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Research Article

Influence of Pre and Per-Exercise Nutritional Supplementation on Working Dogs Biological Markers Evolution during a Standardized Exercise

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Abstract

The authors investigated the effects of a pre/per-exercise nutritional supplementation containing fat and anti-oxidants on physiological, biochemical, inflammatory parameters and stress oxidative markers of dogs performing an endurance exercise. Ten working Belgian Shepherds dogs were randomly divided into two groups. They consumed 2 g/kg body weight of the supplement (SG), or nothing (CG), one hour before and in the middle of the exercise (two times 20 minutes runs at 14 km/h, separated by 5 minutes rest). The trial was repeated fifteen days after by switching the two groups. Physiological parameters were measured before exercise (T0) at the end of the first 20 minutes run (T1), immediately after the run (T2) and then at 10, 20, 30 minutes and 24 hours (T3) later. Blood samples were performed at T0, T2 and T3.

A slower increase of the heart rate during exercise and a faster return to rest heart rate (10 min vs 20 min) after exercise were observed in SG vs CG. Respiratory rate peak was lower in SG and return to rest respiratory rate was faster (20 min vs 30 min) in SG vs CG. Thermal load tended to be lower at T1 and at T2 in SG vs CG (39.2°C vs 39.7°). In SG, blood triglycerides were higher and decreased during exercise. Advanced Oxidation Proteins Products (AOPP) was significantly higher at T2 and T3 in CG. Interleukins IL1 β and IL8 were higher at T2 in CG vs SG, and even at T3 for IL-8.

The results tend to indicate a better tolerance to exercise after the supplementation.

Keywords: *Working dogs; Nutrition; Supplementation; Oxidative stress; Cytokines; Short and medium-chain fatty acids*

Abbreviations: SG: Supplemented Group; CG: Control Group; FFA: Free Fatty Acids; ROS: Reactive Oxygen Species; H2O2: Hydrogen Peroxide; GSH-PX: Glutathione Peroxidise; SOD: Superoxide Dismutase; SMCT: Short and Medium chains triglycerids; AOPP: Advanced Oxidation Protein Product

Introduction

Mild intensity endurance exercise induces an increase in energy consumption, mainly covered in dogs by lipid oxidation [1,2]. In addition to having a direct effect on the quantity and quality of energy requirements, muscle work also has a great influence on the nutritional balance of food intake via induced stress. When exercise is prolonged (more than a few minutes) and of a moderate intensity 50 to 70 p100 of the maximal oxygen consumption, still poorly quantified in dogs but very higher than in humans [3] aerobiosis is the metabolic pathway that covers 90 p100 of the muscular energy requirements. Prolonged muscle work is therefore responsible for a marked increase in plasma FFA [4-6] and both FFA mobilization ability and the level of fat in the diet are directly correlated to endurance performance in the dog [1,7-11]. Inflammation and ROS productions are also increased during such exercise.

The quality of the energy supplied to dogs during an effort is therefore of primary importance, and, hence, optimum energy criteria have been defined for racing/working dogs:

- The energy (fat) must be readily and rapidly available at the site of use (muscle);
- The balance of the energy components must be such that their combustion is accomplished with a maximum efficiency, minimum waste and without a risk of metabolic blockade.

For this purpose, short or medium chain fatty acids (coprah and palm fats, etc…) have been extensively used for their qualitative advantages in the dog: their digestion is enhanced since it occurs passively and without the need of bile salts solubilisation [8] and the digestive action of pancreatic lipase is facilitated by their low molecular weight. Triglycerides containing short or medium chain fatty acids one thus hydrolysed more rapidly and more completely than those with long chains, and they are even released preferentially if the triglyceride is mixed [12]. In metabolic terms, a second advantage comes from the low incorporation of these fatty acids into adipose tissue [13]. In addition, they do not require L-carnitine to penetrate through mitochondrial membranes [14] intramuscular oxidation is then faster and easier for short than for long chain fatty acids.

But in such a case of high lipid oxidation during stamina in the dog, high oxygen intake and the important increase in free fatty acids oxidation are responsible for a high level of production of free radicals (ROS).

ROS, such as the superoxide anion, the hydroxyl radical, H_2O_2 and many others, are constantly produced by metabolic reactions [15]. When they are not « removed » through the action of biological antioxidants, they are harmful to cells, induce membrane lipid peroxidation and damage proteins and nucleic acids. ROS are counteracted by a wide range of antioxidants synthesized in the cell, including glutathione peroxidase (GSH PX) and reductase, superoxide dismutase (SOD) and catalase or supplied by the diet (vitamins E and C, polyphenols, flavonoids, etc…). Antioxidant adjunction in the working dog food has already been shown as an effective limiting factor of oxidative stress cellular consequences [16-19]. However, other studies did not demonstrate any protective effect of nutritional antioxidants on the contracting muscle [20].

The aim of this study was to measure the effects of a nutritional supplement containing SMCT, anti-oxidants, and group B vitamins on physiological and biological markers of mild-intensity endurance exercise in working dogs.

