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Water restriction and bone metabolism in camels

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Summary — 'Krafft disease', occurring in camels living in the very arid areas of North Africa, is characterized by spontaneous fractures of costal and/or appendicular bones. To better understand the mechanisms of this, we studied the influence of water restriction on plasma and urinary markers of bone metabolism in camels. Eight 2-year-old nonpregnant, nonlactating camels were studied at the research station of Laâyoune (Morocco). After a 10 day period of daily watering, five animals were watered only every 10th day over a 50 day period, then again watered daily for a final 10 day period (rehydration). The three control animals were watered daily throughout the whole experimental period (70 days). Each camel was fed a ration of straw, lucerne hay and barley, resulting in a daily intake of 25 g calcium and 11 g phosphorus. Water restriction induced a decrease in daily urinary volume and an increase in plasma osmolality. These symptoms of dehydration were not associated with any significant change either in the markers of osteoblastic activity (plasma alkaline phosphatase activity and osteocalcine concentration) or in the markers of bone resorption (urinary excretion of calcium, hydroxyproline pyridinoline and deoxypyridinoline). Thus, in well-fed camels, water restriction did not affect bone metabolism. However, no conclusions were possible regarding the influence of dehydration or calcium and/or phosphorus deficiency in the etiology of 'Kraft disease'.

dromedary camel / dehydration / bone resorption / urinary deoxypyridinoline excretion / osteocalcine

Résumé — Inflence d'une restriction hydrique sur le métabolisme osseux du dromadaire. *La «maladie du Krafft», qui sévit parfois chez les dromadaires vivant dans les zones très arides du sud de la Tunisie, de l'Algérie et du Maroc, est caractérisée par des fractures spontanées des os costaux et/ou appendiculaires. Nous avons donc observé l'éventuelle influence d'une restriction hydrique sur les marqueurs biochimiques du remodelage osseux de ces animaux. À la station expérimentale de Laâyoune (Maroc), après une période de 10 jours pendant laquelle huit jeunes dromadaires femelles*

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non gestantes et non allaitantes étaient abreuvées quotidiennement, cinq d'entre elles ne l'ont plus été qu'une seule fois tous les 10 jours pendant une période de 50 jours, puis à nouveau quotidiennement pendant une période de 10 jours (réhydratation), comme l'ont été les trois animaux témoins pendant toute la période expérimentale (70 jours). Les huit dromadaires recevaient une alimentation à base de paille, foin de luzerne et orge, leur apportant quotidiennement 25 g de calcium et 11 g de phosphore. Chez les cinq animaux soumis à la restriction hydrique, celle-ci entraîne une diminution du volume d'urine émise quotidiennement, associée à une élévation de l'osmolalité plasmatique. Ces symptômes de déshydratation n'étaient pas associés à des modifications significatives de l'activité phosphatase alcaline du plasma ou de l'ostéocalcinémie, marqueurs de l'activité ostéoblastique. L'excrétion urinaire de calcium, d'hydroxyproline et des agents de pontage du collagène (pyridinoline et désoxypyridinoline), marqueurs de la résorption osseuse, n'ont pas non plus significativement varié chez ces animaux déshydratés. Ces résultats, qui démontrent qu'une déshydratation modérée n'a pas d'effet important sur le métabolisme osseux du dromadaire convenablement alimenté, ne permettent pas de préciser le rôle d'une déshydratation plus ou moins intense, éventuellement associée à une carence phospho-calcique plus ou moins marquée, dans l'étiologie de la «maladie du Krafft».

dromadaire / déshydratation / résorption osseuse / désoxypyridénolinurie / ostéocalcinémie

