Effect of dietary fat on sympathetic nervous system activity in the rat.

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Research Article

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Effect of Dietary Fat on Sympathetic Nervous System Activity in the Rat

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ABSTRACT Previous studies from our laboratory have demonstrated that dietary intake affects the sympathetic nervous system (SNS); carbohydrate intake, in particular, has been shown to stimulate sympathetic activity. The present studies were undertaken to characterize the effect of dietary fat on SNS activity in the rat. Sympathetic activity was assessed by measurement of norepinephrine (NE) turnover in heart, interscapular brown adipose tissue (IBAT), and pancreas and by excretion of NE in the urine. When fed a fat-enriched diet (50% chow, 50% lard), fractional NE turnover in heart (k) increased from 6.3±0.6% h in ad lib. fed controls to 14.7±1.3% h in the high-fat group ($P < 0.001$); calculated NE turnover rate increased from 24.5±2.4 ng/heart per h to 36.8±3.5 ($P < 0.05$). Urinary NE excretion more than doubled after 6 d of the same high fat diet ($P < 0.001$). Ganglionic blockade produced a greater effect on NE turnover in fat-fed, as compared with chow-fed animals, consistent with increased sympathetic activity in the fat-fed group. When fat absorption was blocked with a bile acid binding resin (cholestyramine), the same high-fat diet did not increase cardiac NE turnover, indicating that fat absorption is required for the stimulatory effect on sympathetic activity. In another series of experiments, in which chow (and hence protein) intake was held constant, the effect of fat and isocaloric sucrose supplements on NE turnover was assessed in heart, IBAT, and pancreas. The caloric value of the supplements was 50, 100, and 335% of the chow in the different experiments. An effect of fat on NE turnover in heart and IBAT was demonstrable at the lowest level of fat supplement. Fat increased pancreatic NE turnover when added in amounts sufficient to double the caloric intake. The stimulatory effect of sucrose and fat on NE turnover in heart and IBAT was similar. These experiments demonstrate that fat increases SNS activity in the rat and that the magnitude of the effect is similar to that of sucrose. The results imply that fat may contribute to dietary thermogenesis in this species.

INTRODUCTION

Studies from our laboratory have demonstrated that dietary intake exerts an important effect on sympathetic nervous system (SNS)[1] activity in the rat. Norepinephrine (NE) turnover techniques have shown that fasting suppresses SNS (1), whereas overfeeding sucrose has been shown to stimulate sympathetic activity in heart (2) and other tissues of the rat (3). An important role for carbohydrates in the relationship between dietary intake and SNS activity has been inferred from data indicating that hypoglycemia (4, 5) and 2-deoxy-D-glucose administration (6) suppress sympathetic activity, whereas hyperinsulinemia, in the absence of hypoglycemia, has a stimulatory effect (7, 8). Little is known about the effects of noncarbohydrate nutrients on SNS activity. The demonstration that overfeeding mixed high-caloric diets with substantial fat content increases sympathetic activity (9) suggested that nutrients other than sucrose may influence the SNS as well. The studies described in this report were undertaken to assess the effect of dietary fat on sympathetic activity in the rat.

Sympathetic activity was estimated in heart, pancreas, and interscapular brown adipose tissue (IBAT) by the [^{3}H]NE turnover technique (3, 10). The results demonstrate that dietary fat, after absorption from the gut, increases centrally mediated SNS activity in these

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[1] *Abbreviations used in this paper:* E, epinephrine; IBAT, interscapular brown adipose tissue; NE, norepinephrine; SNS, sympathetic nervous system.

organs. The effect of fat on SNS activity, furthermore, is of the same order of magnitude as that of sucrose.

METHODS

Animals. Female CD (Sprague Dawley-derived) rats, 110–130 g (Charles River Breeding Laboratories, Wilmington, MA) were housed four to six per double cage or two to three per single cage in a constant-temperature animal room (22°C). For urinary catecholamine measurements, rats were housed individually in metabolic cages. The animals were allowed free access to water and to their respective diets, except as noted, before and during the experiments. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication No. [NIH] 78-23, revised 1978).

Diets. The various dietary mixtures were prepared from the following ingredients: standard laboratory chow (Charles River Chow R-M-H 3200; Agway Country Foods, Agway Inc., Syracuse, NY), sucrose, and lard (ICN Nutritional Biochemicals, Cleveland, OH). Food intake was estimated for each cage of animals and converted to energy equivalents according to the following factors: chow, 3.7 kcal/g (manufacturer's estimate of "digestible" energy); sucrose, 4.0 kcal/g; and lard, 9.0 kcal/g.

Pharmacologic agents. Chlorisondamine (Ecolid; CIBA Pharmaceutical Co., Summit, NJ) was dissolved in saline to a final concentration of 10 mg/ml and injected intraperitoneally at a dose of 15 mg/kg. Cholestyramine powder (Questran; Mead Johnson and Co., Evansville, IN) was mixed directly into the chow or chow-lard preparations, in a ratio of 0.05 g cholestyramine/g of diet.

