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Hair and faecal cortisol level's variations during the training in Schutzhund dogs

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The aim of this study was to determine the cortisol levels in Schutzhund dogs during training, in order to highlight a relationship between different work programmes and adrenocortical activity. Fifteen Schutzhund dogs (12 males and 3 females) were used: six dogs with the highest level of training (title IPO-3, group IPO-3), and nine animals without title IPO (group IPO-0). Animals of the two groups followed two different training programmes. The programme followed by IPO-3 dogs consisted of a period of intense work for each section (section A - tracking phase, section B - obedience phase and section C - protection phase; period 1) and a period of reduction in the intensity of the training (period 2). On the other hand, IPO-0 dogs underwent a constant work intensity throughout the experiment. The faeces and hair cortisol content was measured by RIA. In all dogs the exercise induced a significant increase of faecal cortisol concentrations as compared with the levels at rest; the faecal levels of this hormone were higher in IPO-3 than in IPO-0 dogs both on days when animals were not doing physical activity and on days of work, indicating a greater exercise-related adrenocortical stimulation. Training intensity induced a modification in faecal cortisol concentrations while these were not affected by the type of work (Sections A, B and C). Correlation between hair and faecal cortisol levels was also checked; hair cortisol levels correlated positively with those observed in faeces.

Keywords - Schutzhund dogs, cortisol, hair, faeces, training.

INTRODUCTION

Haematological and hormonal parameters are important to assess both health status and welfare of the dog. These parameters also can be used in sport medicine for the evaluation of stress induced by different training methods.

Markers are needed to assess the fitness of the dogs: a well-established stress marker in this species is cortisol.[1-3] In training, as well as in sport practice, several biological effects that could modify organism homeostasis can be observed: the related response of the animal is defined "stress response" and it implies physiological, endocrine and behavioural changes. The animal adaptation to environmental conditions depends on the neuroendocrine system, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathic-adrenomedullary axis, through the liberation of a variety of hormones.[4] The interaction between catecholamines and glucocorticoids help animals to bear muscular work and interact with their environment. They allow animals to focalize their attention on events and orient their resources for decision-making process

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and action. Indeed, glucocorticoids have an important role in cognitive operations. Their interaction with norepinephrine influences attention and emotional memory, while decision-making process involve glucocorticoids and dopamine[5]. According to what was previously described, the positive meaning of moderate stress (eustress) during training can be perceived. [5-6] However, chronic high glucocorticoids levels are negative for cognitive/emotional process and might lead to behavioural and physical pathologies.[7-8-9]

Cortisol is a useful marker in the evaluation of stress caused by training.

There are many physiological and behavioural studies on measuring chronic stress in both laboratory[1,10-14] and shelter dogs.[15,16] Unfortunately, few studies deal with chronic welfare problems of working dogs.[17,18] Among these, many researchers studied the endocrine and haematological changes in Schutzhund dogs during/after exercise,[19-21] but data concerning these modifications during race training are lacking.

The determination of these parameters in trained dogs could be very important. In fact, over-physical work or training conducted with coercive methods that physically force dogs to make exercises can lead these subjects to manifestations of acute fatigue, which may subsequently determine chronic strain with incomplete functional recovery, early tiredness and lower performance[22]. This may be related to several factors, including stress, with an excessive release of glucocorticoids.

A stress response could be indicative of a work overload and, consequently, of an ineffective training; furthermore, some parameters could be out of the reference range for trained dogs, depending on the different characteristics of the work.

Schutzhund is a sport in which the man-dog duo performs tests that have the purpose to assess the dog's aptitudes and physical characteristics. This sport consists of three phases: tracking (A), obedience (B) and protection (C). These working tests are divided into three levels of increasing difficulty: IPO-1, IPO-2 and IPO-3 (IPO = International Prüfungsordnung). The tracking phase consists in searching a smelling track generated by a person trampling the ground and in discovering objects abandoned along the same track. The obedience phase consists of a series of exercises that the man-dog duo exhibits in a specific sequence, as free heeling, sitting during the march, lying down with recall, standing during the race, retrieving (on flat surface, jumping over an obstacle and over an A-frame), sending forward and lying down on command, and lying down with distraction. The protection phase, tests the dog's courage to protect itself and its handler and its ability to be controlled while doing so. There are a series of exercises, like finding the hidden helper and prevent him from moving only by barking at him, stopping the helper attack or escape by biting the padded sleeve on the helper arm. When the attack or escape stops, the dog is commanded to "out," or release the sleeve. The dog must out or it is dismissed. At all times the dog must show the courage to engage the helper and the temperament to obey the handler while in this high state of drive.

