

Some Effects of a Low Sodium Diet High in Potassium on the Renin-Angiotensin System and Plasma Electrolyte Concentrations in Normal Dogs

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Pedersen, H. D., J. Koch, A. L. Jensen, K. Poulsen, A. Flagstad: Some effects of a low sodium diet high in potassium on the renin-angiotensin system and plasma electrolyte concentrations in normal dogs. Acta vet. scand. 1994, 35, 133-140. – Eight normal male Beagle dogs received 0.7 mmol Na⁺/kg/day for 5 weeks and 4.0 mmol Na⁺/kg/day in one 3 week control period preceding and another similar period following the low sodium period. The dogs received 6.8 mmol K⁺/kg/day throughout the study.

The median plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were higher in the low sodium period than in the following control period (0.67 versus 0.28 ng/ml/h, $p < 0.0001$) and (204 versus 31 pg/ml, $p < 0.0001$). PRA and PAC quickly stabilized on a new steady level in response to altered intake of sodium chloride. The angiotensin-converting enzyme (ACE) activity was not changed by the altered intake of sodium chloride.

The plasma concentrations of sodium and chloride were increased during the low sodium period. This could be due to an indirect effect of the high potassium intake of the dogs. Potassium leads to an increased secretion of aldosterone and thereby to an increased retention of sodium and chloride in the kidney. The possible implications of a high potassium content in a low sodium diet are discussed.

Beagle dogs; physiology; plasma renin activity; plasma aldosterone concentration; angiotensin-converting enzyme activity; chloride; magnesium.

Introduction

A low sodium diet is often used as an adjunctive treatment in dogs with congestive heart failure (CHF) (Ross 1984). Sodium restriction helps by reducing the retention of salt and water (Pensinger 1964) caused mainly by aldosterone and angiotensin II (Ang II), a component of the renin-angiotensin system (RAS). Increased activity of the RAS is a major part of the pathophysiology of CHF (Watkins *et al.* 1976). Therefore, it seems paradoxical that sodium restriction acts as a very potent stimulus for the RAS. Sodium restriction is known to

cause an increase in plasma renin activity (PRA), Ang I, Ang II and plasma aldosterone concentration (PAC) (Krieger *et al.* 1990). A recent study in rats also showed an increase in serum angiotensin-converting enzyme (ACE) activity in response to strict sodium restriction (Fox *et al.* 1992). Knowing that sodium restriction has a major impact on the RAS makes it important to know the sodium intake of the patient when evaluating the different parameters within the RAS.

Studies focused on the impact of low sodium diets on the RAS are scarce in the veterinary

literature. The purpose of this study was to assess the effect of a clinically relevant low sodium diet on PRA, PAC and serum ACE activity in normal dogs. In a clinical situation, it is important to know the time it takes for these parameters to reach a new steady state after introducing a diet with a certain sodium content, for instance a low sodium diet. The study was therefore designed to give information concerning the numeric increase as well as the long term dynamics of PRA, PAC and ACE activity.

Practically all dogs with CHF are treated with a diuretic. Treating heart failure in dogs with furosemide has been shown to cause a reduction in the plasma concentration of potassium (Cobb & Michell 1992). A high potassium content in low sodium diets would be desirable in helping to avoid hypokalaemia in such patients. It was decided in this study to evaluate the effects of a low sodium diet with a high potassium content.

Methods

Dogs and protocol

Eight clinically healthy non-related male Beagle dogs aged 1 to 1½ years old and weighing 12-16 kg (mean 14 kg) were included in the study. The dogs were housed individually in their usual cages and were fed once a day between 9.00 and 10.30 a.m. according to the equation (Legrand-Defretin 1993): kcal metabolisable energy per dog per day = 125 (bodyweight)^{0.75}. A minor weight loss during the initial control period, probably caused by a high level of activity, promoted a 10% increase in the daily allowances for the rest of the study to ensure a stable body weight. Each dog ate all of the provided food each day. The dogs were offered tap water ad libitum containing approximately 0.2 mmol potassium and 1.3 mmol sodium per litre. The dogs re-

ceived no drugs 2 weeks prior to or during the study. Furthermore, no abnormalities were revealed on routine hematological and biochemical analyses of the dogs prior to the experiment.

