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A high-fat, refined-carbohydrate diet affects renal NO synthase protein expression and salt sensitivity

CHRISTIAN K. ROBERTS,[1,2] NOSRATOLA D. VAZIRI,[2]
RAM K. SINDHU,[2] AND R. JAMES BARNARD[1]
[1]*Department of Physiological Science, University of California, Los Angeles 90095; and*
[2]*Division of Nephrology and Hypertension, Departments of Medicine, Physiology, and Biophysics, University of California, Irvine, California 92697*

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Roberts, Christian K., Nosratola D. Vaziri, Ram K. Sindhu, and R. James Barnard. A high-fat, refined-carbohydrate diet affects renal NO synthase protein expression and salt sensitivity. *J Appl Physiol* 94: 941–946, 2003. First published October 25, 2002; 10.1152/japplphysiol.00536.2002.—Chronic consumption of a high-fat, refined-carbohydrate (HFS) diet causes hypertension. In an earlier study, we found increased nitric oxide (NO) inactivation by reactive oxygen species (ROS) and functional NO deficiency in this model. Given the critical role of NO in renal sodium handling, we hypothesized that diet-induced hypertension may be associated with salt sensitivity. Female Fischer rats were fed an HFS or a standard low-fat, complex-carbohydrate (LFCC) rat chow diet starting at 2 mo of age for 2 yr. Arterial blood pressure, renal neuronal NO synthase (nNOS), endothelial NO synthase (eNOS), and inducible NO synthase (iNOS) protein and nitrotyrosine abundance (a marker of NO inactivation by ROS), and urinary NO metabolite excretion were measured. To assess salt sensitivity, the blood pressure response to a high-salt (4%) diet for 1 wk was determined. After 2 yr, renal nNOS and urinary NO metabolite excretion were significantly depressed, whereas arterial pressure, eNOS, iNOS, and nitrotyrosine were elevated in the HFS group but remained virtually unchanged in the LFCC group. Consumption of the high-salt diet resulted in a significant rise in arterial pressure in the HFS, but not in the LFCC, group. Thus chronic consumption of an HFS diet results in hypertension and salt sensitivity, which may be in part due to a combination of ROS-mediated NO inactivation and depressed renal nNOS protein expression.

endothelial dysfunction; hypertension; oxidative stress; blood pressure; nitric oxide

IN A SERIES OF RECENT STUDIES, we documented that a high-fat, refined-carbohydrate (HFS) diet, one similar to that consumed in Westernized societies, induces insulin resistance (1), endothelial dysfunction (25), and other characteristics of the metabolic syndrome. We also showed that prolonged HFS diet consumption results in hypertension, which is accompanied by enhanced vascular nitric oxide (NO) inactivation in genetically normotensive Fischer rats (30).

Increased dietary salt intake raises arterial pressure in salt-sensitive, but not salt-resistant, humans (10) and animals (21, 22). The mechanism(s) to explain why salt sensitivity occurs in some animals, but not others, is unclear. Tolins and Shultz (38) documented that high-salt feeding induces an increase in NO production and enhances natriuresis without raising blood pressure in genetically normotensive Sprague-Dawley rats. However, in the presence of an NO synthase (NOS) inhibitor, increased dietary salt intake results in a rise in blood pressure, which helps restore sodium balance via pressure natriuresis. Similarly, others have found no significant change or a mild rise in blood pressure with salt loading in genetically normotensive animals (18, 23). An earlier study by our group demonstrated that salt sensitivity in Dahl salt-sensitive rats may be linked to downregulation of renal and vascular NOS expression (21). As reviewed by Majid and Navar (16), the tubuloglomerular feedback-mediated afferent arteriolar vasoconstrictive response to luminal sodium concentration is attenuated by NO derived from neuronal NOS (nNOS) in the macula densa. Moreover, pharmacological inhibition of NOS has been shown to augment the tubuloglomerular feedback response (16, 47). Inhibition of nNOS has been reported to confer salt sensitivity in experimental animals (47). On the basis of evidence that insulin resistance is associated with salt sensitivity in humans (11, 12), diets that cause insulin resistance may also induce salt sensitivity. Because chronic consumption of an HFS diet causes insulin resistance (1) and a functional NO deficiency (30), we hypothesized that it may lead to salt sensitivity and that diminished renal NO availability plays a role in conferring salt sensitivity in this model.