The dog, during training and athletic activity, is subjected to "psychological stress", generated mainly by strong, instinctive, motivational impulses, and to "physical stress", generated by the intense muscular work.

No specific studies have been conducted so far to assess the activity of the HPA axis in dogs engaged in training for the Schutzhund tests at a competitive level. Based on these considerations, the purpose of this study was to determine some endocrine parameters (cortisol) relating to levels of stress the Schutzhund dogs undergo during training. At the same time, we investigated the relationship between different work programmes and adrenal cortex activities. Unlike other authors, we used non-invasive techniques when assessing cortisol levels, such as detecting the content of this hormone in faeces and hair.

This study aims to evaluate the activity of the HPA axis in Schutzhund dogs, a sport in which the man-dog duo undergoes tests of utility and defence.

MATERIALS AND METHODS

Animals

Fifteen intact dogs (12 males and 3 females), of three different pure breeds (German Shepherd n=12, Rottweiler n= 2 and Dobermann n=1) aging between 2 and 7 years were used. All the subjects belonged to private owners and were trained for Schutzhund competition: at the time of the study, six dogs had the highest level of training (group IPO-3), and nine dogs were at the beginning of the training (group IPO-0) (Table 1).

Structures

The study was carried out at a training field officially recognised by the German Shepherd club (Verein für Deutsche Schäferhunde) and under the control of ENCI (Ente Nazionale della Cinofilia Italiana). The training field, measuring 80 x 60 m and covered with

Table 1 - Characteristics of dogs used in experimentation (average ± S.E.)				
	TOTAL	German Shepherd	Rottweiler	Dobermann
Total dog (n°)	15	12	2	1
Male not sterilized	12	10	1	1
Female not sterilized	3	2	1	0
Age (years)	4,30 ± 0,43	4,56 ± 1,54	3,37 ± 0,48	3,02 ± 0,00
Group IPO				
IPO 3 (n°)	6	4	1	1
Male not sterilized	3	2	0	1
Female not sterilized	3	2	1	0
Age (years)	2,67 ± 0,67	3,00 ± 0,91	1,00 ± 0,00	3,00 ± 0,00
IPO 0 (n°)	9	8	1	0
Male not sterilized	9	8	1	0
Female not sterilized	0	0	0	0
Age (years)	5,11 ± 0,20	5,13 ± 0,23	5,00 ± 0,00	0

Figure 1 - Picture of training field equipped with Schutzhund typical equipment. It measures 80 x 60 m and is covered with a grassy mantle.

a grassy mantle, was equipped with Schutzhund typical equipment: palisades, hiding places for helper - the "bad guy" (Figure 1).

Training

The period of the study was between September and December of the same year when the animals of the two groups, IPO-3 and IPO-0, followed two different training programmes, in relation to their preparation's level.

The training of IPO-3 dogs consisted of a period of 2 months of intense work, in preparation for the Italian Schutzhund Championship (period 1 - pre-agonistic period), followed by a period of 2 months characterized by a reduction in training intensity (period 2 - post-agonistic period). During the pre-agonistic period, dogs were trained three or more times per week, while in the post-agonistic period dogs were trained once a week. The training of IPO-0 dogs was constant throughout the 4 months' experimental period during which dogs had three or more trainings per week.

Training sessions of IPO-3 dogs were more intense for speed required, physical effort and psychological

The study, carried out in an official Schutzhund training field, involved 15 dogs divided into two groups, divided based on the difficulty of the trials and the intensity of the training (IPO-3 and IPO-0).

stress, due to the repetition of some exercises rather than those of IPO-0 dogs. Agonistic performance were asked to IPO-3 dogs, whereas IPO-0 group was composed by young dogs that are in a state of preparedness to that activity, so too long or physical and psychological stressing sessions would have created a demotivation toward the work.
Each training session lasted about 15 minutes and included tracking, obedience and protection exercises, both for IPO-3 and IPO-0 dogs.
An operator (expert trainer) constantly evaluated the intensity of work. To assess the degree of work intensity ("light" or "intense") and psycho-physical fatigue at the end of the work, the dogs' respiratory and heart rate after work were clinically detected and considered as well: an increase of over 50% of the baseline frequency of both parameters designated an intense work.

