

## The effect of sodium chloride ingestion on food intake and on fat deposition in male rats

By D. DRORI

*Institute of Animal Science, Agricultural Research Organization,  
PO Box 6, Bet Dagan, Israel*

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1. A 21 d slaughter trial was done using weanling male rats offered sodium chloride added at 10, 20 and 30 g/kg to a nutritionally adequate, control diet containing 13 g NaCl/kg, or added to the drinking-water at 5, 10, or 15 g NaCl/l. Food and drinking-water with or without NaCl were offered *ad lib.* and their intakes were measured.
2. NaCl in the food reduced food intake by 10–15%; NaCl in solution reduced food intake only when added at the 15 g/l level; the reduction was 25%.
3. NaCl increased the fluid intake of the rats; for NaCl in the food the increments ranged from 23 to 44%; for NaCl in the drinking-water they ranged from 44 to 229%.
4. Body fat was significantly reduced by 20 and 30 g NaCl/kg added to the control diet, indicating a reduction in the efficiency of fat synthesis. Comparable amounts of NaCl taken in the drinking-water had a negligible effect on fat deposition.
5. It is suggested that NaCl in the food produces hypodipsia, i.e. a failure to drink an optimal amount of water, causing changes in the electrolyte balance which reduce food intake and progressively reduce the efficiency of fat synthesis.

Sodium chloride induces thirst, and in experiments with rats using high levels of NaCl in the diet (Richter & Mosier, 1954) it has been shown that free access to water is necessary to maintain a food intake compatible with survival and health. Nevertheless, there is no evidence to indicate that animals offered NaCl in their diet take a sufficient amount of water to maintain normal water and electrolyte balance and unimpaired energy utilization. In the tissues of rats given diets high in Na and having free access to water there was a slight increase in Na with a concomitant increase in potassium (Meyer, Grunert, Zepplin, Grummer, Bohstedt & Phillips, 1950). Hypodipsia (subliminal thirst) and resultant hypohydration are often found in many species, even when animals consume diets with a normal NaCl content (Wolf, 1958; Greenleaf, 1966). The effects of hypohydration or Na retention on the efficiency of energy utilization have not been studied.

Kromann & Meyer (1966), Kromann & Ray (1967) and Jackson, Kromann & Ray (1971) have reported that sodium bicarbonate and NaCl in the diet of lambs reduced fat deposition, energy gain and weight gain to a greater extent than could be explained by reduced food intake. The authors suggested no explanation of these results.

Preliminary experiments in our laboratory have indicated that NaCl in the diet of rats produces the same effects as those described in lambs. Furthermore, NaCl in the diet of the domesticated white Norway rat produces relatively less drinking than in the wild Norway rat (Richter & Mosier, 1954); on the other hand, the

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domesticated white rat can be induced to drink large amounts of water if dilute NaCl solution (saline) is offered instead of water (Weiner & Stellar, 1951). The experiment reported here was designed to test the hypothesis that increased water consumption induced by drinking saline prevents the adverse effects of NaCl ingestion.

#### EXPERIMENTAL

A 21 d comparative slaughter trial was done using weanling male rats (*Rattus norvegicus*) of a random-bred colony to determine the effects of adding different concentrations of NaCl (Analar grade; BDH Chemicals, Poole, Dorset, UK) to a basal diet or to the drinking-water, on food and water intake and on the retention of body fat, fat-free dry matter (DM) and energy. The experiment was done in an air-conditioned room with a mean temperature of 22°.

The basal diet was one which supported growth and reproduction for more than 10 years; it contained (g/kg): maize meal 617, soya-bean meal 219, white fish meal 119, soya-bean oil 22, Ca<sub>2</sub>(PO<sub>4</sub>)<sub>3</sub> 10, NaCl (food grade) 10, and a commercial vitamin supplement 3. The latter provided the following amounts of vitamins (/kg food): retinol equivalent 3.1 mg, cholecalciferol equivalent 42 µg, riboflavin 3.6 mg, pantothenic acid 3.6 mg, nicotinic acid 12 mg, choline chloride 480 mg, cyanocobalamin 10 µg. The diet contained 13 g NaCl/kg. Distilled water was given.

Seven dietary treatments were compared: 1, basal diet and water (control); 2-4, basal diet with 10, 20 and 30 g added NaCl/kg diet respectively, and water; 5-7, basal diet and water with 5, 10 and 15 g added NaCl/l respectively. Food and water or saline solution were offered *ad lib.* throughout the experiment.

