Test of the hypothesis that eating high-salt food leads to obesity by stimulating sugar-solution consumption

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Running head: Salt, sucrose, and obesity

Abstract:

High salt intake has been linked to obesity in humans and rodents, although the direction of causation and underlying mechanisms are unclear. One hypothesis suggests that consuming salt stimulates thirst, which is assuaged by drinking sugar-sweetened beverages, leading to excess energy intake and thus obesity. We attempted to test this hypothesis using a mouse model. Adult male C57BL/6J mice ate semisynthetic diet with either low (0.56 g Na⁺/kg diet) or high (5.62 g Na⁺/kg diet) salt content for 8 weeks. Half the mice fed each diet could drink water; the other half could drink both water and a 16% sucrose solution. Mice fed the high-salt diet with water to drink ingested ~25% more water than did those fed the low-salt diet with water to drink, demonstrating that salt stimulated thirst. However, there was no influence of dietary salt on water or sucrose intake in the groups with access to both water and sucrose. This was probably because sucrose intakes were near-maximal in both groups; mice apparently do not require salt to encourage them to drink sucrose. Dietary salt level had no effect on body weight. Relative to mice that drank only water, those that drank sucrose had a net increase in energy intake but, surprisingly, gained less body weight, perhaps because they consumed too little protein to thrive. In sum, our results do not support the hypothesis that salt increases sugar-sweetened beverage consumption, leading to obesity; however, the simple mouse model used here may not provide a competent test of this hypothesis.

Highlights:

- Mice ate low- or high-salt diets and drank water or water and sucrose
- High-salt diet increased water intake by ~25%
- Dietary salt did not influence sucrose intake
- High dietary salt did not increase body weight
- Sucrose-drinking mice increased energy intake, but gained less weight

Keywords: Salt diet; Sucrose intake; Sugar-sweetened beverages; Obesity; Taste

1. Introduction

There is strong epidemiological evidence for a positive association between salt consumption and body weight [1–6]. Salt is an ingredient of many high-fat and high-energy dense foods, so salt consumption may merely be incidental to the consumption of other ingredients that render individuals obese [7–10]. However, some investigators have argued that consuming salt is a causative contributor to the obesity epidemic, although a confirmed mechanistic basis for this is lacking. Hypotheses include salt enhancing adipocyte glucose uptake [11], modulating leptin [12,13], or suppressing the activity of the renin-angiotensin-aldosterone system, which modulates fat stores [14–17]. It has also been argued that salty foods are addictive, leading to overconsumption and obesity [18].

One hypothesis with popular appeal links salt consumption to sugar-sweetened soda consumption [19]. The argument is that salt consumption heightens thirst, which is assuaged by drinking sugar-sweetened beverages. This increases energy intake and thus leads to excess body weight. Consistent with this hypothesis, per capita salt sales correspond with carbonated soft drink consumption (see [19]). Moreover, large epidemiological studies report that intake of sodium is positively associated with intake of sugar-sweetened beverages by children [20–22]. However, this evidence is entirely correlational. To conduct a causative experiment with humans would be expensive and might raise ethical concerns. There is nothing in the hypothesis to preclude the same underlying mechanisms from being present in rodents. Consequently, here we investigated whether mice fed a high-salt diet increased their (i) water intake (i.e., thirst) (ii) sugar solution intake, and (iii) body weight.

2. Methods

2.1. Subjects, maintenance, diets, and measurements

Subjects were 48 male C57BL/6J mice, aged 13 weeks (at the beginning of the experiment), purchased from The Jackson Laboratory (Bar Harbor, Maine). The mice were individually-housed in plastic “tub” cages (26.5 cm × 17 cm × 12 cm) with stainless steel grid lids, and wood shavings for bedding. The vivarium was maintained at 23°C on a 12:12 h light/dark cycle, with lights off at 1800 h. The mice had been used previously in an experiment in which they were food-deprived for 2 h and then fed in the presence of an innocuous odor for 1 h. They were undisturbed with *ad libitum* access to Teklad 8604 chow and water for 8 days before beginning the experiment described here.

