by $P < 0.05$. Statistical analysis was performed using GraphPad Prism, demo version 7 (GraphPad Software Inc, San Diego, CA), and the results were expressed as mean \pm SD. T wave's polarity was analyzed qualitatively.

Results

Glucose, lactate, and cTnI levels

There was no significant difference between the pre-simulation (rest moment) glucose levels and the other evaluation times (exercise and recovery phases). The critical difference with an increase in lactate level was observed at the end of the search simulation (60'EXER) ($p = 0.0073$), as well as 30 min ($p = 0.0416$) and 60 min ($p = 0.0452$) of recovery time compared to baseline level (rest moment) (Figure 2).

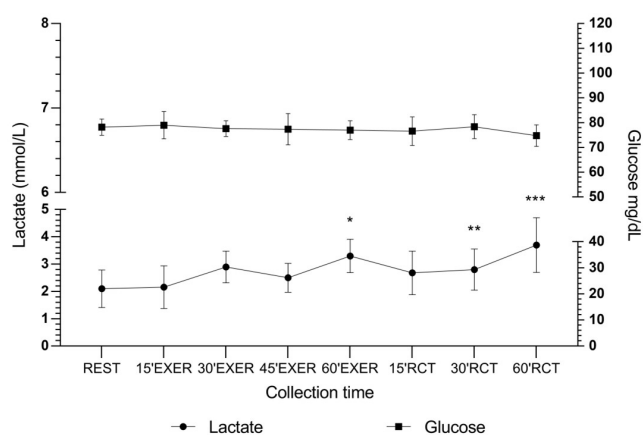


Figure 2 – Mean \pm SD of lactate (mmol/L) and glucose levels (mg/dL) of five adult healthy dogs from the Rescue and Recovery Service of Military Firefighters Corps of Espírito Santo, Brazil, submitted to a 60-min search operation simulation. Measurements were performed before (REST), during (EXER), and after (RCT) simulation. There is a significant difference in lactate level compared to baseline values: * $p = 0.0073$; ** $p = 0.0416$; *** $p = 0.0452$. Paired Samples *t*-test.

Table 1 – Electrocardiographic assessment (mean \pm SD) of five adult healthy dogs from the Rescue and Recovery Service of Military Firefighters Corps of Espírito Santo, Brazil, submitted to a 60-min search operation simulation. Measurements were performed before (REST), during (EXER), and after (RCT) simulation.

	Collection Time				
	REST (Baseline) n=5	60'EXER n=5	15'RCT n=5	30'RCT n=5	60'RCT n=5
P wave (s)	0.047 \pm 0.002	0.056 \pm 0.004*	0.051 \pm 0.002*	0.051 \pm 0.001*	0.049 \pm 0.005
P wave (mV)	0.191 \pm 0.025	0.239 \pm 0.028	0.230 \pm 0.021	0.201 \pm 0.044	0.212 \pm 0.032
Q wave (mV)	0.130 \pm 0.043	0.156 \pm 0.138	0.168 \pm 0.104	0.170 \pm 0.126	0.194 \pm 0.163
R wave (mV)	1.084 \pm 0.167	0.999 \pm 0.182	1.086 \pm 0.147	1.068 \pm 0.192	1.084 \pm 0.241
S wave (mV)	0.025 \pm 0.045	0.031 \pm 0.030	0.034 \pm 0.067	0.025 \pm 0.026	0.025 \pm 0.026
T wave (mV)	0.233 \pm 0.123	0.295 \pm 0.197	0.293 \pm 0.139	0.253 \pm 0.179	0.269 \pm 0.172
PR interval (s)	0.102 \pm 0.017	0.104 \pm 0.015	0.101 \pm 0.013	0.098 \pm 0.013	0.102 \pm 0.016
QRS complex (s)	0.061 \pm 0.008	0.068 \pm 0.011	0.059 \pm 0.008	0.070 \pm 0.012*	0.062 \pm 0.007
QT segment (s)	0.194 \pm 0.013	0.196 \pm 0.008	0.208 \pm 0.017	0.208 \pm 0.012	0.206 \pm 0.013
ST segment	0.051 \pm 0.017	0.033 \pm 0.015*	0.025 \pm 0.021*	0.018 \pm 0.022*	0.012 \pm 0.011*

*Significant value compared to rest moment ($p < 0.05$); Paired Samples *t* test.

At the end of the search simulation (60'EXER), there was no significant increase in cTnI level compared to baseline ($p = 0.0992$). In recovery time, there was a considerable increase in cTnI at 60'RCT ($P = 0.0161$), 120'RCT ($p < 0.0001$), and 4hRCT ($p = 0.0326$). After 12 h of recovery time, the cTnI level began to decline, remaining low until 24 h after the end of the search simulation (Figure 3).

