

Changes in Plasma and Muscle Creatine Concentration after Increases in Supplementary Dietary Creatine in Dogs¹

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EXPANDED ABSTRACT

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Creatine (Cr)³ is an important component of the energy delivery process in tissues with a high and/or fluctuating energy demand. In the phosphorylated form, phosphocreatine (PCr) is directly involved in maintaining low adenosine diphosphate concentrations at the sites of energy utilization. Consequently, the maintenance of an adequate supply of PCr at the contractile site of the muscle is important for the perpetuation of muscle performance during intense exercise and exertion.

In normally active animals and humans, a sufficient supply of PCr is considered to be synthesized from the amino acids arginine and glycine (Harris and Lowe 1995). It has been observed in humans, however, that supplementary dietary Cr can, over time, increase the normal muscle content of 100–130 mmol/kg dry weight by between 2 and 40 mmol/kg (Harris et al. 1992). Dietary Cr supplementation has been shown to have a positive effect on short-lasting maximal exercise performance and sustained intense exercise with reduced metabolic effort (Balsom et al. 1993, Greenhaff et al. 1993, Harris et al. 1993); therefore, such Cr loading might be considered beneficial to the working and/or sporting dog.

In the wild, the intake of Cr by the dog from freshly killed prey can be regular and substantial, 1.23–3.0 mmol/(kg body weight · d) (Rohrs 1987). However, a recent review of the dietary intake of Cr in the domestic dog indicated that dietary supply may be limited (Harris et al. 1997). Although this should not be regarded as a deficiency of the diet, it is certainly possible that an increase in supply may influence performance as with human athletes. A recent study in humans showed that long-term, low dose supplementation of the diet with Cr was ultimately as effective in raising muscle Cr concentration as a short-term, high dose supplementation (Hultman et al. 1996). In dogs, it has been shown that Cr is equally well absorbed into the plasma from either fresh meat or synthetic Cr (Harris and

Lowe 1995). It remains unknown, however, which of the feeding regimens is the most appropriate to maximize muscle Cr concentration in dogs.

A preliminary study was therefore conducted to investigate the effect of increasing dietary supplemental Cr on muscle and plasma Cr concentrations in dogs before a further more detailed study of the accumulation of muscle Cr over time.

Materials and methods. Twelve adult Beagle dogs (13.7 kg, SD 2.59) were housed in 2.4 m² (1.7 m × 1.4 m) concrete block pens with an open-mesh steel gate to the front allowing them visual access to kennel-mates and the central walkway/exercise area. Bedding was provided in the form of soft-wood shavings. The kennel building was heated and ventilated to maintain a temperature between 16 and 24°, 30–70 % relative humidity with 12 h of light in a 24-h period. All dogs were monitored throughout the day and pens cleaned once daily. The study protocols were appropriately approved and the animals maintained under the care of a veterinary surgeon for the duration of the study in compliance with the 1986 EC directive (86/609/EEC) regarding the protection of animals used for experimental and other scientific purposes. All dogs had been fed a basal diet (Lowe et al. 1994), which contained 0.156 mmol Cr/kg diet for at least 2 mo. The dogs were then randomly allocated to four treatment groups (n = 3 per group) and the diet supplemented with dry crystalline creatine monohydrate to provide the equivalent of 0.38, 0.76, 1.53 or 3.05 mmol Cr/kg body weight daily for 28 d. Muscle biopsy samples were taken under anesthesia from the biceps femoris muscle of each dog using a percutaneous needle biopsy technique on d 0 (the day preceding Cr supplementation), 14 and 28; samples were frozen immediately. Subsequently, the samples were freeze-dried, dissected from visible connective tissue, powdered and analyzed for Cr, creatinine (Cn) and PCr, using reverse-phase ion-pairing high performance liquid chromatography (Dunnett et al. 1991). Blood samples were taken immediately before feeding and then at 0.5, 1, 2, 3, 4 and 5 h after feeding on d 0, 14 and 28. All samples were immediately centrifuged and the plasma separated and frozen for subsequent Cr and Cn analysis.