Materials and Methods

Animals

Ten Belgian Shepherds Malinois, members of the Paris Fire Brigade k9 Search and Rescue Unit, were included in the study and divided into two groups according to body weight and age. They were all males, with similar Body Condition Scores ranging from 2 to 3 on a 5 degrees scale, aged from 3 to 7 years. The animals were in perfect health condition (with a regular clinical and biochemical routine survey as for all military service dogs in France) followed the same daily physical training program including endurance training for one year and were all fed the same specialized complete balanced dry food (« 4300 », Royal Canin®, Table 1). During the whole study, all the dogs were housed in the same kennel and had exactly the same physical activity program. During resting days, a basic clinical examination was performed daily, including heart and respiratory rates, body temperature control and paw-check.

Nutritional Supplement

In the supplemented group (SG), each dog received a nutritional supplement at two different times during the exercise day:

- One hour before the start of the test run
- During the 5 minutes break period after 20 minutes of run (corresponding to half-time of the total running test).

Humidity	8 p100	Vitamin A	2500 UI/kg
Crude proteins	28 p100	Vitamin E	650 mg/kg
Crude fat	21 p100	Vitamin C	1000 mg/kg
Crude fibers	2.9 p100	Vitamin B9	20.3 mg/kg
Ash	7.5 p100	Vitamin B12	0.09 mg/kg
Nitrogen Free Extract	32.6 p100	L-carnitine	150 mg/kg
Energetic density	4140 kcal/kg		

Table 1: *Analytic composition of the daily food (Royal-Canin 4300®) (vs crude matter).*

The supplement (copyright Royal Canin®) contains 40 p100 of fat based on dry matter (short and medium chain fatty acids extracted from coprah) a mixt of anti-oxidants (vitamin A, E, C and green tea polyphenols), B group vitamins and L-carnitine. Its energy content is 5300 kcal/kg. It is processed as a "pillow", so that active nutrients are protected by an external layer of extruded starch, and tested for palatability in the dog. Water is provided as usual ad-libitum for the two groups during the test period.

Humidity	7 p100	Vitamin A	26600 IU/kg
Crude proteins	27 p100	Vitamin E	1400 mg/kg
Crude fat	36 p100	Vitamin C	1600 mg/kg
Crude fibers	2 p100	Vitamin B9	45 mg/kg
Ash	3.6 p100	Vitamin B12	30 mg/kg
Nitrogen Free Extract	24.4 p100	L-carnitine	400 ppm
Energetic density	5296 kcal/kg	Polyphenols	150 mg/kg

Table 2: *Analytic composition of the tested supplement (vs crude matter).*

Endurance Test

The test is made of two times twenty minutes of run, separated by five minutes of passive recovery. The speed of each dog during the test is constant: 14 km/h. Each dog runs aside of his handler laterally linked to a bicycle without ever pulling and speed is maintained constant by using a portable GPS device (Keymaze Kalenji 7000). In order to perform the test without pulling or being pulled, the dogs have been trained this way twice weekly during one year.

Experimental design

The protocol is designed as a cross-over study so that each dog is his own control and performs the run test twice, once in the supplemented group (SG), once in the control group (CG). The two tests were performed at fourteen days of interval in order to respect a washout period.

On the test day, all dogs were fed precisely three hours prior to their own test-starting time, receiving one third of their regular daily intake (« 4300 », Royal Canin®). They received (SG), or not (CG), the supplement one hour before their starting time, and during the five minutes stop at half-run after twenty minutes. The last two third of the daily intake was given 7 hours after the exercise. The day after the endurance trial, dogs were fed exactly three hours before T3. Registration of physiological parameters, blood sampling and on site treatments of blood samples were performed in standardized conditions by five veterinarians of UMES-ENVA (Unite de Medecine de l'Elevageet du Sport-EcoleNationaleVeterinaired'Alfort).