METHODS

Animals and diet. All protocols were conducted in accordance with the University of California, Los Angeles, Animal Research Committee. Female Fischer rats were obtained from Harlan Sprague Dawley (San Diego, CA) at 2 mo of age. We used this rat model in our previous studies, inasmuch as

Address for reprint requests and other correspondence: R. J. Barnard, Dept. of Physiological Science, UCLA, 2204 Life Science Bldg., 621 Charles E. Young Dr. South, Los Angeles, CA 90095-1606 (E-mail: jbarnard@physci.ucla.edu).

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the female Fischer rat normally shows little weight gain after its maturation phase (1, 30). The animals ($n = 8$ per group) were housed four per cage with a 12:12-h light-dark cycle starting at 0700 at 75–76°F and were allowed to acclimatize to their environment for 1 wk before the dietary intervention was initiated. The animals were randomly assigned to the low-fat, complex-carbohydrate (LFCC) or HFS diet and fed the diets and water ad libitum. Our laboratory previously noted that both groups consume a similar number of calories (26). The diets were prepared in powder form by Purina Test Diets (Richmond, IN) and contained a standard vitamin and mineral mix and all essential nutrients. The LFCC diet (Purina 5001) is low in saturated fat and contains mostly complex carbohydrate (starch); the HFS diet is high in saturated and monounsaturated fat (primarily from lard plus a small amount of corn oil) and high in refined sugar (sucrose), as previously reported (28) (Table 1).

Before death at 2 yr on the diets, animals from both groups were placed on a high-salt (4.0%) diet for 1 wk while others were continued on the standard 0.4% salt diets. Blood pressure was measured by tail cuff plethysmography, as previously described (30). Briefly, the rats were placed in a constant-temperature (29°C) chamber for ≥15 min on 2–3 separate days for acclimatization to the chamber environment. Subsequently, several recordings were made while the animals were quietly resting. During the initial experiments, blood pressure was measured on 2 days, and if recordings were not similar, measurements were taken on a 3rd day. The daily means from five to six viable measurements were calculated to obtain a value for each rat.

Urinary NO metabolite excretion. Urinary excretion of the total nitrite and nitrate (NOx) was determined after the rats were placed in metabolic chambers and fasted for 2 days during urine collection, as previously described (30).

Nitrotyrosine measurement. Renal tissue (25% wt/vol) was homogenized in a solution containing 50 mM Tris·HCl (pH 7.4); 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EGTA; aprotinin, leupeptin, and pepstatin (1 μg/ml each); 1 mM Na_3VO_4; and 1 mM NaF at 0–4°C using a Polytron homogenizer. Homogenates were centrifuged at 12,000 *g* at 4°C for 5 min to remove tissue debris and nuclear fragments. The supernatant was processed for determination of total protein concentration by a protein assay kit (Bio-Rad, Hercules, CA), and tissue nitrotyrosine abundance was determined by Western blot, as previously described (40), using an anti-nitrotyrosine monoclonal antibody (Upstate Biotechnology, Lake Placid, NY).

NOS protein determination. Western blot was performed on renal tissues of both groups at 2 yr to quantify endothelial NOS (eNOS), inducible NOS (iNOS), and nNOS protein levels using anti-eNOS, anti-iNOS, and anti-nNOS monoclonal antibodies (Transduction Laboratories, Lexington, KY), as previously described (42).

Statistical analysis. Statistical analyses were performed with GraphPad Prism software (GraphPad, San Diego, CA). Data were analyzed using an ANOVA when more than two groups were compared and a *t*-test when two groups were compared. When significant *F* values were noted, post hoc analyses were performed using a Newman-Keuls multiple-comparison test. Differences were considered statistically significant at $P < 0.05$. Values are means ± SE with seven to eight rats per group unless otherwise indicated.

