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Effects of exercise on urinary biochemical parameters and proteins in a group of well-trained military working dogs

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ABSTRACT

Exercise-induced proteinuria has been widely investigated in humans, also in relation to intensity and duration of activity. Instead, there are only limited publications regarding urinary biochemical parameters and urinary proteins before and after physical activity in dogs. This paper aimed to investigate the effects of exercise on urinary biochemistry and proteins in military dogs. Twenty-four dogs were enrolled in this study. All the dogs were clinically sound, and they were examined before and after activity. Pulse rates (PR) and respiratory rate (RR) were monitored. Urine was sampled before and after a training session of search activity. Standard urinalysis was carried out, urine total proteins and creatinine were measured and the urinary protein:creatinine ratio was calculated; finally, the urinary proteins were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Clinical examination before and after activity did not reveal any pathological finding. After activity, the PR was slightly increased, while the RR was notably increased ($p < 0.05$). Total proteins, albumin, and their ratio with creatinine were significantly higher after exercise when considering all the dogs included or only the females while, when considering only the males no significant difference was detected. The clinical relevance of this study was related to the possibility of using urine as a non-invasive sample for monitoring health status after training activity and exercise in dogs. An increase in microalbuminuria after search activity, measured using SDS-PAGE could be considered an early biomarker of renal function during training sessions.

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Introduction

Military working dogs (MWDs) play a fundamental role together with the army in managing humanitarian missions on both national and international territories. Their function is well recognized by intergovernmental organizations, such as the United Nations (UN) and the North Atlantic Treaty Organization (NATO), both of which have the aim of maintaining international peace and security. Training carried out correctly can lead to highly specialized fitness in these dogs, resulting in the prevention of professional traumatic injuries (Spinella et al. 2022).

Human sport medicine recommends that adults should engage in moderately-intense cardiorespiratory exercise for 150 min per week (30 min sessions 5 days per week) in order to have a proper level of physical wellness (Hesketh et al. 2020). Adult dogs should also undergo similar activities; however, while conditioning, it is of utmost importance to prevent work overload and muscle fatigue.

Changes in physiological, hematological and metabolic parameters during exercise contribute to the internal load, also defined as 'the relative biological stressors imposed on the athlete during training or competition' (Bourdon et al. 2017). The internal load depends on several factors, including the typology of the exercise and the extent of the canine training as well as on environmental factors (Cerqueira et al. 2018; Spinella et al. 2021). Depending on the type of stimulus, regular exercise leads to changes in the proteome and metabolome in an extremely complicated network of signalling and metabolic pathways (McGlory et al. 2017).

To monitor the correct execution of the training, clinical and hematological examinations should be carried out frequently. However, hematological monitoring

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requires repeated blood samplings and could potentially stress dogs. To avoid the invasiveness of sequential blood samplings, urine could be considered an interesting alternative for investigating the health status and pre- and post-exercise changes in athletic and working dogs.

Urine represents an ideal biological sample as it can easily be obtained with non-invasive procedures and repeated after a short time. In veterinary medicine, the application of proteomics to urine samples has been reported in the literature, for the most related to companion animals (Ferlizza et al. 2020; Miller 2020). Urinary protein profiles represent an interesting opportunity for monitor pathophysiological adaptations of kidney function or the possible site of renal damage. For example, decreased urinary uromodulin is related to tubular dysfunction in dogs and cats (Ferlizza et al. 2015, De Loor et al. 2013) while an abundance of proteins with a high and intermediate molecular mass (MM) is indicative of glomerular proteinuria (Hokamp et al. 2018).

The aim of this prospective study was to investigate the effects of exercise on urinary biochemical parameters and urinary proteins separated using SDS-PAGE electrophoresis in well trained MWDs.

Materials and methods

A standing agreement between the Italian Army and the Department of Veterinary Medical Sciences of Bologna University for the use of their data and for the development of the present study was stipulated (f.n. M_D SSMD REG2020 0051733 – 27/03/2020 SMD –IGESAN). The study was carried out according to European Union Directive 2010/63/EU and was approved by the Animal Welfare Committee of the University of Bologna (Project ID 914).