Sample collection

Faeces were collected every day for the whole experimental period, obtaining samples from both rest and training days. Faeces were collected immediately after evacuation, cleaned up from litter or debris, put in plastic bags and frozen within few minutes.
Hair was collected from every dog at the beginning and at the end of each experimental period (1 and 2) for dogs IPO-3 and at the beginning and at the end of research (*sampling period*) for dogs IPO-0. The hair was cut from a sternal or lateral thoracic region and it was taken from an area with a diameter of about 10 cm. Hair was trimmed using small scissors, as this was the less invasive method. Re-sampling was taken from the same anatomical region of each dog. The observer collected between 10 and 90 mg of hair from each animal on each sampling session; hair was put into plastic bags with hermetic locking.
All samples (faeces and hair) were labeled (animal name, day, evacuation time or sample anatomy region) and stored at -20° C until analysis.

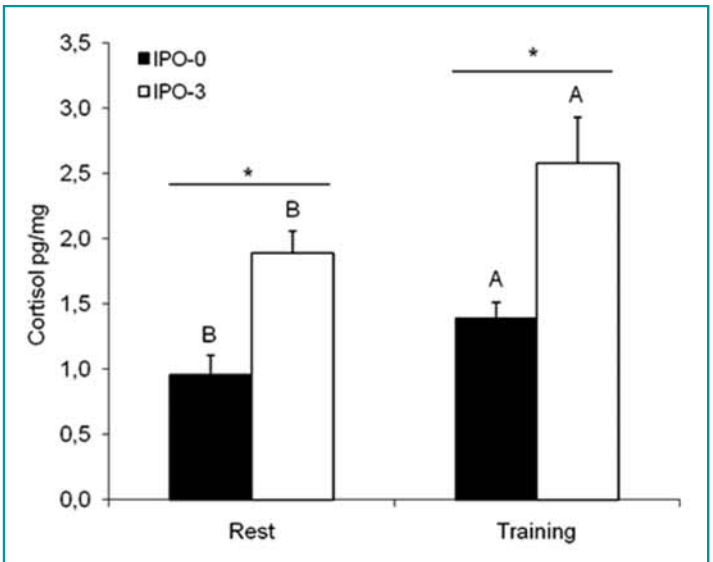

Figure 2 - Faecal cortisol concentrations in dogs from the two groups (rest and training) in relation to the activity/training program (IPO-0 and IPO-3) (mean ± S.E.). A, B: P<0.01 rest day vs training.
*: P<0.01 IPO-0 vs IPO-3

Faecal samples were collected daily while the hair samples were collected four times in the IPO-3 group and twice in the IPO-0 group; cortisol was analysed with RIA and a final statistical analysis was performed.

Cortisol analysis

Extraction from faeces was performed as described by Schatz and Palme (2001)[23]. Briefly, a methanol: water (v/v 4:1) solution was added to faeces in capped glass tube vials. Vials were then vortexed for 30 min using a multitube pulsing vortexer. Following centrifugation (1500g for 15 min), ethylic ether and NaHCO3 (5%) were added to 1 ml supernatant. This preparation was then vortexed for 1 min on multitube pulsing vortexer and centrifuged for 5 min (1500g). The ether portion was then separated and evaporated to dryness under air-stream suction hood at 37°C; dry residue was finally re-dissolved into phosphate-buffered saline (PBS) 0.05M, pH 7.5.
Extraction from hair was performed as described by Accorsi et al. (2008).[24] Hair was first minced into 1-3 mm length fragments and trimmed hair was put in a glass vial. Methanol was added and vials were incubated at +50°C with gentle shaking for 18 h. The content of the vials was then filtered to separate the liquid phase. The latter was evaporated to dryness under air-stream suction hood at 37°C and dry residue was then dissolved into PBS 0.05M, pH 7.5.
Assay in both faeces and hair was carried out according to Tamanini et al. (1983).[25] Analysis was performed in duplicate. Parameters for the analysis validation were: sensibility 0.26 pg/mg, assay variability 6.8%, variability between assays 9.3%.

Statistical analysis

All concentrations are expressed in picograms of cortisol relative to milligram of faecal or hair shaft matter (pg/mg). Data are expressed as means ± standard error (X ± SE) after checking for normal distribution of the residues.
An analysis of variance for repeated measures was carried out to detect any variations in cortisol concen-

trations of faecal and hair samples of IPO-3 and IPO-0 dogs between periods 1 and 2. A Duncan's post hoc test was carried out.
Correlation between individual cortisol contents found in the hair and mean individual faecal cortisol levels over the period of hair growth was tested using Pearson Rank Correlation Test.
For all analysis values was set = 0.05.