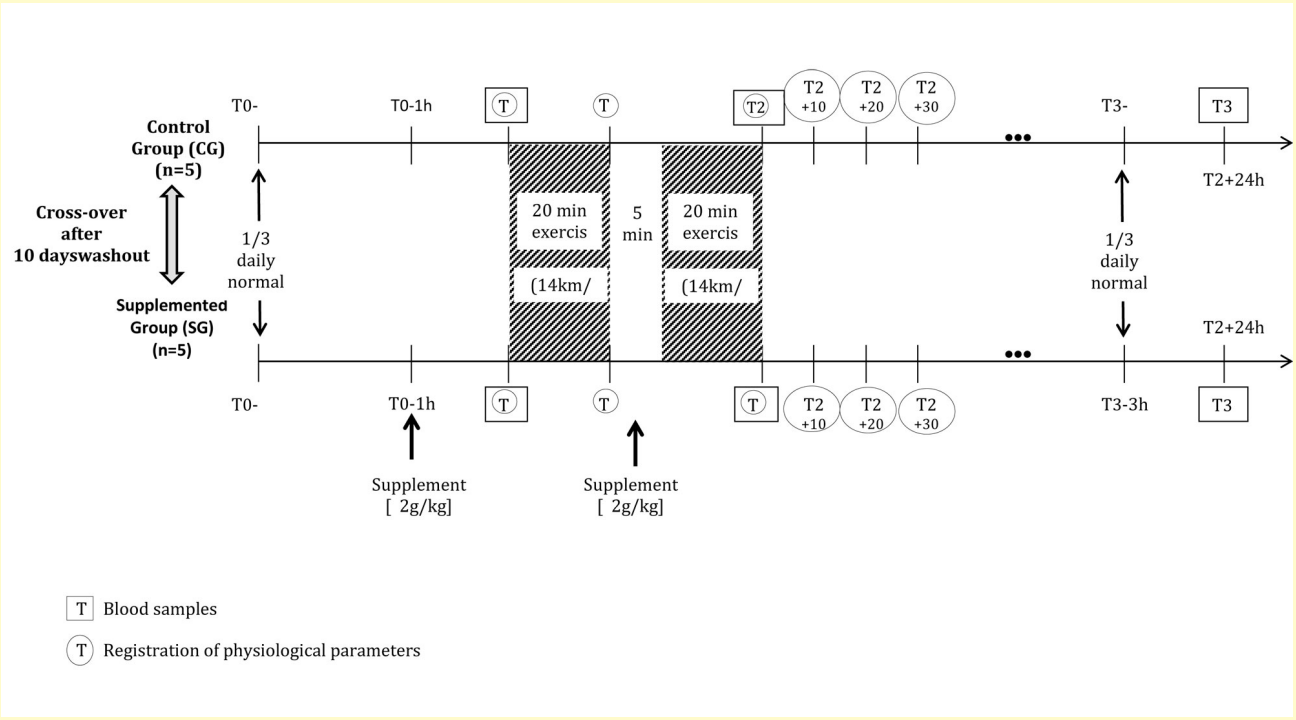

Figure 1: *Design of the cross-over field protocol.*

Biological parameters

Physiological parameters

Heart rate, respiratory rate and rectal temperature were registered three minutes prior to the test start (T0), immediately after the first twenty minutes run (T1), immediately after finishing the second twenty minutes run (T2) and during the recovery period at 10, 20, 30 minutes and 24 hours (T3) post run.

All measurements were obtained directly and by the same veterinarian for each of the three parameters: Heart rate (stethoscope and femoral pulse), Respiratory rate (count of thoracic respiratory movements) and Body temperature (rectal, at the same depth).

Blood samples

Venous blood samples were collected from the jugular vein (easy, quick, and without risk of hematoma when samples are repeated) five minutes prior to test start (T0), immediately after the whole test (T2) and 24 hours post-test (T3). Three tubes were collected at each time (dry, heparined and EDTA).

Dry tubes were centrifuged on site, thirty minutes after sampling (1500 rpm x 10 minutes) and stored in an ice-cooler. Heparin tubes were immediately centrifuged (1500 rpm x 10 minutes). Plasma was aliquoted in Eppendorf tubes protected from UV rays and stored in an ice-cooler. One aliquot of each sample was treated on site for further plasma vitamin C analysis with 1.2 ml of a 10 p100 trichloracetic acid solution added to 0.3 ml of plasma; after gently shaking, it was centrifuged again (1500 rpm x 20 minutes), protected from UV rays and stored in an ice-cooler. EDTA tubes were simply immediately stored in an ice-cooler.

- Lactates were analysed on site, as poorly reliable results are obtained when analysis are delayed, immediately after blood sampling, with a Lactate Pro Analyser (Roche®).
- Standard blood biochemical parameters including glucose, triglycerides, vitamins B_9 and B_{12}, were measured with an automatic analyser (Vista 2, Siemens healthcare Diagnosis).
- Blood vitamins A, E and C were measured using High Performance Liquid Chromatography with Spectrofluorometric detection.
- Advanced Oxidation Proteins Products (AOPP), reduced glutathione (GSH), isoprostanes were analysed by colorimetric reactions in order to evaluate induced oxidative stress.
- Interleukins IL-1β, IL-8, IL-10, IL-15 were assessed by q RT-PCR.

Statistical analysis

Statistical analysis was performed according to data distribution using Tanagra® and Statistical Analysis System® (SAS) softwares. Non parametric tests (Friedman and paired Wilcoxon tests) were used when data were not normally distributed (Physiological parameters, triglycerides, urea, creatinine, vitamin B9, AOPP, IL-1β, IL-8, IL-10, IL-15). Variance or covariance analysis was performed on normally distributed data (Lactates, glucose, vitamins A, E, C and B12).

Results and Discussion

Results

Physiological parameters

A lower increase of the heart rate during exercise was observed for SG (CG vs SG: 133 vs 110 median values in beats/min at T1; $p < 0.05$). No difference between the groups was recorded on the maximum heart rates measured at the end of the test. A ten minutes faster recovery time (to rest heart frequency) was observed in the supplemented group compared to the control group. Return to basic respiratory rate was 10 minutes faster in SG than in CG group. Consequently, thermal load tended to be lower at T1, T2, T2 + 10 min and T2 + 20min in SG compared to CG group.