For the 8-week duration of the experiment, the mice were randomly assigned to four groups according to a 2 x 2 design: Low-salt + water, High-salt + water, Low-salt + sucrose, High-salt + sucrose (12 mice per condition). They were fed a modified AIN-76A diet (see Table 1) prepared by Dyets, Inc. (Bethlehem, PA) with either a low-salt concentration of 0.56 g Na⁺/kg diet (Catalog # 104560) or a high-salt concentration of 5.6 g Na⁺/kg diet (Catalog # 104564). Half the mice fed each diet received deionized water to drink *ad libitum* from a plastic bottle with a stainless steel sipper tube. The other half of the mice were provided with a 16% sucrose solution to drink *ad libitum* in addition to deionized water. The sucrose was obtained from Sigma-Aldrich (Catalog # S9378), dissolved in deionized water, and presented to mice in a glass bottle with a stainless steel sipper tube.

Food, water, and sucrose intakes were assessed weekly and used to calculate total energy consumption, based on the sum of energy provided by the diet (3.79 kcal/g) and the sucrose solution (0.64 kcal/mL). Pelleted food was available through the bars of the cage lid. The cage lids and food were weighed weekly (correcting for the weight of the cage lid itself and any spillage) and these values were subtracted from those collected the previous week to derive the total weight of food consumed. Mice were weighed weekly by placing them on a top-loading balance with 0.1g precision. Body composition was assessed at the end of the experiment. To do this, each mouse was carried to an adjacent room housing a Bruker Minispec LF110; the mouse was placed into a plastic restraining tube, and then inserted into the core of the machine for ~90 s while its body composition was assessed by magnetic resonance technology. This protocol of experiments were approved by the IACUC of The Monell Chemical Senses Center. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Statistical Analyses

Weekly measurements were analyzed using repeated measures analyses of variance with factors of diet (low or high- Na^+), sucrose (absent or present), and time (weeks). If the main effects or interactions were significant, the source of the effect was determined with Fisher's LSD post-hoc tests. Statistical significance was determined at the 95% confidence level ($\alpha \leq 0.05$). All statistical analyses were performed using Statistica (Version 13).

3. Results

3.1. Dietary salt increases water but not sucrose intake

Over the 8-week experiment, mice fed the high-salt (5.6 g Na⁺/kg) diet consistently drank more water than did those fed the low-salt diet (0.56 g Na⁺/kg; Table 2, Figure 1; results of statistical analyses are presented in Table 3). As expected, all mice given 16% sucrose solution drank it copiously and they displayed a strong preference for sucrose over water, drinking ~20-times more sucrose than water. There was no significant effect of dietary salt on sucrose intake. Towards the end of the experiment, the high-salt diet + sucrose-drinking mice tended to drink more sucrose than did the low-salt diet + sucrose-drinking mice, but this was not significant (Figure 1).

3.2 High dietary salt fails to increase body weight

Neither low- nor high-salt diets had an impact on body weight or cumulative body weight gain during the 8-week experiment (Table 2 and 3, Figure 1).

3.3 Sucrose option increases energy intake

Both groups of mice given sucrose to drink increased overall daily energy consumption (Table 2 and 3, Figure 1). They ate significantly less food than did the groups with only water to drink but they nevertheless consumed significantly more energy, demonstrating a consistent decrease in energy intake from food and a consistent increase in energy intake from sucrose over the length of the experiment (Figure 1).

3.4 Sucrose slows weight gain and decreases lean mass

Sucrose solution slowed body weight gain during the 8-week experiment. Both groups of mice receiving sucrose solution gained significantly less weight than did the mice that had only water to drink (Table 2 and 3). Mice that drank sucrose had less lean mass in comparison with those that did not have sucrose. Mice fed the high-salt diet had a higher body water content than did those fed the low-salt diet (Table 4).