The feeding experiment consisted of 3 periods of 3, 5 and 3 weeks duration respectively. Initially, the dogs were fed a canned control diet for 3 weeks, then a canned low sodium diet for 5 weeks and finally the canned control diet again for 3 weeks. The control diet was made by adding sodium chloride to half the batch of low sodium diet just before it was canned. Hence only the content of sodium chloride differed between the 2 diets. This protocol provided each dog with 4.0, 0.7 and 4.0 mmol Na⁺/kg/day in the first, second and third period, respectively (not including sodium in the drinking water, approximately 0.08 mmol Na⁺/kg/day). The diets provided each dog with approximately 6.8 mmol K⁺/kg/day.

Sample collection

Blood samples were collected by jugular venipuncture at the end of the first control period and every 7 days for the rest of the trial. Additionally, PRA and PAC were measured 3 days into the low sodium period and 2 days into the following control period. All samples were taken between 9.00 and 10.00 a.m. after overnight fast. Great care was taken to minimize stress during the blood sampling procedure and all the blood samples were drawn by the same veterinarian. Blood for PRA and PAC determination was transferred to iced EDTA-containing tubes (Becton-Dickinson) immediately and centrifuged at 4°C within 1 h. Serum was also separated within 1 h. Plasma and serum were kept at -20°C until the end of the trial. The samples were analysed for PRA, PAC, ACE-activity in serum and for the concentration of sodium, potassium, chloride, calcium and magnesium in plasma.

Analytical procedures

PRA was assayed according to the radioimmunoassay of *Poulsen & Jørgensen* (1974). The PAC was determined with a RIA-kit (Coat-A-Count®, Diagnostic Products Corporation). The ACE-activity in serum was measured spectrophotometrically with a kit (Angiotensin Converting Enzyme (ACE)®, Sigma) applied to a Cobas Fara® centrifugal analyzer (Roche) as described by *Jensen & Koch* (1992). The concentrations of sodium, potassium, chloride, calcium and magnesium in plasma were measured by the electrolyte module of the Cobas Fara® centrifugal analyzer. Each component was analysed in one run, and all measurements were done in duplicate.

Statistical analysis

Wilcoxon signed rank test for paired observations was used to compare the values of the different components before and after each of the 2 changes in sodium intake. Wilcoxon rank sum test was used to compare pooled data from the low sodium period with pooled data from the following control period. ANOVA for repeated measurements was used to look for changes within the low sodium period and within the following control period. The null hypothesis was rejected at $p < 0.05$. Data are presented as medians and 25/75 percentiles.

Results

Fig. 1 summarizes the changes in PRA, PAC and ACE-activity in serum during the feeding trial. Introducing the low sodium diet caused no change in PRA ($p = 0.55$), but withdrawing the low sodium diet after 5 weeks decreased PRA significantly ($p = 0.008$). The median PRA during the low sodium period was higher than during the following control

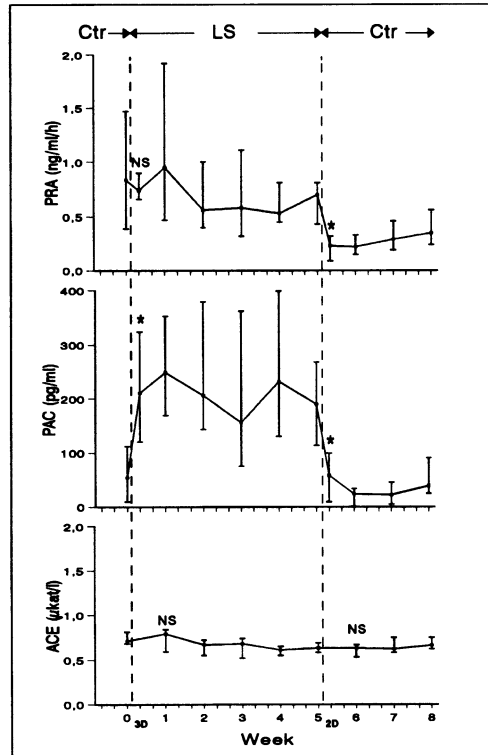


Figure 1. Plasma renin activity (PRA), plasma aldosterone concentration (PAC) and serum angiotensin-converting enzyme activity (ACE) in 8 normal Beagle dogs fed a control diet (Ctr) for 3 weeks, a low sodium diet (LS) for 5 weeks and the control diet again for 3 weeks. Values are medians and 25/75 percentiles. The first values after each change in diet were compared to the values obtained before the diet-change. * = Significant change ($p \leq 0.05$), NS = Non-significant changes ($p > 0.05$). 3D and 2D indicates 3 respectively 2 days after the diet changes.

