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Pressor Effects of Fat and Salt in Rats (40972)[1]

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Abstract. The role of dietary lipids in the development of salt-induced hypertension was studied. Sprague–Dawley rats were fed a high-fat (HSAT) (5% corn oil–15% coconut oil), a high-carbohydrate (HCARB) (5% corn oil), or a commercial rat diet. Within the HSAT and HCARB groups, subgroups were randomly chosen and the level of salt was varied in the following progression: high salt (HS) (8% NaCl); medium salt (MS) (6% NaCl); and basal salt (BS) (0.15% NaCl). Rats were maintained at each level of salt for 2 weeks. Systolic blood pressure was measured from the tail artery using an electrosphygmomanometer. No significant elevation in blood pressure was demonstrated by rats maintained on the HCARB–BS or commercial laboratory diet during a 6-week experimental period. An intermediate elevation in blood pressure was seen in rats fed the HCARB–HS or HSAT–BS diets. Rats fed the HSAT–HS diet demonstrated the most significant elevation in blood pressure, becoming hypertensive within 1 week of diet introduction. Blood pressure dropped to normotensive levels when the level of salt in the HCARB and HSAT groups was reduced to 6% of the diet. Results from this short-term experiment suggest that the amount of fat in the diet, as well as the level of salt consumed, plays a key role in the regulation of blood pressure in rats.

Hypertension is a major health problem which afflicts 23–25 million Americans (1–3). Up to 90% of all reported cases in hypertension are classified as essential hypertension, or hypertension for which the mechanisms sustaining the hypertensive state are unknown (2, 3). Hypertension has been identified as one of the most significant risk factors in heart disease (1–3).

Numerous clinical (4, 5), epidemiological (6, 7), and experimental studies implicate high salt intake in the development of essential hypertension. Recent evidence suggests that the hypertensive effect of dietary salt may be modified by varying the level of dietary fat (8–10). However, the relative importance of the interaction between the amount of salt and fat required to achieve a hypertensive response still needs to be studied. The purpose of the present investigation was to examine how the amount of dietary fat interacts with the level of dietary salt to affect blood pressure in rats.

Materials and methods. Male Sprague–Dawley rats (ARS, Sprague–Dawley, Madison, Wisc.), approximately 6 weeks of age, were housed individually in hanging wire cages with a 12-hr light/dark cycle. Rats were equilibrated on a high-carbohydrate (HCARB) (5% corn oil) basal-salt (BS) (0.15% NaCl) diet for a period of 1 week before subgroups of rats were randomly chosen and exposed to the dietary regimens as shown in Fig. 1. HCARB diets were based on diets containing 5% corn oil and high-saturated-fat diets (HSAT) were based on diets containing 5% corn oil–15% coconut oil. Rats were fed high-salt diets (HS) (based on 8% NaCl) for 2 weeks before being exposed to medium-salt diets (MS) (based on 6% NaCl).

After a 2-week exposure to MS diets, rats were once again switched from MS to BS diets (0.15% NaCl). A separate group of rats was maintained on a laboratory diet

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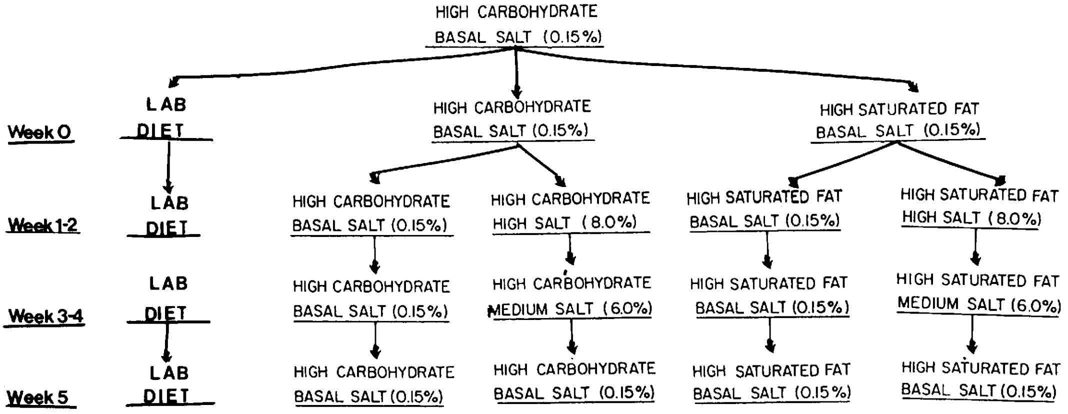

