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The concentration of creatine in meat, offal and commercial dog food

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SUMMARY

The concentrations of creatine (Cr), phosphorylcreatine (PCr) and creatinine (Cn) were determined in a variety of meats, before and after cooking by boiling, in a range of commercially available canned dog foods, in rendered and dried meat products and in commercially available dry dog foods. None of the samples contained PCr. Uncooked chicken, beef and rabbit meat contained approximately 30 mmol kg^{-1} of Cr. Ox-heart and ox-liver had Cr concentrations of 22·5 and 2·3 mmol kg^{-1}, respectively. Canned dog foods had Cr concentrations of 0·5 to 2 mmol kg^{-1}. Dried meat samples had Cr concentrations of 90 to 100 mmol kg^{-1} dry weight. In contrast, the Cr concentration of dried rendered meat meal was 3 mmol kg^{-1} dry weight or less. Dry dog foods contained 0·5 to 4 mmol kg^{-1} dry weight of Cr. The results indicate that in the canned dog foods, the dried meat samples and the dried rendered meat meal creatine had been degraded to variable extents to creatinine.

CREATINE (Cr) is an important component of the energy delivery process in several tissues, particularly those characterised by a high and/or fluctuating energy demand. In its phosphorylated form, phosphorylcreatine (PCr), Cr is directly involved in maintaining low ADP concentrations at sites of energy utilisation, and in the transfer of high energy phosphate from mitochondria. The highest concentrations of Cr are found in skeletal muscle, which accounts for 95 per cent of the total body pool. Lower concentrations are found in cardiac and smooth muscle, brain, retina, macrophage cells and spermatozoa.

Creatine is synthesised in the body from the amino acids arginine and glycine, with a final transmethylation step involving methionine. Creatine is also found in the diet of meat eating animals. One kg of raw meat may contain up to 35 mmol of Cr. The loss of Cr by non-enzymatic conversion to creatinine (Cn) has been estimated in human beings to be 1·6 per cent of the total body pool per day (Hoberman et al 1948, Crim et al 1976). A similar rate of turnover may be expected in dogs. Larger breeds (with bodyweights of the order of 35 kg and a muscle mass of 15·4 to 20·0 kg) may be expected to lose 9 to 11 mmol of the endogenous Cr pool daily as Cn in the urine, although the total excreted will include also Cn derived from the diet (Watson et al 1981, Epstein et al 1984).

In human beings, Cr has been shown to be readily absorbed from the gut (Crim et al 1976, Harris et al 1992) and repeated doses of 4 x 34 mmol Cr monohydrate for three or more days have been shown to increase its content in skeletal muscle by 10 to 40 per cent (Harris et al 1992). Several studies (Harris et al 1992, Greenhaff et at 1993, Söderlund et al 1994) have suggested an upper threshold for the total Cr store in human muscle of approximately 150 mmol kg^{-1} dry muscle, although still higher values of up to 180 mmol kg^{-1} dry muscle, have been observed after dietary supplementation combined with exercise (Harris et al 1992). The administration of smaller amounts of Cr for longer periods, for example 20 mmol Cr monohydrate daily for 30 days, results in similar increases in the total muscle Cr store (Balsom et al 1994). The creatine supplementation of people has been shown to result in significant increases in the individual's capacity for sustained or intermittent intense exercise (Harris et al 1993) with reduced metabolic effort, as indicated by a reduction in the loss of muscle adenine nucleotide to inosine monophosphate (IMP) (Balsom et al 1993a, Greenhaff et al 1993, Birch et al 1994). However, creatine supplementation appears to have less effect on exercise endurance at submaximal work loads (Balsom et al 1993b, Stroud et al 1994).

The authors have recently reported that Cr is rapidly absorbed by dogs when it is supplied in the diet, either as a constituent of freeze-dried uncooked meat or as a supplement added to a cereal-based diet (Harris and Lowe 1995), and to approximately the same extent. An adequate supply of Cr may be important for the performance of dogs, and its content in meat and offal and its modification by cooking, as well as its content in commercially available dog foods, have therefore been investigated.