Twenty-four trained military working dogs (MWDs) belonging to the Italian Army were enrolled in this study (Table 1): 23 German Shepherd dogs and one Belgian Shepherd Malinois. The inclusion of the one dog of a different breed is not expected to influence the results. Fifteen dogs were female (14 intact females and one spayed) and 9 were male (6 intact, one neutered and 2 monorchid). The dogs, which were selected among those available at the military centre (not on mission), were those capable of carrying out search activities. All the dogs had a level of training established by a military veterinarian and appropriate for search activity. The dogs were fed with premium (Protein: 26% – Fat content: 17% – Crude ash: 6.4% – Crude fibers: 1.4%) or super-premium (Protein: 32% – Fat content: 30% – Crude ash: 7.8% – Crude fibers: 1.8%) commercial food. The dogs were randomly divided into two groups according to typology of activity: ten dogs performed only a standard 20-minute training of search activity (Group A) and 14 dogs additionally exercised 10 min on a treadmill after the search activity (Group B). The activity on the treadmill was performed at trot with a mean velocity of 12 km/h. Mean environmental temperature and relative humidity were recorded: the mean environmental air temperatures ranged from 26.5 °C on the 1st day to 23.5 °C on the 2nd and the 3rd days of the study, and the relative humidity from 71% on the1st day to 75% on the 2nd and 3rd days of the study.

All the dogs were routinely monitored by military veterinary personnel throughout their activity of MWDs. However, before each work session, all the dogs underwent complete signalment and physical examination to ensure their current healthy status. None of the dogs had received any medication with steroids or non-steroidal anti-inflammatory drugs (NSAIDs) within 30 days before the study. All the dogs that participated in the study were normothermic before performing the exercise. Pulse rate (PR), respiratory rate (RR) and rectal body temperature (BT) were recorded before the activity; the PR and RR were also evaluated after the activity. The PR was detected by palpation of the femoral artery, and the RR was measured by thoracic visual observation. In both the male and the female dogs, ten mL of mid-stream urine were sampled during spontaneous voiding into sterile urine cups before the daily feeding and watering activities, and with the animals at rest (T0). Within 30 min after the search activity a second sample of urine was obtained using the same procedure (T1); this second sample represented the first urination after the exercise. Moreover, in 12 dogs, the lactate concentration was also randomly evaluated using a rapid lactometer (Roche Diagnostics spa, Germany) to monitor the potential changes induced by exercise.

Table 1. Age, weight, breed, and type of activity of the dogs enrolled in the study.

	All the dogs enrolled	Females (intact/ spayed)	Males (intact/ neutered/monorchid)
Specimens	24	14/1	6/1/2
Age (months)	32 ± 18 (24)	33 ± 22 (24)	30 ± 10 (24)
Weight (Kg)	30 ± 4.5 (29.5)	27.4 ± 2.6 (27)	34.4 ± 1.9 (35)
Breed	23 GS; 1 BSM	15 GS	8 GS; 1 BSM
Search activity (Group A)	10	5	5
Search activity and treadmill (Group B)	14	10	4

The data are reported either referring to all the dogs enrolled in the study, or divided into males and females. The data for age and weight are reported as mean ± SD with the median in parentheses.
GS: German Shepherd; BSM: Belgian Shepherd Malinois

Urinalysis

All the urine samples were kept refrigerated (+4 °C) and were processed on a routine basis within 2 h after collection. In particular, the urinalysis consisted of a macroscopic examination evaluating the color and turbidity. Urine specific gravity (USG) was measured using a manual refractometer (Giorgio Bormac, 41012 Modena, Italy), the chemical evaluation was carried out using a semi-quantitative dipstick test (Combur10Test, Roche Diagnostic, Mannheim, Germany). After centrifugation at 1500 g for 10 min, urine sediment at T0 was observed under both high

(400x) and low microscopic fields (100x). Urine supernatants were divided into aliquots and stored in part at −20 °C for a maximum of 7 days for total protein and creatinine determination, and in part at − 80 °C for the subsequent proteomic analysis.

Urine protein to creatinine ratio

Urine total proteins (uTP) and creatinine (uCr) were measured using commercial kits (Urinary/CSF Protein, OSR6170, and Creatinine OSR6178, Olympus/Beckman Coulter, Atlanta, GE, USA) on an automated chemistry analyzer (AU 480, Olympus/Beckman Coulter, Atlanta, GE, USA). The urine protein:creatinine ratio (UPC) was calculated using the following formula: UPC = uTP (mg/dL)/uCr (mg/dL).

