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Effect of Water Intake on Nitrogen Metabolism in Dogs[1,2]

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ABSTRACT Nitrogen balance studies in dogs were carried out to determine the effect of variable water intake on nitrogen metabolism. The results showed that increased water intake resulted in lower retention of nitrogen at low or high levels of nitrogen intake. In most cases urinary urea decreased when water intake increased. Apparent nitrogen absorption was not influenced by water intake. The urinary sulfur did not change with changes in water intake. Total serum protein and albumin concentration, albumin-to-globulin ratio and urea levels did not change significantly when higher volumes of water were given. It is concluded that in nitrogen balance work, water intake volume should remain as constant as possible to reduce the variability in nitrogen retention. Also, with a constant water intake, when protein intake is decreased, it is possible to learn when the nitrogen balance of the experimental subject becomes stable by measuring urine volume.

In studies of nitrogen retention in dogs, it was observed that the volume of urine decreased with amino acid imbalances.[3] In theory, the feeding of such diets should also have given a lower or even a negative nitrogen balance (1, 2), but this was not always the case. In these studies[3] the amino acid imbalances were induced by adding essential amino acids other than the most limiting amino acid, to a casein diet, and it was thought that variable water intake might cause the differences in excreted urine volume, which in turn, might explain the lack of effect observed. Studies by other investigators have shown that changes in water intake caused changes in nitrogen excretion in the urine (3–6). The recent studies of Konishi and McCay (7) in dogs showed that a decrease in water intake resulted in lower nitrogen excretion and that an increase in water intake had the reverse effect. The problem of water intake was, therefore, studied further.

MATERIALS AND METHODS

In experiments to be described, groups of 2 or 3 dogs of about 5 months of age, of the same sex, and from the same litter were used in each study. The animals were placed in individual metabolism cages and fed a diet consisting of gelatin, 15.0; casein (vitamin-free), 8.0; DL-methionine, 0.30; DL-tryptophan, 0.20; mineral mixture (8), 2.0; hydrogenated vegetable fat, 10.0; cod liver oil, 1.0; cellulose,[4] 2.7; sucrose, 15.0; dextrin, 22.8; dextrose, 23.0% and 5 ml of a complete B-vitamin solution (9) per 100 g of diet. The diet contained 3.36% nitrogen and 435 kcal/100 g. Depending on the experiment, the dogs were fed from 0.4 to 1.0 g of nitrogen and 100 to 150 kcal/kg of body weight/day. When necessary, the intake of calories was adjusted by feeding a protein-free diet of the following composition: (in grams) cornstarch, 20; dextrose, 40; sucrose, 24; cellulose, 3; hydrogenated vegetable fat, 10; mineral mixture (8), 2; cod liver oil, 1, and 5 ml of a complete B-vitamin solution (9) per 100 g of diet. The animals were weighed and fed daily and their nitrogen intake adjusted every 4 days. The specified amount of water was mixed with the food; in all experiments the food was consumed in less than 30 minutes. Therefore, corrections were not made for loss of drinking water by evaporation. The temperature of the metabolism room varied from 23 to 24°C and the relative humidity from 77 to 84%.

The experimental periods lasted 4 days and feces and urine were collected twice

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a day. At the end of each period, the feces were homogenized, weighed and analyzed for nitrogen. The urine was collected in amber bottles containing 1 cm^3 of concentrated acetic acid and stored under refrigeration (4°C). The 4-day volume was measured and nitrogen determined in aliquots. The nitrogen content of the food, feces and urine was determined by the macro-Kjeldahl method. Urinary urea nitrogen and ammonia nitrogen were determined by nesslerization (10). Urinary sulfur was determined following the method of Folin (6) for total sulfur. In some experiments, blood samples were taken at the end of each 4-day period. Total serum protein was determined by the method of Lowry et al. (11, 12), blood urea by the method of nesslerization (10) and total α-amino nitrogen by the method of Albanese (13) except that tetraethylenepentamine was used to form a deeper-colored complex which was read on an Evelyn colorimeter.