Electrocardiographic assessment

Two dogs presented sinus rhythm at all moments of the study. One animal showed respiratory sinus arrhythmia at rest moment and started to present sinus rhythm after the search simulation (60'EXER), returning with respiratory sinus arrhythmia at 60 min of recovery time (60'RCT). Sinus tachycardia was found in two dogs that presented sinus rhythm at rest moment. In one of them, the tachycardia was observed at 30 min of recovery time (30'RCT), and in another dog, 60'EXER and 30'RCT.

In electrocardiographic assessment (Table 1), P wave duration was significantly higher ($p = 0.008$) immediately after the search simulation (60'EXER), at 15 min (15'RCT) ($p = 0.0064$) and 30 min (30'RCT) ($p = 0.007$) of the recovery phase, compared to baseline values in rest moment. No significant differences were observed in the evaluated moments regarding P wave amplitude. The QRS complex substantially increased at 30'RCT compared to the rest moment ($p = 0.03$). No significant differences were found in Q, R, and S wave amplitudes ($p > 0.05$). The PR interval didn't present a substantial difference compared to the baseline value.

The QT segment showed no significant difference between evaluated moments and baseline values. About the ST segment, it was observed elevation that presented a substantial difference at 60'EXER ($p = 0.017$), 15'RCT ($p = 0.003$), 30'RCT ($p = 0.003$), and 60'RCT ($p = 0.01$) compared to rest moment. There was no significant difference between T wave amplitude ($p > 0.05$). The T wave had amplitude above normality ($T > 25\%$ of R wave) at 60'EXER, 15'RCT, and 60'RCT. Change in T wave polarity was observed in one animal, which was biphasic at rest and became positive at the second moment of evaluation.

Heart rate

Heart rate was obtained before, during, and after the search simulation. There was no significant difference when the different evaluation moments were confronted with baseline value (rest moment) (Figure 4). Furthermore, at 60'EXER, the mean HR was the higher value of all moments of the study, and the animals showed no signs of exhaustion during or after the search simulation.

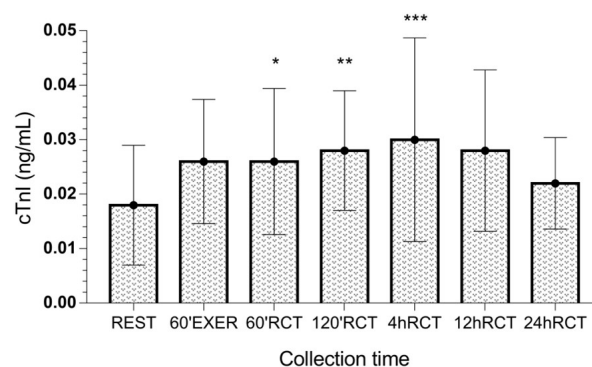


Figure 3 – Mean \pm SD of cardiac troponin I (ng/mL) of five adult healthy dogs from the Rescue and Recovery Service of Military Firefighters Corps of Espírito Santo, Brazil, submitted to a 60-min search operation simulation. Measurements were performed before (REST), during (EXER), and after (RCT) simulation. * $p = 0.0161$; ** $p < 0.0001$; *** $p = 0.0326$. Paired Samples *t*-test.

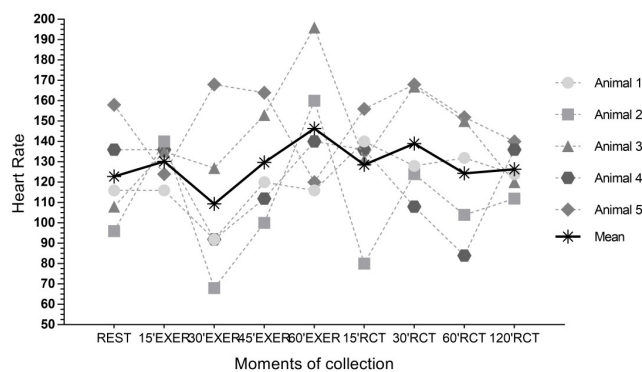


Figure 4 – Individual values and mean heart rate (HR) of five adult healthy dogs from the Rescue and Recovery Service of Military Firefighters Corps of Espírito Santo, Brazil, submitted to a 60-min search operation simulation. Measurements were performed before (REST), during (EXER), and after (RCT) simulation.

Discussion

The results showed that physical exercise induced significant increases in lactate levels at the end of the activity and 30 and 60 min of the recovery phase and a progressive rise in cTnI levels during the recovery phase. It is essential to point out that the performed activity was as close as possible to the actual working situation of these dogs. They could run, jump over obstacles, trot, and walk. Therefore, the physical effort was sufficient to identify variations in physiological biomarkers and myocardial injury levels.