One-D-Electrophoresis

After thawing and centrifugation at 3000×g for 10 min, the urinary proteins in the supernatants were separated using a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system (NuPAGE, Thermo Fisher Scientific, Waltham, MA, USA) as previously described (Ferlizza et al. 2015); SDS-PAGE is commonly used to obtain high resolution separation of complex mixtures of proteins. The method initially denatures the proteins which will undergo electrophoresis and then separates them based on their molecular mass. Briefly, three µg of protein were loaded on 4-12% polyacrylamide gel in MOPS buffer with SDS (Thermo Fisher Scientific, Waltham, MA, USA). If the uTP concentration was lower than 0.100 µg/µL, urine was concentrated using spin columns with a molecular mass cut-off of 3 kDa (Vivaspin 500, Sartorius, Goettingen, Germany), following the manufacturer's instructions. Each gel was also loaded with standard proteins of known molecular mass (Precision Plus Protein Standard, Biorad, Hercules, CA, USA). The gels were stained with Coomassie brilliant blue (PageBlu protein staining solution; Thermo Fisher Scientific, Waltham, MA, USA). After staining, each gel was digitalized (ChemidocMP, BioRad, Hercules, CA, USA), and pherograms were obtained using commercial software (ImageLab, BioRad, Hercules, California, USA). The bands at 100, 67 and 18 kDa were identified on the basis of the molecular mass as previously reported by Ferlizza et al. (2020). The quantification of bands at 100 and 67 kDa was carried out using an internal standard of quantity as described by Ferlizza et al. (2020).

Statistical analysis

The data regarding the blood and urine chemistry were analyzed using statistical software (R version 3.4.4). Normal distribution was tested graphically and using the Shapiro-Wilk normality test, the data were expressed as mean ± standard deviation (SD) or standard error (SE) with the median in parentheses. The variables between the T0 and T1 samples were compared using the Wilcoxon or the *t* Student test depending on their distribution, assuming $p < .05$ as a significant probability.

Results

Twenty-three German Shepherd dogs and one Belgian Shepherd Malinois were enrolled (Table 1) in this study. Clinical examination before activity started at 8.30 am of each day with an interval of 10 min for each dog. All the dogs enrolled were found to be completely sound after a physical examination carried out by two licensed veterinarians. The clinical values for BTs, RRs, PRs and blood lactate are reported in Table 2.

Table 2. Clinical data, serum and urinary analytes in specimens grouped as all dogs and as females and males ($n = 24$; except for blood lactate, $n = 12$) at rest (T0) and within 30 min after the search activity (T1).

	All the dogs enrolled		Female		Male	
	T0	T1	T0	T1	T0	T1
RR	77 ± 35(1)	136 ± 35(1)	75 ± 31(2)	138 ± 39(2)	82 ± 43(3)	133 ± 29(3)
PR	81 ± 15	85 ± 18	82 ± 18	82 ± 18	80 ± 17	90 ± 17
Blood lactate	2.45 ± 0.61 (2.4)	2.04 ± 0.52 (1.8)	2.68 ± 0.53 (2.4)	2.0 ± 0.65 (1.7)	2.22 ± 0.65 (2.1)	2.08 ± 0.43 (1.8)
Urine biochemistry from the dipstick analysis						
pH	6.4 ± 0.6 (6.0)(1)	7.1 ± 0.9 (7.0)(1)	6.4 ± 0.6 (6.0)(2)	6.8 ± 0.9 (6.5)(2)	6.3 ± 0.7 (6.0)(3)	7.5 ± 0.7 (7.0)(3)
USG	1051 ± 11 (1052) (1)	1044 ± 16 (1048)(1)	1051 ± 14 (1052)(2)	1044 ± 17 (1042)(2)	1050 ± 7 (1048)	1043 ± 16 (1048)
Pro mg/dL	25 ± 10 (30)	28 ± 7 (30)	22 ± 12 (30)	27 ± 9 (30)	30 ± 0 (30)	30 ± 0 (30)
UBG µmol/L	2 ± 6 (0)	neg	2 ± 6 (0)	neg	2 ± 5 (0)	neg
Bil mg/dL	neg	neg	neg	neg	neg	neg
Glu mmol/L	neg	neg	neg	neg	neg	neg
Ket mmol/L	neg	neg	neg	neg	neg	neg
Ery (+/number of specimens)	++/2	++/2	++/2	++/2	neg	neg
Leu (+/number of specimens)	+/3	+/5	neg	neg	+/3	+/5

The The data for RRs and PRs are reported as mean ± SD. The data for blood lactate and urine biochemistry from the dipstick analysis are reported as mean ± standard deviation (SD) with the median in parentheses. In the same row and for each group, the same superscript number indicates a significant difference between T0 and T1 ($p < 0.05$).