Turnover procedure. L-[ring-2,5,6-^{3}H]NE (sp act 40–50 Ci/mmol; New England Nuclear, Boston, MA) was purified before use by column chromatography with alumina as described below. In the turnover experiment, [^{3}H]NE was diluted to an appropriate concentration (~50 μCi/ml) with isotonic saline and injected intravenously into the tail veins of unanesthetized rats, followed by a saline flush of 0.9–1.0 ml. At preselected times, four to six animals from each group within a given experiment were killed by cervical dislocation. The tissues were quickly removed, frozen on dry ice, and stored at −20°C for later processing (usually within 2 wk).

Extraction and isolation of catecholamines. The tissues were homogenized in ice-cold 0.4 N perchloric acid using a ground-glass homogenizer (Duall-Kontes Glass Co., Vineland, NJ), and centrifuged to separate the protein precipitate. NE was extracted from the perchloric acid supernatants by adsorption onto alumina previously purified by the method of Anton and Sayre (11). To 2 ml of the perchloric acid supernatant were added 1.5 ml of 2 M Tris buffer (pH 8.7; Sigma Chemical Co., St. Louis, MO) containing 2% disodium EDTA (Fisher Scientific Co., Fair Lawn, NJ), 50 μl of reduced glutathione (0.05 M; Sigma), 3.0 nmol of dihydroxybenzylamine (DHBA; Aldrich Chemical Co., Milwaukee, WI) as an internal standard, and 60 mg of alumina. The resultant mixture was shaken for 10 min and then transferred quantitatively to columns fashioned from Pasteur pipettes plugged with glass wool. The columns were washed two to three times with 1–2 ml of doubly distilled water, and the catecholamines were eluted with 0.40 ml of 0.1 N perchloric acid.

Determination of endogenous NE levels. The alumina eluates were analyzed for NE by liquid chromatography with electrochemical detection (12). The chromatographic system consisted of a pump (M45; Waters Associates, Milford MA), an automatic sample injector (WISP 710B; Waters Associates), a reverse-phase 5-μm ODS column (13 cm × 4.6 mm i.d.; Brownlee Labs, Santa Clara, CA), and an electrochemical detector (LC-4A; Bioanalytical Systems Inc., West Lafayette, IN) equipped with a glassy carbon electrode. Detector response was quantitated by peak-height utilizing an integrating recorder (HP3390A; Hewlett-Packard Co., Avondale, PA). Mobile phase consisted of either a sodium phosphate buffer (0.1 M, pH 5.8) containing 0.1 mM EDTA, 150 mg/liter sodium octyl sulfonate (Eastman-Kodak Co., Rochester, NY), and 5% methanol or an acetate/citrate buffer composed of 100 mM sodium acetate, 40 mM citric acid, 60 mM NaOH, 0.1 mM EDTA, 1.0 mM sodium octyl sulfonate, and 10% methanol. In either case, flow rate was set at 1.5 ml/min. Detector potential was set at +0.65 V vs. a Ag/AgCl reference electrode. Detection limits (signal/noise > 5) for NE standards injected onto the system were 15–20 pg. Results were corrected for recovery, which ranged between 65 and 80%; intraassay coefficients of variation for NE were usually <1%. Within a given experimental group, tissue NE levels did not vary over the time course of the turnover measurement.

Measurement of [^{3}H]NE. Aliquots of the alumina eluates were counted for [^{3}H]NE by scintillation spetrometry in a Packard 460C liquid scintillation counter (Packard Instrument Co., Downers Grove, IL). Efficiency for ^{3}H in this system is 30–35%.

Urine collection and analysis. Urine from rats in individual metabolic cages was collected in acid under paraffin oil. After collection, urines were stored at −20°C until processing. After addition of 0.5 ml of 2% EDTA and 10 mg sodium metabisulfite, the catecholamines were extracted from the urine samples by adsorption onto a cation exchange resin (AG-50W × 4, 100–200 mesh; Bio-Rad Laboratories, Rockville Center, NY) and eluted with 2 N HCl. Before analysis, the catecholamines in the acid eluate were concentrated by passage over alumina. 5.0 ml of the acid eluate were combined with an equal volume of 2 M Tris buffer (pH 8.7 containing 2% EDTA), 0.05 ml of 0.05 M reduced glutathione, and 60 mg of alumina. Catecholamines were eluted from the alumina with perchloric acid and analyzed amperometrically as described above.

Data analysis. Data are presented as the mean±SEM unless otherwise noted. In NE turnover studies, data were plotted semilogarithmically. The slope of the decline in NE specific activity with time (the fractional turnover rate, k) was estimated by the method of least squares. Statistical significance of the linear regression was assessed by analysis of variance (ANOVA), routinely achieving significance at $P < 0.0001$. Endogenous levels of NE did not vary significantly (ANOVA) over the 24 h of the turnover measurement in any of the tissues analyzed in either the control or experimental groups. Comparisons among fractional turnover rates were made by analysis of covariance (13). The calculated NE turnover rate was computed as the product of the fractional turnover rate (k) and the mean endogenous NE content [NE]. Standard errors and 95% confidence intervals for calculated turnover rates were estimated by a modification of propagation-of-errors analysis (14). Urinary NE measurements were compared by ANOVA for blocked data. Comparisons among mean body weights and caloric intakes were made by the t test or by analysis of variance as appropriate. In experiments requiring multiple comparisons,

the presence of statistically significant variation was established among all groups simultaneously before individual comparisons between any two groups. Two-group comparisons then utilized either repeat analysis of covariance (to compare fractional turnover rates) or the Newman-Keuls multiple-range test (for all other data).