RESULTS

Figure 2 shows faecal cortisol levels in dogs of the two groups (IPO-0 and IPO-3) during training days and during rest days. The psycho-physical exercise determined, in both groups, a significant increase in faecal cortisol concentrations (IPO-0: 0.96 ± 0.15 *vs* 1.39 ± 0.12 and IPO-3: 1.89 ± 0.17 *vs* 2.58 ± 0.35; P<0.01). Cortisol levels, during rest and training days, were different, depending on the level of training: animals with the highest Schutzhund titles and that followed the IPO-3 program presented, both in rest and working days, a significantly higher cortisol concentrations (P<0.01) as compared to animals that practiced the IPO-0 program.
Figure 3 shows faecal cortisol levels in two groups of dogs (IPO-0 and IPO-3) for period 1 and 2 during training and rest days. IPO-3 dogs, presented a significant decrease (P<0.01) of faecal cortisol concentrations in period 2, both in rest and training periods. In IPO-0 dogs, no significant difference in faecal cortisol concentrations was observed between the two periods, both in rest and training days.
The influence of the psychophysical work intensity on faecal cortisol concentration can be observed in Figure 4. In the IPO-3 subjects, a significant increase of faecal cortisol levels (1.97 ± 0.39 *vs* 3.03 ± 0.52; P<0.01) occurred during the intense work, but not in IPO-0 dogs, subjected to a constant intensity of work in all training sessions (1.37 ± 0.14 *vs* 1.44 ± 0.22).
However, the kind of work performed in training sessions did not affect faecal cortisol concentrations: in fact, no significant differences were found in relation to the tracking, obedience and protection phase (Figure 5).
Figure 6 shows hair cortisol concentrations at the beginning of the experiment, immediately after the Italian championship and at the end of the experimental period. IPO-3 animals presented the highest levels of hair cortisol concomitantly with the national championship; these values were significantly higher (13.60 ± 3.71; P<0.01) than those recorded at the beginning (2.65 ± 0.46) and at the end (3.80 ± 0.96) of the experimental period. Furthermore, at the end of the study, cortisol concentration was significantly higher (P<0.01) than that observed at the beginning. The IPO-0 animals, which did not perform the national championship of

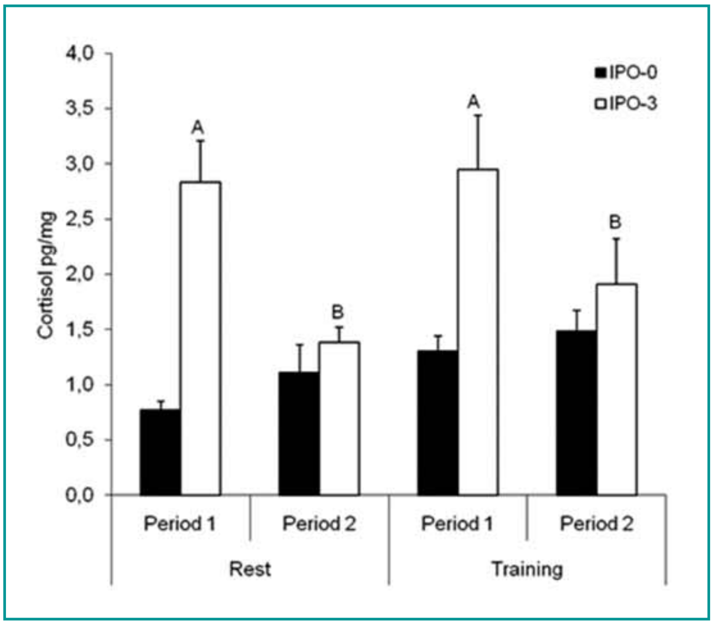

Figure 3 - Cortisol concentrations in faeces collected from dogs of the two groups after days with no physical activity and after days of physical activity during the periods 1 and 2 in relation to the training program (IPO-0 and IPO-3) (mean ± S.E.). A, B: P<0.01 period 1 vs period 2.

Hair cortisol exhibited a different pattern in the two groups; the same was true for faecal cortisol during training and rest. With exercise, faecal cortisol increased in both groups.

