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# Effects of Dietary Salt, Fat, and Carbohydrate on Fluid and Salt Consumption of Rats[1] (41901)

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*Abstract.* Studies were conducted to investigate the influence of dietary sodium chloride (NaCl), fat, and carbohydrate on NaCl preferences of rats. Both long-term (48 hr) and short-term (20 min) two-bottle preference tests were used. Rats were fed either a basal salt (1.17%) diet or high salt (8.37 to 8.59%) diets in which the level of sucrose was modified by substitution with either corn oil or cornstarch. Water and NaCl consumption was measured in 48-hr two-bottle preference tests using various concentrations (0.005 to 0.5 *M*) of NaCl paired with water. The results of these studies suggest that changes in fluid consumption were accounted for by an increase in water consumption which was inversely related to the sucrose to starch ratio and directly related to the NaCl content of the diets. A reduction in NaCl consumption was observed only during the short-term (20 min) tests.

An increase in consumption of a sodium chloride (NaCl) solution can be elicited in rats, as well as in other species, by maintaining the animals on a sodium deficient diet (1–3). Adrenalectomized rats also show a marked increase in intake of NaCl solutions (4–6). However, sodium consumption is not totally regulated by a metabolic requirement for sodium; e.g., rats that are in sodium balance consume quantities of sodium salt solutions that are in excess of physiologic need when given a choice between a NaCl solution and water (2, 7). Thus, it is clear that gustatory factors contribute to the amount of sodium consumed.

The influence of dietary constituents on the regulation of sodium intake has not been well characterized. Smith-Barbaro *et al.* (8) demonstrated that rats maintained on high salt diets containing 20% saturated or unsaturated fat exhibited a decrease in preference (defined as milliliters saline consumed/total milliliters fluid consumed) for either 0.15 or 0.30 *M* NaCl, when given a choice between a NaCl solution and deionized water, compared with rats maintained on 5% fat, high salt diets. These investigators suggested that the changes in salt acceptability may be attributed to dietary postingestional feedback mechanisms that are dependent on the level of fat in the diet, rather than to the accompanying changes in blood pressure (9). However, the level of fat was adjusted at the expense of sucrose to obtain isocaloric diets and it is not clear whether the observed changes in preference were due to alterations in the level of dietary fat or sucrose.

The purpose of the present studies was to extend earlier observations that dietary constituents, in addition to NaCl, contribute to changes in sodium consumption, and to examine these dietary effects in more detail. Specifically, the goals of the present studies were (i) to investigate the effects of dietary fat, carbohydrate, and NaCl on long-term (48 hr) NaCl and water consumption; and (ii) to investigate the effect of dietary constituents on short-term (20 min) NaCl and water consumption in an attempt to separate postabsorptive from stimulus-derived factors. The results indicate that the changes in fluid consumption observed during the long-term tests were due predominantly to an increase in water intake which was directly related to the level of dietary NaCl and inversely related to the sucrose to starch ratio of the diet. In addition, analysis of the short-term tests revealed

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a decrease in the amount of NaCl consumed and it appeared that this reduction was dependent on the level of dietary NaCl.

**Methods.** *Experiment 1.* Forty-two-day-old male Sprague–Dawley rats (Harlan Sprague–Dawley, Madison, Wisc.) were housed individually in hanging stainless-steel cages with a 12-hr light–dark cycle. Rats were randomly divided into six groups (12 rats/group) and were adapted to a 5% fat, basal salt (1.17% NaCl, BS) diet for 2 weeks before each group was assigned at the beginning of the third week of the study to one of the six dietary regimens described in Table I. Rats adjust their food consumption to maintain caloric intake, regardless of the caloric density of the diet (11). Diets were formulated according to Newberne *et al.* (11) to ensure that all animals were consuming similar quantities of caloric as well as noncaloric dietary constituents (Table I). The 5% fat, HS–low sucrose diet was designed to investigate the effects of low levels of sucrose in a HS diet without adjustments in fat content. The 5% fat, HS–low starch diet was used to examine the effects of raising the level of sucrose at the expense of starch. Lastly, the 20% fat, HS–low starch diet was designed to separate the effects of high levels of fat from the effects of lowering both the sucrose and starch content of the diets. All constituents used to prepare the diets were obtained from ICN Nutritional Biochemicals (Plainview, N.Y.). Each group of animals was fed the assigned experimental diet for a period of 8 weeks (Weeks 3–10). Food and water were provided *ad libitum.* Water was presented in two, glass graduated drinking tubes similar to those described by Richter (12). Throughout the entire study, body weights of all rats from each dietary group were recorded at the end of each week.