RESULTS

Table 1 shows the effect on nitrogen balance and on other parameters of changing water intake from 400 to 1000 ml/day and back to 400 ml/day in one group, and from 1000 to 400 ml/day to 1000 ml/day in the second. Nitrogen intake and apparent nitrogen absorption did not change. Nitrogen balance and the urea nitrogen-to-total nitrogen ratio was lower when the higher water intake was given whether between 2 low water intake periods, or having a low intake of water in between. The urine-to-water intake ratio was highest when the intake of water was higher for both groups of dogs. The urinary total sulfur excretion did not change with changes in water intake. No consistent changes in body weight gain between water treatments was observed. Table 1 also shows the results of the next experiment carried out with 3 dogs. Water intake, high (1000 ml/day) and low (400 ml/day), was kept constant for 12 days to learn of variations in nitrogen balance for a longer period of time. Nitrogen retention was again lower when larger volumes of water were given and increased with the lower water intake. In both cases nitrogen balance remained constant within water treatment, the changes observed being small. Other determinations on the urine gave results similar to those shown for previous experiments, except urea nitrogen-to-total nitrogen ratio, which was in general high irrespective of water intake.

In the second experiment, the results of which are shown in table 2, the same 2 groups of dogs were used. One was given increasing and the other decreasing water intakes. Urea nitrogen-to-total nitrogen ratio followed the same tendency

TABLE 1

Effect of changing and of constant water intake on nitrogen balance, urine sulfur and urea nitrogen excretion in dogs [1]

Water intake [2]	Nitrogen					Urine/water intake ratio	Urine sulfur	Urea N/total N ratio
	Intake	Fecal	Urine	Absorbed	Retained			
ml/day	*mg/kg/day*						*mg/kg/day*	
400	692	41	374	651	277	0.51	14.0	0.76
1000	701	57	517	644	127	0.73	14.5	0.40
400	703	51	443	652	209	0.50	14.4	0.72
1000	665	28	450	637	187	0.74	15.8	0.46
400	668	36	388	632	244	0.61	16.2	0.78
1000	673	42	464	631	167	0.78	17.0	0.50
1000	719	35	498	684	186	0.71	17.1	0.95
1000	720	38	521	682	161	0.73	16.3	0.93
1000	719	40	514	679	165	0.72	17.6	0.89
400	723	55	482	668	186	0.57	17.0	0.61
400	722	49	467	673	206	0.61	16.8	0.82
400	722	34	471	688	217	0.65	15.8	0.91

[1] Number of dogs used, and average intitial weight: upper section 3, 10.25 kg; middle section 2, 13.46 kg; and lower section, 3, 11.42 kg.
[2] Each balance period is of 4-day duration.

TABLE 2

Effect of increasing and decreasing water intake on nitrogen balance, urine, sulfur and urea nitrogen excretion and on serum proteins and urea levels in dogs[1]

Water intake[2]	Nitrogen					Urine/water intake ratio	Urine sulfur	Urea N/total N ratio	Serum		
	Intake	Fecal	Urine	Absorbed	Retained				Total protein	Albumin	Urea
ml/day			*mg/kg/day*				*mg/kg/day*		*g/100 ml*	*g/100 ml*	*mg/100 ml*
400	714	42	401	672	271	0.44	13.1	0.89	6.62	2.89	17.40
600	712	36	459	676	217	0.61	15.0	0.67	6.75	2.97	17.69
800	715	53	451	662	211	0.68	15.8	0.87	6.48	3.04	17.73
1000	717	45	485	672	187	0.72	15.8	0.92	6.97	3.05	20.10
1000	717	45	481	672	191	0.65	15.2	0.95	—	—	—
1000	678	44	469	634	165	0.77	15.2	0.63	6.58	4.60	11.85
800	680	34	447	646	199	0.73	16.0	0.75	5.79	3.98	15.48
600	683	47	435	635	201	0.70	17.7	0.86	6.06	3.66	14.71
400	687	40	434	647	213	0.62	15.7	0.87	5.90	3.82	18.25
400	687	45	440	642	202	0.59	15.3	0.88	—	—	—

[1] Number of dogs used and average initial weight: upper section, 3, 10.68 kg; lower section, 2, 13.80 kg.
[2] Each balance period is of 4-day duration.

as previously shown in only the group of animals receiving decreasing levels of water intake. The urine-to-water intake ratio increased with increases in water consumption and decreased with decreases in water intake. Urine sulfur behaved as indicated for the results of the first experiment. Table 2 also shows the effect of changing water intake on total serum proteins, albumin, and serum urea. Total serum protein and albumin concentration showed no change when higher volumes of water were given. The albumin-to-globulin ratio remained constant within the group of dogs, and the higher values observed in the animals given decreasing intakes of water are probably characteristic of the breed of dogs used. The serum urea concentration did not change consistently with changes in water intake in either group of dogs.