Ninety-six male weanling rats aged 30 d were divided into eight similar groups by weight. The mean weight of the groups was 75.5 g (SEM 2.6). One group was killed to determine the initial empty body-weight (EBW) and body composition. The remaining seven groups of twelve rats were each assigned to one of the dietary treatments; they were housed in groups of six rats/cage. Food and water intake were measured at 24 or 48 h intervals throughout the experiment. After 21 d the rats were weighed and killed using chloroform anaesthesia, the gastrointestinal contents and the urine in the bladder were quickly removed and whole EBW was measured. The bodies were frozen until analysed for moisture, fat and fat-free DM contents.

Body composition analyses were done according to the methods for meat and meat products of the Association of Official Agricultural Chemists (1960) modified to treat whole, empty rats. The modifications have been checked and found to produce satisfactory results. Moisture content was determined by placing the opened bodies backside down on stainless-steel trays and drying them to constant weight in a mechanical convection oven at 105°. After determination of moisture content, the dried bodies were transferred to cotton bags, crushed and extracted in light petroleum (b.p. 60-80°) using a Soxhlet apparatus with approximately fifty changes of solvent during 24 h.

The initial EBW, body fat and fat-free DM of each animal was estimated from regressions of these values *v.* live weight obtained from the group of twelve rats

killed at the beginning of the experiment. The regression lines and the respective  $SE(b)$  of the estimated regression slopes were:

$$\text{EBW } v. \text{ live weight: } Y = 0.920X (\pm 0.051),$$

$$\text{body fat } v. \text{ live weight: } \log Y = 1.72 \log X - 2.49 (\pm 0.090),$$

$$\text{fat-free DM } v. \text{ live weight: } Y = 0.197X + 0.162 (\pm 0.038),$$

where  $Y$  is EBW, body fat, fat-free DM respectively;  $X$  is live weight.

The increase in EBW, body fat and fat-free DM for each animal was the difference between its final value as determined directly and its initial one calculated using the above regression equations.

Energy retention, i.e. the energy content of the tissue stored, was calculated as the heat of combustion of the increase in body fat plus that of fat-free DM. The specific heats of combustion of fat and protein used in the calculation were 39.767 and 23.860 kJ/g respectively (Armsby, 1917), and the average protein content of fat-free DM was assumed to be 855 g/kg, as determined previously in our laboratory.

The analysis of variance of all variables was based on cage means with a total of thirteen degrees of freedom and seven for error.

#### RESULTS

The results are given in Table 1. The food intake of the rats offered NaCl added to the food (treatments 2-4) was significantly reduced ( $P < 0.05$ ). In contrast the food intake of rats offered a similar amount of NaCl in the drinking-water (treatments 5 and 6) was not reduced. The latter consumed more water than the rats offered NaCl in the food. The rats offered a hypertonic solution of NaCl (treatment 7) increased their fluid intake more than threefold compared with that of the controls, but their food intake was reduced to 75% of that of the controls.

Mean increments in EBW, body fat and energy retention indicate a severe reduction with increasing levels of NaCl in the food. Although at the level of 10 g added NaCl/kg (treatment 2) the reduction in the increment of body fat (23%) and in energy retention may be accounted for by the decrease in food intake, which was about 10%, the difference between treatment 2, on the one hand and treatments 3 and 4, on the other, is not accounted for by a reduction in food intake. Whereas the further reduction in food intake was only 3 and 5% for treatments 3 and 4 respectively and statistically non-significant, the reduction in fat deposition was 23 and 34% respectively and statistically significant ( $P < 0.05$ ). In contrast the retention of fat-free DM on the same treatments was only slightly reduced.

The adaptation of food and water intake of the rats ingesting NaCl during the experiment was limited. The ratio, water intake:food intake was relatively stable except on treatment 4, where the ratio increased 30% from the 1st to the 3rd week but was not accompanied by an increase in food intake. Only on treatment 7 did the rats adapt their food intake with time; relative to the control, food intake on this treatment was 68, 73 and 81% during the 1st, 2nd and 3rd weeks of the experiment respectively.

Table 1. Mean intake of food, water and sodium chloride, increase in empty body-weight (EBW), body fat and fat-free dry matter (DM) and energy retention by rats during 21 d of dietary NaCl supplementation

(Mean values for twelve rats/treatment)

No.	Dietary treatment*		Intake			Increase in:			Energy retention (kJ)
	NaCl added		Food† (g)	Water (ml)	NaCl (g)	EBW (g)	Body fat (g)	Fat-free DM (g)	
	In food (g/kg)	In water (g/l)							
1	13	0	365	450	4.75	117.8	16.4	28.0	1224
2	23	0	330	554	7.59	102.1	12.5	25.0	1007
3	33	0	319	632	10.53	96.4	9.5	24.7	882
4	43	0	312	646	13.42	92.1	8.1	24.3	816
5	13	5	355	649	7.86	112.0	14.7	27.6	1147
6	13	10	361	831	13.00	116.0	15.5	27.8	1182
7	13	15	273	1481	25.76	73.9	5.2	19.5	603
SE of treatment differences			9	52		6.8	1.2	1.2	63

\* For details, see p. 196.