During the initial period when all animals were receiving the 5% fat, BS diet, the animals were tested for NaCl preference using 0.1 *M* NaCl paired with deionized water. Consumption of water and saline was measured by means of two-bottle, 48-hr preference tests as previously described (8). Experimental diets were initiated and after 1 week (beginning Week 4), all animals from each group were

TABLE I. COMPOSITION OF EXPERIMENTAL DIETS

| Diet constituent | Dietary groups | | | | | |
|---|---|---|---|---|---|---|
| | 5% fat, BS[a] | 5% fat, HS[b] | 20% fat, HS | 5% fat, HS–low sucrose | 5% fat, HS–low starch | 20% fat, HS–low starch |
| Protein (casein) | 20.00 | 18.54 | 22.82 | 18.54 | 18.54 | 22.82 |
| Sucrose | 47.77 | 44.29 | 16.03 | 16.03 | 50.20 | 24.08 |
| Cornstarch | 10.00 | 9.27 | 11.41 | 37.53 | 3.36 | 3.36 |
| Fat (corn oil) | 5.00 | 4.64 | 22.82 | 4.64 | 4.64 | 22.82 |
| Choline chloride | 0.20 | 0.19 | 0.23 | 0.19 | 0.19 | 0.23 |
| Mineral mixture[c] | 4.10 | 3.80 | 4.68 | 3.80 | 3.80 | 4.68 |
| Vitamins[d] | 2.49 | 2.31 | 2.85 | 2.31 | 2.31 | 2.85 |
| Cellulose | 10.00 | 9.27 | 11.41 | 9.27 | 9.27 | 11.41 |
| DL-Methionine | 0.29 | 0.27 | 0.33 | 0.27 | 0.27 | 0.33 |
| NaCl | 0.15 | 7.42 | 7.42 | 7.42 | 7.42 | 7.42 |
| NaCl (total)[e] | 1.17 | 8.37 | 8.59 | 8.37 | 8.37 | 8.59 |
| Sucrose:starch[f] | 4.78 | 4.78 | 1.40 | 0.43 | 14.94 | 7.17 |
| Caloric density (cal/g food) | 3.59 | 3.33 | 4.10 | 3.33 | 3.33 | 4.10 |

[a] BS is basal salt (1.17% NaCl).

[b] HS is high salt (8.37 to 8.59% NaCl).

[c] Rogers and Harper's (10) mineral mix. This mix contributes NaCl in addition to the amount added.

[d] The vitamin mix contains (per kg of diet)—vitamin A concentrate, 19822 IU; Vitamin D concentrate, 2200 IU; $\alpha$-tocopherol, 110 mg; ascorbic acid, 991 mg; inositol, 110 mg; choline chloride, 1.65 g; menadione, 49.5 mg; *p*-aminobenzoic acid, 110 mg; niacin, 99.0 mg; riboflavin, 22 mg; pyridoxine hydrochloride, 22 mg; thiamine hydrochloride, 22 mg; calcium pantothenate, 66 mg; biotin, 0.44 mg; folic acid, 1.98 mg; vitamin $B_{12}$, 0.03 mg.

[e] Computed value of the total NaCl present in the diet; includes NaCl contributed by the mineral mixture.

[f] Computed value of the sucrose to corn starch ratio of the diets.

tested with six different concentrations of NaCl (0.005, 0.05, 0.10, 0.15, 0.30, and 0.5 *M*). Rats were randomly exposed to one of the six concentrations of NaCl in one drinking tube and deionized water in the other. These tests were conducted throughout a 6 week period (Weeks 4–9) with at least 3 days between each 48-hr test. All animals were presented with each NaCl solution only once.

At the beginning of the eleventh week of the study, six of the rats from each group were sacrificed and blood collected to obtain measurements of blood urea nitrogen (BUN), serum electrolytes, and osmolality (described below). The remaining six animals from each dietary group were returned to the 5% fat, BS diet and preference for either 0.15 or 0.30 *M* NaCl was measured. All remaining rats were tested with both concentrations of NaCl paired with deionized water. The preference tests were conducted 1 and 3 weeks (Weeks 12 and 14) after the animals were returned to the 5% fat, BS diet. The amount of water and saline consumed during the 48-hr preference tests was determined by volume differences and fluid consumption/100 g body wt was calculated.