Energy utilization related biochemical parameters

Glucose

No statistically significant difference was observed between CG and SG groups concerning glycemia. Therefore, glycemia of the two groups were compiled at T0, T2 and T3. A significant increase of blood glucose was observed at T2 compared to T1 (increase) or T3 (return to basic levels) ($p < 0.001$) in both groups.

Lactates

The median value of blood lactates before exercise was 1.3 mmol/L in both groups. No significant differences were observed at any time between SG and CG.

Triglycerides

Triglycerides blood concentration was significantly higher in SG before exercise (SG vs CG: 1.05 g/L vs 0.6 g/L at T0 ($p < 0.05$)). During exercise, their concentration increased in CG (0.75g/L at T2 vs 0.6g/L at T0 ($p < 0.05$)), whereas it decreased in SG (0.8g/L at T2 vs 1.05g/L at T0 ($p < 0.05$)). At T3 (24 hours post exercise) both groups were back to the basic value.

	Control group (n = 10)	Supplemented group (n = 10)
	M (Q1-Q3)	M (Q1-Q3)
HR (beats/min)		
T0	87.5 (81-93.5)	94 (82.2-103.7)
T1	133 (128-160)^{A}	110 (102-126.7)^{b}
T2	136 (130-177.5)^{a}	122.5 (120-140)^{a}
T2 + 10 min	106 (94.5-127.7)^{A}	100 (87.2-110)$^{A, b}$
T2 + 20 min	88 (84-99)^{a}	87.5 (77-98.7)
T2 + 30 min	86 (74-98.5)	87 (75.5-100.5)
T3	85 (81-98.5)	93 (78.7-100.5)
RR (beats/min)		
T0	58 (42.5-93.5)	80 (49-117.5)
T1	145 (121-188.5)^{A}	140 (134-155)^{A}
T2	174 (162-195)^{a}	155 (142.5-160)^{a,b}
T2 + 10 min	130 (120-144.5)^{A}	120 (120-160)
T2 + 20 min	102 (90-120)^{A}	90 (72.5-115)^{A}
T2 + 30 min	98 (82.5_116)	72 (46.7-100)^{b}
T3	54 (34.5-75.5)^{a}	48 (42-115.5)
T (°C)		
T0	38.3 (37.9-38.5)	38.2 (38.0-38.5)
T1	39.7 (39.3-40.3)^{A}	39 (38.9-39.2)^{A}
T2	39.7 (39.2-40.4)	39.1 (38.9-39.6)^{a}
T2 + 10 min	39.3 (38.9-39.7)^{a}	38.9 (38.3-39.5)^{b}
T2 + 20 min	38.8 (38.5-39.2)^{A}	38.6 (38.2-39)^{a}
T2 + 30 min	38.4 (38.3-38.7)^{A}	38.4 (38-38.7)
T3	38.3 (38.2-38.6)	38.6 (38.4-38.7)

Table 3: *Evolution of physiological parameters during exercise and during the recovery period in the two groups.*

Q1: quartile 1, M: Mean, Q3: Quartile 3.

Evaluation of time effect within groups and group effect within time:

^{a}*: $p < 0.05$ (T_n vs T_{n-1}).*

^{A}*: $p < 0.01$ (T_n vs T_{n-1}).*

^{b}*: $p < 0.05$ (CG vs SG).*

Vitamins B9 and B12

At T0 and T2, vitamins B9 and B12 blood concentrations were statistically higher ($p < 0.05$) in SG than in CG. No significant differences between groups were observed at T3.

Oxidative stress parameters

Among the analysed oxidative stress related parameters, only AOPP Figure 2 showed statistically significant variations (figure 2). It was higher at T2 (6.3 Chloramine equivalent (Cl.eq)) and T3 (8.5 Cl.eq) than at T0 (5.4 Cl.eq) in CG ($p < 0.05$), and statistically lower in SG than in CG at T2 (5.7 Cl.eq vs 6.3 Cl.eq ($p < 0.05$) and T3.

	Control Group (n = 10)	Supplemented Group (n = 10)
	M (Q1-Q3)	**M (Q1-Q3)**
Triglycerids (g/L)		
T0	0.61 (0.4-0.8)	1.05 (1-1.1)[b]
T2	0.75 (0.4-1.5)[a]	0.8 (0.5-1.1)[a]
T3	0.65 (0.4-1.1)	0.5 (0.4-1.6)
Vitamin B9 (ng/mL)		
T0	426 (417-531)	617.5 (604-649.7)[B]
T2	471 (425-543)	622.5 (600.7-658.7)[B]
T3	527 (456.2-602.7)	610 (601.2-638.7)
	M ± SD	**M ± SD**
Vitamin B12 (pg/L)		
T0	497.1 ± 90.4	671 ± 80.3[B]
T2	503.4 ± 100.7	638 ± 74.3[B]
T3	532.5 ± 87.8	568.7 ± 97.6
Lactates (mmol/L)		
T0	1.3 ± 0.8	1.3 ± 0.7
T2	2.0 ± 1.1	2.1 ± 1.1
T3	1.5 ± 1.1	497.1 ± 90.4
Glucose (mmol/L)		
T0	5.1 ± 0.7	4.6 ± 0.9
T2	5.7 ± 0.6[A]	6.0 ± 0.9[A]
T3	5.0 ± 1.1[A]	4.9 ± 0.4[A]