Table 1. *LFCC and HFS diet composition*

	Diet	
	LFCC	HFS
%Energy as carbohydrate	59.81	40.12
%Energy as fat	12.14	39.09
%Energy as protein	28.05	20.72
Fiber, g/100 g	5.3	1.6
Sucrose, g/100 g	3.68	45.10
%Cholesterol	0.0200	0.0175
%Saturated fat	1.50	7.91
%Monounsaturated fat	1.58	8.22
%Polyunsaturated fat	0.71	3.04
Physiological energy, kJ/g	13.97	19.63

LFCC, low-fat complex-carbohydrate; HFS, high-fat refined-carbohydrate.

RESULTS

Blood pressure and body weight. The HFS group exhibited a gradual rise in systolic blood pressure during the 2-yr study period ($P < 0.008$, ANOVA). In contrast, blood pressure remained unchanged in the LFCC control group throughout the course of the study (147.3 ± 3.7 vs. 123.0 ± 3.9 mmHg, $P < 0.01$; Fig. 1). The HFS group weighed significantly more than the LFCC group (374.0 ± 9.0 vs. 260.0 ± 6.0 g, $P < 0.001$).

Blood pressure increased significantly in the HFS rats after 1 wk of high-salt feeding (160.0 ± 4.1 vs. 147.3 ± 3.7 mmHg). In contrast, the high-salt diet had no effect on blood pressure in the LFCC rats (123.8 ± 4.8 vs. 123.0 ± 3.9 mmHg; Fig. 1).

Renal nitrotyrosine, eNOS, iNOS, and nNOS protein abundance and urinary NOx excretion. Compared with the LFCC group, the HFS animals exhibited a marked increase in renal nitrotyrosine abundance (Fig. 2). This was accompanied by a significant reduction of renal nNOS protein abundance (Fig. 3), pointing to increased NO inactivation and decreased nNOS-derived NO production capacity in HFS-fed animals at 2 yr. This was coupled with a compensatory upregulation of renal eNOS and iNOS proteins in the HFS group (Fig. 3). Long-term HFS diet consumption resulted in a marked reduction in urinary NOx excretion (540 ± 170 vs. 10 ± 10 μmol/g creatinine; Fig. 3), pointing to reduced NO availability.

DISCUSSION

HFS animals exhibited a marked increase in nitrotyrosine, pointing to increased NO inactivation. This was accompanied by a significant reduction in urinary NOx excretion, denoting reduced NO availability. To test the effect of diet and NO on salt sensitivity, salt loading studies were performed. After ~2 yr on the LFCC or the HFS diet when hypertension had been induced in the HFS group, the rats were placed on a high-salt diet for 1 wk by addition of NaCl to the food for a final concentration of 4.0%. The reduction in NO availability in the HFS diet-fed animals was associated with marked salt sensitivity, as evidenced by a significant rise in blood pressure on the high-salt diet. In contrast, the LFCC group exhibited resistance to the high-salt diet, as evidenced by a lack of change in arterial pressure, despite high salt intake. These observations point to renal NO deficiency as a likely cause of salt sensitivity in the HFS animals.