The baseline lactate levels were within the limit of normality reported for dogs (Allen & Holm, 2008) and were close to the values described by other authors in police working dogs (Alves et al., 2012; Alves & Santos, 2016).

No change in lactate levels was observed before the search and rescue activities, and the changes observed during the experiment were strictly due to physical activity.

A significant increase in lactate levels in search dogs at the end of physical activity was also observed by other authors in a 20-min physical exercise experiment, showing that lactate is an indicator of anaerobiosis and exercise intensity, where strenuous exercise culminates with higher values of this metabolite, which changes as the individual becomes more conditioned to the exercise performed (Rovira et al., 2008). Therefore, we suggest that animals in this study are adapted to 60 min of physical activity since the difference with the baseline lactate occurred only at the end of the search and rescue simulation.

The significant increase in lactate levels in recovery phases (30'RCT and 60'RCT) may be associated with the anticipation of a new exercise, a physiological condition observed in animal athletes, which results in changes even before the activity starts (Angle et al., 2009).

However, another theory to explain this condition, believed to be the most plausible one, is the strong hormonal stimulus for glycogenolysis/gluconeogenesis for sparing and replenishment of muscle glycogen that remains increased during later rest periods and for several days after the conclusion of exercise (Davis et al., 2020).

Our study also showed that 60 min physical activity performed by rescue and recovery dogs induced significant increases in serum cTnI levels in the recovery phase (60'RCT, 120'RCT, and 4hRCT). These results corroborate studies that found that cTnI is released into the circulatory system later during exercise (Wakshlag et al., 2010; Wall et al., 2018).

Although variations were observed between the different evaluated moments, the obtained results did not exceed the average values for the species (<0.07 ng/mL) (Sleeper et al., 2001), indicating that the physical activity performed by the dogs in our study did not lead to the development of subclinical myocardial injury, in addition to confirming the absence of chronic cardiac damage (Spratt et al., 2005).

The set adjustments in the cardiovascular physiology after the onset of physical activity can lead to the injury of cardiomyocytes with cTnI extravasation (Nie et al., 2016; Shave et al., 2010). Our study's lack of cTnI value elevation suggests that the dogs were not subjected to a workload higher than they could bear.

The absence of results that extrapolated the normal values of cTnI in dogs has also been reported in a study with sled dogs undergoing endurance activity for approximately 60 min, where the authors suggested that non-strenuous physical activities performed by clinically healthy animals

didn't induce relevant elevations of this contractile protein levels, possibly reflecting an increase in cardiac membrane permeability, characterized by a discrete increase in cTnI levels, but within the average values (Wakshlag et al., 2010).

Considering the kinetic behavior of this biomarker of myocardial injury in our study, we infer that the dogs didn't suffer from cardiac deleterious effects associated with endurance activity. Therefore, considering the duration and intensity of the activity practiced, the degree of physical effort required was insufficient to interfere with cardiac homeostasis and myocardial oxygenation.

We identified that the 60 min search simulation did not result in significant differences in HR compared to the baseline value. The physical effort performed by these animals during the search simulation was not intense enough to generate substantial increases in HR. They are conditioned to this intensity and duration of activity, so they do not need activating compensatory mechanisms to increase cardiac output, which would result in increased HR (Piccione et al., 2012; Rathore et al., 2011; Rovira et al., 2008). Moreover, physical activities that do not require so much of the animals – especially those conditioned to exercise – do not cause considerable increases in HR (Allen & Holm, 2008).

Since these dogs work freely to perform the type of activity necessary to sniff the environment – alternating between trotting, galloping, running, and walking – the intensity of physical exercise was determined by each individual, explaining the significant variation of HR between the animals during the activity, that presented no relation with the elapsed time of physical activity. These results differ from cardiovascular responses to exercise observed in animals submitted to incremental treadmill tests, whose HR increases gradually as the exercise intensity increases (Piccione et al., 2012; Queiroz et al., 2018). Therefore, our animals did not develop an incremental physical activity but a biological activity of random intensity and individual variation.

Regarding the parameters evaluated by ECG, the types of rhythm observed were all considered normal, being only physiological variations in dogs, such as respiratory sinus arrhythmia and sinus tachycardia (Kraus et al., 2016; Lima et al., 2016).

It is important to note that no rhythm formation or conduction system disorders in any evaluated animal were identified. The occurrence of arrhythmias (ventricular extrasystoles and ventricular tachycardia) and electrical alternation was demonstrated in race dogs that reached their maximum capacity (Ponce Vázquez et al., 1998), besides being reported in other studies with dogs submitted to physical activity (Moraes et al., 2017; Santos et al., 2018).