period (0.67 ng/ml/h and 0.28 ng/ml/h, $p < 0.0001$). PAC increased in response to the decreased sodium intake ($p = 0.008$) and decreased when the control diet resumed ($p = 0.008$). Accordingly, the median PAC was higher during the low sodium period than during the following control period (204 pg/ml and 31 pg/ml, $p < 0.0001$). Both PRA and PAC

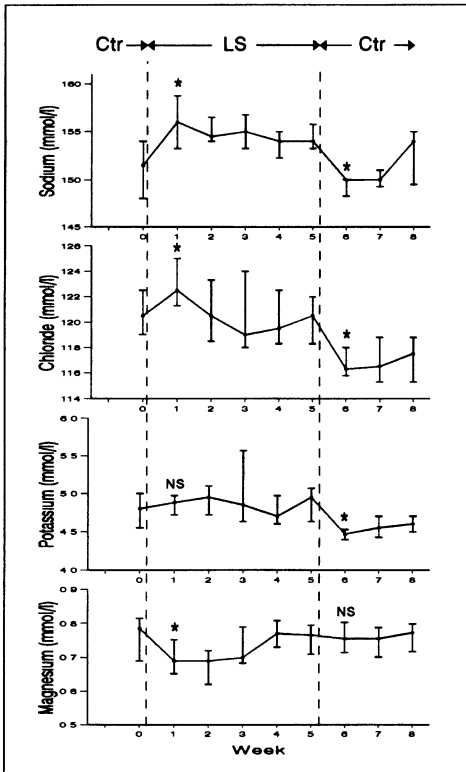


Figure 2 Plasma concentrations of sodium, chloride, potassium and magnesium in 8 normal Beagle dogs fed a control diet (Ctr) for 3 weeks, a low sodium diet (LS) for 5 weeks and the control diet again for 3 weeks. Values are medians and 25/75 percentiles. The values one week after both changes in diet were compared to the values obtained before the diet-change. * = Significant changes ($p \leq 0,05$), NS = Non-significant changes ($p > 0,05$).

were constant during the 5 week low sodium period ($p = 0.17$ and $p = 0.18$) and during the following 3 week control period ($p = 0.18$ and $p = 0.06$). The ACE-activity in serum was unchanged at both the first and the second change in sodium intake ($p = 0.69$ and 0.84). The median ACE-activity in serum during the low sodium period equaled the level of the following control period ($0,64 \mu\text{kat/l}$ and $0,635 \mu\text{kat/l}$, $p = 0,92$).

Fig. 2 summarizes the changes in the plasma electrolytes during the feeding trial. Both the plasma sodium and the plasma chloride concentration increased in response to the low sodium diet ($p = 0.016$ and 0.047) and decreased again when the control diet resumed ($p = 0.008$ and 0.008). The plasma potassium concentration did not change when the low sodium diet was introduced ($p = 0.30$) but decreased when the dogs were fed the control diet again ($p = 0.016$). The median plasma potassium concentration was higher during the low sodium period than during the following control period (4.9 mmol/l and 4.5 mmol/l , $p < 0,0001$). The plasma magnesium concentration decreased at the first change in diet ($p = 0.008$) but not at the second change ($p = 0.69$). The plasma calcium concentration (not shown) were unchanged by the decrease as well as the increase in sodium intake ($p = 0.74$ and 0.08).