INTRODUCTION

Physiological and behavioral adaptations allow camels to survive in very high air temperatures despite a lack of drinking water (Yagil, 1985; Wilson, 1989). For example, over a period of 2 to 3 weeks, camels can lose more than one-third of their body water without any apparent ill effects and have been used in studies for water and electrolyte metabolism (Macfarlane, 1968; Yagil and Berline, 1976; Ben Goumi et al, 1993; Riad et al, 1994). Water deprivation has been shown to induce wide differences in bone structure and remodeling in *Meriones shawi,* a sub-desert rodent (Sahni et al, 1987). In normally watered camels, the endocrine regulation of calcium and bone metabolism does not differ from that demonstrated in other ruminant animals (Riad, 1995). Some nutritional disorders observed in camels are of mineral origin (Blajan and Lasnami, 1989). The first report concerning 'Krafft disease' (Durand and Kchouk, 1958) indicated that this osteopathy affects underfed adult camels living in the south of Tunisia. More recently this syndrome has also been reported in camels from southern Algeria and Morocco (Blajan and Lasnami, 1989). Among the various clinical symptoms, spontaneous fractures of costal and/or appendicular bones characterize this disease. Its etiology remains poorly understood (Bouassida, 1986). In this study we observed the influence of a water deprivation at the limit of animal care practice on bone metabolism in camels.

MATERIALS AND METHODS

Experimental design

This study was carried out at the research station of Laâyoune (Morocco) during February and March 1995, on eight 2-year-old nonpregnant, nonlactating female camels *(Camelus dromedarius),* weighing 263 ± 37 kg (mean ± SEM). The animals were kept confined in a shelter. They were fed a daily ration of 1 kg straw, 1 kg lucerne hay and 1 kg barley, resulting in a daily intake of 25 g calcium (Ca) and 11 g inorganic phosphorus (P).

The experimental period (70 days) consisted of three phases. During the first phase (10 days), five animals received water ad libitum once a day (control period). During the following 50 days, they were watered ad libitum only once every 10th day (water restriction). During dehydration (third phase; 10 days) they were watered ad libi-

tum once a day, as were the three control camels during the whole experimental period. Blood samples were collected into chilled EDTA-coated tubes by puncture of an external jugular vein on days 1, 5, 10 (control period), 15, 20, 25, 30, 35, 40, 45, 50, 56 (dehydration) and 60, 65 and 70 (rehydration). Each blood sample was taken at 0800 hours to overcome possible nyctohemeral variations. After centrifugation, plasma was frozen at –20 °C until analysis.

The urine excretion of each animal was measured during the 24 h before each blood sampling on days 1, 10, 20, 30, 40, 50, 60, 65 and 70. The urine was collected using plastic bags designed for the urinary tract of female camels (Ben Goumi et al, 1993). For each animal, the urine from a 24 h period was pooled and a sample frozen at –20 °C until analysis.

Analysis

The osteoblastic activity was evaluated by measuring plasma alkaline phosphatase activity (ALP) and osteocalcine (OC) concentration (Delmas, 1990). ALP was measured colorimetrically, using the hydrolysis of *p*-nitrophenol phosphate which yielded phosphate and *p*-nitrophenol and was converted to a yellow complex which was mea-

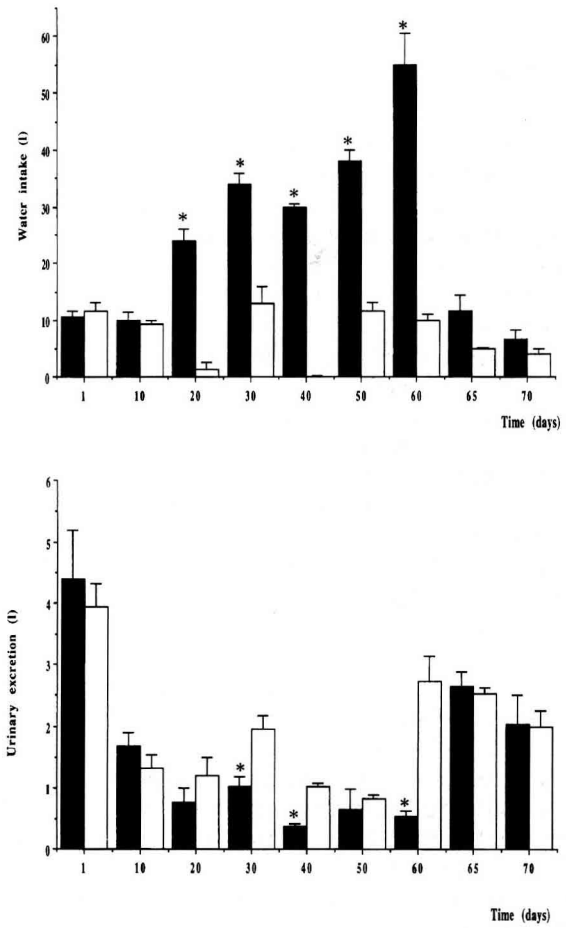

Fig 1. Daily water intake and urine excretion in water-restricted (black bars) and control camels (white bars) (means ± SEM; * $P < 0.05$, comparison with controls).