During the weeks in which the animals were sacrificed (Weeks 11 and 15), animals were exsanguinated and trunk blood collected and centrifuged to obtain serum. The heart, kidneys, and adrenal glands from each animal were removed, cleaned of extraneous tissue, inspected for gross pathological changes and weighed. Blood urea nitrogen (BUN) was measured by the Feron reaction whereby urea was condensed with diacetylminoxime in acid medium to form a chromagen (No. 535, Sigma Chemical Co., St. Louis, Mo.). Serum sodium (Na) and potassium (K) concentrations were determined by atomic absorption spectroscopy (Perkin–Elmer Model 14000, Norwalk, Conn.). Serum chloride (Cl) concentration was assessed by a mercurimetric titration using diphenylcarbazone as indicator (No. 830-T, Sigma Chemical Co.). Serum osmolality was determined by freezing point depression using a micro-osmometer (Precisions Systems, Inc., Sudbury, Mass.).

Experimental data collected from the entire study were analyzed by analysis of variance (13). Significance of differences between group means for each parameter was determined by the Tukey test with the confidence limit set at 95%.

*Experiment 2.* Forty-eight (42 days old) Sprague–Dawley rats (Harlan Sprague–Dawley) were maintained as before (Experiment 1) except that a 12-hr, reversed light–dark cycle was used. The animals were allowed to adjust to the reversed light–dark cycle for 3 weeks and were subsequently placed on the 5% fat, BS diet for an additional 4 weeks. After this period, the animals were divided into four equal groups and each group (12 rats per group) was fed one of the following experimental diets: 5% fat, BS; 5% fat, HS; 5% fat, HS–low sucrose (Table I), or 5% fat, BS–low sucrose. The composition of the 5% fat, BS–low sucrose diet was identical to the 5% fat, BS diet except that the percentage of sucrose and cornstarch was equivalent to that present in the 5% fat, HS–low sucrose diet. The animals remained on their respective diets for 8 weeks (Weeks 5–12).

All animals were trained to consume fluid in a short-term, two-bottle NaCl preference test during the first 2 weeks of the study, before initiating dietary treatment. Food and water were removed from each cage at the beginning of the dark cycle, on the day of the test. After 1 hr of deprivation, the drinking bottles containing fresh deionized water were positioned on the cages. After 10 min, the positions of the drinking bottles were reversed. After another 10-min period, the drinking bottles were removed, and both food and water were returned to the cages. Rats usually consumed a total of approximately 6 ml of fluid during the 20 min tests. Four training sessions were conducted in this fashion over the first week of the study.

Beginning the second week of the study, NaCl preference tests were conducted before initiation of dietary treatment. The rats were randomly exposed to one of two concentrations of NaCl (0.05 and 0.10 *M*) in one drinking bottle and deionized water in the other. All rats were tested three times (Weeks 2–4) with both concentrations of NaCl. These concentrations of NaCl were chosen on the basis of the results obtained from Experiment 1 where the most dramatic differences in fluid consumption among the dietary groups were observed. During the course of dietary treatment, each animal was exposed to each con-

centration of NaCl only once during a test week and was tested every week throughout the study. The amount of each fluid consumed during the two, 10 min periods of the 20-min test was determined by bottle weight differences and fluid consumed per 100 g body wt was determined.

Two-way analysis of variance (13) was used to determine the effects of both dietary NaCl and the sucrose to starch ratio on the short-term preferences for 0.05 and 0.10 *M* NaCl. Significance was set at the 95% confidence level.

**Results.** *Experiment 1.* The body weights of rats were measured weekly except during Week 10 (Fig. 1). No significant differences in body weight were observed during the equilibration period (Weeks 1 and 2) when all rats were fed the 5% fat, BS diet (Panel A). Significantly lower body weights were observed for the animals maintained on the 5% fat, HS diet during weeks 4, 5, 6, 7, and 9, relative to the animals maintained on the 5% fat, BS group (Panel B). Significantly lower body weights were also observed for the rats receiving the 20% fat, HS diet at Weeks 7–9 and for the rats receiving the 5% fat, HS–low sucrose diet at Weeks 3 and 8. In addition, significantly lower body weights were observed for rats on the 5% fat, HS–low starch diet at Weeks 3–5 and 7–9. No significant differences were observed in body weights when the animals from each dietary regimen were returned to the 5% fat, BS diet (Panel C).