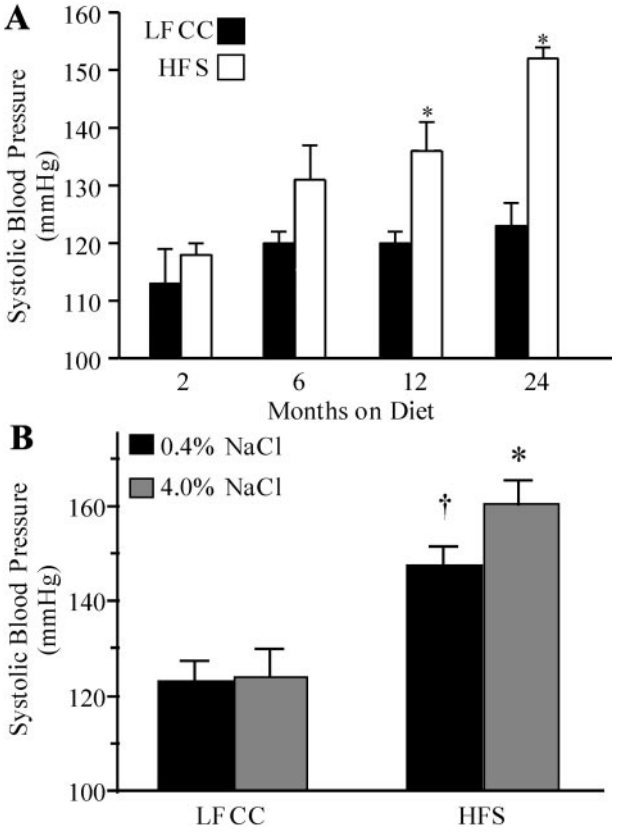

Fig. 1. *A*: effect of high-fat refined-carbohydrate (HFS) and low-fat complex-carbohydrate (LFCC) diets on systolic blood pressure over the course of 2 yr. $*P < 0.01$ vs. LFCC. *B*: effect of 1 wk of a high-salt diet on blood pressure at 2 yr. High-salt feeding for 1 wk resulted in a further increase in blood pressure in the HFS group. Values are means ± SE. $*P < 0.05$ vs. LFCC on high-salt diet. $\dagger P < 0.01$ vs. LFCC on normal-salt diet.

Nitrotyrosine is the stable footprint of the interaction between NO, ROS, and tyrosine residues of proteins, and, as such, its accumulation in the renal tissue of animals with diet-induced hypertension reflects increased ROS-mediated NO inactivation in this model (9, 13). Oxidation and sequestration of NO by ROS, such as superoxide, decrease NO availability (2, 46). In addition to inactivating NO, ROS and their by-product of interaction with NO, peroxynitrite, can oxidize and deplete tetrahydrobiopterin, an essential NOS cofactor, which can augment NO deficiency and oxidative stress by uncoupling NOS from an NO- to a superoxide-producing enzyme (3). Shinozaki et al. (34) demonstrated impaired endothelium-dependent vasorelaxation in rats fed a high-fructose diet. This was associated with impaired eNOS activity, increased superoxide production, and tetrahydrobiopterin depletion in endothelial cells.

Increased production of NO results in renal vasodilation and both natriuresis and diuresis. A defect in the production of NO might be responsible for the salt sensitivity found in many hypertensive patients (4). Administration of competitive NOS inhibitors reduces sodium excretion and increases renovascular resistance (15, 24). In the normotensive animal, dietary sodium loading results in increased NO production (as demonstrated by an increase in NO metabolite excretion), which facilitates natriuresis and preserves blood pressure homeostasis (6, 35). Furthermore, Chen and Sanders (5) showed that increases in dietary salt increased NO production in salt-resistant, but not salt-sensitive, Dahl rats, suggesting that decreased NO production may cause salt-sensitive hypertension. It appears that, in the presence of defective NO metabolism, an increase in blood pressure is necessary to maintain sodium balance. Furthermore, when the NO system is intact, increases in dietary sodium do not increase blood pressure. Data showing that rats that are resistant to the effects of salt become salt sensitive in the presence of an NOS inhibitor support this contention (38). Long-term HFS diet consumption induced a marked reduction in renal nNOS abundance, which