RR: respiratory rate; PR: pulse rate; Pro: Dipstick Urine Proteins; UBG: Dipstick Urine Urobilinogen; Bil: Dipstick Urine Bilirubin; Glu: Dipstick Urine Glucose; Ket: Dipstick Urine; Ketones; Ery: Dipstick Urine Erythrocytes; Leu: Dipstick Urine Leukocytes; USG: urine specific gravity; Neg: negative; nd: not determined..

Urinalysis and urinary protein characterization

The color of the urine samples ranged from light yellow to yellow and turbidity was clear or slightly cloudy. The urinary biochemistry data in the specimens grouped as both sexes, females and males are reported in Table 2. The activity did not influence body temperature, pulse rate or serum lactate concentration. The pH ranged from 6.3 ± 0.7 at T0 to 7.5 ± 0.7 at T1 in males and a significant increase after exercise was present in all groups ($p = 0.00015$ both sexes group, $p = 0{,}008$ females; $p = 0.006$ males). The USG values for samples collected at both times were within the normal reference range reported for canine species (Rudinsky et al. 2019). However, the USG at T0 was significantly higher than the values measured at T1 in the group of both sexes (24 dogs) and in the female group (15 dogs).

Bilirubin, glucose and ketones presented non-detectable values in all the sample examined. The dipstick was positive for protein in 19 dogs at T0 (25 ± 10 mg/dL) and in 20 at T1 (28 ± 7 mg/dL); when considering sex, all the males had dipstick positive for protein. Urobilinogen resulted positive (lowest level of positivity) in 3 samples collected at T0 regardless of sex. The microscopic urine sediment evaluation carried out at T0 showed the presence of occasional epithelial cells and erythrocytes in 4 samples, and leucocytes in 8 samples. Calcium oxalate crystals were detected in 5 samples collected both at T0 and at T1.

Urinary protein separation in 1-DE

Urine samples collected before and after exercise presented similar profiles characterized by the presence of the two most abundant bands at an apparent molecular mass (MM) of 103 and 67 kDa in the females and the neutered males (Figure 1 and 2). The band profile was similar to that reported by Ferlizza et al. (2020) who identified the proteins as uromodulin, albumin and arginine esterase at 103, 67, and 18 kDa, respectively, using mass spectrometry. Therefore, the identity of the bands obtained in the present study could be hypothesized based on the apparent MM, and comparing data with those reported by Ferlizza et al. (2020).

Albumin was present in the majority of the samples while uromodulin was present in all the sample examined regardless of the sex. The intensity of albumin band was more pronounced in the urine collected after exercise. In addition, the urine samples from the intact male dogs presented two additional evident bands having apparent MMs of 18 and 12 kDa. The band at 18 kDa could be identified as arginine esterase (Figure 2).

Concentrations of total proteins, uromodulin and albumin, and their ratio with creatinine (uTP, UPC, uUC and uAC) in specimens grouped as both sexes, females and males are reported in Table 3.

Urine sampled before exercise presented a low concentration of albumin (1.2 ± 0.4 mg/dL) and a high concentration of uromodulin (11.4 ± 2.6 mg/dL). In the group of both sexes, the total protein concentration