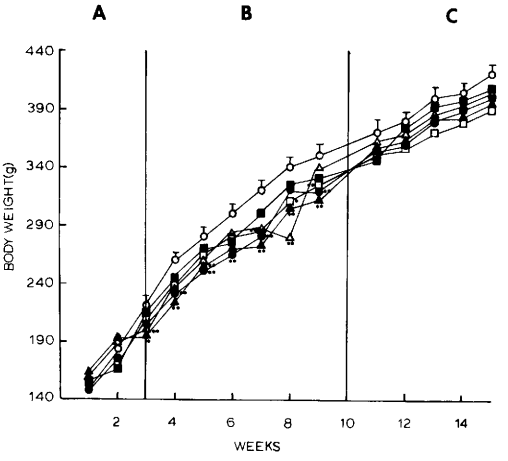


FIG. 1. Effect of varying diet on body weight. One SEM is indicated at each mean value for only the 5% fat, BS group for the sake of clarity. (A) All animals in each group were maintained on the 5% fat, BS diet ($N$ = 12 for each group). (B) Animals were maintained on their respective diets ($N$ = 12 for each group). (C) All animals were returned to the 5% fat, BS diet ($N$ = 6). Significance at $P < 0.05$ level designated as * and at $P < 0.01$ level as **. ○, 5% fat, BS; ●, 5% fat, HS; □, 20% fat, HS; △, 5% fat, HS–low sucrose; ▲, 5% fat, HS–low starch; and ■, 20% fat, HS–low starch.

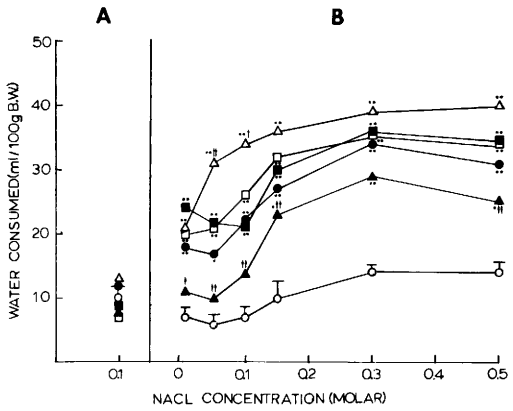


FIG. 2. Effect of diet on water consumed during the long-term NaCl preference tests. One SEM is indicated only at each mean value for the 5% fat, BS group for the sake of clarity. (A) All groups ($N$ = 12 per group) were maintained on the 5% fat, BS diet. (B) Animals were grouped ($N$ = 12) and maintained on their respective diets. Significance: different from the 5% fat, BS group, *$P < 0.05$ and **$P < 0.01$; different from the 5% fat, HS group, †$P < 0.05$ and ††$P < 0.01$; different from the 5% fat, HS–low sucrose group, ‡$P < 0.05$ and ‡‡$P < 0.01$. ○, 5% fat, BS; ●, 5% fat, HS; □, 20% fat, HS; △, 5% fat, HS–low sucrose; ▲, 5% fat, HS–low starch; and ■, 20% fat, HS–low starch.

Figure 2 shows the effect of the various dietary treatments on water consumption during the long-term preference tests. While receiving the 5% fat, BS diet (Panel A) no significant differences in water consumption were apparent during the 0.10 *M* NaCl preference test among all dietary groups. Animals fed the HS diets (except the rats maintained on the 5% fat, HS–low starch diet) drank significantly more water ($P < 0.01$) than the 5% fat, BS dietary group at each concentration of NaCl tested. Significant increases in water intake by the 5% fat, HS–low starch group, relative to the 5% fat, BS group were observed during the tests employing the 0.15 *M* ($P < 0.05$), 0.3 *M* ($P < 0.01$), and 0.5 *M* ($P < 0.05$) NaCl solutions. The rats fed the 5% fat, HS–low sucrose diet consumed significantly more wa-