MATERIALS AND METHODS

Creatine content of meat

Samples of stewing beef, chicken breast, ox heart and ox liver were purchased from local retail outlets on the morning of analysis. No attempt was made to determine the history of the meat samples. Portions of hind limb muscles of rabbits shot locally were obtained within 15 hours of death. Samples of meat (150 to 300 mg) and liver (300 to 500 mg) were homogenised in 1 ml of water for two periods of 30 seconds, and 750 µl of the homogenate were deproteinised by the addition of 22·5 µl of 11·0 mol $litre^{-1}$ perchloric acid. The extracts were mixed intermittently for two minutes while standing in ice, centrifuged and 200 µl of the supernatant was loaded on to 100 mg ml^{-1} Isolute C18 solid-phase extraction cartridges (International Sorbent Technology, Hengoed, UK) previously conditioned with 2 ml

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of methanol and 1 ml of 1 mol litre^{-1} perchloric acid. The sample was pushed slowly through the cartridge and collected in a 1·8 ml tube. The retained sample was washed through with 400 µl of 0·6 mol litre^{-1} sodium hydrogen phosphate buffer, pH 2·4. Cr, PCr and Cn were assayed by reverse-phase ion-pair high performance liquid chromatography (HPLC) (Dunnett et al 1991) using a 15 cm Spherisorb ODS 2 (5 µi) (Jones Chomatography, Hengoed, UK) fitted with a 2 cm Sperisorb ODS 2 (5 µ) guard column. Cr, PCr and Cn were detected by their ultra violet absorbance at 210 nm. When necessary, the extracts were further diluted with the mobile phase. The assays were calibrated against mixed 0·5 mmol litre^{-1} standards of Cr, PCr and Cn prepared in the mobile phase. The initial estimates of concentration were adjusted for losses during extraction by using appropriate standards. A separate 400 mg sample of meat was freeze-dried to determine its water content. This value was subsequently used in the calculation of the total homogenate volume.

Samples of air-dried meat (biltong) identified only as beef, ostrich or kudu were purchased from retail outlets in Johannesburg. No information was available on the cut of the meat or its age after slaughter. Powdered biltong was obtained by filing the meat samples. Three 20 to 25 mg portions of the powder were extracted with 0·5 mol litre^{-1} perchloric acid and neutralised with 2·1 mol litre^{-1} potassium hydrogen carbonate (Harris et al 1974). Interfering substances were removed by extraction of 200 µl with 100 mg ml^{-1} Isolute C18 solid-phase extraction cartridges as previously described, and the extracts were assayed for Cr, PCr and Cn. When necessary the extracts were further diluted with mobile phase.

Stability of creatine in meat to cooking

Homogenates of stewing beef, chicken breast, ox heart and ox liver, prepared as before and contained in 1·8 ml screw-cap micro-tubes, were heated in a boiling water bath for 10, 20, 40 or 60 minutes. The samples were removed at the appropriate time, frozen in liquid nitrogen, thawed and extracted with 11 mol litre^{-1} perchloric acid as before.

Creatine in canned dog food

Eight varieties of canned dog food (A to H) were purchased from a range of supermarkets. Three examples of each type of canned feed were purchased, each example being from a different batch. The contents of each can were emptied into a household blender and homogenised to a slurry by frequently mixing the contents with a spatula, followed by 30 second bursts of homogenisation. A weighed sample of approximately 1 *g* was mixed with 10 ml of water and extracted by mixing at 0°C for five minutes. The extracts were filtered through a Millipore GS 0·22 µm filter. Interfering substances were removed by extraction of 200 µl with 100 mg ml^{-1} Isolute C18 solid-phase extraction cartridges as previously described, and the extracts were assayed for Cr, PCr and Cn. When necessary the extracts were further diluted with mobile phase.

Creatine in dry dog food

Four varieties of complete dry dog food (I to L) were purchased. Three examples of each variety, each from different batches, were obtained. Fifty to 100 g samples of each feed were milled to a fine powder. A weighed sample of approximately 1 g was then mixed with 20 ml of water and extracted by vigorous shaking for 15 minutes at 0°C. The extracts were filtered through a Millipore GS 0·22 µm filter. Further cleaning of the sample by solid phase extraction was not necessary.