Table 3 shows the results of another experiment obtained by feeding a group of dogs 2 levels of protein at a constant intake of calories of 140 kcal/kg/day. Water intake was varied from 300 to 900 ml/day and back to 300 ml/day with the same sequence at each level of nitrogen intake. During the transition in nitrogen intake, water intake remained constant at 300 ml/day. Apparent nitrogen absorption was not affected by water intake at either level of protein feeding when expressed as percentage of nitrogen intake. The nitrogen retention was again lower at the higher water intake volume independent of nitrogen intake. Nitrogen balance at the lower level of intake was about one-half the nitrogen retained at the higher nitrogen intake in milligrams per kilogram per day, and similar when expressed as percentage of intake. The urea nitrogen-to-total nitrogen in the urine was lower at the higher water intake volume for both levels of protein. The changes were more marked at the lower level of protein intake. Although not shown in the table, a corresponding change in ammonia nitrogen was observed in both experiments, and creatinine in milligrams per kilogram per day decreased slightly at the higher water intake volume, the change being not significant. It was the same at both levels of protein feeding.

TABLE 3

Effect of changing water and nitrogen intake on nitrogen balance and serum proteins in 3 dogs [1,2]

Water intake [3]	Nitrogen					Urine/water intake ratio	Urine sulfur	Urea N/total N ratio	Serum		
	Intake	Fecal	Urine	Absorbed	Retained				Total protein	Albumin	Urea
ml/day	*mg/kg/day*						*mg/kg/day*		*g/100 ml*	*g/100 ml*	*mg/100 ml*
300	1036	54	675	982	307	0.67	31.7	0.92	6.46	2.94	10.6
900	1031	48	731	983	252	0.83	35.3	0.72	6.75	3.13	12.0
300	1042	47	680	995	315	0.68	29.7	0.88	6.45	3.24	11.1
300	533	41	384	492	108	0.67	22.3	0.83	—	—	—
300	548	33	338	515	177	0.63	21.3	0.97	6.47	3.13	8.9
300	570	42	368	528	160	0.64	22.7	0.96	—	—	—
300	564	54	363	510	147	0.63	30.1	0.74	5.94	2.81	12.9
900	518	42	387	476	89	0.69	26.8	0.30	5.97	2.82	15.4
300	539	42	348	497	149	0.47	23.6	0.76	6.04	2.81	17.9

[1] Average initial weight: 5.98 kg.
[2] Average urinary *creatinine excretion: mg/kg/day*: 28.06.
[3] Each balance period is of 4-day duration.

Table 3 also shows that total serum protein and albumin remained constant, within levels of protein, but lower at the lower protein intake. Urea concentration in the serum did not change with changes in water intake within level of protein feeding, but was higher at the lower level of protein intake.

Table 4 shows the effect of water changes on nitrogen balance when nitrogen intake is decreased from about one to around 0.5 g/kg/day and back to about 1.0 g/kg/day. The intake of calories remained constant at 140 kcal/kg/day. During changes from high to low and from low to high intake of nitrogen, water intake remained constant at 900 ml/day. Nitrogen retention was highest when water intake was lowest, for both high and low levels of nitrogen feeding. Although not shown in the table, the α-amino nitrogen values in urine were slightly higher for the higher water intakes at both levels of nitrogen feeding, and a decrease was observed when nitrogen intake decreased. As in previous studies, urinary urea nitrogen-to-total nitrogen ratio was higher for the lower intakes of water, and lower for the higher water consumption. Ammonia nitrogen changes in the opposite direction. Table 4 also shows the values for blood protein and serum urea which varied as in previous experiments.

DISCUSSION

The lower retention of nitrogen with higher water intakes or the higher retention with lower intake of water was evident at all levels of nitrogen intake. These results are in agreement with the results of Konishi and McCay (7), who reported a small increase in nitrogen retention by dogs upon water restriction. Black et al. (3) and Grande et al. (4), working with humans observed that water deprivation increased urinary nitrogen output in subjects given a low caloric diet devoid of protein. The results presented by these investigators are not comparable nor do they agree with those presented in this manuscript or published by Konishi and McCay (7).