RESULTS

Effect of dietary fat on cardiac NE turnover. In the initial experiments, the effect of dietary fat and sucrose supplements on NE turnover in heart was assessed. 6 d before the experiment, a large group of animals was fed ~25% of their normal dietary intake as powdered chow. 5 d before the experiment, the animals were divided into three groups: One continued to receive the restricted chow ration for the remainder of the experiment (control group); the second received the chow ration supplemented by sucrose to provide approximately a threefold increase in caloric content; the third group received the same chow ration supplemented with lard in an amount isocaloric to the sucrose supplement (Table I). The experimental diets were continued for 5 d before and during the day of the turnover study. The effect of the sucrose and fat supplements on cardiac NE turnover is shown in Fig. 1; the data for the experiment are summarized in Table I. Addition of both fat and sucrose to the chow ration substantially increased cardiac NE turnover. Both supplements produced a similar (threefold) elevation in fractional NE turnover in heart which increased from 4.1±0.8%/h in controls to 13.1±1.3%/h in sucrose fed rats and to 15.5±1.1%/h in the rats receiving the fat supplement ($P < 0.0001$). As compared with the restricted chow control, fat supplementation significantly reduced cardiac NE content (Table I); calculated NE turnover rate (the product of the endogenous

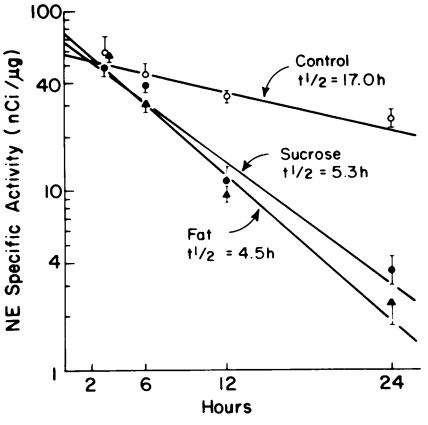

FIGURE 1 Effect of fat and sucrose supplements on NE turnover in rat heart. Data are plotted as the mean±SEM for specific activity of hearts from four to six animals from each group at each time point. Open circles denote rats fed reduced-chow ration (control); closed circles, the sucrose-fed rats; and closed triangles, the fat-fed rats. The slope, k, of each turnover line is significant at $P < 0.0001$. Feeding protocol was as described in legend to Table I. On the day of the experiment (after 5 d of dietary preparation), animals were injected with tracer [^{3}H]NE (50 µCi/kg) and killed after 3, 6, 12, and 24 h. Specific activity of cardiac NE is plotted semilogarithmically as a function of time. Both fat and sucrose, when added to chow for 5 d, significantly increased cardiac NE turnover, in comparison with restricted chow control animals.

TABLE I
Effects of Dietary Sucrose and Fat on Cardiac NE Turnover

Group	Final body weight	Dietary intake			Heart		NE turnover rate		
		Chow	Supplement	Total calories	Weight	NE content	Fractional (slope, k)	Calculated	
	g	*g/d*	*g/d*	*kcal/d*	*g*	*ng*	*%/h*	*ng/h*	*95% confidence limits*
Control (restricted chow) (n = 20)	94.6±2.1	3.6	—	13.3	0.275±0.007	324±11	4.1±0.8	13.2±2.8	8.8–19.9
Sucrose supplemented (n = 20)	109.4±1.6*	3.6	7.8	44.5	0.373±0.008*	285±22	13.1±1.3‡	37.2±4.7§	29.0–47.7
Fat supplemented	104.2±1.6*	3.6	3.5	44.4	0.356±0.006*	211±10‖	15.5±1.1‡	32.8±2.7§	27.8–38.6

Data are presented as means±SEM. All animals received a restricted chow ration (25% normal intake) for 5 d before and including the day of the NE turnover study. The supplemental groups received, in addition, isocaloric sucrose or fat supplement for the same time period. Protein intake was the same in all groups. Fat and sucrose supplemented groups differed only with respect to final body weight ($P < 0.05$) and cardiac NE content ($P < 0.001$).

* $P < 0.01$.

‡ $P < 0.0001$.

§ $P < 0.05$.

‖ $P < 0.001$.

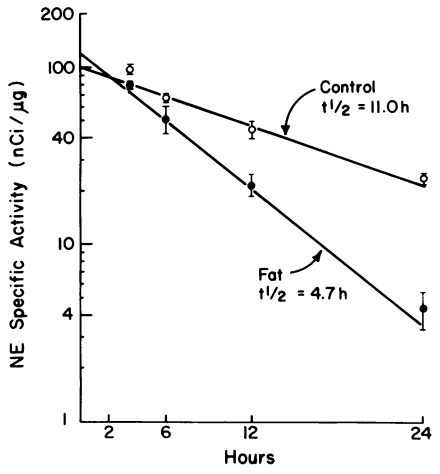

FIGURE 2 Effect of a fat-enriched diet on NE turnover in rat heart. Data are plotted as the mean±SEM for specific activity of hearts from four to six animals from each group at each time point. Open circles denote chow-fed rats (control), whereas closed circles represent fat-fed animals. The slope, k, of each turnover line is significant at $P < 0.0001$. Experimental protocol was as described in the legend to Table II and the legend to Fig. 1. 4 d of a high-fat diet significantly increased cardiac NE turnover.