Table 4: *Evolution of physiological parameters during exercise and during the recovery period in the two groups. Results are presented as M (Q1-Q3) for triglycerids and vitamin B9.*

Q1: quartile 1, M: Mean, Q3: Quartile 3

Results are presented as M (+/-SD) for vitamin B12, lactates and glucose. Evaluation of time effect within groups and group effect within time:

[a]: P < 0.05 (T_n vs T_{n-1})

[A]: p < 0.01 (T_n vs T_{n-1})

[b] : p < 0.05 (CG vs SG).

[B]: p < 0.05 (CG vs SG).

	Control group (n = 10)	Supplemented group (n = 10)
	M ± SD	M ± SD
Vitamin A (µmol/L)		
T0	6.57 ± 0.29	6.10 ± 0.29
T2	6.34 ± 0.29	6.27 ± 0.29
T3	6.57 ± 0.29	6.59 ± 0.29
Vitamin E (µmol/L)		
T0	113.37 ± 2.98	103.35 ± 2.98
T2	104.63 ± 2.98	110.29 ± 2.98
T3	105.84 ± 2.98	110.83 ± 2.98
Vitamin C (µmol/L)		
T0	1270.70 ± 506.4	591.4 ± 506.4
T2	1147.3 ± 506.4	612 ± 506.4
T3	1188.2 ± 506.4	1763.7 ± 506.4
GSH (µmol/mL)		
T0	1036.85 ± 44.45	1006.02 ± 44.45
T2	1033.1 ± 44.45	983.45 ± 44.45
T3	959.61 ± 44.45	911.84 ± 44.45
Isoprostane (ng/L)		
T0	55.24 ± 3.66	51.78 ± 3.66
T2	53.16 ± 3.66	51.17 ± 3.66
T3	64.65 ± 3.66	57.49 ± 3.66

Table 5: *Evolution of inflammatory markers during exercise and during the recovery period in the two groups. Results are presented as M (+/-SD). Evaluation of time effect within groups and group effect within time was performed after a log transformation.*

$_a$: $p < 0.05$ (T_n vs T_{n-1}).

$_b$: $p < 0.05$ (CG vs SG).

Inflammatory markers

Table 6 regroups the results for IL-1β, IL8, IL10 and IL15 gene expressions. IL10 and IL15 were not affected by exercise test nor by supplement intake. IL-1β (Figures 3) and IL-8 gene expressions were statistically ($p < 0.05$) increased in CG compared to SG post-stamina at T2. No significant differences were observed at T3 between the two groups and to T0.

Discussion

The result obtained in our field cross-over study can be discussed in relation with three metabolic areas deeply involved in the exercise performance and eventual clinical consequences in the dog:

- Nutritional quality of the energy provided in the food.
- Nutritional management of exercise induced oxidative stress.
- Possible impact of nutrition on post exercise induced inflammation consequences.

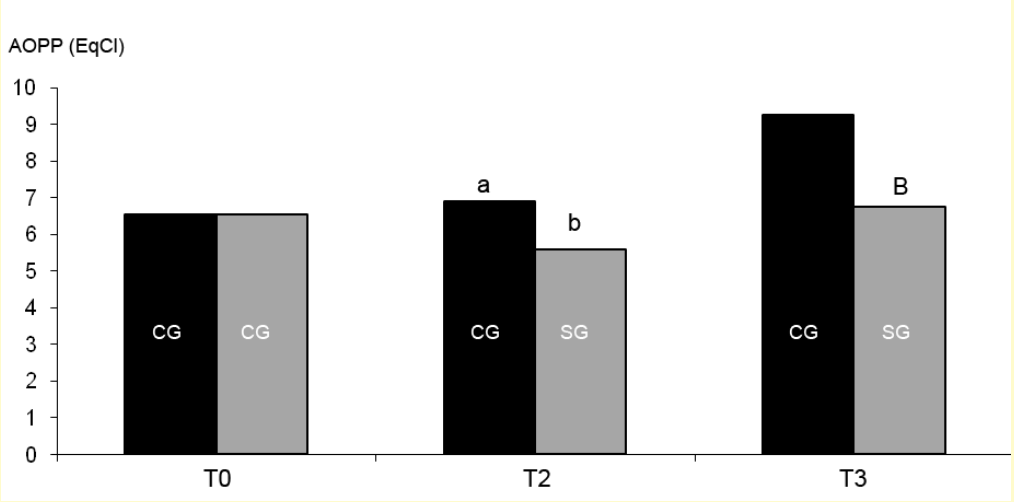

Figure 2: *Evolution of AOPP during exercise and during the recovery period in the control group (CG in black) and the supplemented group (SG in grey).*

Evaluation of time effect within groups and group effect within time was performed after a log transformation:

[a]: $p < 0.05$ (T_n vs T_{n-1}).