Plasma Cr and Cn were analyzed by ANOVA using the area under the plasma curve for 5 h after feeding (AUC₅) within each collection day. With the use of ANOVA, muscle

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³ Abbreviations used: AUC₅, the area under the plasma curve, calculated by integration, for the 5 h after feeding for plasma creatine and/or creatinine content in mmol; Cn, creatinine; Cr, creatine; PCr, phosphocreatine; TCr, the sum of Cn plus Cr plus PCr in muscle expressed in mmol/kg.

TABLE 1

The mean and SEM for AUC₅ values for plasma Cr and Cn in dogs together with the time of occurrence of peak plasma Cr and Cn concentration in hours after feeding dietary supplemental Cr for 14 or 28 d at 0.38, 0.75, 1.53 or 3.05 mmol/(kg body weight · d)¹

	Plasma creatine (Cr)				Plasma creatinine (Cn)			
	Mean	SEM	P	Peak	Mean	SEM	P	Peak
Day 14								
0.38 Treatment	227.2	49.76	0.009	1	111.7	22.04	0.41	2
0.75 Treatment	289.6			1	146.5			2
1.53 Treatment	366.0			1	115.3			1
3.05 Treatment	639.6			1	132.1			1
Day 28								
0.38 Treatment	253.2	64.64	0.005	0.5	88.5	39.2	<0.001	1
0.75 Treatment	294.1			0.5	113.1			1
1.53 Treatment	482.1			1	201.6			1
30.5 Treatment	849.0			3	348.2			3

¹ AUC₅, the area under the plasma curve for the 5 h after feeding.

Cr concentration was examined by regression on Cr intake within a sample day. It is acknowledged that simple ANOVA between the two chosen time points is inappropriate; therefore, changes in muscle Cr concentration over time within the experiment were examined by a regression model.

Results. Significant differences were observed in the AUC₅ for plasma Cr on d 14 ($P = 0.009$) and on d 28 ($P = 0.005$) (Table 1). The 1.53 and 3.05 mmol/kg treatments substantially increased the AUC₅ and peak plasma values for Cr. The profiles for plasma Cr were similar in shape for both d 14 and 28. Curves for the pooled data are shown for illustration in Figure 1. The plasma Cn profile for each treatment closely followed the plasma Cr profile with no apparent time lag. Although the plasma Cn profile was similar in shape, there was greater variability in value. There was a correlation between the AUC₅ for plasma Cn and the AUC₅ of Cr, which could be explained by the following equation:

$$\text{Plasma Cn} = [0.32(\text{SEM} = 0.096) \times \text{Plasma Cr}] - 21.1 (\text{SEM} = 47.32) \quad R = 0.81$$

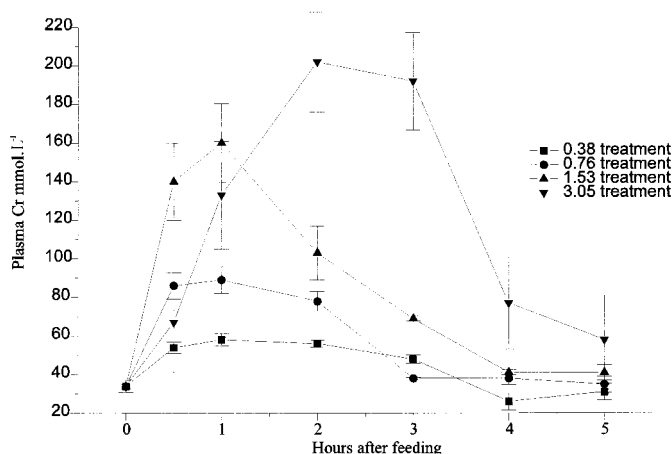


FIGURE 1 The composite plasma creatine profiles from d 14 and 28 expressed as mean (\pm SEM) plasma creatine (mmol/L) in dogs (3 per treatment) over the 5 h after a meal supplemented with 0.38, 0.76, 1.53 or 3.05 mmol creatine/kg body weight.