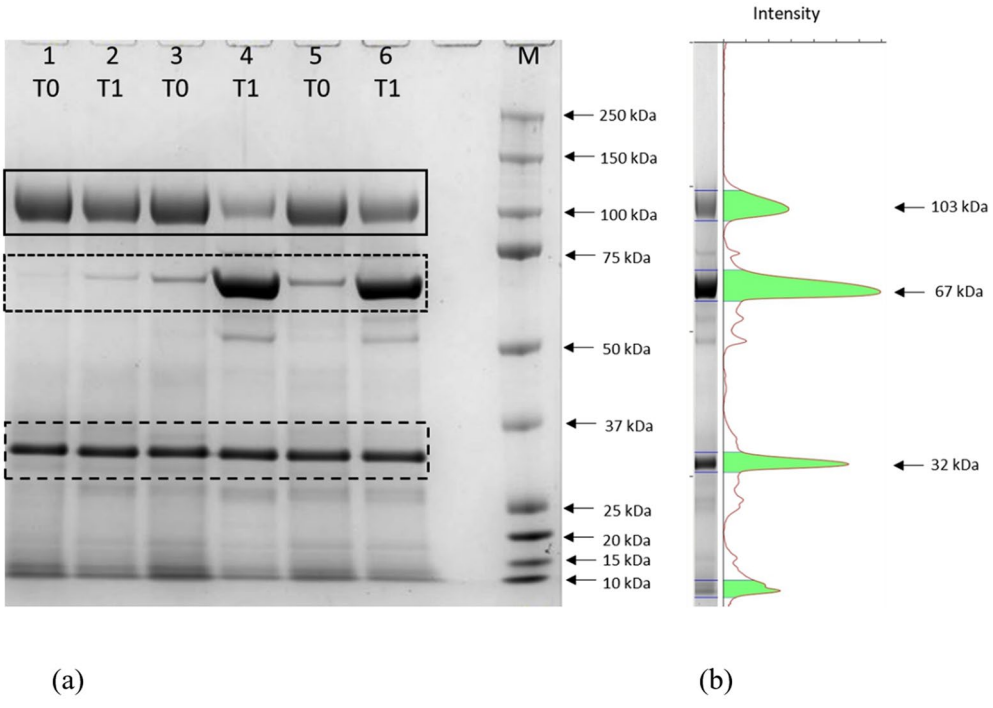

Figure 1. Representative SDS-PAGE gel of the proteins in theurine sampled at T0 and T1 from three female dogs (a). the black continuous box indicates uromodulin (103 kDa); the black dotted box indicates albumin (67 kDa); and the black dashed box indicates the internal standard of quantity (1 µg). a molecular mass marker (M) was also loaded. A representative pherogram (lane 6) is reported in (b).

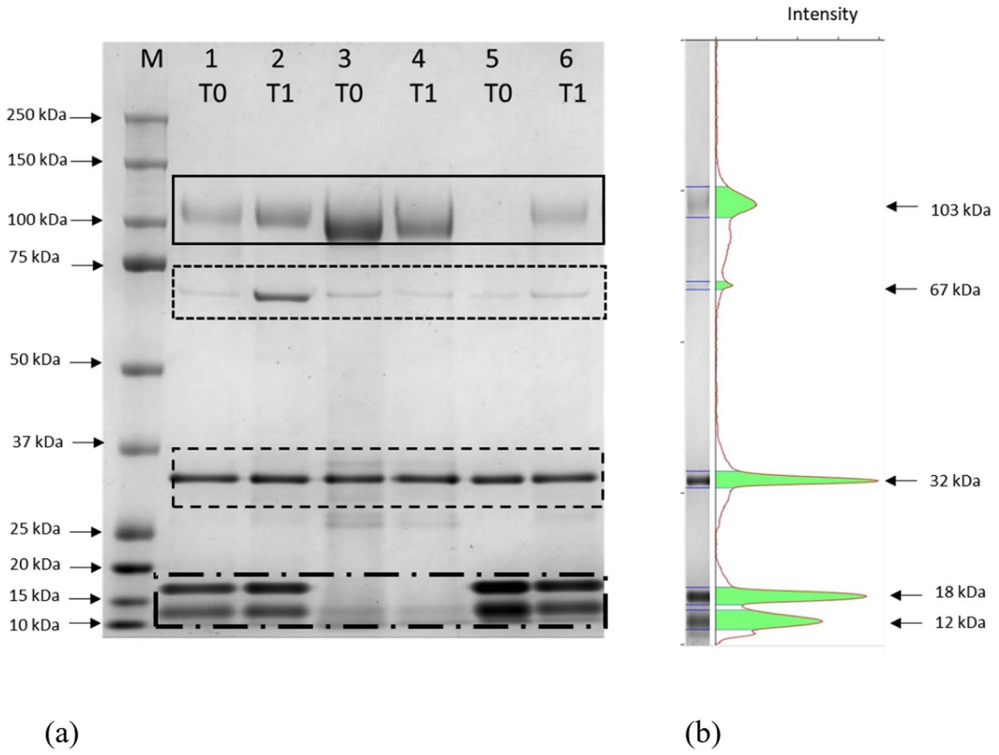

Figure 2. Representative SDS-PAGE gel of the proteins in the urine sampled at T0 and T1 from two intact male dogs (lanes 1, 2, 5 and 6) and one neutered male dog (lanes 3 and 4) (a). the black continuous box indicates uromodulin (103 kDa); the black dotted box indicates albumin (67 kDa), and the black dashed box indicates the internal standard of quantity (1 µg); black dashed and dotted box indicates arginine esterase (18 and 12 kDa). a molecular mass marker (M) was also loaded. A representative pherogram (lane 6) is reported in (b).