Discussion

The PRA-values in the present study are lower than in other studies both on low sodium intake and on medium/high sodium intake (Villarreal et al. 1990, Nii et al. 1987, Krieger et al. 1990). However, the dogs in the 3 forementioned studies had a lower sodium intake per kg. It is well established that the lower the sodium intake is, the higher is the increase in PRA caused by further reducing the sodium intake. Further, different methodology makes it difficult to compare PRA-values in different studies. Sealey (1991) reported that PRA values varied up to a factor 2.1 when evaluated by different laboratories and kits. A discussion of different methods for measuring PRA including the method used in the present study is given elsewhere (Poulsen & Nielsen 1993).

The PRA value at the end of the initial con-

tol period were higher than the PRA level in the final control period. The dogs were not accustomed to the blood sampling procedure, and therefore, might have had a stress-induced renin release via the β -receptors in the kidney. The results of the present study show that PRA and PAC reach new steady levels within 2 to 3 days after moderate changes in sodium intake. These observations show that 2-3 days after a moderate change in sodium intake, blood sampling for measuring PRA or PAC can take place at any time – the change in diet will not influence the results further.

Villarreal et al. (1990) found that PAC in 5 normal dogs increased from 25 pg/ml when the dogs were fed approximately 40 mmol Na⁺/day, to 103 pg/ml after 1 week and 163 pg/ml after 2 weeks of feeding the dogs < 6 mmol Na⁺/day. *Krieger et al.* (1990) found that PAC averaged 170 pg/ml in 6 normal dogs on a low sodium diet (8 mmol Na⁺/day), decreased to undetectable levels when the dogs received 120 mmol Na⁺/day, and increased to 210 pg/ml when the low sodium diet resumed. The PAC levels in the present study were higher than in the 2 forementioned studies considering that these 2 studies used more restrictive low sodium diets. This cannot be due to Ang II stimulating the aldosterone synthesis, since the PRA-values if anything were low in the present study.

The high potassium intake of the dogs is a more likely explanation for the high PAC-levels in the present study. The dogs received approximately 95 mmol K⁺/day whereas the dogs in the studies of *Villarreal et al.* (1990) and *Krieger et al.* (1990) received respectively 50-60 and 30 mmol K⁺/day. Potassium is a powerful stimulus for the secretion of aldosterone. The associated sodium retention will restrain the secretion of renin, the end result being an increase in the PAC/PRA ratio. A positive potassium balance and an increase in

PAC/PRA ratio has been reported in humans when reducing the sodium intake at a constant potassium intake (*Laragh* 1985). *Laragh* (1985) suggests, that potassium might be retained because of reduced levels of tubular sodium causing reduced activity of the sodium-potassium exchange mechanism. A positive potassium balance is not necessarily reflected in the plasma potassium concentration. Neither sodium nor potassium balance were evaluated in this study, and it is therefore not known whether there was any potassium retention during the low sodium period. However, the increase in aldosterone/PRA ratio (from 111 to 304), and the decrease in plasma potassium when the low sodium diet was replaced by the control diet, indicated that it might have been the case. Furthermore, the median plasma potassium concentration was higher during the low sodium period than during the following control period. None of the dogs in the present study developed hyperkalemia at any time during the study.

In the only veterinary study published so far on the RAS in dogs with spontaneously developed heart failure, *Knowlen et al.* (1983) measured the PAC in 23 dogs with CHF. The following PAC-values were obtained (mean \pm SD): clinically normal dogs: 76 \pm 33 pg/ml, dogs in New York Heart Association (NYHA) class III: 202 \pm 102 pg/ml and dogs in NYHA class IV: 420 \pm 223 pg/ml. It appears that the PAC in dogs in NYHA class III was at a level corresponding to the level obtained in the present study, when feeding normal dogs the low sodium diet. On the basis of these findings, the increase in PAC observed by *Knowlen et al.* (1983) can probably be ascribed in part to a dietary induced increase, since many of the patients in the study, as opposed to the control dogs, were fed low sodium diet for therapeutic reasons (*Knowlen et al.* 1983).

The ACE-activity in serum was not changed by the low sodium diet. Fox *et al.* (1992) found, that dietary sodium deprivation in rats increased the ACE-activity in plasma by 30%, but they also found an inexplicable increase when the rats were sodium loaded. On the basis of these findings and the findings in the present study, it is probably safe to say that the ACE-activity in serum is only influenced to a minor degree by changes in sodium intake. This is not surprising for 2 reasons: the conversion of Ang I to Ang II is quick and is not a rate limiting step, because ACE is present in a high concentration *in vivo* (Poulsen & Jacobsen 1993). Furthermore, ACE is a rather non specific peptidase, and it is therefore likely, that the ACE-activity in serum is regulated according to the need for conversion/degradation of other peptides as well as Ang I.