[b]: $p < 0.05$ (CG vs SG).

[B]: $p < 0.01$ (CG vs SG).

Interest of short and medium chain fatty acids

The energy supplied to a working dog must be qualitatively adjusted to the required physiologic goal and the type of energy involved. Likewise, the muscle energy yield can be considerably reduced by an accumulation of metabolic waste or by metabolic blockade. Adjustment of qualitative energy intake should offset these risks and the use of certain nutrients present in the complete balanced diet or in the studied supplement in our study improves the muscle energy yield:

- L-carnitine transports long-chain fatty acids across the mitochondrial membrane and did show efficiency in racing sled dogs [21,22];
- (n-3) fatty acids improve erythrocyte deformability and the permeability of the cell membrane to oxygen [23];
- B complex vitamins play a recognized role in the correct functioning of the cell energy mechanisms [24,25].

A high concentration of saturated long-chain fatty acids in the diet allows the dog to build or to rebuild stores of adipose tissue from empty calories. Activation of aerobic metabolism during endurance exercise leads to adipolysis; this increases plasma triglycerides and free fatty acids concentrations, which are finally oxidized in the muscle cells.

Short and medium chain fatty acids (SMCT) can be defined as a mixture of fatty acids with 6, 8 and 12 carbon [26], and are of obvious value to working dogs [21]. The low molecular weight of SMCT facilitates the digestive action of pancreatic lipase, and they can be absorbed without solubilisation by bile [13]. Triglycerides containing SMCT are more rapidly and more completely hydrolysed than those with long-chain fatty acids. They are released preferentially if the triglyceride is mixed [12]. In metabolic terms, another advantage is that SMCT are minimally incorporated into adipose tissue [13], and they do not require carnitine to penetrate mitochondrial membranes [14], and thus generate a faster muscular oxidation. Finally, SMCT have a restraining effect on the de novo synthesis of fatty acids in adipose tissue [27].

	Control Group (n = 10)	Supplemented Group (n = 10)
	M ± SD	M ± SD
IL8 (Δct)		
T0	30.9 ± 22.4	26.7 ± 12.6
T2	223.6 ± 97.9[a]	30.3 ± 17.3[b]
T3	44.4 ± 45.5	23.4 ± 12.6
IL1β (Δct)		
0	2.7 ± 1.9	2.3 ± 0.9
T2	4.4 ± 2.4[a]	1.1 ± 0.9[b]
T3	2.3 ± 2.2	1.3 ± 0.5
IL10 (Δct)		
T0	0.27 ± 0.49	0.15 ± 0.11
T2	0.15 ± 0.15	0.16 ± 0.11
T3	0.1 ± 0.08	0.14 ± 0.10
IL15 (Δct)		
T0	0.21 ± 0.11	0.24 ± 0.09
T2	0.24 ± 0.06	0.22 ± 0.4
T3	0.23 ± 0.08	0.24 ± 0.4

Table 6: *Evolution of inflammatory markers during exercise and during the recovery period in the two groups. Results are presented as M (+/-SD). Evaluation of time effect within groups and group effect within time was performed after a log transformation.*

[a]: $P < 0.05$ (T_n vs T_{n-1}).

[b]: $p < 0.05$ (CG vs SG).

- The choice of physically trained search and rescue dogs lead, for such an inframaximal exercise, to an almost exclusive use of lipids oxidation to cover muscles energy requirements (no increase in blood lactates, statistically significant increase of glycemia);
- The distribution of SMCT to the SG dogs induces a normal increase of blood triglycerides at T0 [28]. But their drop during exercise in this group shows the preferential use of SMCT by muscle, confirming previous work attesting that at least 25 p100 of the energy required by exercise comes in the dog from circulating lipids [29].
- In the CG dogs, an increase in blood triglycerides was observed, confirming other studies already performed on dogs [30]; metabolic fuels are supplied to locomotory muscles mitochondrias from nearby intramuscular stores and from remote stores via the circulation;
- No digestive adverse effect was observed in our dogs, where some abdominal cramping and intestinal complains were observed on exercising humans ingesting MSCT [31];
- The consumed B complex vitamins in the SG group (B9 and B12) showed blood concentrations correlated to their ingestion, attesting no alteration of digestive functions in the supplemented dogs.