NE level and the slope) was nonetheless significantly increased (~250%), as shown in Table I. This experiment demonstrates a definite and unequivocal effect of fat on cardiac NE turnover. Other data (not shown) indicate that an effect of fat on cardiac NE turnover is demonstrable during the first day of fat feeding.

The effect of a fat-enriched diet on cardiac NE turnover in animals feeding ad lib. is shown in Fig. 2. In this experiment, the control animals consumed a normal chow diet ad lib.; the experimental group consumed, also ad lib., a high-fat diet consisting of 50% chow and 50% lard (by weight) for 4 d before and during the turnover study. The data are summarized in Table II. Although total caloric intake was not significantly different in the two groups, the fat-fed animals displayed a marked increase in cardiac NE turnover. Fractional NE turnover was increased >200% from 6.3±0.6%/h in controls in 14.7±1.3%/h in fat-fed group ($P < 0.001$); despite a fall in endogenous NE content in the fat-fed group, calculated NE turnover rate was significantly increased (Table II). This experiment demonstrates that a high-fat diet fed ad lib. significantly increases cardiac NE turnover when compared with normally fed controls, even in the absence of a significant increase in total caloric intake.

The effect of ganglionic blockade on NE turnover in hearts of fat-fed rats. The relationship between cardiac NE turnover and centrally mediated sympathetic activity is demonstrated by the effect of ganglionic blockade on the retention of tracer [^{3}H]NE. When SNS activity is increased, as in cold exposure or sucrose feeding, the effect of ganglionic blockade on NE turnover is correspondingly greater than in control animals (3), since impulse traffic at the level of the ganglion is increased. In the experiment shown in Fig. 3, control animals ate chow ad lib.; fat-fed animals received a high-fat diet consisting of 50% chow and 50% lard by weight for 4 d before the experiment. After the administration of tracer, half the animals from each group received the long-acting ganglionic blocking agent chlorisondamine. 10 h after the administration of tracer, the specific activity in control and fat-fed chlorisondamine-treated animals was similar (Fig. 3); since NE turnover was increased in fat-fed animals (lower specific activity of NE in hearts of fat-fed control group), this represents a substantially greater effect on retention of [^{3}H]NE in fat-fed as compared with control groups (281% increase in tracer

TABLE II
Effects of a Fat-enriched Diet on Cardiac NE Turnover

Group	Final body weight	Dietary intake			Heart		NE turnover rate		
		Chow	Fat supplement	Total calories	Weight	NE content	Fractional (slope, k)	Calculated	
	g	*g/d*	*g/d*	*kcal/d*	*g*	*ng*	*%/h*	*ng/h*	*95% confidence limits*
Control (n = 22)	145.3±1.6	15.0±0.2	—	55.5±0.7	0.460±0.007	389±11	6.3±0.6	24.5±2.4	20.2–29.8
Fat supplemented	135.5±1.2	5.1±1.5	5.1±1.5	65.2±19.4	0.465±0.005	250±11	14.7±1.3	36.8±3.5	30.5–44.4
P vs. control	<0.0001	—	—	NS	NS	<0.0001	<0.001	<0.05	—

Data are presented as means±SEM. Fat supplemented group consumed ad lib. a diet 50% fat by weight for 4 d before and including the day of the turnover study; controls consumed chow ad lib. Initial body weight did not differ (126.0±1.5 g, chow; 129.1±1.9 g, fat). Weight gain over the 4–5-d experiment was significantly less in the fat-fed group (19.3±1.0 g control; 6.4±1.1 g fat; $P < 0.001$).

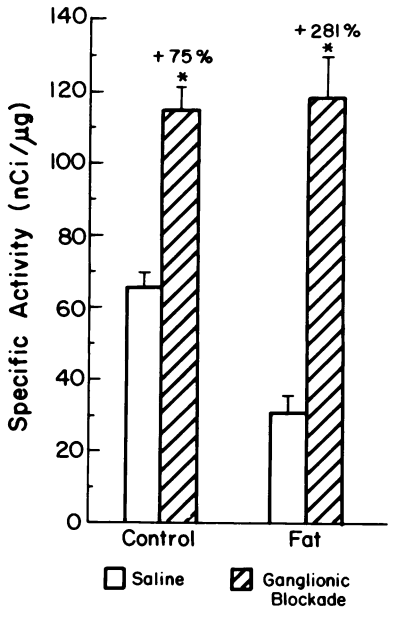

FIGURE 3 Effect of ganglionic blockade on retention of [^{3}H]NE in hearts of control and fat-fed rats. Data are presented as specific activity (mean±SEM). Control animals ate chow ad lib.; fat-fed animals received fat supplemented chow (50% by weight) ad lib. for 4 d before the experiment. On the day of the experiment, all animals received tracer [^{3}H]NE (50 μCi/kg); half of each group received chlorisondamine (15 mg/kg i.p.) 5 min and again 5 h after tracer. Controls received saline diluent at the same times. All animals were killed 10 h after the injection of tracer. Open bars denote saline-treated rats; shaded bars represent chlorisondamine-treated animals. The number over the bar is the percentage increase over saline-treated control. *, $P < 0.0001$ comparing ganglionic blockade and saline treatment.