ter than the 5% fat, HS dietary group during the tests employing 0.05 $M$ ($P < 0.01$) and 0.10 $M$ ($P < 0.05$) NaCl. The 5% fat, HS–low starch group consumed significantly less water than the 5% fat, HS–low sucrose dietary group during the tests using 0.005 $M$ ($P < 0.05$), 0.05 to 0.15 $M$ ($P < 0.01$), and 0.5 $M$ NaCl ($P < 0.01$). By employing orthogonal component analysis (14) for all HS groups, it was determined that the amount of water ingested during the long-term tests using 0.05 $M$ ($F = 19.86$, $P < 0.01$), 0.10 $M$ ($F = 21.94$, $P < 0.01$), and 0.15 $M$ ($F = 10.71$, $P < 0.01$) NaCl was inversely correlated with the sucrose to starch ratio of the diets. Subsequent linear regression analysis, demonstrated significant inverse relationships between the sucrose to starch ratio of the HS diets and water consumed during the 0.05 $M$ ($Y = 26.24 - 1.08X$; $N = 5$, $r = 0.85$, $P < 0.05$), 0.1 $M$ ($Y = 30.18 - 1.16X$; $N = 5$, $r = 0.93$, $P < 0.05$), and the 0.15 $M$ ($Y = 34.15 - 0.75X$; $N = 5$, $r = 0.89$, $P < 0.05$) NaCl preference tests. The amount of NaCl consumed was not statistically different among the dietary groups during any of the long-term preference tests conducted (Fig. 3).

There were no significant differences in water consumption (during preference tests employing 0.15 and 0.30 $M$ NaCl) among any of the groups 1 and 3 weeks after returning the animals to the 5% fat, BS diet, relative to animals that had been maintained on the 5% fat, BS diet throughout the study. No significant differences in consumption of the 0.15 $M$ NaCl solution by any of the dietary groups were detectable after returning the animals to the 5% fat, BS diet. A significant reduction ($P < 0.05$) in the consumption of the 0.3 $M$ NaCl solution after 1 week on the 5% fat, BS diet was observed, however, in the 5% fat, HS–low sucrose (40 mg NaCl consumed/100 g body wt) and the 5% fat, HS–low starch (37 mg NaCl consumed/100 g body wt) group relative to the 5% fat, BS group (155 mg NaCl consumed/100 g body wt). There were no significant differences in consumption of the 0.3 $M$ NaCl solution by any of the groups after 3 weeks on the 5% fat, BS diet.

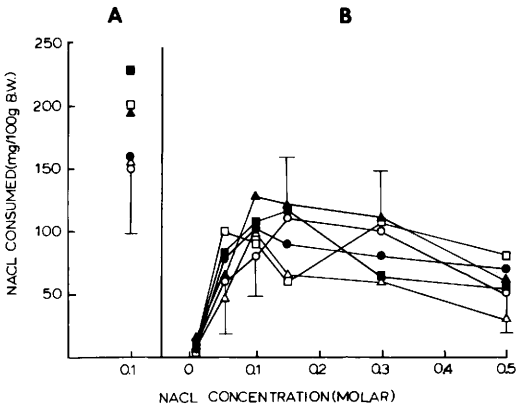


FIG. 3. Effect of diet on NaCl consumed during the long-term NaCl preference tests. One SEM is indicated only at each mean value for the 5% fat, BS group for the sake of clarity. (A) All groups ($N = 12$ per group) were maintained on the 5% fat, BS diet. (B) Animals were divided into groups ($N = 12$) and maintained on their respective diets. ○, 5% fat, BS; ●, 5% fat, HS; □, 20% fat, HS; △, 5% fat, HS–low sucrose; ▲, 5% fat, HS–low starch; and ■, 20% fat, HS–low starch.

Although significant differences in body weight occurred when the animals had been maintained on the various experimental diets for this period of time, no significant differences were observed in organ weights, BUN, serum Na, K, CL concentrations, or serum osmolality after 8 weeks of dietary treatment (Table II). It is worth noting that the body weight differences were reversible because when the rats were returned to the 5% fat, BS diet no significant differences were observed in any of the parameters measured, including body weight.

*Experiment 2.* Mean water consumption of the 5% fat, BS–low sucrose group, during the 0.05 $M$ short-term tests conducted while these animals were on the control diet (Table III, see column A) was significantly greater ($P < 0.05$) than that of the 5% fat, BS group. However, no significant differences in water consumption of any of the dietary groups were detectable during Test 1 which was conducted after the animals had been on their respective diets for 3 days (during Week 5). A significant interaction ($P < 0.05$) between the effects of dietary NaCl and the sucrose to starch ratio of the diets on mean water consumption during the 0.05 $M$ NaCl preference tests was observed during the six short-term tests (mean value of tests 2–7).