sured at 405 nm (Bretaudière et al, 1977). Plasma OC concentration was measured by radioimmunoassay (RIA) using the Osteocalcin ^{125}I RIA kit from Incstar (Incstar Corporation, Stillwater, MN, USA). This assay employs the simultaneous addition of the sample, rabbit antibovine OC antibody and ^{125}I bovine OC followed by an overnight incubation at 4 °C. Phase separation was accomplished by the addition of a complex composed of goat antirabbit serum, carrier rabbit serum and polyethylene glycol. This assay was centrifuged and decanted after a 2 h incubation at 4 °C. Dilution of the camel plasma samples paralleled the standard curve. Under our conditions, intra- and interassay variability was 7 and 10%, respectively. The minimum detectable amount was 0.15 μg/L.

Bone resorption was evaluated measuring total hydroxyproline (HYP), pyridinoline (PYD) and deoxypyridoline (DPD) in acid hydrolysates of urine.

HYP was measured by colorimetry according to the Kivirikko method (1970). The pyridinium cross-links were measured by high-performance liquid chromatography with an automated prefractionation using cellulose CC3I columns and a synthetic internal standard as described previously (Pratt et al, 1992). The overall variation coefficient was less than 3% for PYD and less than 6% for DPD. Creatinine was measured by

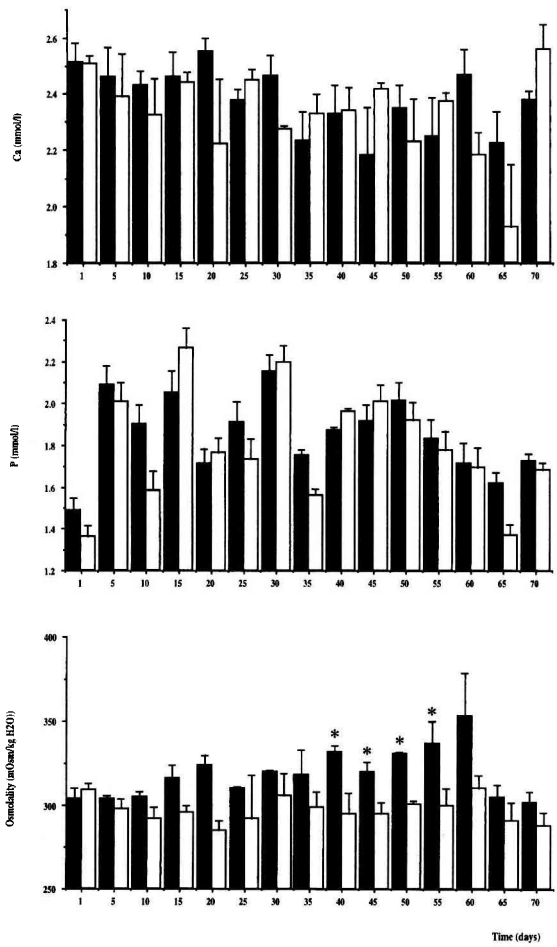

Fig 2. Plasma calcium (Ca) and inorganic phosphorus (P) concentrations and plasma osmolality in water-restricted (black bars) and control camels (white bars) (means ± SEM; * $P < 0.05$, comparison with controls).

colorimetry: after hydrolysis of creatinine to creatine by creatinine aminohydrolase, the creatine was converted to a dye whose rate of formation was proportional to the concentration of the creatinine in the urine.

In the urine and plasma samples, Ca and P concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer 400, Bodenseewerk Perkin Elmer, Uberlingen, Germany) and by colorimetry, respectively. The plasma osmolality was measured by freezing point depression (Fiske OS osmometer, Fiske Associates, Needham Heights, MA, USA).

Results are presented as means ± SEM. The Mann-Whitney U test was used to compare water-restricted and control animals. The influence of water restriction was determined using one-way analysis of variance.