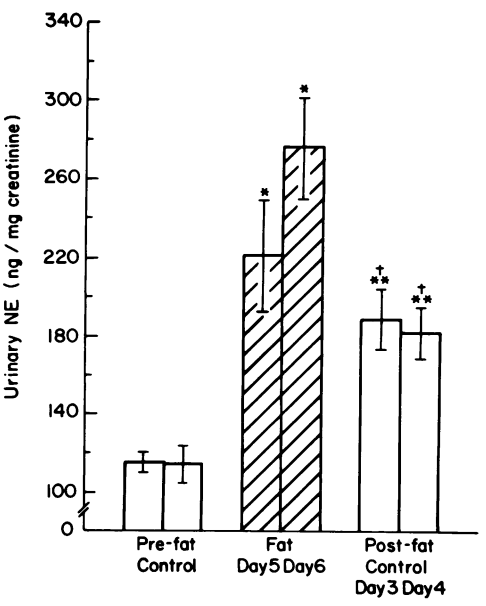

FIGURE 4 Effect of a high-fat diet on urinary NE excretion. Data are presented as nanograms NE per milligram creatinine (mean±SEM for 24-h urine samples from eight animals). After acclimation to metabolic cages, the animals were fed chow ad lib. for 4 d (prefat control), fat-supplemented diet (50% by wt) for 6 d, and returned to chow for 4 additional days (postfat control). Two consecutive urine collections were analyzed on the last 2 d of each dietary regimen. Open bars denote chow (control), while hatched bars represent the high-fat regimen. Urinary NE excretion varied significantly among the three treatment periods ($P < 0.0001$): *, $P < 0.001$ comparing fat feeding to prefat control; **, $P < 0.005$ comparing postfat to prefat controls; †, $P < 0.001$ comparing fat feeding (day 6) with postfat control.

retention at 10 h, as compared with 75% in chow-fed controls). The greater effect of chlorisondamine on cardiac NE turnover in fat-fed animals is consistent with enhanced sympathetic outflow to the hearts of these animals.

Effect of high-fat diet on urinary NE excretion. To clarify further the effect of dietary fat on SNS activity, urinary catecholamine excretion was measured before and after the imposition of a high fat diet (50% lard by weight). After acclimation to metabolic cages, eight rats were fed powdered chow for 4 d, followed by 6 d of the high-fat diet (chow and lard) fed ad lib. The animals were then returned to the chow diet for an additional 4 d. Urine was collected for the 2 final days in each period and analyzed for catecholamines and creatinine. The results are shown in Fig. 4. Fat feeding was associated with a significant increase in NE excretion; on day 5 of fat feeding, NE excretion was 93% greater than control and on day 6, 140% higher. NE excretion diminished during the postfat control days, although it did not return to the level of the initial control period. Epinephrine (E) excretion, by contrast, was unchanged by fat feeding, averaging 31.4±5.5 ng/mg of creatinine during basal collections, 37.7±6.3 during fat feeding and 44.6±3.0 after fat feeding ($F[2, 20] = 1.62$; P = NS), suggesting that fat does not increase adrenal medullary activity. In the absence of a rise in E excretion, the increased urinary NE can be attributed to increased SNS activity. The absence of a change in urinary E argues against a change in renal catecholamine excretion as an explanation for the increase in urinary NE.

This experiment demonstrates that the increase in NE turnover demonstrated in the previous experiments cannot be attributed to a fall in endogenous NE concentration. If the increased turnover were due entirely to diminished pool size, no change in urinary NE would be anticipated once steady-state had been achieved. The urinary NE excretion data, therefore, are consistent with increased sympathetic nervous system activity.

Effect of cholestyramine-induced fat malabsorption on cardiac NE turnover in fat-fed rats. Although the experiments described above demonstrate an increase in SNS activity after fat feeding, the mechanisms involved are obscure. The fact that animals fed a fat-enriched diet ad lib. gained less weight than chow-fed controls (Table II) raised the possibility that diarrhea or malabsorption induced by the high-fat diet might activate the SNS in response to volume depletion or sequestration of extracellular fluid within the intestinal lumen. To distinguish the effect of fat in the gut from postabsorptive processes relating to fat metabolism, fat malabsorption was induced with cholestyramine, a bile acid binding resin, in both fat-fed and chow-fed animals. The rats in this experiment were divided into four groups: One group received chow only, the second group received chow containing cholestyramine (5% by weight), the third group received the high-fat diet consisting of chow and lard (50% by weight), and the fourth group received the high fat diet with cholestyramine. The animals ate their respective diets for 4 d before and including the day of the NE turnover study. The results are summarized in Table III and Fig. 5. Cholestyramine-induced malabsorption was confirmed by the marked weight loss (10% of body weight) in the fat-fed group treated with the bile-acid binding resin. As in the previous experiments, animals fed the high-fat diet had a marked increase in cardiac NE turnover (Fig. 5, Table III). Fractional NE turnover increased from 9.3±0.9%/h in chow-fed animals to 18.7±1.3%/h in the fat fed group ($P < 0.001$). Cholestyramine treatment, which was without effect in the chow-fed rats, resulted in a marked suppression of SNS activity in animals fed the high-fat diet (fractional turnover 6.2±0.9% h), despite the presence of considerable fat malabsorption and steatorrhea (Fig. 5, Table III). This experiment clearly demonstrates that fat within the gut cannot account for the increase in NE turnover demonstrated during fat feeding. The results demonstrate that absorption (and presumably metabolism) of the fat is required for fat-feeding to stimulate SNS activity.