Creatine in rendered meat meal

Fifty to 100 g samples of a commercially available rendered meat and bone (MBM) (50 per cent crude protein, 10 per cent oil and 32 per cent ash) and poultry meat meal (PMM) (62 per cent crude protein, 14 per cent oil and 18 per cent ash), which are commonly used as ingredients in the commercial manufacture of dry dog feed, were extracted as described for the dry feeds. Samples from three different batches of the MBM and PMM were analysed.

Data and statistics

The results are presented as mean (SD). To test the effects of cooking, the combined data from chicken breast, stewing beef and ox heart were analysed by analysis of variance for repeated measures. This assumes minimal variance between the meat homogenates in terms of the change in Cr with cooking, which seems reasonable. Significance was accepted at $P<0·05$. When a significant difference was detected a multiple comparison test, Fischer's Protected Least Significant Difference (PLSD) was used to compare the means.

RESULTS

PCr was not detected in any of the samples analysed, including the rabbit meat removed from the carcase 15 hours after death. Samples of fresh, uncooked meat, including those purchased from retail outlets, showed only slight degradation of Cr to Cn (Table 1). Ox heart contained less Cr than skeletal muscle, and ox liver contained only trace amounts of Cr. Prolonged heating of the meat homogenates at 98°C resulted in the progressive degradation of Cr to Cn (Table 1). The loss of Cr in chicken breast, stewing beef and ox heart was statistically significant ($P<0·5$) after 10 minutes cooking. After 60 minutes cooking the degradation to Cn in these samples averaged 27·7 per cent.

Only trace amounts of Cr and Cn were found in canned dog food (Fig 1); the highest concentration (1·93 [0·36] mmol kg^{-1} canned dog) was found in sample A. The concentration of creatinine was also low in canned dog food. The mean water contents of the canned feeds analysed ranged from 75·3 (1·0) per cent (feed A) to 83·5 (0·4) per cent (feed G).

Freeze-drying had no effect on the Cr content of rabbit meat, apart from the apparent increase due to the loss of water. Air-dried meat or biltong still retained most of its Cr, although its lower ratio of Cr/Cn indicated that significant degradation to Cn had occurred (Table 2).

The combined Cr + Cn contents in samples of MBM and PMM were approximately 20 per cent of those expected for dry meat (Table 2). The highest Cr concentrations occurred in MBM (3·07 [0·68] mmol kg^{-1}). The moisture contents of MBM and PMM were 6·79 (0·10) per cent and 2·78 (0·33) per cent, respectively.

Of the four varieties of meat-based dry dog food analysed, three (I, J and K) had a Cr concentration of less than 0·76 mmol kg^{-1}, but Cn contents of 2·83 to 4·03

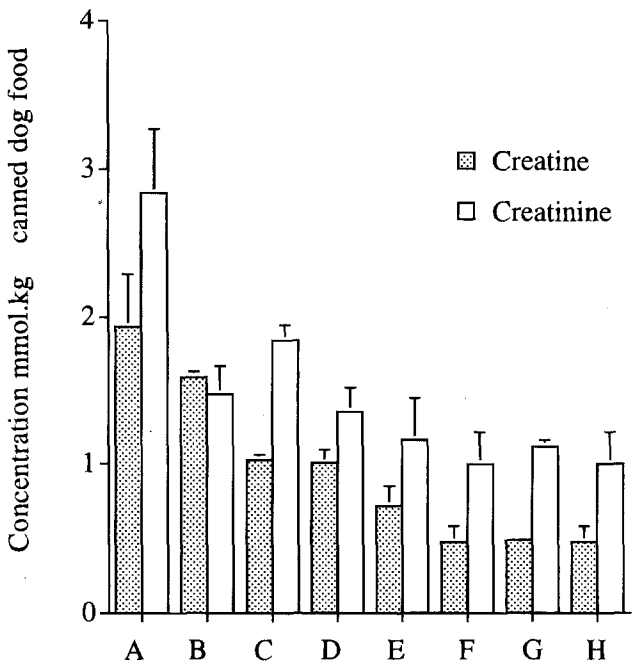

FIG 1: Mean (SD) concentrations (mmol kg^{-1}) of creatine (Cr) and creatinine (Cn) in samples of eight commercially available canned dog foods (A to H). Three examples from different batches of each dog food were analysed