RESULTS

The daily water intake levels (L) (8 ± 3) did not vary significantly in the control camels during the whole experimental period. In the five animals watered only once every 10 days, the water intake level increased from 10 ± 1.6 on day 10 to 55 ± 6 ($P < 0.01$) on day 60 (fig 1). Daily urine excretion (L) in the control camels did not vary significantly between day 1 (3.9 ± 0.4) and day 60 (2.7 ± 0.4). In the water-restricted camels it simultaneously decreased from 4.4 ± 0.8 to 0.5 ± 0.1 ($P < 0.05$), then returned to 2 ± 0.5 on day 70, where it was not different from that measured in the controls (2 ± 0.2; fig 1). The

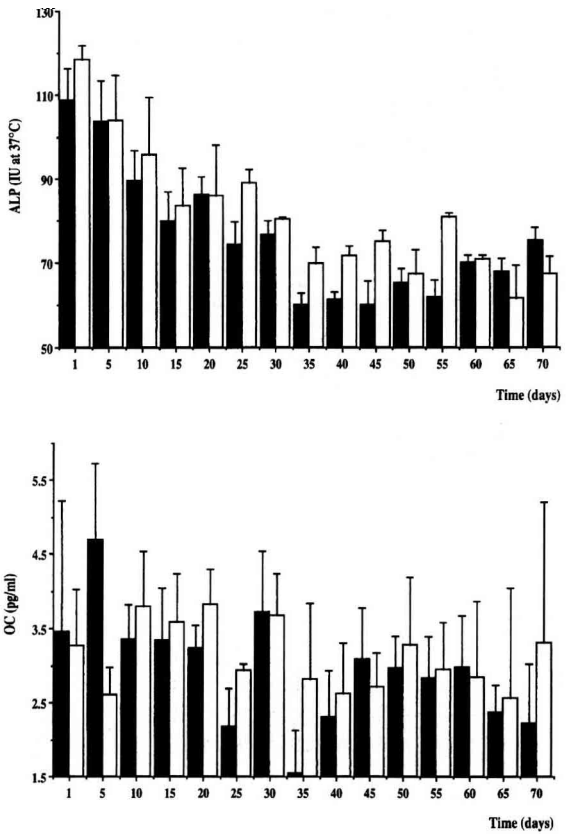

Fig 3. Plasma alkaline phosphatase activity (ALP) and plasma osteocalcine (OC) concentration in water-restricted (black bars) and control camels (white bars) (means ± SEM; no significant difference was observed between groups).

plasma osmolality (mOsm/kg H_2O) never varied significantly in the control camels (297 ± 8; $n = 45$), while it increased from 304 ± 6 on day 1 to 353 ± 26 ($P < 0.05$) on day 60 in the water-restricted animals (fig 2). The plasma Ca and P concentrations did not vary significantly in either group (fig 2).

There were no significant differences in plasma ALP activity (IU at 37 °C) between the water-restricted and control camels. Between days 1 and 60 activity decreased from 118 ± 4 to 71 ± 1 ($P < 0.05$) and from 109 ± 8 to 70 ± 2 ($P < 0.05$) in the control and water-restricted camels, respectively. The plasma OC concentrations also did not vary in either group during the course of the experiment and no difference was observed between groups (fig 3).

In the water-restricted camels, the daily urinary P excretion (mmol) decreased from 0.50 ± 0.09 on day 1 to 0.08 ± 0.02 ($P < 0.05$) on day 60, and then returned to 0.48 ± 0.11 on day 70. The daily urinary Ca excretion (mmol) increased from 1.2 ± 0.4 on day 1 to 21.2 ± 8.3 ($P < 0.05$) on day 65 and to 7.5 ± 0.8 ($P < 0.05$) on day 70. The daily urinary Ca and P excretion did not vary in the controls (fig 4).

The daily urinary HYP (mmol) excretion remained essentially unchanged in the controls. In the water-restricted camels it decreased from 509 ± 129 on day 1 to 109 ± 30 ($P < 0.05$) on day 40, and remained lower than in the controls until day 60. The mean value for urinary HYP excretion in the water-restricted camels (216 ± 56) was about half that in control animals (438 ± 121; $P < 0.05$) (fig 4); however, there was no significant difference in the daily urinary PYD and DPD excretion between control and water-restricted camels (fig 5). Thus, the mean values for the ratios PYD/creatinine, DPD/creatinine and PYD/DPD measured in the water-restricted animals (184 ± 28; 30 ± 7; 6.3 ± 0.2) were similar to the corresponding values for the controls (191 ± 29; 32 ± 5; 6.1 ± 0.2), respectively.