Comparative effects of fat and sucrose supplements on NE turnover in heart, IBAT, and pancreas. Additional experiments were performed to compare the effects of fat and sucrose on NE turnover in heart and other organs of interest. In the first experiment (Fig. 6), the effect of smaller supplements of sucrose and fat on NE turnover in heart, IBAT, and pancreas was studied. At the beginning of the experiment, animals were divided into three groups: control (chow only), sucrose supplemented, and fat supplemented. The sucrose and fat supplements were isocaloric and provided a 50% increase in caloric intake. Animals in all three groups were fed the same amount of chow, averaging ~8 g/100 g body wt per d. The experimental diets were fed

TABLE III
Effects of Cholestyramine-induced Fat Malabsorption on Cardiac NE Turnover in Fat-fed Rats

Group	Final body weight	Dietary intake			Heart		NE turnover rate		
		Chow	Fat supplement	Total calories	Weight	NE content	Fractional (slope, k)	Calculated	
	g	*g/d*	*g/d*	*g/d*	*g*	*ng*	*%/h*	*ng/h*	*95% confidence limits*
Chow only (n = 19)	146.2±2.4	15.4±1.1	—	57.0±4.1	0.471±0.010	342±14	9.3±0.9	31.7±3.5	25.6–39.2
Chow + cholestyramine (n = 20)	142.1±1.8	17.1±1.7	—	66.2±6.7	0.448±0.006	343±12	9.6±1.2	32.9±4.2	25.6–42.2
Fat 50% (n = 16)	120.2±1.1	3.7±0.7	3.7±0.7	47.4±9.4	0.448±0.001	273±16	18.7±1.3	50.9±4.7	42.5–61.0
Fat 50% + cholestyramine (n = 15)	108.4±1.7	3.6±0.5	3.6±0.5	46.6±6.8	0.333±0.009	408±23	6.2±0.9	25.2±4.0	18.4–34.4

Data are presented as means±SEM. Animals were assigned to one of four treatment groups: chow, chow + cholestyramine (5% of diet by wt), fat (lard 50% of diet by wt), or fat + cholestyramine. Initial weights of animals in each group were similar. All animals ate the diet ad lib. for 4 d before and including the day of turnover study. Significant differences included the following. For k: chow vs. fat, $P < 0.001$; fat vs. fat cholestyramine, $P < 0.0001$. For endogenous NE content: chow vs. fat, $P < 0.005$; fat vs. fat-cholestyramine, $P < 0.0001$. For calculated NE turnover rate: fat vs. fat cholestyramine, $P < 0.05$. The amount of weight gained was significantly different in all the treatment groups ($P < 0.01$), as reflected in the final weight (initial weight averaged 120 g). Although caloric intake appeared lower in the fat-fed groups, the difference was not statistically significant.

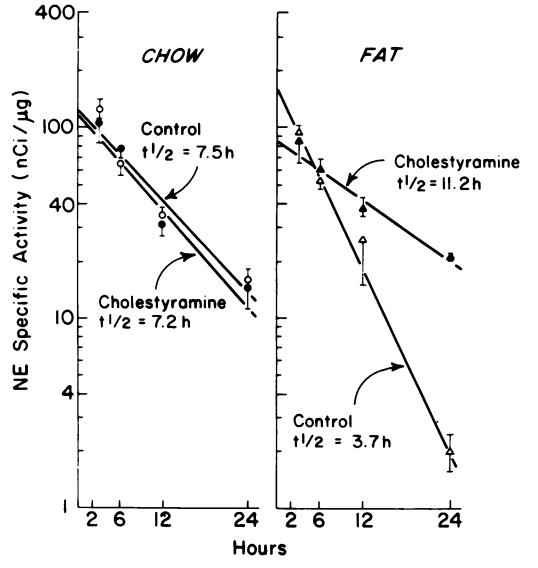

FIGURE 5 Effect of fat malabsorption on cardiac NE turnover in fat-fed rats. Data are plotted as the mean±SEM for specific activity of hearts (four to five animals from each group at each time point). Circles denote chow-fed animals; triangles indicate fat feeding (50% by weight). Open symbols represent the control groups; closed symbols represent cholestyramine treated animals (5% by weight). Each group ate the respective diet ad lib. for 4 d before and including the turnover study. The slope k of each turnover line is significant at $P < 0.0001$. Statistical comparison of the slopes (k) is presented in the legend to Table III. Cholestyramine completely blocked the increase in cardiac NE turnover associated with the high-fat diet.