No significant differences in saline consumption (mg/100 g body wt) during the 0.05 $M$ NaCl short-term tests were observed either

TABLE II. EFFECT OF VARYING DIET ON BODY WEIGHT, ORGAN WEIGHTS, SERUM ELECTROLYTES, AND OSMOLALITY

| | Dietary group | | | | | |
|---|---|---|---|---|---|---|
| | 5% Fat, BS ($N$ = 5) | 5% Fat, HS ($N$ = 6) | 20% fat, HS ($N$ = 5) | 5% Fat, HS–low sucrose ($N$ = 6) | 5% Fat, HS–low starch ($N$ = 6) | 20% Fat, HS–low starch ($N$ = 6) |
| Body weight (g) | 362 ± 27[a] | 306 ± 22[b] | 314 ± 15[b] | 324 ± 23 | 309 ± 20[b] | 323 ± 36 |
| Organ weight (mg/100 g body wt) | | | | | | |
| Heart | 314 ± 16 | 329 ± 14 | 306 ± 23 | 312 ± 24 | 321 ± 25 | 304 ± 6 |
| Kidney (combined) | 670 ± 43 | 711 ± 54 | 727 ± 28 | 714 ± 56 | 668 ± 39 | 699 ± 54 |
| Adrenal (combined) | 11 ± 1 | 12 ± 1 | 14 ± 1 | 12 ± 1 | 13 ± 1 | 13 ± 1 |
| BUN (mg%) | 12 ± 2 | 18 ± 3 | 20 ± 5 | 23 ± 4 | 17 ± 9 | 21 ± 4 |
| Serum analysis | | | | | | |
| Osmolality (mosm/kg) | 293 ± 14 | 298 ± 16 | 293 ± 10 | 297 ± 8 | 294 ± 12 | 286 ± 7 |
| Na (meq/liter) | 155 ± 8 | 165 ± 10 | 157 ± 2 | 157 ± 4 | 159 ± 10 | 156 ± 9 |
| K (meq/liter) | 4.8 ± .7 | 5.0 ± .7 | 5.2 ± .6 | 4.6 ± .4 | 4.9 ± .5 | 4.7 ± .6 |
| Cl (meq/liter) | 105 ± 4 | 105 ± 16 | 108 ± 10 | 111 ± 9 | 106 ± 11 | 109 ± 7 |

[a] Mean ± SEM for animals sacrificed during Week 10.
[b] Significantly different ($P < 0.05$) from animals maintained on the 5% fat, BS diet.

when all the animals were receiving the 5% fat, BS diet or during Test 1 when the animals had received their respective diets for 3 days (Table IV). However, a significant effect of dietary NaCl on mean NaCl consumption was demonstrated in the three tests conducted during Weeks 6–8 ($P < 0.05$) and over all six tests conducted during Weeks 6–12 ($P < 0.05$). No significant changes in either water or saline consumption occurred during any of the 0.10 $M$ NaCl short-term preference tests.

**Discussion.** Very little is known concerning the effects of diet composition on sodium consumption. Smith-Barbaro *et al.* (9) demonstrated that dietary constituents may influence preferences of rats for NaCl solutions relative to water. The results of the present studies indicate that the alterations in NaCl

TABLE III. EFFECT OF DIET ON WATER CONSUMED DURING SHORT-TERM PREFERENCE TESTS: 0.05 $M$ NaCl VERSUS DEIONIZED WATER

| | Water consumed (ml/100 g body wt) | | | | |
|---|---|---|---|---|---|
| | | B[b] | | | |
| Dietary group | A[a] | Test 1 | Tests 2–4 (mean value of three tests) | Tests 5–7 (mean value of three tests) | Tests 2–7 (mean value of six tests) |
| 5% fat, BS | 0.52 ± 0.04 | 0.41 ± 0.07 | 0.47 ± 0.04 | 0.47 ± 0.06 | 0.45 ± 0.04 |
| 5% fat, BS–low sucrose | 0.70 ± 0.04[c] | 0.48 ± 0.03 | 0.54 ± 0.03 | 0.61 ± 0.07 ($N$ = 11) | 0.58 ± 0.04 |
| 5% fat, HS | 0.66 ± 0.06 | 0.39 ± 0.05 | 0.57 ± 0.06 | 0.65 ± 0.09 | 0.61 ± 0.07 |
| 5% fat, HS–low sucrose | 0.53 ± 0.02 | 0.39 ± 0.05 | 0.48 ± 0.03 | 0.61 ± 0.07 ($N$ = 11) | 0.54 ± 0.03 |
| | | | ANOVA analysis | | |
| | Salt | ns | ns | ns | ns |
| | Sucrose:starch | ns | ns | ns | ns |
| | Interaction | ns | ns | ns | $P < 0.05$ |