4. Discussion

We attempted to test the hypothesis that consuming a high-salt diet leads to increased thirst, which stimulates sugar solution intake, leading to excess energy intake and thus obesity. This complex hypothesis involves three sub-hypotheses: consumption of a high-salt diet must increase (i) thirst, (ii) sucrose consumption, and (iii) body weight gain (only when sucrose is available). In support of the first sub-hypothesis, we found that mice fed a high-salt diet drank ~25% more water than did those fed a low-salt diet. However, contrary to the second sub-hypothesis, the level of dietary salt had no effect on consumption of a 16% sucrose solution. The mice drank large volumes of sucrose whether their diet was high or low in salt. The simplest interpretation of this finding is that the ingestion of sucrose solution is driven exclusively by its good taste, and the hydration provided by drinking sucrose solution overwhelms any effects of salt-induced thirst.

Contrary to the third sub-hypothesis, consuming a high-salt diet had no effect on body weight. This was true whether or not the mice had access to sucrose solution. Although high-salt diets have been suggested to be obesogenic (e.g., [3,8,23] see

introduction), our results are consistent with many other studies that show high-salt diets do not increase body weight or adiposity (e.g., [24–27]).

Sucrose-drinking mice gained *less* body weight than did mice with only water to drink, irrespective of the level of dietary salt. This finding was unexpected because it contrasts with many studies showing that rodents with a sucrose solution option increase body weight and body fat deposition [28–32]. We note that these studies typically use concentrations of sucrose much higher than the 16% we used, which we chose as a compromise between the level in human soft drinks (~10%) yet providing concentrated energy. We are also aware of a finding in rats similar to ours [29]. An explanation offered in that report was that drinking sucrose activates brown fat metabolism, leading to heightened energy loss. However, we think a more likely explanation, at least for our results, is that the mice decreased their solid food intake to compensate for the excess energy provided by sucrose, and this led to protein malnourishment. This is consistent with the retarded weight gain and reduced lean mass of the mice given sucrose, and it perhaps can also account for the unexplained deaths of two mice that had sucrose to drink. There are two arguments to be made against this explanation. First, if the mice were really protein-deficient they could have assuaged their deficiency by eating more food. This is an easy objection to counter because there are many examples of rodents failing to satisfy their protein requirements when a more palatable food is available [33–36]. Second, even though mice with sucrose to drink substantially reduced food intake, and thus protein intake (i.e., by ~50%), the diets they had available were fairly high in protein by most standards (i.e., protein comprised 20% of calories) so, at least by values from experiments with rats, it

is surprising that obtaining 10% of calories as protein should fail to support growth. To our knowledge, there are no studies of the protein requirements of mice so the possibility that our mice consumed too little protein to thrive remains speculation.

Our demonstration that mice fed high-salt diet drank consistently more water than did those fed the low-salt diet conforms to the classical narrative of dietary salt inducing thirst [37]. The sodium requirements of the mouse have not been established, so what is considered a “low-“ or “high-“ salt diet is open to question [38,39], particularly when trying to impute relevance for humans. Our low-salt diet (0.56 g Na⁺/kg) contained sodium at a level below what is typically found in standard rodent chow diets (i.e., ~3-4 g/Na⁺/kg; [39–41]), yet not so low that it would stunt growth [42]. The high-salt diet (5.6 g Na⁺/kg) provided exactly 10-times more sodium than did the low-salt diet and so significantly more than the level in typical rodent maintenance chows, which should probably also be considered “high-salt” [43]. To give some human context, we applied the FDA-recommended method to compare drug doses between species, which is based on equating body surface area [44]. Using an estimated energy intake of ~13.5 kcal/day [45] for our mice to convert “dose” to daily consumption, the low-salt diet we used was equivalent to a human “dose” of 0.6 g/day Na⁺, which is ~40% of the ideal limit advocated by the American Heart Association; the high-salt diet provided 10-fold higher sodium, equivalent to ~6.1 g/day Na⁺ for humans, which is well above current recommendations.