FIG. 1. Schematic of the dietary treatment groups used to study the effect of fat and salt on the development of hypertension in rats in experiment 1. Each dietary treatment group contained 6–10 animals. Animals were maintained on the HS and MS diets for 2 weeks.

(Purina Laboratory Chow, St. Louis, Mo.) for the entire 5-week experimental period.

It has been found in our laboratory and those of others that rats adjust their food intake so that similar caloric intake is maintained despite the fact that diets may differ substantially in caloric density (11). Hence, animals will eat quantitatively less high-fat diet and quantitatively more high-carbohydrate diet. Consequently, unless the proportions of the other components in the diet are adjusted, animals fed a high-fat diet will take in substantially different amounts of protein, fiber, vitamins, and minerals than those fed a high-carbohydrate diet. Our experimental diets were manipulated so that all experimental animals were consuming a similar quantity of caloric as well as noncaloric dietary constituents (Table I).

Food and water were available *ad libitum*. Water consumption, weight gain,

TABLE I. DIET COMPOSITION

	Amount (g/100 kcal finished diet)					
Diet constituent	HCARB-BS	HCARB-MS	HCARB-HS	HSAT-BS	HSAT-MS	HSAT-HS
Casein	5.47	5.37	5.36	5.36	5.34	5.37
Sucrose	13.20	13.38	13.33	4.42	4.29	4.40
Fat						
Corn	1.36	1.35	1.35	1.34	1.33	1.34
Coconut	0.0	0.0	0.0	4.42	4.10	4.03
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05
Salt mix[a]	1.12	1.06	1.06	1.07	1.07	1.07
Vitamins[b]	0.68	0.71	0.72	0.58	0.61	0.62
Corn starch	2.73	2.68	2.71	2.68	2.67	2.68
Cellulose	2.73	2.68	2.71	2.68	2.67	2.68
DL-Methione	0.07	0.08	0.08	0.08	0.08	0.08
NaCl	0.04	1.61	2.15	0.04	1.59	2.15
Kcal/g	3.65	3.46	3.39	4.47	4.19	4.09

[a] The salt mix contained (in %) sodium chloride, 13.9325; potassium phosphate, 38.8967; magnesium sulfate, 229.2; calcium carbonate, 38.1442; ferrous sulfate, 2.696; potassium iodide, 0.079; magnesium sulfate, 0.4453; zinc chloride, 0.0259; copper sulfate, 0.0475; cobalt chloride, 0.0022.

[b] The vitamin mix contained (per kilogram of diet) Vitamin A concentrate, 19822 IU; Vitamin D concentrate, 2200 IU; α-tocopherol, 110 mg; ascorbic acid, 991 mg; inositol, 110 mg; choline chloride, 165 g; menadione, 49.5 mg; *p*-aminobenzoic acid, 110 mg; niacin, 99 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}, 0.3 mg.

and food consumption were determined weekly.

Blood pressure and heart rate determinations. Blood pressure and heart rate were determined weekly between the hours of 8 AM and 12 PM. Rats were placed in a preheated chamber (Peninsula Laboratories, San Carlos, Calif.) at 38°C for 10 min. Rats were then transferred to a temperature-controlled rat holder unit (Narco Bio-Systems, Houston, Tex.) calibrated to 38°C. A metal tubular cuff, 7/16 in., was placed around the tail of each rat. Cuff inflation/deflation rates (25 mm Hg/sec) and maximum cuff pressure (225 mm Hg) were controlled by a programmed Electro-Sphygmomanometer PE-300 (Narco Bio-Systems). Animals were allowed to adjust to the cuff for 3 min before at least two consecutive blood pressure readings were recorded on a recording physiograph.