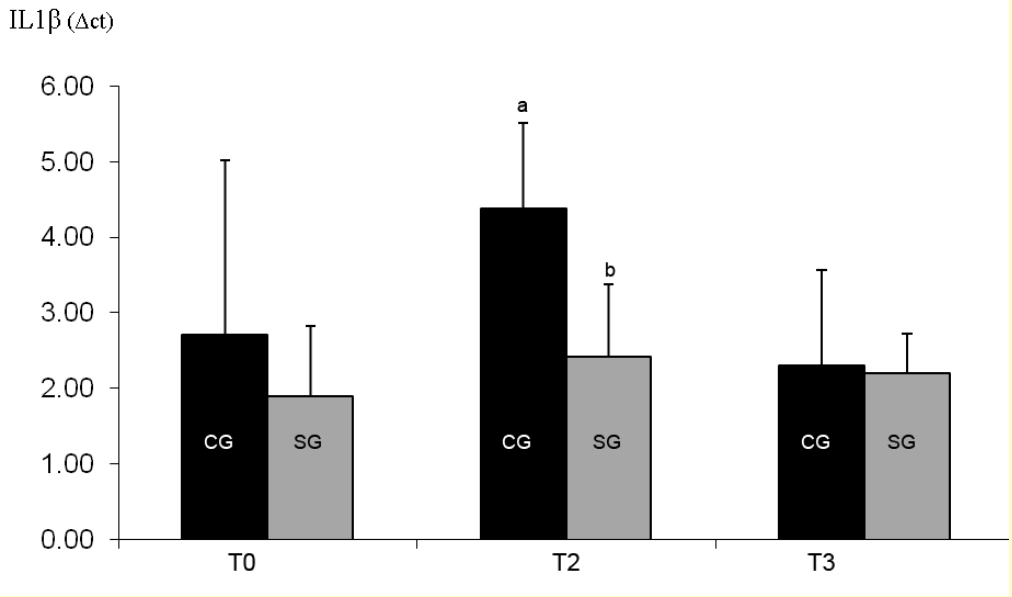

Figure 3: *Evolution of IL1β during exercise and during the recovery period in the control group (CG in black) and the supplemented group (SG in grey).*

Results are presented as M (+/-SD).

Evaluation of time effect within groups and group effect within time was performed after a log transformation.

[a] : $p < 0.05$ (T_n vs T_{n-1}).

[b] : $p < 0.05$ (CG vs SG).

[B] : $p < 0.05$ (CG vs SG).

Management of induced oxidative stress

During exercise a number of potential sources exist for the production of reactive oxygen species such superoxide anions, hydrogen peroxide and hydroxyl radicals, can be triggered. This over production can exceed antioxidant defences, to cause oxidative stress. Oxidative stress has been defined as a disturbance in the pro-oxidant-anti-oxidant balance in favour of the former, leading to potential cellular damages [32]. Oxidative stress does not always result in clinical damages, but it may however result in oxidative damages to membrane lipids, proteins and DNA; and consequently alter athletic performance [33]. Oxidative stress has already been described in the exercising dog, particularly sled dogs [34], hunting dogs [35] and search and rescue dogs [36]. Numerous studies had been conducted in the past on working dogs [37] and demonstrated that stress is linked to the appearance of anemia [38], which can sometimes be serious, stress-diarrhea-dehydration or even sudden death syndrome [39].

As a result from this study, the content in antioxidant has been increased in complete balanced dry foods dedicated to working dogs, through adjunctions of vitamin E, vitamin C, selenium and numerous other antioxidant sources [40,41]. Only Marshall, Scott, Hill, Lewis, Sundstrom, Jones and Harper [42] stated that supplemented vitamin C could reduce the speed of racing Greyhounds.

In our study, daily consumption of antioxidants was elevated and might be sufficient to prevent their degradation during the test. Indeed, we have found any significant variation in blood parameters as vitamin E, vitamin C and reduced glutathione. To better explore a suspected oxidative stress in performing dogs we measured another plasma marker such as AOPP (Advance Oxidation Protein

Products). Elevated levels of oxidized protein have been described in animals following their exposure to various conditions of oxidative stress, including physical exercise, AOPP had never been studied in exercising dogs but has been shown as increased in humans after endurance stamina [43] and appears to be an interesting parameter as it increased in both groups during stamina and even 24 hours post-exercise. Advanced oxidation protein products are uremic toxins created during oxidative stress through the reaction of plasma proteins with chlorinated oxidants such as chloramine [44]. Regarding the mechanism of generation of AOPP, Witko-Sarsat, Gausson and Deschamp-Latscha [45] also pointed out the importance of myeloperoxidase and the subsequent generation of chlorinated oxidants in the formation of AOPP. For these authors, AOPP appear to act as true inflammatory mediators since they are able to trigger the oxidative burst and the synthesis of inflammatory cytokines in neutrophils as well as in monocytes.

In our study, the differences observed between the two groups show statistically significant lower levels of AOPP after exercise in SG than in CG indicating that diet supplementation with MCT during the race might confer some degree of protection towards oxidative stress. This protective effect could be due to increased yield of energy transformation within the mitochondria and to the immediate delivery of ergogenic antioxidants.