If the salt->sugar intake->obesity hypothesis is incorrect then what is responsible for the positive association between salt consumption and body weight observed in epidemiological studies? One likely possibility is that the obese eat more food and thus

incidentally consume more salt. Another is that foods that are likely to promote obesity (i.e., high-fat and high-energy foods) are incidentally high in salt [4,46].

In summary, our findings do not support the hypothesis that salt causes obesity by increasing the ingestion of sugar-sweetened beverages. We concede that different results may have been obtained had we tested other mouse strains, other diets, or other sugar solutions. However, our results raise an important issue. Mice apparently do not need the motivation imparted by salt-induced thirst to coax them to drink large volumes of a strongly preferred sugar solution. One wonders if the same is not also true for humans.

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References

- [1] F. He, G. MacGregor, A comprehensive review on salt and health and current experience of worldwide salt reduction programmes | *Journal of Human Hypertension*, *J. Hum. Hypertens.* 23 (2009) 363–384.
- [2] S.C. Larsen, L. Ångquist, T.I.A. Sørensen, B.L. Heitmann, 24h urinary sodium excretion and subsequent change in weight, waist circumference and body composition, *PLOS ONE*. 8 (2013) e69689. doi:10.1371/journal.pone.0069689.
- [3] H. Song, Y. Cho, H. Lee, Dietary sodium intake and prevalence of overweight in adults, *Metabolism*. 62 (2013) 703–708. doi:10.1016/j.metabol.2012.11.009.
- [4] C.A. Grimes, D.P. Bolhuis, F.J. He, C.A. Nowson, Dietary sodium intake and overweight and obesity in children and adults: a protocol for a systematic review and meta-analysis, *Syst. Rev.* 5 (2016). doi:10.1186/s13643-015-0175-3.
- [5] S.P. Moosavian, F. Haghighatdoost, P.J. Surkan, L. Azadbakht, Salt and obesity: a systematic review and meta-analysis of observational studies, *Int. J. Food Sci. Nutr.* 68 (2017) 265–277. doi:10.1080/09637486.2016.1239700.
- [6] X. Zhang, J. Wang, J. Li, Y. Yu, Y. Song, A positive association between dietary sodium intake and obesity and central obesity: results from the National Health and Nutrition Examination Survey 1999–2006, *Nutr. Res.* 55 (2018) 33–44. doi:10.1016/j.nutres.2018.04.008.
- [7] L.K. Dahl, L. Silver, R.W. Christine, The role of salt in the fall of blood pressure accompanying reduction in obesity, *N. Engl. J. Med.* 258 (1958) 1186–1192.
- [8] I.S. Hoffmann, L.X. Cubeddu, Salt and the metabolic syndrome, *Nutr. Metab. Cardiovasc. Dis.* 19 (2009) 123–128. doi:10.1016/j.numecd.2008.02.011.
- [9] L. Hulthén, M. Aurell, S. Klingberg, E. Hallenberg, M. Lorentzon, C. Ohlsson, Salt intake in young Swedish men, *Public Health Nutr.* 13 (2010) 601–605. doi:10.1017/S1368980009991431.
- [10] C. Morris, Effect of dietary sodium restriction on overall nutrient intake, *Am. J. Clin. Nutr.* 65 (1997) 687–691.
- [11] M.H. Fonseca-Alaniz, J. Takada, S. Andreotti, T.B.F.D. Campos, A.B. Campaña, C.N. Borges-Silva, F.B. Lima, High sodium intake enhances insulin-stimulated glucose uptake in rat epididymal adipose tissue, *Obesity*. 16 (2008) 1186–1192. doi:10.1038/oby.2008.69.
- [12] M.H. Fonseca-Alaniz, L.C. Brito, C.N. Borges-Silva, J. Takada, S. Andreotti, F.B. Lima, High dietary sodium intake increases white adipose tissue mass and plasma leptin in rats, *Obesity*. 15 (2012) 2200–2208. doi:10.1038/oby.2007.261.
- [13] M.A. Lanaspa, M. Kuwabara, A. Andres-Hernando, N. Li, C. Cicerchi, T. Jensen, D.J. Orlicky, C.A. Roncal-Jimenez, T. Ishimoto, T. Nakagawa, B. Rodriguez-Iturbe, P.S. MacLean, R.J. Johnson, High salt intake causes leptin resistance and obesity in mice by stimulating endogenous fructose production and metabolism, *Med. Sci. (n.d.)* 7.
- [14] A. Saiki, M. Ohira, K. Endo, N. Koide, T. Oyama, T. Murano, H. Watanabe, Y. Miyashita, K. Shirai, Circulating angiotensin II is associated with body fat accumulation and insulin resistance in obese subjects with type 2 diabetes mellitus, *Metabolism*. 58 (2009) 708–713. doi:10.1016/j.metabol.2009.01.013.
- [15] M.L. Mathai, N. Chen, L.C. and R.S. Weisinger, The Role of Angiotensin in Obesity and Metabolic Disease, *Endocr. Metab. Immune Disord. - Drug Targets*. (2011). <http://www.eurekaselect.com/88486/article> (accessed December 17, 2018).