It should be noted that the electrosphygmomanometer is an indirect means of measuring systolic blood pressure in rats. The advantage of using this method for blood pressure analysis is twofold. First, it is used by numerous investigators, thus enabling rapid means of comparison of results. Second, it allows for long-term study of animals since it does not involve surgical catheterization of the rats. In addition, studies by Bunag (12) have shown a 0.97 correlation between direct and indirect methods of systolic blood pressure determinations at both hypertensive and normotensive values.

At the end of Week 1, rats exhibiting the two highest and two lowest blood pressures within a dietary treatment group were eliminated from further analysis. This was done to eliminate the influence of genetic predisposition to salt-induced hypertension that has been reported previously in rats (6).

Statistical analysis. Experimental data were statistically compared using Student's t test. Significance of the differences of the means at the 95% confidence interval was determined by comparison to the HCARB−BS group at a particular weekly time period.

Results. Weight gain, calorie consumption and water consumption. Figure 2 shows the effect of varying diet on weekly calorie

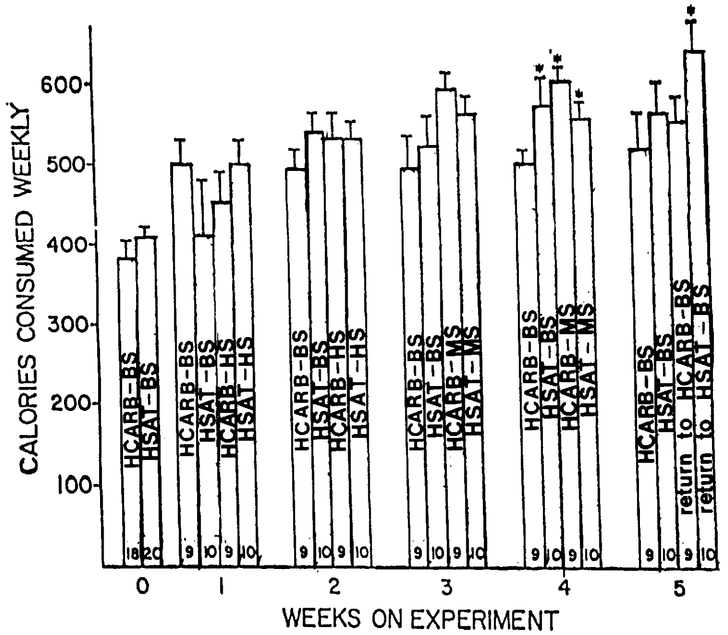

FIG. 2. The effects of varying diet on weekly calorie consumption of rats in experiment 1. Each bar represents the mean weekly caloric intake ± SEM for the number of animals indicated within each bar. Student's t test was used for statistical analysis of the 95% confidence interval. Significant differences ($P \leq 0.05$) when compared to the HCBS diet within the respective time period are designated by the asterisks.

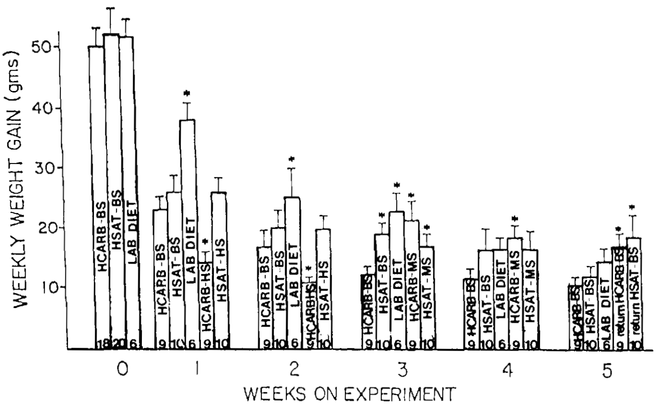

FIG. 3. The effects of varying diets on weekly weight gain in rats in Experiment 1. Each bar represents the mean weekly caloric intake ± the standard error of the mean for the number of animals indicated within each bar. Student's *t* test was used for statistical analysis at the 95% confidence interval. Significant differences ($P < 0.05$) when compared to the HCBS diet within the respective time period are designated by the asterisks.

consumption. Rats generally consumed a similar quantity of calories per week, except at Week 4, where the HCARB–MS, HSAT–BS, and HSAT–HS groups consumed significantly more calories per week than the HCARB–BS group. When rats were returned to the BS diets, weekly calorie consumption remained significantly elevated in the HSAT–BS group (Fig. 2, Week 5).