($p=0.004$), albumin concentration ($p=0.002$), UPC ($p=0.015$), and uAC ($p=0.002$) were significantly higher after exercise. When considering the group of males, the urine sampled after exercise did not present significant differences from that sampled before exercise while, in the female group, the total protein concentration ($p=0.01$), albumin concentration ($p=0.005$), UPC ($p=0.0011$), and uAC ($p=0.004$) were significantly higher in the urine sampled after exercise.

Regarding the type of activity, no significant differences were found between Groups A and B (Table 1S).

Discussion

The aim of this study was to examine the effects of routine exercise on urinary biochemical parameters and urinary proteins in well-trained MWDs. To the Author's knowledge, studies aimed at integrating the different aspects of urinalysis when monitoring the health status of athletic dogs are lacking in veterinary medicine in the literature. As reported by Zhao et al. (2020), urinalysis and proteomics may provide the clinician with fundamental information regarding health status, since changes in the blood may even be magnified in the urine inasmuch, as urine does not have the homeostatic mechanisms of blood.

In the present study, significant differences were observed for uTPs, the UPC, albumin concentration, and the uAC before and after exercise both in the entire group (both sexes and both activities) or in the female alone group, although clinical examination of the MWDs before and after activity did not reveal any pathological findings.

Both respiratory and pulse rates showed a physiological increase after exercise; however, respiratory rate showed a persistent elevation, probably related to the environmental air temperatures (≥ 23.5 °C) and relative humidity, which led the dogs to a physiological increase in panting. Similar results have previously been observed in working and sports dogs carrying out specific activities (Rovira et al. 2008; Spoo et al. 2015; Diverio et al. 2016; Cerqueira et al. 2018; Lopedote et al. 2020). A return to normal physiological ranges is generally observed in well-trained dogs within 30 min after exercise. Specific and correct conditioning is an essential requirement for trained dogs in order to produce a physiological adaptation to the activity and to decrease the risk of musculoskeletal injury (Pellegrino et al. 2018; Spinella et al. 2021). In particular, this is mandatory for military dogs, which generally perform missions in foreign countries with highly and repeatedly modifiable environmental conditions (Spinella et al. 2022).

A constant concentration of blood lactate is considered to be the gold standard for evaluating aerobic activity in humans, dogs, and horses (Miranda et al. 2014; Alves and Santos, 2016). In this study, no significant differences were observed for lactate blood concentration before and after exercise, providing proof of adequate training as also reported in

Table 3. Concentrations of total proteins, albumin, and uromodulin in the urine samples at rest (T0) and within 30 min after the search activity (T1).

	All the dogs enrolled		Females		Males	
	T0	T1	T0	T1	T0	T1
uTP mg/dL	38.2 ± 12.2 (23.5)[1]	44.5 ± 7.2 (35.0)[1]	20.8 ± 2.8 (22.1)[2]	29.6 ± 3.9 (27.1)[2]	67.2 ± 53.5 (53.6)	69.5 ± 26.3 (63.6)
uCr mg/dL	288 ± 17 (275)	295 ± 17 (280)	291 ± 26 (295)	303 ± 18 (295)	284 ± 32 (270)	283 ± 62 (265)
UPC	0.13 ± 0.04 (0.08)[1]	0.16 ± 0.03 (0.12)[1]	0.07 ± 0.01 (0.07)[2]	0.09 ± 0.01 (0.09)[2]	0.24 ± 0.19 (0.13)	0.27 ± 0.13 (0.17)
Uromudulin mg/dL	11.4 ± 2.6 (8.8)	12.2 ± 3.0 (7.3)	15.4 ± 3.8 (9.4)	17.3 ± 4.4 (10.5)	5.6 ± 2.9 (5.3)	4.8 ± 4.1 (0.7)
uUC	0.035 ± 0.007 (0.023)	0.038 ± 0.011 (0.018)	0.043 ± 0.011 (0.033)	0.052 ± 0.017 (0.034)	0.021 ± 0.012 (0.019)	0.015 ± 0.013 (0.004)
Albumin mg/dL	1.2 ± 0.4 (0.4)[1]	19.1 ± 9.9 (1.3)[1]	1.5 ± 0.6 (0.3)[2]	28.3 ± 15.9 (1.4)[2]	0.8 ± 0.5 (0.5)	5.8 ± 5.3 (1.2)
uAC	0.037 ± 0.001 (0.001)[1]	0.065 ± 0.035 (0.004)[1]	0.004 ± 0.001 (0.001)[2]	0.090 ± 0.055 (0.003)[2]	0.003 ± 0.002 (0.001)	0.023 ± 0.021 (0.004)