- [16] N.S. Kalupahana, N. Moustaid-Moussa, The renin-angiotensin system: a link between obesity, inflammation and insulin resistance, *Obes. Rev.* 13 (2012) 136–149. doi:10.1111/j.1467-789X.2011.00942.x.
- [17] W.-H. Liao, C. Suendermann, A.E. Steuer, G.P. Lopez, A. Odermatt, N. Faresse, M. Henneberg, W. Langhans, Aldosterone deficiency in mice burdens respiration and accentuates diet-induced hyperinsulinemia and obesity, (2018). doi:10.1172/jci.insight.99015.
- [18] J.A. Cocores, M.S. Gold, The salted food addiction hypothesis may explain overeating and the obesity epidemic, *Med. Hypotheses.* 73 (2009) 892–899. doi:10.1016/j.mehy.2009.06.049.
- [19] H. Karppanen, E. Mervaala, Sodium intake and hypertension, *Prog. Cardiovasc. Dis.* 49 (2006) 59–75. doi:10.1016/j.pcad.2006.07.001.
- [20] C. Grimes, L. Ridell, K. Campbell, C.A. Nowson, Dietary salt intake, sugar-sweetened beverage consumption, and obesity risk, *Pediatrics.* 131 (2013) 14–21.
- [21] C. Grimes, J. Wright, K. Liu, C.A. Nowson, C. Loria, Dietary sodium intake is associated with total fluid and sugar-sweetened beverage consumption in US children and adolescents aged 2-18 y: NHANES 2005-2008, *Am. J. Clin. Nutr.* 98 (2013) 189–196.
- [22] F. He, N. Marrero, G. MacGregor, Salt intake is related to soft drink consumption in children and adolescents, *Hypertension.* 51 (2008) 629–634. doi:10.1161/HYPERTENSIONAHA.107.100990.
- [23] H. Zhu, N.K. Pollock, I. Kotak, B. Gutin, X. Wang, J. Bhagatwala, S. Parikh, G.A. Harshfield, Y. Dong, Dietary Sodium, Adiposity, and Inflammation in Healthy Adolescents, *PEDIATRICS.* 133 (2014) e635–e642. doi:10.1542/peds.2013-1794.
- [24] T. Ogihara, T. Asano, K. Ando, Y. Chiba, N. Sekine, H. Sakoda, M. Anai, Y. Onishi, M. Fujishiro, H. Ono, N. Shojima, K. Inukai, Y. Fukushima, M. Kikuchi, T. Fujita, Insulin Resistance With Enhanced Insulin Signaling in High-Salt Diet-Fed Rats, *Diabetes.* 50 (2001) 573–583. doi:10.2337/diabetes.50.3.573.
- [25] V.C. DeClercq, J.S. Goldsby, D.N. McMurray, R.S. Chapkin, Distinct Adipose Depots from Mice Differentially Respond to a High-Fat, High-Salt Diet, *J. Nutr.* 146 (2016) 1189–1196. doi:10.3945/jn.115.227496.
- [26] K. Kitada, S. Daub, Y. Zhang, J.D. Klein, D. Nakano, T. Pedchenko, L. Lantier, L.M. LaRocque, A. Marton, P. Neubert, A. Schröder, N. Rakova, J. Jantsch, A.E. Dikalova, S.I. Dikalov, D.G. Harrison, D.N. Müller, A. Nishiyama, M. Rauh, R.C. Harris, F.C. Luft, D.H. Wasserman, J.M. Sands, J. Titze, High salt intake reprioritizes osmolyte and energy metabolism for body fluid conservation, *J. Clin. Invest.* 127 (2017) 1944–1959. doi:10.1172/JCI88532.
- [27] D. Pitynski-Miller, M. Ross, M. Schmill, R. Schambow, T. Fuller, F.W. Flynn, D.C. Skinner, A high salt diet inhibits obesity and delays puberty in the female rat, *Int. J. Obes.* 41 (2017) 1685–1692. doi:10.1038/ijo.2017.154.
- [28] R.B. Kanarek, N. Orthen-Gambill, Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats, *J. Nutr.* 112 (1982) 1546–1554. doi:10.1093/jn/112.8.1546.
- [29] S. Rattigan, M.G. Clark, Effect of sucrose solution drinking option on the development of obesity in rats, *J. Nutr.* 114 (1984) 1971–1977. doi:10.1093/jn/114.10.1971.
- [30] T. Kawasaki, A. Kashiwabara, T. Sakai, K. Igarashi, N. Ogata, H. Watanabe, K. Ichiyanagi, T. Yamanouchi, Long-term sucrose-drinking causes increased body weight and glucose