DISCUSSION

Dehydration in camels following 14 days of water deprivation in a hot environment induces a decrease in the volume of excreted urine and an increase in plasma osmolality, so that during the rehydration period the animals can drink 75 L of water within 5 min (Ben Goumi et al, 1993). As shown by the increase in water intake and plasma osmolality and the decrease in urine volume (figs 1 and 2), watering only once every 10th day induced dehydration in the animals used in this experiment.

In these five dehydrated camels no significant change in plasma Ca and P concentration was observed (fig 2). In growing *Meriones,* as well, water deprivation after weaning had no significant effect on the plasma levels of either Ca or P. However, compared to controls, wide differences in bone structure and remodeling were observed in these rodents, including a decrease in the cortical thickness of compact bone and a reduction in the cancellous bone mass. These differences might result from an increase in osteoclastic resorption. The processes of bone formation were also greatly altered, as observed in the cancellous bone by the accumulation of osteoid tissue and in the cortical bone by differences in the orientation of the bone layers (Sahni et al, 1987).

It has been reported that dehydration in camels induces histological evidence of increased parathyroid activity (Charnot, 1963). Examination of the biochemical markers of bone turnover, ie, plasma ALP and plasma OC concentration for osteoblastic activity and urinary excretion of HYP, PYD and DPD for bone resorption (Delmas, 1992), did not show such as increase. Alkaline phosphatases are glycoproteins coded for by at least four different gene loci which catalyze the hydrolysis of phosphate esters at alkaline pH. Tissue nonspecific (liver, bone and kidney), intestinal and placental