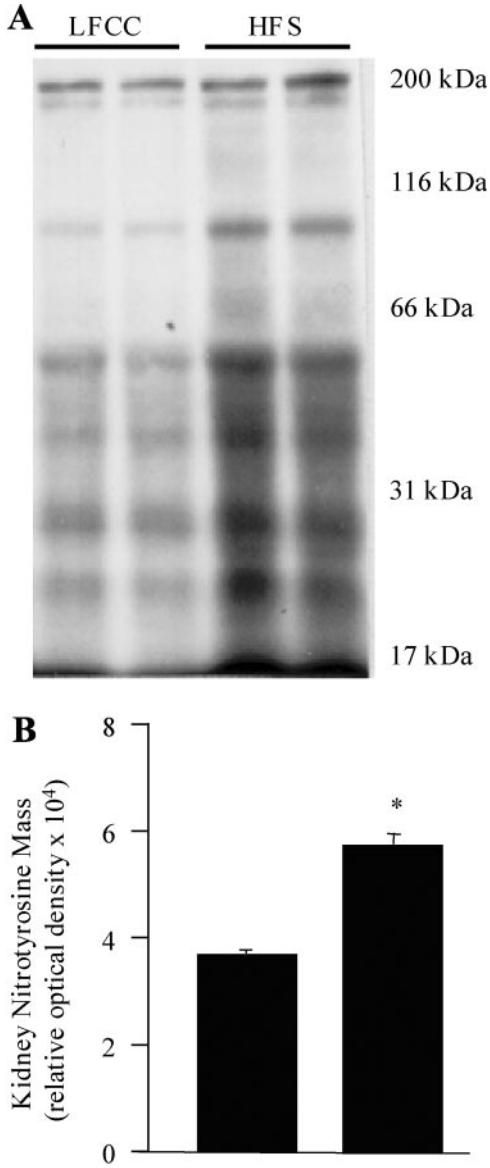

Fig. 2. Effect of diet on renal nitrotyrosine abundance of 2 LFCC and 2 HFS rats at 2 yr. *A*: immunoblot. *B*: corresponding group data. Values are means ± SE ($n = 4$). $*P < 0.05$ vs. LFCC.

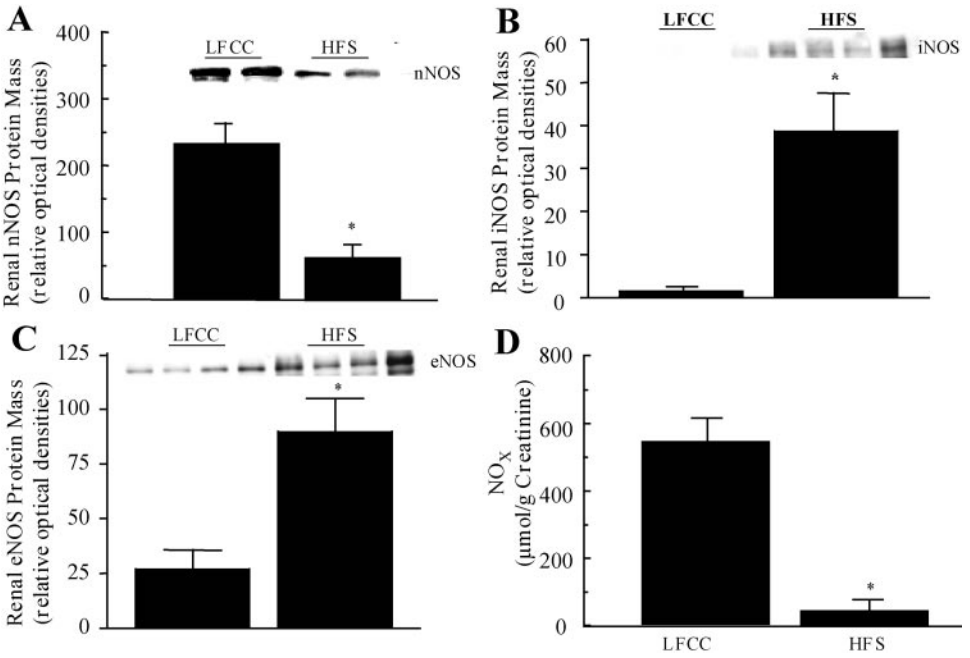

Fig. 3. Effect of diet on renal nitric oxide synthase (NOS) protein expression and urinary total nitrite + nitrate (NOx). Western blots show neuronal NOS (nNOS, *A*), inducible NOS (iNOS, *B*), and endothelial NOS (eNOS, *C*) protein mass of 2 LFCC and 2 HFS rats at 2 yr. Corresponding group data are illustrated below immunoblots. *D*: effect of diet on urinary NOx. Values are means ± SE ($n = 4$). *$P < 0.05$ vs. LFCC.

may have contributed to the salt sensitivity noted in this model. In the kidney, nNOS is prominently expressed in the macula densa and, to a lesser extent, in the efferent arteriole, mesangial cells, and intrarenal neurons (16). NO produced by the macula densa attenuates tubuloglomerular feedback-mediated afferent arteriolar vasoconstriction in response to the luminal salt content (37, 47). Thus downregulation of renal nNOS, ROS-mediated inactivation of NO, or pharmacological inhibition of NOS can promote salt sensitivity. In contrast to the control group, which showed no discernable rise in blood pressure on the high-salt diet, the HFS group exhibited a marked rise in blood pressure with increased dietary salt intake, pointing to an acquired salt sensitivity mediated, at least in part, by a reduction in renal nNOS abundance and increased ROS-mediated NO inactivation. This NO deficiency would result in an exaggerated tubuloglomerular feedback response, leading to salt retention, volume expansion, increased renovascular resistance, and hypertension. Thus it appears that the NO deficiency in the HFS group may have contributed to the salt sensitivity in this group.