Figure 3 shows the effect of varying diet on weekly weight gain. The HCARB–HS group gained significantly less weight per week than the HCARB–BS group at Weeks 1 and 2. Significant elevations in weekly weight gain were found in the HSAT–BS, commercial laboratory diet, HCARB–MS, and HSAT–MS groups at Week 3 compared to the HCARB–BS group.

Weekly water consumption in the various experimental groups is shown in Fig. 4. Rats consuming the commercial laboratory diet began to drink significantly more water

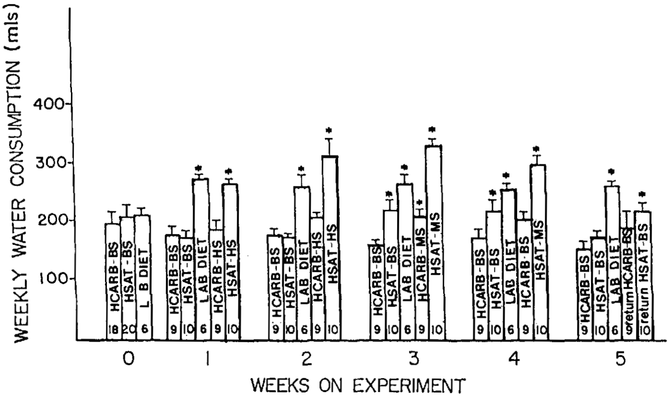

FIG. 4. The effects of varying diet on weekly water consumption of rats in Experiment 1. Each bar represents the mean weekly water consumption ± the standard error of the mean for the number of animals indicated within each bar. Student's *t* test was used for statistical analysis at the 95% confidence interval. Significant differences ($P < 0.05$) when compared to the HCBS diet within the respective time period are designated by the asterisks.

at Week 1 than the HCARB–BS group. This trend persisted throughout the entire 5-week experimental period. Significant elevations in water consumption were found in the HSAT–HS groups (Fig. 4, Weeks 1 and 2) and the HSAT–MS groups (Fig. 4, Weeks 3 and 4) when compared to the HCARB–BS group. At Weeks 3 and 4, the HSAT–BS groups consumed significantly elevated amounts of water compared to the HCARB–BS group.

Blood pressure and heart rate determinations. The effect of varying the fat and salt content of the diet on blood pressure is shown in Fig. 5. Rats consuming the HCARB–HS diet demonstrated significant elevations in blood pressure within 1 week of diet introduction (Fig. 5, Week 1). When the level of salt in the HCARB–HS diet was lowered to the MS level, blood pressure decreased sharply to values which were not significantly different from the values observed for the HCARB–BS group (Fig. 5, Weeks 3, 4). Significant elevations in blood pressure were found throughout the entire 5-week experimental period in the HSAT–BS group. The greatest increase in blood pressure was found in the HSAT–HS group. Significant elevations in blood pressure were found in the HSAT–HS group within 1 week of diet exposure and persisted during the second week of HSAT–HS feeding (Fig. 5, Weeks 1, 2). When the level of salt in the HSAT–HS diet was decreased to 6%, blood pressure decreased to values that were not significantly different from the group fed the HSAT–BS over the 5-week period (Fig. 5).