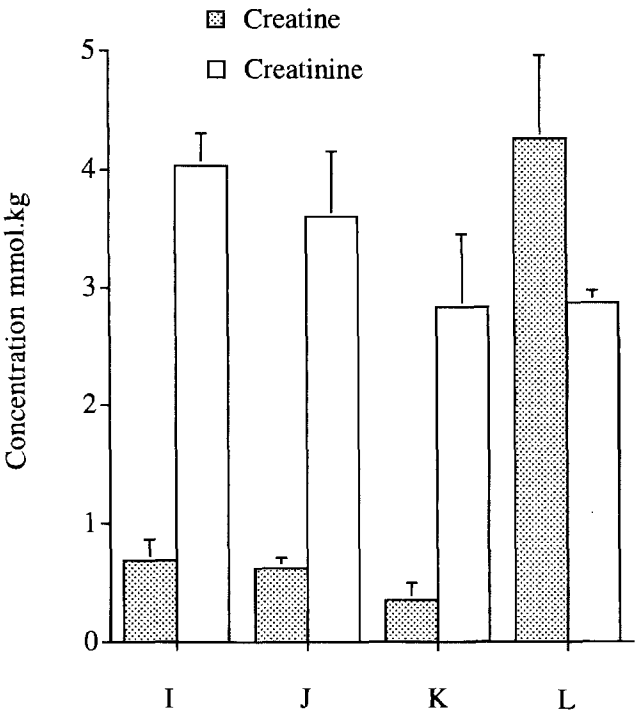

FIG 2: Mean (SD) concentrations (mmol kg^{-1}) of creatine (Cr) and creatinine (Cn) in samples of four commercially available dry dog foods (I to L). Three examples from different batches of each dog food were analysed

mmol kg^{-1}, consistent with their containing approximately 25 per cent of MBM or PMM. The Cr content of the fourth sample (L) was higher (4·25 [0·70] mmol kg^{-1}) and its Cn content lower, indicating a different source material.

DISCUSSION

The importance of Cr lies in its role in the maintenance of adenine nucleotide homeostasis at cellular sites with a high and/or fluctuating energy demand (Funk et al 1989, Kammermeier 1987). Other guanidino phosphagens have evolved to undertake a similar role to Cr and PCr, but Cr is the only such compound found in the muscles of vertebrate animals (Watts 1975). Its content in skeletal muscle varies relatively little between species, the highest values are possibly found in herring flesh although high values are not necessarily found in other fish (Söderlund et al 1994). In skeletal muscle the inadequate expression of Cr-PCr function, both in the transfer of energy from mitochondria and in the buffering of ADP released from the hydrolysis of ATP, ultimately leads to local muscle fatigue. An adequate supply of Cr, whether from synthesis or from the diet, is thus a prerequisite for the development of good athletic or working ability or, in the wild, hunting potential.

Creatine is a natural component of the diet of all meat-eating animals living wild. Wild canids are highly opportunistic feeders, decreasing their meal size and increasing the frequency of feeds with increased availability of food (Röhrs 1987). Their food consists mainly of carrion, large ungulates in winter with an increased proportion of smaller prey in summer. Eighteen to 30 per cent of the wild canid diet may consist of insects and plants. Wolves weighing between 15 and 60 kg consume on average 100 to 130 g meat kg bodyweight^{-1} per day, and in extreme conditions may gorge up to one-fifth of their bodyweight in one sitting. For a 35 kg wolf a normal average meat consumption will amount, therefore, to 3·5 to 4·5 kg of meat per day, with a Cr content of 120 to 160 mmol kg^{-1}. This will be principally in the form of Cr rather than PCr, particularly if the prey has been hunted close to exhaustion.

Although it is an ancestor of the modern dog the wolf may not be typical of the domestic dog, even when this is returned to the feral state. The meat fed domestic dog has an average dietary intake of meat less than half that of the wolf, between 30 and 40 g kg bodyweight^{-1} per day. In a 35 kg domestic dog this amounts to 1·25 kg of meat which, if eaten raw, would have a Cr content of 43 mmol kg^{-1}. It is evident from the data presented that dietary intakes of Cr by domesticated dogs fed commercial foods, or fed heavily cooked meat, particularly if the stock has been discarded, will be very much less than this. However, dogs, like other species, can synthesise adequate amounts of Cr for the maintenance of normal function and are able to maintain normal health even when fed a Cr-free vegetarian diet.