for 4 d before and including the day of the turnover study. NE turnover in heart and IBAT (Fig. 6) was significantly increased by both fat and sucrose. In heart, fractional turnover rate went from 5.4±0.7%/h in control animals to 11.4±0.8%/h in the fat supplemented group ($P < 0.0001$) and to 11.1±1.1%/h in the sucrose-fed group ($P < 0.001$). Endogenous NE level was significantly reduced in heart by both fat and sucrose feeding from 533.4±19.2 ng to 429.0±15.1 ng in fat and to 450.0±26.4 ng in the sucrose group ($P < 0.01$ for both vs. control). Nonetheless, calculated NE turnover rates were significantly different from control in fat- and sucrose-fed groups with values of 28.5±8.5 ng/h in control compared with 48.9±8.3 ng/h in fat fed and 50.1±12.4 ng/h in the sucrose supplemented animals (means±95% confidence intervals). In IBAT (Fig. 6 *B*), fractional turnover rate was significantly increased by both fat and sucrose feeding from 7.8±1.0%/h in control animals to 11.7±0.9%/h in fat-fed ($P = 0.005$) and to 11.1±0.8%/h in the sucrose-fed animals ($P < 0.02$). Endogenous IBAT NE content was not changed significantly by fat or sucrose feeding. NE turnover in pancreas in the same study (not shown) revealed no significant change with fat or sucrose feeding. The effects of fat and sucrose on NE turnover in heart and IBAT were similar. This experiment demonstrates that modest supplements of sucrose and fat increase NE turnover in heart and IBAT and that isocaloric sucrose and fat supplements increase SNS activity to the same extent.

To ascertain the comparative effects of larger dietary supplements on NE turnover in these organs, a similar protocol was employed substituting a lower chow ration (~6 g/100 g body wt per day) and greater supplements of sucrose and fat, the latter representing, in this experiment, a 100% increase in caloric intake above that in chow-fed controls. In this experiment, both fat and sucrose increased NE turnover in heart and IBAT, as expected. In heart, fractional turnover (k) was increased by 78% in sucrose-fed animals and by 111% in animals fed the fat-supplemented diet; k in chow-fed controls was 3.83±5.4%/h, as compared with 6.81±0.82%/h in sucrose-fed rats ($P < 0.005$) and 8.07±0.92%/h in the fat-supplemented group ($P < 0.005$). Calculated turnover rates were similarly increased 52% in the sucrose-fed animals and 93% in the fat-fed group. There was no significant difference between the fat-fed and the sucrose-fed groups; in IBAT, fat and sucrose increased fractional NE turnover 92 and 113%, respectively, from a k value of 5.2±1.1%/h in control animals to 10.2±0.9%/h in the fat fed group and to 11.3±1.2%/h in those receiving the sucrose supplement ($P < 0.001$ vs. control for each supplemented group). Calculated NE turnover rate was increased 60% in the fat-supplemented group and 115% in those fed sucrose. There was no difference between the fat-supplemented and the sucrose-supplemented group.

In pancreas, NE turnover was also significantly increased by the larger fat supplement (k = 5.8±0.9%/h in control-fed; 9.0±0.9%/h in the fat-fed group, an increase of 55%; $P = 0.016$). Endogenous pancreatic NE was unchanged in the different diet groups (223±9 ng in control, 204.8±8 ng in fat-fed, and 232±9 ng in the sucrose-fed group). Calculated NE turnover was increased 89% in the fat-fed group. The sucrose-fed animals did not differ significantly from controls (k = 6.8±0.8%/h, P = NS). This experiment demonstrates a significant effect of fat feeding on pancreatic NE turnover at the higher level of fat consumption.

DISCUSSION

Diet-induced changes in SNS activity were first described in fasting (1) and sucrose-fed (2) rats in studies utilizing NE turnover techniques to measure sympathetic activity in different organs (9, 15). Subsequent

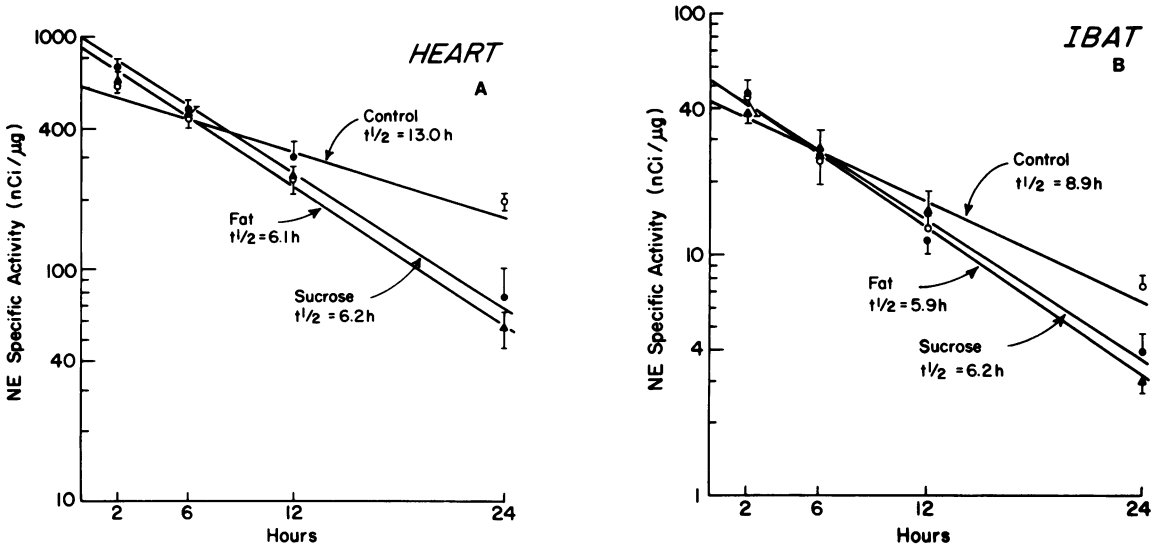

FIGURE 6 Effect of fat and sucrose supplements on NE turnover in heart and IBAT. Data are plotted as the mean±SEM for specific activity of NE from four to six animals from each group at each time point. Open circles denote (control) chow-fed animals; closed circles, the sucrose-supplemented group; and closed triangles, the fat-fed animals. The fat and sucrose supplements were isocaloric and added in sufficient quantity to increase caloric intake by 50%. The respective diets were fed for 4 d before and including the NE turnover study. Fractional turnover was significantly increased by fat and sucrose feeding in both heart (*A*) and IBAT (*B*).