- intolerance in normal male rats, *Br. J. Nutr.* 93 (2005) 613–618.
doi:10.1079/BJN20051407.
- [31] A. Lindqvist, A. Baelemans, C. Erlanson-Albertsson, Effects of sucrose, glucose and fructose on peripheral and central appetite signals, *Regul. Pept.* 150 (2008) 26–32.
doi:10.1016/j.regpep.2008.06.008.
 - [32] R.B. Kanarek, J.R. Aprille, E. Hirsch, L. Gualtiere, C.A. Brown, Sucrose-induced obesity: effect of diet on obesity and brown adipose tissue, *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 253 (1987) R158–R166. doi:10.1152/ajpregu.1987.253.1.R158.
 - [33] E.M. Scott, Self selection of diet I. Selection of purified components, *J. Nutr.* 31 (1946) 397–406. doi:10.1093/jn/31.4.397.
 - [34] E. Scott, E. Quint, Self selection of diet; appetite for protein, *J. Nutrition.* 32 (1946) 293–301.
 - [35] B.G. Galef, A contrarian view of the wisdom of the body as it relates to dietary self-selection., *Psychol. Rev.* 98 (1991) 218–223. doi:10.1037/0033-295X.98.2.218.
 - [36] D. Reed, M. Friedman, M.G. Tordoff, Experience with a macronutrient source influences subsequent macronutrient selection, *Appetite.* (1992) 223–232.
 - [37] R.J. Wood, E.T. Rolls, B.J. Rolls, Physiological mechanisms for thirst in the nonhuman primate, *Am. J. Physiol.-Regul. Integr. Comp. Physiol.* 242 (1982) R423–R428.
doi:10.1152/ajpregu.1982.242.5.R423.
 - [38] A.R. Michell, Physiological Aspects of the Requirement for Sodium in Mammals, *Nutr. Res. Rev.* (1989). doi:10.1079/NRR19890012.
 - [39] National Research Council, *Nutrient Requirements of Laboratory Animals.*, Fourth, National Academies Press, Washington, 1995.
<http://public.eblib.com/choice/publicfullrecord.aspx?p=3377738> (accessed November 28, 2018).
 - [40] L. Bell, L. Hurley, Ultrastructural effects of manganese deficiency in liver, heart, kidney, and pancreas of mice., *Lab Invest.* 29 (1973) 732–736.
 - [41] Teklad rodent diet 8604, (2015). <https://www.envigo.com/resources/data-sheets/8604-datasheet-0915.pdf> (accessed December 17, 2018).
 - [42] W. Beierwaltes, W. Arendshorst, P. Klemmer, Electrolyte and water balance in young spontaneously hypertensive rats. - PubMed - NCBI, *Hypertension.* 4 (1982) 908–915.
 - [43] W. Martus, D. Kim, J.L. Garvin, W.H. Beierwaltes, Commercial rodent diets contain more sodium than rats need, *Am. J. Physiol.-Ren. Physiol.* 288 (2005) F428–F431.
doi:10.1152/ajprenal.00310.2004.
 - [44] S. Reagan-Shaw, M. Nihal, N. Ahmad, Dose translation from animal to human studies revisited., *Faseb.* 22 (2008) 659–661.
 - [45] A.A. Bachmanov, D.R. Reed, G.K. Beauchamp, M.G. Tordoff, Food Intake, Water Intake, and Drinking Spout Side Preference of 28 Mouse Strains, *Behav. Genet.* 32 (2002) 435.
 - [46] L. Cordain, S.B. Eaton, A. Sebastian, N. Mann, S. Lindeberg, B.A. Watkins, J.H. O’Keefe, J. Brand-Miller, Origins and evolution of the Western diet: health implications for the 21st century, *Am. J. Clin. Nutr.* 81 (2005) 341–354. doi:10.1093/ajcn.81.2.341.