In aqueous solution Cr loses a molecule of water to form Cn. The rate of this conversion to the anhydro-form is increased at low pH, and by an increase in temperature (Fuller and Elia 1988). The extensive cooking of meat by boiling, frying or microwaving will thus cause a significant degradation of Cr to Cn (Hughes 1960, Snider and Baldwin 1981) (Table 1) though the combined pool of Cr + Cn remains essentially unaltered (Hughes 1960). The levels of Cr will also be reduced if the stock in which the meat is cooked is discarded and, in meat-based pet foods, if a relatively low ratio of muscle to offal is included as the meat fraction of the formulation. The low Cr + Cn contents and low Cr/Cn ratios of MBM and PMM are consistent with a high offal content and with the draining of stock during processing, and with the thermal degradation of Cr to Cn. As a result MBM and PMM are relatively poor sources of dietary Cr. The present results, even without a detailed knowledge of the ingredients and methods used in prepara-

TABLE 1: Mean (SD) creatine (Cr) and creatinine (Cn) contents (mmol kg^{-1}) of different meats at time of purchase and the changes during prolonged heating at 98°C of homogenates prepared from 150 to 300 mg of meat or 300 to 500 mg liver in 1 ml of water

	Boiling time (minutes)									
	0		10		20		40		60	
Meat	Cr	Cn	Cr	Cn	Cr	Cn	Cr	Cn	Cr	Cn
Chicken-breast	32·3 (0·38)	0·41 (0·15)	30·5[a] (1·18)	2·23 (0·31)	29·5[b] (0·97)	4·67 (1·10)	25·4[b] (2·10)	7·76 (1·00)	22·7[b] (1·03)	11·4 (0·95)
Stewing-beef	27·8 (1·31)	0·69 (0·29)	26·4[a] (1·32)	2·31 (0·18)	26·0[b] (1·55)	4·21 1(0·45)	24·6[b] (1·18)	7·63 (1·04)	21·8[b] (2·23)	10·5 (2·27)
Ox-heart	22·5 (2·57)	0·60 (0·28)	21·6[a] (2·88)	0·60 (0·28)	19·4[b] (2·41)	2·97 (0·32)	17·7[b] (1·75)	5·8 (0·43)	15·4[b] (1·00)	7·23 (0·57)
Ox-liver	2·38 (1·13)	ND	2·12 (1·06)	ND	1·85 (0·81)	ND	1·84 (0·97)	ND	1·54 (0·62)	ND
Rabbit meat	29·7 (1·18)	10·9 (0·59)								

ND Not determined
[a] Mean at 0 versus mean at 10 minutes $P<0·05$
[b] Mean at 0 versus mean at all other times $P<0·001$

tion, also show that both the canned and dry dog foods have low ratios of Cr/Cn, again indicating extensive degradation of Cr to Cn.

The highest levels of Cr were preserved in the dry meat samples, including biltong. Air-drying was a common form of meat preservation up to the 20th century, and in the Nordic countries it is still used for preserving fish. In these dried foods Cr is essentially stable at ambient temperatures and further loss is minimal.

It has been shown that in human beings an additional dietary supply of Cr results in further uptake into muscle (Harris et al 1992) despite the possibility that this may reduce endogenous synthesis by the feedback inhibition of arginine-glycine transamidinase (Walker 1961). In subsequent studies, increasing the muscle Cr content by dietary means was shown to improve an individual's ability to perform sustained or intermittent intense exercise (Balsom et al 1993, Greenhaff et al 1993, Harris et al 1993, Birch et al 1994). The authors have shown that dogs rapidly absorb Cr when it is supplied as meat (Harris and Lowe 1995) but it is not known whether the Cr is taken up into muscle. However, any such change in the muscle content could well be of importance in working dogs, particularly those exercising in difficult terrain throughout the day, or in sprint dogs. However, an increase in the availability of Cr to the muscles above the levels sustained by normal synthesis is unlikely to be of similar importance in normally active domesticated pet dogs, although dietary Cr could be important if the capacity for endogenous synthesis should fall below that required for normal function.

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