[a] Values represent means ± SEM of three short-term preference tests conducted while all animals were maintained on the 5% fat, BS diet.
[b] Means ± SEM for 12 animals, unless otherwise stated in parentheses; values obtained when animals were grouped and maintained on their respective diets.
[c] Significantly different ($P < 0.05$) from the 5% fat, BS group.

TABLE IV. EFFECT OF DIET ON NaCl CONSUMED DURING SHORT-TERM PREFERENCE TESTS: 0.05 *M* NaCl VERSUS DEIONIZED WATER

| | NaCl consumed (mg/100 g body wt) | | | | |
|---|---|---|---|---|---|
| | | B[b] | | | |
| Dietary group | A[a] | Test 1 | Tests 2–4 (mean value of three tests) | Tests 5–7 (mean value of three tests) | Tests 2–7 (mean value of six tests) |
| 5% fat, BS | 2.74 ± 0.21 | 2.23 ± 0.34 | 2.41 ± 0.29 | 2.18 ± 0.21 | 2.31 ± 0.22 |
| 5% fat, BS–low sucrose | 3.03 ± 0.29 | 2.30 ± 0.28 | 2.47 ± 0.30 | 2.26 ± 0.29 ($N$ = 11) | 2.45 ± 0.28 |
| 5% fat, HS | 3.21 ± 0.34 | 2.23 ± 0.30 | 1.92 ± 0.24 | 2.13 ± 0.25 | 2.03 ± 0.19 |
| 5% fat, HS–low sucrose | 3.04 ± 0.24 | 2.24 ± 0.29 | 1.89 ± 0.11 | 1.69 ± 0.26 ($N$ = 11) | 1.82 ± 0.15 |
| | | | ANOVA analysis | | |
| | Salt | ns | $P < 0.05$ | ns | $P < 0.05$ |
| | Sucrose:starch | ns | ns | ns | ns |
| | Interaction | ns | ns | ns | ns |

[a] Values represent means ± SEM of three short term preference tests conducted while all animals were maintained on the 5% fat, BS diet.

[b] Mean values ± SEM for 12 animals, unless stated in parentheses; obtained when animals were grouped and maintained on their respective diets.

preferences reported by Smith-Barbaro *et al.* (8) were most likely related to the level of dietary sucrose rather than the level of dietary fat. In addition, the reduction in NaCl preferences observed in the present studies was not related to changes in saline consumption but rather to an increase in water consumption. These conclusions are supported by the dramatic increase in water consumption that was observed when the level of dietary sucrose was reduced (5% fat, HS–low sucrose diet) to the level present in the 20% fat, HS diet. The finding that there were no differences in water ingestion among the 20% fat, HS, the 20% fat, HS–low starch, and 5% fat, HS groups (during any of the preference tests) suggests that the amount of water consumed by the two high-fat groups was primarily the result of high levels of dietary NaCl.

The fact that water consumption by the 5% fat, HS–low sucrose group during the 0.05 and 0.1 *M* NaCl tests was significantly greater than that of the 5% fat, HS group suggests that the sucrose to starch ratio is an important factor in the regulation of water intake during preference tests in which lower concentrations of NaCl are employed. The demonstration that the amount of water consumed by the HS dietary groups was inversely correlated with the sucrose to starch ratio of the diet supports this hypothesis. The dramatic differences in water intake between the 5% fat, HS–low starch group (approaching the level observed in the 5% fat, BS group during some of the tests) and the 5% fat, HS–low sucrose group during the tests employing 0.005 to 0.15 *M* NaCl is consistent with these findings. The similar increases in water intake among the HS dietary groups when tested with 0.3 and 0.5 *M* NaCl paired with water suggest that dietary NaCl was the predominant factor rather than the sucrose to starch ratio when higher concentrations of NaCl were used. Thus, it appears that both the sucrose to starch ratio and the NaCl content of the diet are important determinants of water consumption during tests employing lower concentrations (0.05 to 0.15 *M*) of NaCl whereas dietary NaCl is the deciding factor for increased water consumption during the tests utilizing higher concentrations of NaCl.