The increase in the plasma concentrations of sodium and chloride observed when the dogs were fed the low sodium diet is in contrast to the findings in other studies. Usually no change is found in plasma sodium or plasma chloride in response to low sodium diets (Nii *et al.* 1987, Hamlin *et al.* 1964). The increased concentrations of sodium and chloride in this study are probably related to the potentiated stimulation of aldosterone that was observed. It is known that hyperaldosteronism is associated with an increase in the plasma concentrations of sodium and chloride.

The decrease in plasma magnesium concentration observed for 3 to 4 weeks after introduction of the low sodium diet is not immediately explainable. It is well known that in ruminants, a sudden change to a high potassium / low sodium feed causes a decrease in plasma magnesium concentration via a decreased enteral absorption of magnesium (Leonhard *et al.* 1989). Whether or not such a feed has a similar influence on magnesium ab-

sorption in monogastric animals remains to be established.

In arterial hypertension in humans there is a beneficial effect on blood pressure associated with dietary potassium supplementation (Cappuccio & MacGregor 1991). In hypertensive rats, regression of the associated cardiac hypertrophy has also been found in response to dietary potassium (Mervaala *et al.* 1992). Whether potassium supplementation has beneficial effects in the treatment of CHF is unknown. The findings in the present study indicates that potassium might be a useful means of decreasing the PRA-levels. This will lead to decreased circulating concentrations of Ang II. Ang II is involved in cardiac hypertrophy and have been shown to cause myocyte injury in rats (Tan *et al.* 1991). The angiotensin-converting enzyme inhibitors which prevent the formation of angiotensin II have been proven to prolong the life of both humans and dogs with heart failure (Braunwald 1991, Ettinger 1992). Thus, cardioprotective effects might be obtained by feeding dogs with heart failure a diet high in potassium. A more obvious advantage of such a diet is the reduced risk of hypokalaemia in patients treated with diuretics. Further studies are warranted in order to find the appropriate level of potassium in low sodium diets.