Figure 6 shows the effect of varying diet on heart rate. All groups had similar heart rates at Week 0. At Weeks 1, 2, 4 and 5, the HSAT–BS group had significantly elevated heart rates, as compared to the HCARB–BS group. The HCARB–HS group had a significantly elevated heart rate at Week 1, while at Weeks 3 and 4 the HCARB–MS group had a heart rate significantly greater than that of the HCARB–BS group. Significantly elevated heart rates were found in rats fed the HSAT–HS diet (Fig. 6, Weeks 1 and 2). When the level of salt in the HSAT–HS diet was lowered to 6%,

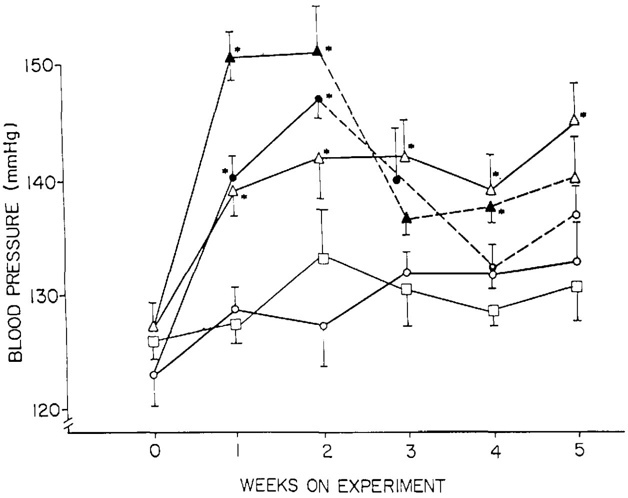

FIG. 5. The effect of varying fat and salt on systolic blood pressure of rats in experiment 1. Each point represents the mean systolic blood pressure of seven to nine animals ± the SEM. ○——○, HCARB–BS; ●——●, HCARB–HS; ●– – –●, HCARB–MS, ○– – –○, return to basal carbohydrate; △——△, HSAT–BS; ▲——▲, HSAT–HS; ▲– – –▲, HSAT–MS; △– – –△, return to basal fat; □——□, laboratory diet. Student's *t* test was used for statistical analysis at the 95% confidence interval. Significant differences ($P \leq 0.05$) comparing the groups to the HCARB–BS diet at different time periods are designated by asterisks.

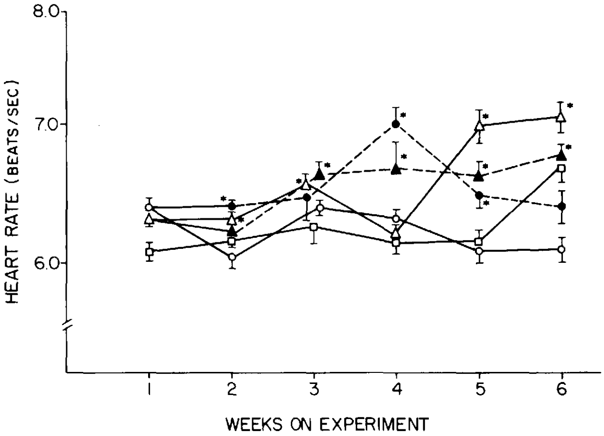

FIG. 6. The effect of varying fat and salt on heart rate of rats in experiment 1. Each point represents the mean heart rate of seven to nine animals ± SEM. ○——○, HCARB–BS; ●——●, HCARB–HS; ●– – –●, HCARB–MS; ○– – –○, return to basal carbohydrate; △——△, HSAT–BS; ▲——▲, HSAT–HS; ▲– – –▲, HSAT–MS; △– – –△, return to basal fat; □——□, laboratory diet. Student's *t* test was used for statistical analysis at the 95% confidence interval. Significant differences ($P \leq 0.05$) comparing the groups to the HCARB–BS diet at the time period indicated are designated by the asterisks.

heart rate in the HSAT–MS group was greater than in the HCARB–BS group at Weeks 3 and 4.

Discussion. Results from this short-term experiment suggest that high levels of saturated fat in the diet play an important role in the development of essential hypertension in rats. High-saturated fat, irrespective of the level of dietary salt, induced a sharp increase in blood pressure which persisted throughout the experimental period (Fig. 5). The percentage of hypertension (systolic blood pressure greater than 140mmHg) in the HSAT–BS group ranged from 72% after Week 1 to 84% after Week 5.