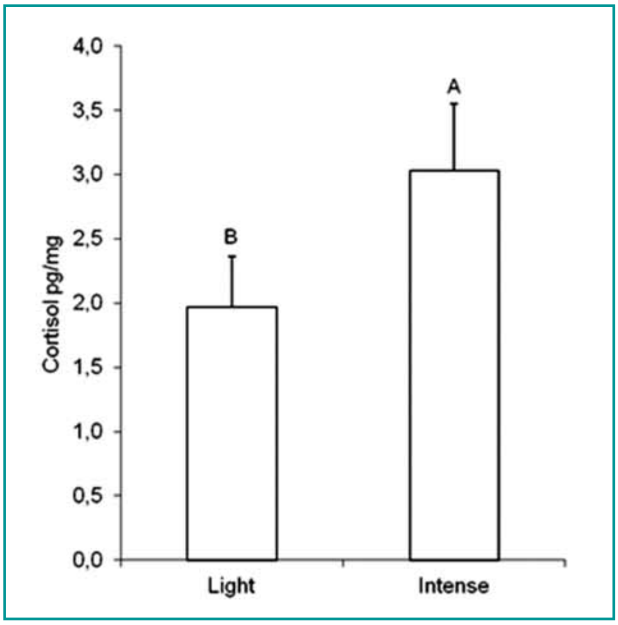

Figure 4 - Faecal cortisol concentrations after a light and an intense training, in relation to the training program (IPO-3) performed by animals (mean ± S.E.). A, B: P<0.01 light vs intense.

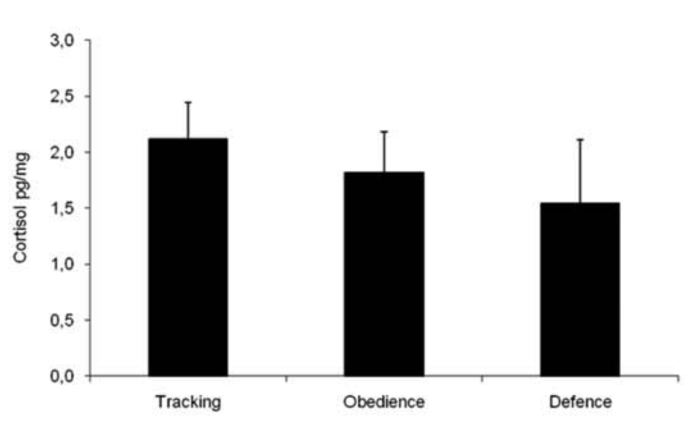

Figure 5 - Faecal cortisol concentrations after work in relation to the kind of activity (tracking, obedience and protection phase) performed (mean ± S.E.).

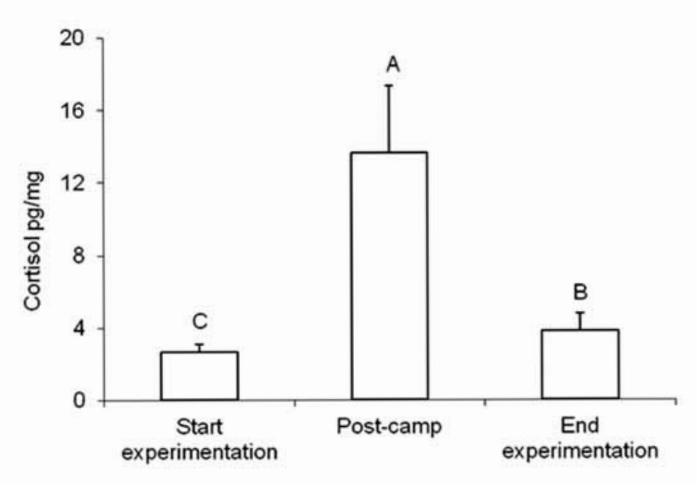

Figure 6 - Hair cortisol concentrations in dogs IPO-3 at the beginning, after national championship of Schutzhund and at the end of the experimental period (mean ± S.E.). A, B, C: P<0,01.