Table 1. List of diet ingredients

	Low-salt Diet Dyets #104560	High-salt Diet) Dyets #104564
Casein	200.0	200.0
DL-Methionine	3.0	3.0
Sucrose	500.0	500.0
Cornstarch	150.0	150.0
Corn Oil	50.0	50.0
Microcrystalline Cellulose	48.58	35.71
Mineral Mix #200073 ^a	35.0	35.0
Sodium Chloride (39.34% Na)	1.42	14.29
Vitamin Mix #300050 ^b	10.0	10.0
Choline Bitartrate	2.0	2.0
Total Energy (kcal/kg)	3787.15	3787.15

^aMineral mix Ingredients (g/kg): Calcium phosphate, dibasic 500, sodium chloride 0, potassium citrate, H₂O 220, potassium sulfate 52, magnesium oxide 24, manganous 3.5, ferric citrate. U.S.P. 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, chromium potassium sulfate. H₂O 0.55, sucrose 192.03. ^bVitamin mix ingredients (g/kg): Thiamin HCl 0.6, riboflavin 0.6, pyridoxine HCl 0.7. niacin 3, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B12 (0.1%) 1, vitamin A palmitate (500,000 IU/g) 0.8. vitamin D3 (400,000 IU/g) 0.25, vitamin E acetate (500 IU/g) 10, menadione sodium bisulfite 0.08, sucrose, finely powdered 981.15.