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References

- Braunwald E*: ACE inhibitors – A cornerstone of the treatment of heart failure. *New Engl. J. Med.* 1991, 325, 351-353.
- Cappuccio FP, MacGregor GA*: Does potassium supplementation lower blood pressure? A meta-analysis of published trials. *J. Hypertens.* 1991, 9, 465-473.
- Cobb M, Michell AR*: Plasma electrolyte concentrations in dogs receiving diuretic therapy for cardiac failure. *J. Small Anim. Pract.* 1992, 33, 526-529.
- Ettinger SJ, Cooperative Veterinary Enalapril (Cove) Study Group*: Evaluation of enalapril therapy in dogs with heart failure in a large multicenter study. *Proc. 17th WSAVA World Congress 1992*, 7-11.
- Fox J, Guan S, Hymel AA, Navar G*: Dietary Na and ACE inhibition effects on renal tissue angiotensin I and II and ACE activity in rats. *Amer. J. Physiol.* 1992, 262, F902-F909.
- Hamlin RL, Smuth RC, Smuth CR, Powers TE*: Effects of a controlled electrolyte diet, low in sodium, on healthy dogs. *Vet. Med./Small Anim. Clin.* 1964, 59, 748-751.
- Jensen AL, Koch J*: Determination of angiotensin-converting enzyme in canine serum. *Clin. Chem. Enzym. Comms.* 1992, 4, 311-317.
- Knowlen GG, Kuttleson MD, Nachreiner RF, Eyster GE*: Comparison of plasma aldosterone concentration among clinical status groups of dogs with chronic heart failure. *J. Amer. vet. med. Ass.* 1983, 183, 991-996.
- Krieger JE, Luard JF, Cowley Jr AW*: Hemodynamics, fluid volume, and hormonal responses to chronic high-salt intake in dogs. *Amer. J. Physiol.* 1990, 259, H1629-H1636.
- Laragh JH*: Atrial natriuretic hormone, the renin-aldosterone axis, and blood pressure-electrolyte homeostasis. *New Engl. J. Med.* 1985, 313, 1330-1340.
- Legrand-Defretin V*: Energy requirements of cats and dogs – what goes wrong. *Int J Obesity* 1994, 18 (Suppl. 1), S8-S13.
- Leonhard S, Martens H, Gabel G*: New aspects of magnesium transport in ruminants. *Acta vet. scand.* 1989, suppl 86, 146-151.
- Mervala EMA, Humberg J, Laakso J, Tuomänen P, Karppanen H*: Beneficial effects of a potassium- and magnesium enriched salt alternative. *Hypertension* 1992, 19, 535-540.
- Nu T, Fouad-Tarazi FM, Ferrario CM, Bravo EL, Czernska B*: Changes in hemodynamics and myocardial contractility during chronic sodium depletion in conscious dogs. *Hypertension* 1987, 9 (suppl III), III-176 – III-180.
- Pensinger RR*: Dietary control of sodium intake in spontaneous congestive heart failure in dogs. *Vet. Med./Small Animal Clin.* 1964, 59, 752-757.
- Poulsen K, Jacobsen J*: Enzymic reactions of the renin-angiotensin system. In: Robertson JIS, Nicholls MG (eds). *The renin angiotensin system*. Gover Medical Publishers, London 1993.
- Poulsen K, Jørgensen J*: An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J. clin. Endocr. Metab.* 1974, 39, 816-825.
- Poulsen K, Nielsen AH*: The measurement of renin. In: Robertson JIS, Nicholls MG (eds): *The renin angiotensin system*. Gover Medical Publishers, London 1993.
- Ross JN Jr*: Heart failure. In: Lewis LD, Morris ML Jr (eds): *Small animal clinical nutrition*, 2nd ed. Mark Morris Associates, Topeka, Texas 1984.
- Sealey JE*: Plasma renin activity and plasma prorenin assays. *Clin. Chem.* 1991, 37, 1811-1819.
- Tan L, Jalil JE, Puck R, Janucki JS, Weber KT*: Cardiac myocyte necrosis induced by angiotensin II. *Circulation Research* 1991, 69, 1185-1195.
- Villarreal D, Freeman RH, Brands MW*: ANF and postprandial control of sodium excretion in dogs with compensated heart failure. *Amer. J. Physiol.* 1990, 258, R232-R239.
- Watkins L Jr, Burton JA, Haber E, Cant JR, Smuth FW, Barger AC*: The renin-angiotensin-aldosterone system in congestive failure in conscious dogs. *J. clin. Invest.* 1976, 57, 1606-1617.

Sammendrag

Nogle effekter af en diæt med lavt natrium indhold og højt kalium indhold på renin-angiotensin systemet og plasma elektrolyt koncentrationer hos raske hunde.

Otte normale beagle hunde indtog 0.7 mmol Na⁺/kg/dag i én 5 ugers periode og 4.0 mmol Na⁺/kg/dag i en 3 ugers kontrol periode før og én efter lav-natrium perioden. Hundene indtog 6.8 mmol K⁺/kg/dag i hele perioden.

Medianværdien af plasma renin aktiviteten (PRA) og plasma aldosteron koncentrationen (PAC) var højere i lav-natrium perioden end i den efterfølgende kontrol periode (0.67 versus 0.28 ng/ml/h, $P < 0.0001$) og (204 versus 31 pg/ml, $P < 0.0001$).

PAC indstillede sig hurtigt på et nyt stabilt niveau efter ændret natrium indtag. Angiotensin-converting enzyme (ACE) aktiviteten ændrede sig ikke som følge af det ændrede natrium indtag.

Plasma koncentrationerne af natrium og klorid var højere i lav-natrium perioden sammenlignet med de

to kontrol perioder. Dette skyldtes muligvis en indirekte effekt af det høje kalium indtag. Kalium fører til en forøget sekretion af aldosteron og dermed til en øget retention af natrium og klorid i nyrene. Mulige betydninger af et højt kalium indhold i lav-natrium diæter diskuteres.

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