The HSAT–HS group demonstrated the greatest increase in blood pressure, with 90% of all animals becoming hypertensive within 1 week of diet introduction and 80% remaining hypertensive during the second week of diet exposure. When the level of salt in the HSAT–HS diet was lowered to 6% the percentage of rats with hypertension decreased to 67% (Fig. 5, Week 4).

The results reported herein extend earlier observations that fat and salt influence blood pressure (9). One major difficulty with previously reported studies is that the interactions between fat and salt were not examined in a quantitative manner. Salt was presented in the drinking water and salt solution consumption was not recorded. Therefore, the amount of sodium chloride consumed daily by the rats could not be determined. We found that the BS groups consumed an average of 29 mg NaCl/day while the HS groups consumed an average of 1500 mg NaCl/day.

Ten Hoor and Van de Graff (9) reported an 8% increase in blood pressure of rats fed a high-saturated-fat/high-salt diet. We found a greater increase in blood pressure (20%) in the HSAT–HS group. This may be attributed to several factors. Ten Hoor and Van de Graff presented salt in the drinking water (9). We chose to present the salt mixed into the food, which is a more natural physiological situation. In addition, we began exposure to the dietary treatments at an earlier age than Ten Hoor and Van de Graff. It has been reported that weanling rats are more susceptible to the hypertensive effect of sodium chloride than older animals (13).

All diets used in the present study contained at least 2% linoleic acid which is considered to be a more than adequate level for this essential fatty acid (13). Therefore, the increased blood pressures observed with the high-fat diets are probably attributable to the high levels of saturated fat and not to inadequate intakes of essential fatty acids (8).

Animals fed the HCARB–HS diet demonstrated a significant elevation in blood pressure within 1 week of diet introduction (Fig. 5). At Week 1, 44% of the animals consuming the HCARB–HS diet were classified as hypertensive or as having a systolic blood pressure greater than 140 mm Hg. Eighty-nine percent of the HCARB–HS group were classified as hypertensive after consuming the diet for 2 weeks. When the level of salt in the HCARB–HS diet was lowered to 6% (Fig. 5, Week 3), blood pressure values decreased sharply to values that were not significantly different from those of the HCARB–BS group.

The Sprague–Dawley rat has been shown to manifest hypertension with any intake of salt greater than that provided by the Hubbel–Mendel–Wakeman mineral mix when the mix is fed at the 3% level (15). The sodium chloride allowance provided by this mixture is 0.14% of the final diet. Our HCARB–BS diet provided 0.15% NaCl. This level of NaCl should allow for the maintenance of normal blood pressure values in the HCARB–BS group.

The significant elevation in blood pressure found in the HCARB–HS group has been demonstrated by previous investigators (6, 16, 17). However, we found extremely rapid increases in blood pressure (15%) in the HCARB–HS group by Week 2 of diet exposure (Fig. 5). This might be attributable to the high sucrose content of our HCARB–HS diet. Beebe *et al.* (18) found that feeding a high-sucrose/high-salt semipurified diet caused a significant increase in blood pressure within 1 week of diet introduction. Rats fed a high-sucrose/basal-salt diet over a period of 10 weeks demonstrated a 12% increase in blood pressure. In our experiment, we found an 8% increase in blood pressure in the HCARB–BS group by Week 5.

It has been suggested that the increased blood pressure of animals fed diets high in saturated fat causes a decrease in urinary excretion of prostaglandins. Renal prostaglandin metabolism has been associated with the synthesis and release of renin as well as sodium conservation mechanisms (9). Since numerous investigators have suggested that misregulation of the renin–angiotensin system may play a role in the development of essential hypertension (19, 20) it is not unreasonable to assume that the effect of dietary fat on blood pressure may be mediated by some aspect of prostaglandin metabolism.

The observed results suggest that high levels of fat may play an important role in the development of hypertension in Sprague–Dawley rats. However, it is not known whether the hypertensive effect of high levels of dietary fat in this study was due to the type of fat fed (coconut oil) or the level of fat in the diet (5% vs 20%). Additional studies are needed to examine the role of the type as well as the amount of dietary fat in salt-induced hypertension in rats. These aspects of fat metabolism add another dimension to the potential interrelationships between fat and salt consumption and heart disease.

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