Table 2. Average daily intakes and weight gain

Measure	No Sucrose		Sucrose	
	Low Na ⁺	High Na ⁺	Low Na ⁺	High Na ⁺
Food Intake (kcal/day)	12.0 ± 0.1	12.3 ± 0.2	5.8 ± 0.2***	6.1 ± 0.2***
Sucrose Intake (kcal/day)	-	-	9.0 ± 0.2	9.6 ± 0.3
Energy Intake (kcal/day)	12.0 ± 0.1	12.3 ± 0.2	14.8 ± 0.2***	15.5 ± 0.2***
Water Intake (ml/day)	3.0 ± 0.04	4.3 ± 0.1	0.5 ± 0.04***	0.4 ± 0.04***
Total Fluid Intake (ml/day)	3.0 ± 0.04	4.3 ± 0.1	14.5 ± 0.4***	15.6 ± 0.4***
BW gain (g/day)	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1**	0.4 ± 0.1**

p < 0.01, * p < 0.001 relative to group fed same diet but not given sucrose to drink

Table 3. Results of ANOVA's

	Na ⁺	Sucrose	Na ⁺ x Sucrose	Weeks	Weeks x Na ⁺	Weeks x Sucrose	Weeks x Na ⁺ x Sucrose
Daily Intakes and Weight Gain							
Body Weight	F(1,42) = 0.02	F(1,42) = 7.32**	F(1,42) = 0.02	F(7,294) = 4.03***	F(7,294) = 2.18*	F(7,294) = 0.53	F(7,294) = 1.44
Gain							
Food Intake	F(1,42) = 0.45	F(1,42) = 205***	F(1,42) = 0.00	F(7,294) = 39.8***	F(7,294) = 2.30*	F(7,294) = 8.27***	F(7,294) = 0.27
Water Intake	F(1,42) = 18.8***	F(1,42) = 519***	F(1,42) = 20.9***	F(7,294) = 1.99	F(7,294) = 2.06*	F(7,294) = 2.15*	F(7,294) = 2.62*
Sucrose Intake	F(1,20) = 1.12	-	-	F(7,140) = 18.2***	F(7,140) = 1.19	-	-
Total Fluid	F(1,42) = 4.73*	F(1,42) = 432***	F(1,42) = 0.02	F(7,294) = 19.6***	F(7,294) = 2.11*	F(7,294) = 15.6***	F(7,294) = 0.76
Intake							
Energy Intake	F(1,42) = 2.94	F(1,42) = 69.8***	F(1,42) = 0.92	F(7,294) = 5.76***	F(7,294) = 1.42	F(7,294) = 3.76**	F(7,294) = 1.50
Body Composition							
Body Fat	F(1,42) = 0.31	F(1,42) = 0.71	F(1,42) = 1.04	-	-	-	-
Lean Fat	F(1,42) = 0.40	F(1,42) = 6.59*	F(1,42) = 0.07	-	-	-	-
Fluid	F(1,42) = 5.75*	F(1,42) = 1.99	F(1,42) = 0.05	-	-	-	-

*p < 0.05, **p < 0.01, *** p < 0.001

Table 4. Analysis of mean body composition data

Measure	No Sucrose		Sucrose	
	Low Na ⁺	High Na ⁺	Low Na ⁺	High Na ⁺
Body Fat (g)	6.2 ± 0.6	6.6 ± 0.8	6.4 ± 1.0	5.1 ± 0.6
Lean Fat (g)	29.5 ± 0.5	29.8 ± 0.5	27.2 ± 1.3*	27.9 ± 0.6*
Body fluid (g)	5.7 ± 0.1	6.5 ± 0.1 [†]	5.4 ± 0.3	6.0 ± 0.6 [†]

*p < 0.05 relative to group fed same diet but not given sucrose to drink

[†]p < 0.05 relative to group fed low Na⁺ diet

Figure Legend

Figure 1. Cumulative body weight gain and food, energy, sucrose, water, and total fluid intakes during 8 weeks with low- or high-salt diet to eat and water or water + 16% sucrose solution to drink. Open circles = Low Na⁺ diet + water, open triangles = Low Na⁺ diet + 16% sucrose solution, filled circles = High Na⁺ diet + water, filled triangles = High Na⁺ diet + 16% sucrose solution.

Figure 1