Dietary NaCl induces thirst and access to water for rats maintained on HS diets is essential for elimination of excess Na (15, 16). The increase in water intake observed during the long-term tests in the present studies was partially a consequence of the increased dietary NaCl levels. The induction of thirst was presumably elicited by undetected transient extracellular osmolality changes. The finding

that serum osmolality, and Na, K, and Cl concentrations were within normal limits and BUN was similar among all dietary groups suggests that the regulation of extracellular fluid and electrolyte balance were operative in animals maintained on the HS diets. In addition, the heart, adrenal, and kidney weights were similar among all dietary groups suggesting that no compensatory enlargements of these organs occurred in response to 8 weeks of HS intake.

The high levels of dietary NaCl may account for some of the differences in body weights observed among the dietary groups (Fig. 1). In this regard, Drori (17) observed that the food intake of rats fed NaCl adulterated food (10, 20, and 30 g NaCl/kg diet) was significantly reduced over a 21-day period. However, in the present studies, significant reductions in body weight were not found in all HS dietary groups, indicating that dietary components other than NaCl may be involved.

The stimulus for elevated water consumption that occurred in groups fed lower levels of dietary sucrose (but higher dietary starch) may be related to the relative rates of hydrolysis and subsequent absorption of the two carbohydrates from the gastrointestinal tract. Normally, the osmotic pressure of the chyme entering the duodenum from the stomach is adjusted to isotonicity by the movement of water between the blood and the gastrointestinal tract (18). Thus, isotonicity of the chyme is maintained throughout the small intestine. Although starch initially exerts relatively little osmotic pressure, the liberation of many particles by hydrolysis leads to a much greater osmotic pressure. Thus water moves from the blood to the intestinal lumen to preserve isotonicity of the intestinal contents. As the hydrolytic products (glucose) are absorbed, the isotonicity of the luminal contents is preserved by the concomitant absorption of water. Therefore, the osmotic pressure exerted by the hydrolytic products of sucrose may be more transient than that exerted by the products of starch hydrolysis. This hypothesis is supported by the report of Cohen and Teitelbaum (19) which demonstrated that the absorption of glucose from the gastrointestinal tract following sucrose feeding is much more rapid compared to that of starch. It has also been reported that rats fed diets containing dextrin consumed considerably more water than rats fed diets containing sucrose (20).

The dietary effects on water consumption during the long-term tests were completely reversed after 1 week on the low fat, BS diet and supports our hypothesis that changes in fluid consumption arise from postingestional factors. Differences in 0.3 *M* NaCl consumption by the 5% fat, HS–low sucrose and –low starch dietary groups were observed after all animals were fed the 5% fat, BS diet for 1 week but completely disappeared after 3 weeks. Studies investigating possible mechanisms underlying these shifts in saline consumption are currently in progress.

The results obtained from Experiment 2 suggest that diet-induced changes in fluid consumption are detectable over a short-term test. During the preference tests employing the 0.05 *M* NaCl solution, alterations in the amount of water ingested as well as a reduction in the amount of NaCl consumed occurred. Although no differences in water intake were detected during Test 1 (when the animals had been fed their respective diets for 3 days), there were differences when all groups were on the 5% fat, BS diet at the beginning of the study (Table III). Thus, interpretation of the water consumption data during the 0.05 *M* NaCl test is not possible.

The decrease in the amount of NaCl consumed during the short-term tests appeared to be related to the high levels of NaCl in the diet (Table IV). It is possible that the dietary NaCl-induced changes in NaCl consumption were not totally dependent upon postingestional events since the short-term tests were designed to minimize postingestional effects of the stimulus. Previous studies suggest that changes in preference behavior and fluid intake may be related to changes in gustatory acuity and that saliva may play a role in these changes (21, 22).

The results of the present studies provide evidence that postingestional feedback derived from dietary constituents influence the amount of fluid consumed by rats during NaCl preference tests. These studies demonstrate that the increase in water ingested is inversely related to the sucrose to starch ratio of the diet and directly related to the level of dietary

NaCl. The mechanism(s) responsible for the shifts in fluid consumption during long- and short-term testing require further study.


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