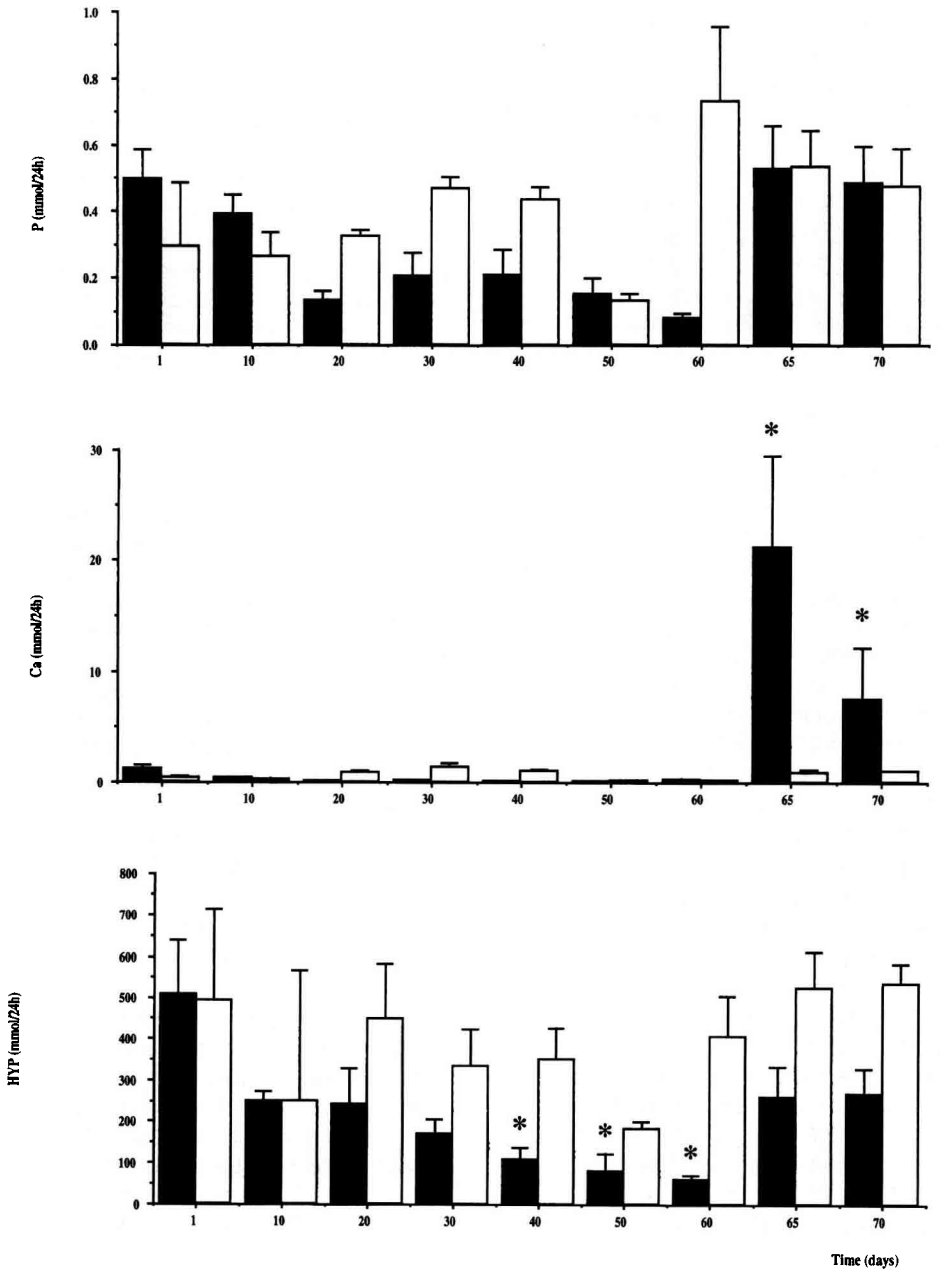

Fig 4. Daily urinary calcium (Ca), inorganic phosphorus (P) and hydroxyproline (HYP) excretion in water-restricted (black bars) and control camels (white bars) (means ± SEM; * $P < 0.05$, comparison with controls).