Human studies also suggest that there is a variation in the blood pressure response to dietary sodium, with some individuals being salt sensitive and others salt resistant. Although ~25% of normotensive individuals exhibit an increase in blood pressure with salt loading, >50% of hypertensive patients experience a blood pressure rise in response to salt loading (45). In addition, several investigators have reported a relationship between insulin resistance and salt sensitivity. Rocchini (31) and Sharma et al. (32, 33) demonstrated that sodium-resistant individuals were more insulin sensitive. It has been established that high-salt feeding is associated with insulin resistance and a blunted NO generation in humans (10). The HFS animals in the present study exhibited insulin resistance (28), and thus salt sensitivity may be an additional manifestation of diet-induced syndrome X.

In contrast to nNOS, which was significantly decreased, eNOS and iNOS were elevated. An upregulation of eNOS and iNOS has been demonstrated in other models of hypertension (39, 41). This is primarily due to avid inactivation of NO by ROS and the resultant attenuation of the negative-feedback regulatory influence of NO on NOS expression (43). The abundance of nitrotyrosine, which is the by-product of the NO-ROS-tyrosine interaction, was elevated in the HFS group as well as in various other forms of hypertension (44). Thus, despite upregulation of iNOS and eNOS, the bioavailability of NO is diminished in this model. To our knowledge, there is only one other study that has also investigated the role of a high-fat diet on NOS isotype expression. Using a diet containing 32% of energy from fat, Dobrian et al. (8) reported the occurrence of obesity, hypertension, oxidative stress, and compensatory upregulation of renal eNOS mRNA in a subgroup of rats designated obesity prone. The HFS diet employed in the present study contained a higher fat content (39%) than the diet used by Dobrian et al. In addition, the fat used in the present study was primarily saturated in nature, whereas the type of fat used by Dobrian et al. was not specified. Moreover, in our study, the HFS diet included sucrose as its main carbohydrate source, which may further contribute to the observed abnormalities, because high-sugar diets in the form of fructose have been shown to induce endothelial dysfunction (14).

Although the role of eNOS in the regulation of cardiovascular function is well characterized, the role of iNOS is less clear. Contrary to the conventional view, iNOS is constitutively expressed in several tissues, such as heart, vascular smooth muscle, and kidney (19,

20). In the present study, iNOS expression was virtually undetectable in the LFCC and increased in the presence of HFS diet consumption, suggesting the possibility of diet-induced inflammation.

The role of obesity in this model of diet-induced hypertension is unclear. It is well established that obesity is associated with hypertension and that reductions in blood pressure are associated with weight loss (7, 17). The HFS group weighed significantly more than the LFCC group. Although the reduction in body weight previously noted in this model after the animals were switched from the HFS to the LFCC diet may have contributed to the reductions in blood pressure and oxidative stress and the increase in NO availability, the animals still weighed significantly more (~45 g) than the control group (29). Recently, using a 3-wk intensive diet-and-exercise intervention, we documented a large reduction in blood pressure in obese men, despite a modest change in body mass index from 37.6 to 36.1, indicating the presence of significant obesity after the program (27). Furthermore, in a recent study, intentional weight loss achieved by gastric surgery yielding a sustained weight loss of ~20 kg had no effect on the incidence of hypertension during an 8-yr observation period (36). Thus the contributing role of obesity in this model requires further study; however, it appears that the diet per se contributed to abnormalities noted in the present study.

Overall, prolonged consumption of the HFS diet induces excessive ROS production, which contributes to hypertension via NO inactivation and decreased NO availability (30). Additionally, the HFS diet induces salt sensitivity, which is associated with a reduction in renal nNOS expression, further contributing to the development of hypertension by augmenting the tubuloglomerular feedback response and diminishing pressure natriuresis. Thus an HFS diet induces salt sensitivity, which is associated with and perhaps mediated, in part, by a renal NO deficiency.

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