Data are reported as mean ± SE with the median in parentheses. In the same row and within each group, the same superscript number indicates a significant difference between T0 and T1 ($p < 0.05$).
uTP: urine total proteins; uCr: urine creatinine; UPC: urine protein:creatinine ratio; uUC urine uromodulin:creatinine ratio; uAC: urine albumin:creatinine ratio.

a previous study on working dogs (Alves and Santos, 2016).

Different scientific studies have demonstrated that exercise produces variations in urinary pH, specific gravity, and proteinuria in human athletes (Pero et al. 2020; Wołyniec et al. 2016). These variations can be considered to be a response to the stress on specific organs, such as kidneys and muscles, caused by exercise, and their identification represents a new and precocious approach to monitoring health status, also in working dogs.

Hydration status in canine athletes can be assessed by the pH and specific gravity of urine (Pasławska et al. 2020). The present data showed that after exercise the pH was slightly increased with significant differences in all the groups considered, reaching a maximum value of 7.5 ± 0.7 in males at T1 (Table 2). A similar trend was reported by Pasławska et al. (2020) in adult dogs which had run 5 km at 25 °C. On the other hand, a decrease in pH to ≤ 5 after physical activity is indicative of excessive hypohydration and lactic acidosis (Pero et al. 2020). Hydration status can also be evaluated by USG in the first morning urine. Intraindividual and interindividual variations in urine concentration capacity were reported by Rudinsky et al. (2019); however, a mean USG of 1.040 ± 0.011 was considered representative of a healthy dog population. In the dogs considered in the present study, the USG values were within the above reported interval. After exercise, in the group of the females and that of both sexes, the USG was significantly lower, indicating a good hydration status. Instead, the concentration of creatinine in the urine did not change after exercise, confirming that there had been no dilution of urine and that the rate of glomerular filtration of the creatinine was not influenced by search activity, regardless of sex.

Exercise determined variations in urinary proteins. The first screening for proteinuria is routinely obtained by dipstick analysis which is an imprecise and inaccurate method. Any positivity for proteins in the dipstick analysis should be interpreted in the light of the specific gravity of the urine which in this study was within the interval reported by Rudinsky et al. (2019) as being representative of a healthy dog population. The proteinuria tested by dipstick was not influenced by exercise.

On the contrary, the uTP, UPC, albumin, and uAC were significantly higher in the urine collected after physical activity in the group of females and that of both sexes, as reported by other authors in humans and dogs (Sentürk et al. 2007; Pasławska et al. 2020). The absence of differences in the male dog group was probably related to the presence of six intact males out of a total of nine dogs. In a previous study involving dogs, Bertieri et al. (2015) reported that the UPC before castration was significantly higher than after. The present study confirmed that the urinary proteome in intact males was different due to the presence of arginine esterase (Figure 2). This dimeric protein of prostatic origin, following the denaturing and reducing treatment of the samples before

SDS-PAGE, appeared in the gel separated into one intense band at 18 kDa and a less prominent one at 12–14 kDa (Miller et al. 2020*)*. When loading a fixed quantity of proteins (three µg) onto the gel, the largest percentage was represented by arginine esterase in the intact males, while in the females and the neutered males it was represented by uromodulin and albumin. The presence of arginine esterase in the urine of the intact male dogs should be taken into account in clinical settings in order to exclude false tubular proteinuria, as previously suggested by Ferlizza et al. (2020).