Impact on post exercise induced inflammation

The relationship between exercise and inflammation can undergo lots of variations. Epidemiological studies [46,47,48] and meta-analysis [49]; Karapis and Thomson [50] have demonstrated a relationship between physical exercise and anti-inflammatory effects. But exercise can also initiate an acute inflammatory response in the short term, known in working dogs where it cumulates with induced oxidative stress to result in muscular affections. C-reactive protein (CRP), for example, may serve as potential marker for exercise-induced inflammation. In a recent study performed on sled dog, [51] conclude that CRP concentrations may serve as a potential marker for exercise-induced inflammation. According to these authors, the exact amount of exercise required to induce such a response is unknown, but dogs apparently have a more robust acute-phase response than do humans. Working on dogs after a strenuous exercise of short duration [52] did not find cardiac troponine values above the reference range for healthy dogs; and although increased after two days of short-duration strenuous exercise, CRP did not reach concentrations suggestive of inflammation as reported previously in the endurance sled-dogs.

For these reasons we chose in our study to focus on the genes expressions of some pro and anti-inflammatory cytokines, such as

- IL-1 β, IL-6, IL-8, TNF α (pro-inflammatory cytokines)
- IL-10 and IL-15 (anti-inflammatory cytokines)

Research through the past twenty years has demonstrated that exercise induces considerable changes in the immune system, and authors like Pedersen and Hoffman-Goetz [53] and others focused on cytokines and their possible roles as a link between muscle contractions and cellular immune changes. This research led to the discovery that exercise provokes an increase in a number of cytokines [54]. Thus, skeletal muscle has to capacity to express cytokines like IL-6, IL-8 and IL-15 [55,56] and muscle contractions play a regulatory role in the muscular expression of these cytokines. Pedersen, Akerstrom, Nielsen, Fischer [57] even proposed to call them myokines for these reasons.

Endurance exercise upregulates the production of IL-6 and IL-8, which leads to elevated circulating concentration of these myokines following exercise [58]. In our study, we unfortunately could not have IL-6 analyzed as scheduled, but IL-8 is highly increased by exercise in CG, where it stays constant in SG. The same statistically different result is obtained for IL-1 β, confirming similar datas obtained on humans [59]. TNF α could not be analysed as scheduled either, but is documented as usually increased following an endurance exercise in humans [60]. Concerning pro-inflammatory cytokines genes expressions, our results show a statistically significant positive effect (no variations induced by exercise in SG); this result is probably linked

- To the lower increase of body temperature during exercise observed in the supplemented group SG [61];
- To the relationship not yet clearly explained of adiponectine (that plays an important role in the hormonal regulation in response to energy expenditure during exercise) with pro-inflammatory cytokines [62,63] as in our study, the provided supplementation decreases exercise induced lipolysis.

Concerning anti-inflammatory cytokines, we did not observe any statistical variation in the genes expressions of IL-10 and IL-15, where increases were found by authors working on human athletes [64] for IL-10, and no or little effects of endurance exercise on IL-15 by others [65]. The question of the sufficiency of duration and intensity of the proposed exercise in our study remains.on human athletes [64] for IL-10, and no or little effects of endurance exercise on IL-15 by others [65]. The question of the sufficiency of duration and intensity of the proposed exercise in our study remains.

The present study was the first one, to our knowledge, examining the interest of a pre-per work nutritional supplementation in the working dog. Its primary goal was to evaluate the eventual interest of SMCT and extra-antioxidants at such times in the search and rescue dog, a category of dogs always subject to exercise periods repetitions in highly stressful conditions. The protocol itself could be handled in optimal conditions due to the high level of professionalism of the Paris Fire Brigade dog handlers and the choice of a cross-over design that made statistical approach possible.

Nevertheless, a field protocol is often submitted to hazards and a series of aliquots was destroyed, unabling some of the scheduled analysis including for example interleukin IL-6, TNF α and plasma free fatty acids that were for us quite important to examine. The results of the study are very encouraging, as the distribution of the tested nutritional supplement to a group of highly professional trained search and rescue dogs showed positive effects

- On the induced modifications of physiological parameters, especially body temperature, a strong limiting factor of stamina in the working dog (highly sensible to heat-stroke)
- On exercise related oxidative stress, acting for a better prevention of related post stressful exercise clinical consequences
- On the potential appearance of post-exercise acute inflammation.

Conclusion

Dietary supplementation improved physiological parameters such as temperature load, respiratory rate peak, in supplemented dogs compared to no supplemented dogs. Plasma cytokines IL 1 B and IL 8, as well as AOPP levels where lower in the supplemented group than in the control group. All together these results show that the test supplement may increase dog's endurance and recovery. This practical approach is of a major importance in search and rescue dogs usually working by shifts of 20 to 40 minutes; and for which a longer « strait » working period can save human lives. It seems possible to consider that the distribution of short and medium chain triglycerides and selected anti-oxidants, before and during exercise, can be beneficial for the working dog. Further experiments are needed in order to determine more precisely the impact of exercise and related nutrition on potentially induced exercise inflammation.

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