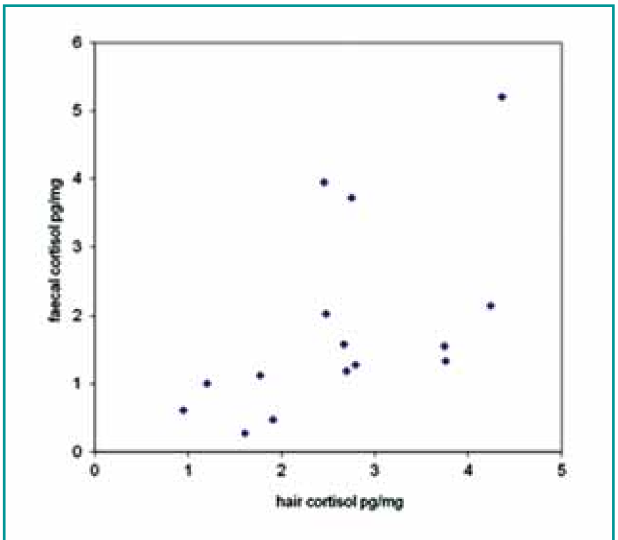

Figure 7 - Pearson Correlation plot. Mean individual faecal cortisol values plotted against individual hair cortisol content for the dog sample (N = 15).

Schutzhund, presented similar values in samples taken from the same area of hair regrowth, both at the beginning and at the end of the proof (2.48 ± 0.55 *vs* 2.85 ± 0,63).

The Pearson Correlation Test revealed a significant positive correlation between hair and faecal cortisol levels in each dog (r_s = 0.68, P< 0.001; Fig 7).

DISCUSSION

The aim of the present study was to determine, for the first time, changes in faeces and hair cortisol concentrations in Schutzhund dogs training.

The results of the present study indicate that the psycho-physical exercise determined in all dogs a significant increase in faecal cortisol concentrations, compared with those at rest. The faecal levels of this hormone are higher in dogs with the highest level of training and performing the IPO-3 program than in those without patents and supporting the IPO-0 program, both on days with and without physical activity, thus indicating a greater adrenocortical stimulation in relation to exercise.

The faecal levels we determined, both at rest and during training, are lower than those reported by other studies;[23,26] these differences could possibly be due to the different living conditions of the subjects (a shelter and garden at the handler's home vs laboratory and shelter) or maybe to the different techniques employed for cortisol determination (RIA vs EIA).

The higher concentration of faecal cortisol in IPO-3 dogs and its increase during exercise are indicative of its impact on adrenocortical activity.

On the other side, works examining faecal concentrations of cortisol in dogs are, to our current knowledge, scarce.

The increased concentration of faecal cortisol we found during physical activity is in agreement with the results found in literature.[27,28] Haverbeke *et al.* (2008)[27] analysed the physiological (cortisol) and behavioral responses of military working dogs. The subjects were submitted twice to environmental challenges composed of social and protection work exercises, visual and auditory stimuli. Similarly, to our study, plasma cortisol concentration was significantly higher after challenge 1 than before it; however, that was not the case after challenge 2. Also, Preziuso and Preziuso (2001)[28] determined blood concentrations of cortisol, as well as lactate, in dogs subjected to physical activities as evidence of attack (similar to the Schutzhund' defence test), race track and hunting. As noted in our subjects, after the phys-

ical effort dogs showed an increase in cortisol concentrations.
Increases in cortisol concentrations after sustained exercise also were observed in horses[29] and human athletes, [30] while in dog, results reported in literature are contradictory. In this species, in fact, increases in cortisol concentrations in response to exercise were identified by Wakshlag *et al.* (2004),[31] and Durocher *et al.* (2007),[32] while no significant changes were observed by Arokoski *et al.* (1993)[33] and Rovira *et al.* (2007).[34]

HPA axis activity is influenced by exercise itself, but also by additional factors: the type of exercise, the relationship between dog and handler, the breed, sex and age of the subjects.