† Excluding the NaCl supplement in treatments 2-4.

#### DISCUSSION

The results indicate that growing male rats given a natural diet containing 43 g added NaCl/kg, deposited 50% less fat, 14% less fat-free DM, and retained 33% less energy than controls given 13 g added NaCl/kg. Rats ingesting similar amounts of NaCl by consuming the control diet (13 g NaCl/kg) and drinking hypotonic (5 g NaCl/l) or slightly hypertonic (10 g NaCl/l) saline showed only negligible effects on growth. The addition of 10 g NaCl/kg to the control diet reduced food intake and body fat commensurately, but further addition of NaCl reduced the deposition of body fat and had only a minor effect on food intake and fat-free DM retention. On the other hand, replacing the drinking-water by saline (5 or 10 g NaCl/l) did not reduce food intake and had only a minor effect on fat deposition.

The fact that NaCl offered in solution significantly increased the intake of water suggests that the reduction in fat deposition caused by NaCl in the food was the result of hypodipsia, i.e. failure to drink optimal amounts of water offered *ad lib*. The results obtained when NaCl was added to the food practically duplicated the effects on fat deposition in lambs mentioned previously (Kromann & Meyer, 1966; Kromann & Ray, 1967; Jackson *et al.* 1971).

This study was not concerned with the physiological mechanism responsible for the reduction in performance with diets containing high NaCl levels. However, since increased water intake prevented the reduction in performance, it must be primarily regarded as a disturbance in water-electrolyte balance. An increased level of Na in tissues of rats given diets high in NaCl has been reported (Meyer *et al.* 1950). Gross dehydration was not apparent in this study, but this is not unexpected because dehydration in the rat is uncommon; Babineau & Pagé (1955) found that the water

content of the fat-free body of rats is remarkably constant under a variety of conditions, including food and water deprivation. The mean water content of the fat-free body of the rats in this study, with the seven dietary treatments, ranged from 734.2 to 739.8 g/kg; the differences were not significant.

Sheep and cattle consume large amounts of NaCl under a variety of circumstances. Saline may be the only available source of water (Peirce, 1957). Ruminants graze saltbush (*Atriplex* spp.) which contains large amounts of NaCl (Wilson, 1966). NaCl is added to protein and concentrate supplements on the range in order to limit intake (Meyer, Weir, Ittner & Smith, 1955), and is used in rations of lambs to prevent the formation of urinary calculi (Elam, Ham & Schneider, 1957), although there is a tendency now to replace NaCl with  $\text{NH}_4\text{Cl}$  or  $\text{CaCl}_2$  (Beck, 1974). The inhibitory effect of NaCl on appetite is well documented, as it is commonly used to achieve that end. However, the proposition that NaCl in the food may fail to induce the intake of an amount of water compatible with maximum efficiency of food utilization has not been hitherto supported by evidence.

Sheep double or triple their water intake when offered solutions containing up to 13 g NaCl/l (Potter, 1961) and there are indications that water intake can be increased further by small amounts of magnesium and sulphate in the water (Peirce, 1957, 1959, 1960, 1962, 1963). In cattle also, water intake increases with increasing NaCl concentration (Weeth & Haverland, 1961). When only saline is available for stock, it is generally regarded as undesirable because its NaCl content is usually higher than 10 g/l and ions other than  $\text{Na}^+$  and  $\text{Cl}^-$  may be present and cause damage. In this study, a hypertonic solution of NaCl indeed produced the most severe reduction in food intake and growth; this was probably related to the excessive amount of solution drunk by the rats. However, offering rats a hypotonic solution of NaCl, or both water and a 10 g/l solution of NaCl, has resulted in only an increased intake of water without any harmful effects (Kaunitz, Geller, Slanetz & Johnson, 1960). It is therefore suggested that under circumstances necessitating increased water intake in domestic animals (high incidence of urinary calculi, heat stress, availability of unpalatable water), offering them a hypotonic to isotonic solution of common salt (NaCl) may be the most efficient method of achieving that purpose.

There is a possible human aspect to the relationship between NaCl, water and energy retention. Weight-conscious persons often maintain that the intake of water aggravates overweight. This notion is commonly dismissed as arbitrary. Although water supplies no energy and its adequate intake should be encouraged because of reasons unrelated to overweight, the evidence in this study suggests that under certain conditions water intake may indeed increase appetite and the efficiency of energy retention.

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