The intensity of the exercise rather than its duration influences the origin of proteinuria (Pero et al. 2020). Moderate exercise has been associated with glomerular proteinuria with an increase in the filtration of proteins having a molecular mass >60 kDa, such as albumin (Sentürk et al. 2007). By contrast, during intense exercise glomerular and tubular (mixed) proteinuria occurs due to excessive filtration or the low reabsorption of albumin and other low-molecular mass proteins by the tubule (Lippi et al. 2012). Various mechanisms have been reported to explain transient post-exercise proteinuria, namely decreased circulation in the renal districts which results in inflammation (Lippi et al. 2012), hypoxia (Joyce et al. 2020) and the increased production of reactive oxygen and nitrogen species (Sentürk et al. 2007). Hemodynamic changes in kidney vessels produce an increase in glomerular membrane permeability and the passage into the ultra-filtrate of macromolecules, such as albumin (Sentürk et al. 2007). The concentration of albumin in urine also depends on tubular function since this protein is also reabsorbed mainly in the proximal tubule by an endocytic mechanism (Christensen et al. 2012).

In dogs, microalbuminuria is defined as a concentration of albumin in the urine of >1 mg/dL which is below the limit of detection of semi-quantitative screening tests (30 mg/dl), while an albumin concentration of >30 mg/dL is defined as overt albuminuria (Whittemoreet al. 2006). In this study, the mean albumin values measured in dogs before activity were slightly above (in the female group and that of both sexes) or under (in the male group) the cut-off of 1 mg/dL while after exercise, they did not exceed the cut-off of 30 mg/dL, despite a significant increase in the female group and that of both sexes.

Albuminuria can also be expressed as the urinary albumin:creatinine ratio (uAC). In veterinary medicine, uAC values <0.03 are considered to be normal, while values between 0.03 and 0.3 are indicative of microalbuminuria, and values >0.3 are indicative of overt albuminuria (Pasławska et al. 2020; Falus et al. 2022). Gary et al. (2004) found a microalbuminuria prevalence of 15% in healthy dogs at T0 and reported that mild exercise on a treadmill for 20 min did not affect urinary albumin concentration. In the present study, the prevalence of microalbuminuria at T0 was 25%, regardless of sex; however, after exercise, the prevalence doubled, indicating that search activity alone or associated with a treadmill led to an increase in post-exercise albuminuria. However, the finding that the mean concentration of albumin after exercise did not exceed the upper limits of microalbuminuria (0.3) gave the Authors the confidence that the training of military dogs and the intensity of exercise were correctly set.

Using SDS-PAGE, it was also possible to measure the urinary uromodulin concentration, obtaining a mean value similar to that reported in healthy dogs by Ferlizza et al. (2020). Uromodulin is a glycoprotein abundantly present in the urine of healthy dogs. Previous research involving dogs showed that serum and urinary uromodulin concentration could be correlated with the glomerular filtration rate and that its decrease was considered to be a promising and early biomarker of renal/tubular dysfunction (Ferlizza et al. 2020; Seo et al. 2022). The concentration of uromodulin in the urine of the dogs analyzed in the present study did not present significant changes after physical activity, suggesting the absence of renal/tubular impairment (Table 3).

Finally, the wide variability of urinary total protein and albumin concentration determined after physical activity was in accordance with the data reported by other authors (Pasławska et al. 2020). In addition to interindividual physiological variability, the development of post-exercise proteinuria could also indicate underlying medical conditions, such as early renal disease or diabetes mellitus, requiring additional investigation. Therefore, the monitoring of proteinuria by SDS-PAGE at regular intervals in canine athletes could be useful in identifying subjects with a higher risk of developing renal failure, as it has been suggested in human medicine (Lippi et al. 2012).

Conclusions

Overall, the results of the present study were in accordance with those of other authors; however, also revealed interesting and remarkable findings. First, the method of separating and quantifying urinary proteins using SDS-PAGE electrophoresis was sensitive enough to find even a slight increase in microalbuminuria after search activity in well trained dogs. Second, albuminuria could be considered an early and non-invasive biomarker for monitoring renal function during training sessions. Finally, the physiological presence of arginine esterase in the urine of intact males could influence UPC, and uromodulin and albumin concentrations and mask the post-exercise proteinuria.

One limitation of the study was the presence of intact and neutered specimens in the male group; additional research is needed to evaluate the effect of castration on the urinary proteome in male dogs. An additional limitation of the study was the limited number of specimens in the male group. Furthermore, additional studies, also carried out with metabolomic techniques, are needed to investigate the presence of other possible urinary biomarkers indicative of the impairment of renal and muscular function after physical activity.

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