The reason of these conflicting results may possibly be due to the different experimental conditions. First, because of the extremely different physical activities, which dogs undergo: in fact, the various sports employ different systems and determine changes in hematologic and biochemical parameters. Aerobic training (i.e., sled dogs) causes emodilution, in order to provide extra total body water to maintain cardiovascular and thermic stability during acute exercise,[35] while maximal exercise training (i.e., Greyhounds competing in racing tracks over distances of 235–800 m, with maximum velocities of 18 m/s) induces increases in blood volume, to achieve higher oxygen uptake.[36]
Another possible cause of discrepancy in results of the HPA axis activity may be due to the breed[28] of the dogs used and human-dog relationship[37] can influence cortisol level. The use of toy or treat as reward by owners and playful owners' interactions with their dogs are negative correlated with level of this hormone. Friendly and encouraging relationships cause the decrease of cortisol levels and stress[37].
In this research, cortisol level seems not to be influenced by age or gender of subjects. All dogs considered are adults and several studies demonstrates that after weaning and during adult life there are not difference in cortisol levels [38-39], thus we may suppose that this parameter does not affect the results. Gender of dogs is different in IPO-3 (all males) and IPO-0 dogs (3 males and 3 females. The current literature report contrast results about gender-influence on cortisol levels. Mongillo *et al.* (2014)[39] observed higher cortisol concentration in plasma of male subjects, but other authors did not report any sex-related differences [40-41]. Cortisol level in male and female dogs of the study does not largely differ and gender seems irrelevant, although the low number of females does not guarantee a significant result about an effective influence of sex.
Results of this research has shown a significant reduction of fecal cortisol concentrations has been observed in IPO-3 dogs in the period 2, both during rest and training sessions. Period 1 corresponds to the particular psychophysically intense phase of work, depending on the participation of dogs to the national Schutzhund championship. On the contrary, Period 2 is finalized at maintaining the degree of "psycho-physical tone" reached by dogs and professionals consider it as "light". During training sessions, dogs were accustomed to the same environmental conditions of those of competition, thus, we may suppose that the higher level of cortisol in Period 1 mainly depends on training intensity and not on changes in their habits.
IPO-0 dogs, which had followed a constant program in two different periods, did not show any significant difference of cortisol concentrations. Therefore, these subjects can be considered as a "control" of cortisol trend, in order to exclude any "seasonal" effect on changes in its concentrations. Indeed, this hormone, presents both a circadian and an annual rhythm. We have hypothesized that in IPO-3 subjects the psycophysical work influences faecal cortisol concentrations. We have observed significantly higher hormone levels in intensive working session, which did not occur in IPO-0 dogs that had a constant work during the daily training session. Similar results were achieved by Radosevich *et al.* (1989)[42]. During low intensity exercise, cortisol plasma levels increased along with the duration of exercise; during high intensity exercise, cortisol increased faster, and the integrated plasma response of these hormones was greater. Thus, peripheral release of cortisol during exercise is dose-related to both time and intensity. We may conclude that exercise is a physiologic regulator of both peripheral and central neuroendocrine systems.
However, high cortisol levels can occur during chronic stress conditions also[43]. Therefore, further investigations should be necessary in order to know if the increase of faecal cortisol levels is related to a high training intensity or to a chronic and repeated stress, due to training sessions closer in the time. Despite these different commitments of the dog, the kind of work (Sections A, B and C), carried out in training sessions, did not affect concentrations of faecal cortisol.
In this study, for the first time, we determined hair cortisol concentration in Schutzhund dogs; these levels agree well with those observed in the faeces. In particular, after the Italian championship and after the trial period, hair cortisol concentration is significantly higher than that recorded at the beginning in IPO-3 ani-

The higher concentration of cortisol found during the most intense training period in IPO3 dogs is suggestive of the impact of either work intensity or of repeated chronic stress.

mals, but not in IPO-0 ones, which presented very similar cortisol values in hair collected at the beginning and end of proof.

In a previous work[24] we evidenced a significant positive correlation between faecal and hair cortisol concentrations. This correlation seems to support the hypothesis that both faecal and hair cortisol measurements reflect the same adrenal activity. Moreover, our results are in agreement with those by Davenport *et al.* (2006)[44] on rhesus macaques obtained under controlled laboratory conditions. In that species, hair cortisol content was found to correlate positively with salivary cortisol and to increase as a result of prolonged stressful conditions. The fact that hair cortisol content correlates with faecal or salivary cortisol across a variety of subjects and living conditions, encourages the employment of this non-invasive technique to monitor stress responses over protracted periods.

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KEY POINTS

- Cortisol is an important biological marker for the evaluation of dog stress, training stress included.
- Moderate stress (eustress) causes an elevation of glucocorticoid levels, which are useful to interact with the environment; together with other hormones and neurotransmitters, glucocorticoids have a positive influence on cognitive, decision-making and emotional processes and support muscle work.
- Chronic and repeated stress, such as overtraining, causes a prolonged release of glucocorticoids, which, if in excess, have a negative impact on cognition and emotion as well as on body functions. Excessive training is therefore counterproductive.
- Exercise as such and its intensity cause an elevation of cortisol. The adrenocortical activity is also influenced by a number of factors such as breed, age and time of year.

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