Contrary to the present study, Konishi and McCay (7) in dogs, as well as Larsen et al. (5) in their work with ruminants,

TABLE 4

Effect of changing water and nitrogen intake on nitrogen balance, and serum proteins in 2 dogs[1]

Water intake[2]	Nitrogen					Urine/water intake ratio	Serum		
	Intake	Fecal	Urine	Absorbed	Retained		Total protein	Albumin	Urea
ml/day	*mg/kg/day*						*g/100 ml*	*g/100 ml*	*mg/100 ml*
900	1026	49	597	977	380	0.77	5.52	2.93	14.6
300	1030	47	477	983	506	0.64	5.47	3.03	16.0
900	1023	46	572	977	405	0.71	5.59	2.86	16.1
900	522	53	393	469	76	0.78	—	—	—
900	540	50	382	490	108	0.70	—	—	—
900	546	52	336	494	158	0.68	—	—	—
900	554	52	365	502	137	0.70	—	—	—
900	554	50	336	504	168	0.72	5.25	2.29	11.3
300	554	52	237	502	265	0.49	5.39	2.45	12.6
900	562	46	285	516	231	0.64	5.41	2.44	16.0
900	970	53	508	917	409	0.60	—	—	—
900	966	60	512	906	394	0.56	5.75	2.85	10.4
900	933	32	497	901	404	0.59	—	—	—
900	943	39	606	904	298	0.62	6.01	3.07	12.9
900	936	46	509	890	381	0.59	6.14	3.28	12.6
300	941	40	418	901	483	0.47	6.65	3.28	18.1
900	937	43	560	894	334	0.54	6.53	2.82	17.3

[1] Average initial weight: 4.34 kg.
[2] Each balance period is of 4-day duration.

observed that nitrogen absorption also increased with water restriction. The disagreement in apparent nitrogen absorption with respect to water intake between the present study and that of others could be accounted for by the high apparent digestibility of the protein used in this study, which would approximate 100% digestibility if corrections were made for endogenous fecal nitrogen.

Two possible mechanisms could explain the effect of high and low water intake on nitrogen balance. First, high intakes of water may have a flushing effect in the kidney and other tissues washing out nitrogen metabolites. Second, high water intakes may have some influence on the formation of urea. When the amino acids have been absorbed, part are excreted in the urine, as suggested by the slight increase in α-amino nitrogen, although the largest part is deaminated. The ammonia produced is then discarded with the urine and not utilized in urea biosynthesis. This is indicated by the lower urea nitrogen-to-total nitrogen ratio, and higher ammonia nitrogen-to-total nitrogen ratio when the higher level of water is consumed. More data are needed, however, before the above explanation can be accepted. Some adaptation by the animal may possibly occur with respect to urea formation when the higher level of water is consumed, since a lower urea nitrogen-to-total urine nitrogen ratio was not always observed. The combined statistical analysis for 22 cases available indicated nonsignificant differences in urea nitrogen excretion according to water intake. However, the 22 cases available were separated according to water intake into a high urea nitrogen excretion group (13) and a low urea nitrogen excretion group (7). A significant difference in urea excretion was detected in the group of 14 but not in the group of eight observations. The decrease in urinary excretion of nitrogen during periods of low water intake is probably also a result of less water available for concentration and solution of the end products of protein metabolism.

The interaction of water intake and amino acid imbalance is being studied to learn whether one affects the other adversely. If so, the lack of response in nitrogen balance when the imbalanced diets were fed will be explained.[5] It has been shown that animals fed amino acid

[5] See footnote 3.

imbalanced diets show a high blood urea concentration (14). If the water intake remains low the animal needs to convert all its amino nitrogen from deamination into urea to protect itself against increases in ammonia; however, if water intake is high, this will dilute the ammonia produced and as a result, nitrogen balance will decrease, since more nitrogen will be present in the urine.

It has been repeatedly reported from work with rats that one of the characteristics associated with the feeding of amino acid imbalanced diets is the almost immediate refusal of the diet (14, 15) by the experimental animal. The studies of Lepkovsky et al. (16) showed that rats fed without water ate less food than rats fed with water. When fed without water, rats regulated their food intake so that it matched the amount of water that they could mobilize from their own tissues, thereby maintaining the proper water-to-food ratio in the gastric contents. Therefore, the work of Lepkovsky et al. (16) confirmed that water intake has a decisive effect on food intake and food intake greatly influences the amount of water ingested. It would be of interest, therefore, to learn whether the decreased food intake due to amino acid imbalance diets can be corrected by increasing water consumption.

On the basis of the results, it is suggested that in nitrogen balance work, water intake volume should remain as constant as possible in order to reduce the variability in nitrogen retention within periods of the same treatment and also when comparing 2 or more treatments. Also of interest is the preliminary observation that when protein intake is decreased, it may be possible to learn when the nitrogen balance of the experimental subject becomes stable by measuring urine volume, provided water intake and environmental conditions have remained constant.

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