Considering that the dogs in this study presented no arrhythmias and the mean HR remained relatively low during physical activity, we can infer that they practiced submaximal physical activity to their athletic capacity (Rovira et al., 2010). Therefore, it is possible to intensify the physical exercises in these animals, increasing their performances and effectiveness in the work.

In ECG exams, a significant increase in P wave duration was observed at 60'EXER, 15'RCT, and 30'RCT. Increased time of the P wave was found in all evaluation moments beyond the normal values for the canine species, which is 0.04 seconds (Ponce Vázquez et al., 1998). However, the value of the P wave of 0.044 ± 0.002 s was described in German Shepherds before being conditioned to a 6-week exercise (Moraes et al., 2017). Even in non-athlete animals, an increase in duration wave P can be observed, which leads us to believe that reference values of electrocardiographic patterns should be specific for each breed since there are significant differences between body structure, weight, and thoracic conformation. Also, we observed a substantial increase in P wave duration compared to the animals' baseline value, which may suggest a left atrial overload (Ponce Vázquez et al., 1998). A study that evaluated sled dogs before and after a physical conditioning program identified that physical exercise induced the increase of the left atrium in echocardiography, emphasizing the relation that physical activity has with the rise of preload (Stepien et al., 1998). This was possibly the cause of increased duration wave P induced by physical activity.

Considering the normality limits of 0.06 s established for dogs (Kosić et al., 2017; Mukherjee et al., 2015), a significant increase in the QRS at 30'RCT was observed. The increased duration of the QRS complex was also described in a study that evaluated sled dogs after a six-month endurance training, and the authors suggested that this change was due to cardiac hypertrophy triggered by the practice of physical activity (Constable et al., 2000). It is worth mentioning that there is no electrocardiographic record of animals before they start activities in the corporation. Thus, we infer that the exercise caused no change in this parameter. The observed QRS increase could be an inherent normality attributed to the breed since the echocardiographic examination identified ventricular overload in only one animal.

The significant differences observed in the ST segment can be explained by assigning the zero value to the isoelectric ST segment observed in some animals, which generated substantial differences between the means of the moments. None of the dogs in our study had ST segment depression, which was also observed in a study where only two of the 12 Belgian Malinois evaluated had ST segment depression

(but within normality limits for the species). All 10 evaluated German Shepherds presented an isoelectric ST segment (Lima et al., 2016).

A change in the polarity of the T wave was only found in one animal. The T wave alternation beat to beat, is reported as a precedent to ventricular arrhythmias. These alternations can be observed in T wave morphology, amplitude, and polarity (Costantini et al., 2000). However, the polarity alternation observed in this study did not occur beat to beat but between different evaluation moments. This condition was previously described in German Shepherd and Belgian Malinois athletes without the authors identifying the cause for this change (Mukherjee et al., 2015; Santos et al., 2018).

Among the causes of the altered polarity of T-wave is the elevation of the diaphragm during respiration (Kumar et al., 2014), and the dogs in this study could present alterations in this parameter due to ventricular overload associated with "athlete's heart," electrolytic disturbances or myocardial hypoxia due to physical activity, or an acute increase in sympathetic activation. However, we could not identify which causes could influence the T wave conformation in these animals.

This study presented some limitations. It was performed under natural conditions of search and rescue activity practiced by dogs, so changes in markers of physical adaptation and some vital information could not be easily collected, as occurs in studies with treadmills or a controlled environment. Furthermore, access to animals was restricted because it is a heterogeneous group with a small number of animals in the State service, whose work is essential for rescuing victims. It did not allow some variables complementary to the results obtained to be evaluated, for example, comparing the same variables in controlled situations of temperature, humidity, different environments, or tracking the time to physical exhaustion of animals, since it would require time for improvement and reassessment of methods, and the removal of animals from work would leave the search and rescue service without support.

The information obtained in this study can help develop more effective training programs, increase the animals' physical fitness, and change the intensity and duration of the training routine according to individual needs.

Conclusion

A 60-min search simulation was sufficient to generate identifiable biochemical alterations in lactate and cardiac troponin I level and a few alterations in ECG parameters and the maintenance of HR. These alterations suggest the adaptation of rescue and recovery dogs to the type, intensity, and duration of physical activity being performed. This

research encourages subsequent new studies to evaluate and define new methods of physical training for rescue and recovery dogs, as well as to follow their adaptive responses to suggested training, which could be identified from increases in specific parameters during training, gradually improving their overall performance.

Conflict of Interest

The authors report no conflicts of interest.

Ethics Statement

All applicable international, national, and/or institutional guidelines for the care and use of animals

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- were followed. The experimental procedures were reviewed and approved by the Animal Ethics and Utilization Committee in accordance with the Protocol 059/2017. Those responsible for the animals signed a Term of Free and Informed Consent, authorizing their participation in the study.

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