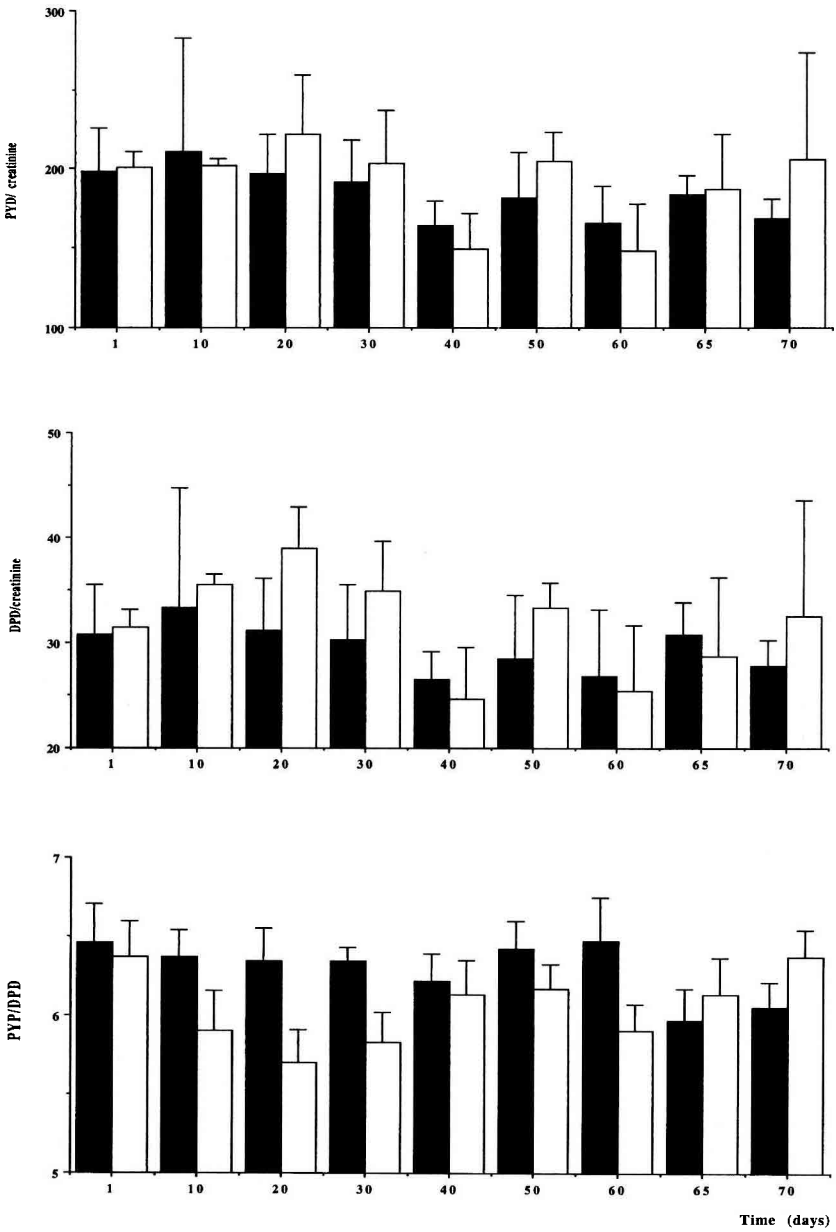

Fig 5. Daily urinary pyridinoline (PYD/creatinine) and deoxypyridinoline (DPD/creatinine) excretion and urinary pyridinoline/deoxypyridinoline ratio (PYD/DPD) in water-restricted (black bars) and control camels (white bars) (means ± SEM; no significant difference was observed between groups).

ALP have been identified (Harris, 1989). ALP from the liver, bone and kidney differ from each other on the basis of electrophoretic motility and heat and urea stability. Bone ALP is localized in the plasma membrane of osteoblasts and is released into the circulation (Rodan et al, 1982). A positive linear relationship has been demonstrated between ALP and bone ALP in ovine plasma (Collignon et al, 1996). For an unknown reason plasma ALP decreased in both groups between days 1 and 35, then did not vary between days 35 and 70. However, plasma ALP and plasma OC concentration measured in water-restricted camels never differed from that measured in the controls (fig 3), indicating that, in our experimental conditions, water restriction had no significant effect on osteoblastic activity.

The measurement of total urinary HYP excretion as an index of collagen metabolism was introduced by Ziff et al (1956) and was later specified as an index of bone collagen metabolism by Klein et al (1964) and by Gruson et al (1967). Although urinary HYP has been widely used as a marker of bone resorption (Kivirikko, 1970) it is not specific for this process. Urinary HYP is derived from all types of collagen and may even derive from collagen synthesis (Hoerlein et al, 1978). A portion of HYP is metabolized in the liver; thus, HYP excreted in the urine represents only about 10% of that produced by collagen catabolism (Prockop, 1964) and can be influenced by diet (Kivirikko, 1970). Several studies have shown that the collagen cross-links PYD and DPD provide good urinary markers of collagen degradation, primarily reflecting bone resorption (Black et al, 1989; Seibel et al, 1992). They are both present in the mature form of collagen (Robins, 1983). PYD is a maturation product of the reducible lysine-derived intermolecular bond that is absent in skin collagen but is a major cross-link of cartilage and, to a lesser extent, of bone. DPD has a more restricted tissue distribution and is primarily located in bone collagen (Robins and Duncan, 1987). PYD and DPD are released into the circulation and are excreted in the urine. Thus, the measurement of their levels in urine is a sensitive index of bone resorption (Delmas, 1992; Seibel et al, 1992). However, daily urine excretion of PYD and DPD measured in water-restricted camels was not different from that measured in controls, and the ratio PYD/DPD also did not vary between the groups (fig 5).

The different findings for the cross-links compared with HYP excretion may be related to changes in intracellular processing of collagen. In addition to the possible contribution of the procollagen N-propeptide to HYP excretion, a proportion of procollagen is known to be degraded intracellularly (Bienkowski et al, 1978). This process, which may account for up to 40% of total procollagen synthesis, can be considerably influenced by a number of factors that are generally mediated by cyclic-AMP (Bienkowski, 1984). It is possible, therefore, that one response of the camel to water restriction is to reduce the level of intracellular procollagen degradation, thus leading to changes in HYP excretion but without entailing any change in the pyridinium cross-links which reflect only the degradation of mature collagen fibrils.

These results demonstrated that the dehydration induced by a restricted watering, as shown by a slight but significant increase in plasma osmolality, does not appear to have a major effect on bone metabolism in these young well-fed camels. No conclusions were possible, however, whether repeated water restriction during periods longer than 10 days might play a role in the etiology of 'Kraft disease' which is observed in camels living in arid areas of North Africa, especially during periods of food and mineral restriction.

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