The concentrations of the individual components, Cr, PCr and Cn found in the muscle samples were different from that expected to be found in vivo. In all probability, this is due to the likely hydrolysis of PCr during sample collection and preparation. The data were therefore analyzed on the basis of the total PCr + Cr + Cn, termed TCr, in the muscle sample. The TCr found in the muscles of supplemented dogs was within the range found in the muscles of Cr-supplemented humans. Variation in sample adenosine triphosphate (mean 23.9 ± 0.45 mmol/kg) indicated that the samples were consistent in their muscle content and did not account for any differences seen in TCr among dogs. Numerical increases, on the order of 8–32 mmol/kg in muscle TCr were observed within individual dogs from d 0 to 14. Further increases on d 28 were not observed, and no consistent effect over time for any treatment could be established. The mean values for each day and treatment are shown in Table 2. Large between-animal variation was observed both pre- and post-treatment, which, together with the small sample size, accounted for the overall large P -values reported for the treatment effects.

Dogs with low initial muscle TCr increased their muscle

TABLE 2

The effect of creatine (Cr) intake on muscle Cr concentration. Means (mmol/kg dry muscle) and SEM of PCr + Cr + Cn (TCr) concentration in the muscle of dogs before (d 0) and 14 and 28 d after dietary supplementation with 0.38, 0.76, 1.53 or 3.05 mmol Cr/kg body weight

	Mean	SEM	P	n
Day 0				
Basal diet	128.6	17.19		12
Day 14				
0.38 Treatment	149.4	19.93	0.23	3
0.76 Treatment	126.5			3
1.53 Treatment	144.1			3
3.05 Treatment	110.8			3
Day 28				
0.38 Treatment	131.8	15.42	0.29	3
0.76 Treatment	117.2			3
1.53 Treatment	134.1			3
3.05 Treatment	106.1			3

TABLE 3

The effect of supplementary Cr on muscle TCr concentration by repeated ANOVA for all data and excluding dogs with high (>140 mmol/kg) initial muscle Cr¹

	Intercept	Slope	SEM	P	R	P	n
All dogs	128.6	-3.7	2.55	0.16	0.27	0.16	12
Dogs <140 mmol Cr/kg	111.7	11.8	3.44	0.003	0.92	0.006	9

¹ Cr, creatine; TCr, the sum of creatinine plus Cr plus phosphocreatinine.

TCr concentration more than those with higher initial muscle TCr concentrations. Within a given day, there were between-treatment differences, with the 3.05 mmol/kg treatment producing consistently lower muscle Cr values.

Elimination of the dogs ($n = 3$) with high (>140 mmol/kg) initial muscle TCr concentrations from the data resulted in a more dramatic treatment effect by reducing the mean and the variability (SEM) of the TCr in the control dogs from 128.6 ± 17.19 to 111.7 ± 5.2 . This resulted in a more definite effect of Cr intake on muscle Cr concentration, (Table 3). The observed increase, similar to that in humans (Hultman et al. 1996), is likely to be sufficient to account for the improvement in lactate threshold observed in dogs fed dietary supplemental Cr (Lowe, unpublished observations).

Discussion. The data indicate that there may be large differences in muscle TCr concentration in dogs despite a constant dietary supply. The effect of feeding dietary supplemental Cr over a 4-wk period is to increase the muscle TCr of those dogs with low (<140 mmol/kg) initial TCr while having a negligible effect on those dogs with higher initial TCr values. Within the confines of the number of animals used, the data appear to suggest that any increase in muscle TCr concentration is maximized within 14 d and that this effect is achieved, in this study, by supplementing the diet with as little as 0.38 mmol Cr/kg body weight. This is consistent with recent data reported for humans (Hultman et al. 1996). There appears to be little benefit from feeding larger amounts of supplemental dietary Cr in terms of further increasing muscle TCr concentration after 14 d. However, on the basis of the data reported by Hultman et al. (1996) for humans, the feeding of supplementary Cr in excess of 0.38 mmol/kg may result in consistently higher muscle TCr concentration being attained sooner than 14 d after the start of the feeding regimen.

The plasma Cr content, by d 28, increased in relation to the amount of supplementary dietary Cr fed and as a proportion (0.39 ± 0.015) of plasma Cr. This increase in plasma Cr observed with increases in supplementary dietary Cr will result in increases in urinary Cr excretion in dogs supplemented with dietary Cr. The results of this preliminary study indicate that further studies are required to expand our knowledge of Cr metabolism in the dog.

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