studies have clearly demonstrated that diet affects SNS activity in human subjects as well (7, 16–21). The effect of carbohydrates, particularly glucose and sucrose, has been studied most intensively; a stimulatory effect on the SNS in rats (2, 3, 22) and humans (18, 19, 23, 24) is generally recognized. Evidence in favor of central nervous system glucose metabolism in coupling changes in dietary intake with changes in SNS activity has been deduced from the observations that hypoglycemia (4, 5) and 2-deoxy-D-glucose (6) suppress sympathetic activity, whereas simultaneous infusions of insulin and glucose, which avoid hypoglycemia, stimulate the SNS (7, 8, 25). The observation that overfeeding a mixed diet stimulates SNS activity in rats (9) and in human subjects (16) suggested, however, that other nutrients, and possibly other mechanisms may be involved.

The present study clearly demonstrates that fat, added to the chow diet in the form of lard, increases sympathetic activity in the rat. Evidence of SNS stimulation during fat feeding includes increased NE turnover in heart, IBAT, and pancreas, and increased urinary NE excretion. The relationship between changes in NE turnover and centrally mediated sympathetic activity is established by the experiment involving ganglionic blockade; the greater effect of ganglionic blockade on NE turnover in the fat-fed animals is consistent with increased impulse traffic at the level of the ganglia. A similar effect of ganglionic blockade on NE turnover has been noted in other situations associated with sympathetic stimulation such as cold exposure and sucrose feeding (3, 5). The increase in urinary NE excretion, without an increase in E excretion is also evidence of SNS stimulation. The fact that the urinary NE did not return to the base-line level after restitution of normal diet for 4 d suggests either that the effect of fat on sympathetic activity is more prolonged than that of sucrose (22) or that the increased size of the rat, owing to growth over the course of the experiment, resulted in an actual change in base line. The increase in urinary NE excretion, coupled with the increase in NE turnover in three different organs suggests that the effect of fat on SNS activity is a generalized one.

The fall in endogenous NE content in the hearts of fat-fed rats (Tables I–III) is not completely understood. Situations associated with increased NE turnover, such as sucrose feeding and cold exposure, often show decreased endogenous NE levels, but rarely to the extent seen here with fat (3). Fat feeding may, therefore, exert an independent effect on NE storage. It should be noted, however, that the decreased NE levels in the hearts of fat fed animals do not invalidate the results of the turnover studies or alter the conclusion that sympathetic activity is increased by fat feeding. Since the endogenous cardiac NE level was constant throughout the turnover in the fat-fed animals, the steady-state

assumption implicit in the turnover technique is not violated. As indicated in Results, the fall in endogenous level cannot account for the increased NE turnover, since both the calculated turnover rate, which takes the fall in endogenous NE level into account, and the urinary NE excretion were increased in fat-fed animals.

Direct comparison between the effects of isocaloric supplements of fat and sucrose over a wide range of intakes (where the supplements represented 50, 100, and 335% of the calories provided as chow) indicates that fat and sucrose stimulate the SNS to the same extent. This is in distinction to acute-feeding studies in humans that demonstrate sympathetic activation with 400 kcal of glucose but not with fat (19); the relationship of these acute-feeding studies in man, however, to the present results in rats, is uncertain.

The mechanisms involved in stimulation of SNS activity by fat are not clear. The present study (Fig. 5, Table III) demonstrates that the stimulatory effect of fat cannot be attributed to undigested lipid within the gut lumen. Fat malabsorption, induced by cholestyramine, completely blocked the stimulatory effect of fat on cardiac NE turnover. Similarly, differences in protein intake cannot explain the effect of dietary fat on sympathetic activity. Although chronic protein restriction (with isocaloric substitution of sucrose for casein) increases SNS activity (26), diminished protein intake cannot explain the effect of fat demonstrated in the present studies, since dietary protein was the same (equal chow rations) in the fat-fed and control rats in the experiments described in Fig. 1 and Table I and Fig. 6.

Evidence in favor of central nervous system glucose metabolism, perhaps stimulated by insulin (7, 27), has been advanced to explain the relationship between dietary intake and SNS activity. It seems unlikely that the effects of fat described here are explicable in terms of an insulin-glucose model, despite the fact that a recent report demonstrates that fat-feeding in the rat is associated with a modest increase in insulin level (28). The involvement of other neural and hormonal mechanisms, perhaps related to vagal afferents from the liver or gut (29), and perhaps involving cholecystokinin seems more likely. Cholecystokinin, as well as glucose, for example, has been shown to decrease afferent hepatic vagal discharge, effects opposite to those of 2-deoxy-D-glucose (29). Different nutrients may, therefore, influence the SNS via different sets of signals that involve afferent neurons and circulating hormones.

The fact that fat stimulates SNS activity is consistent with the demonstrated effects of mixed diets on sympathetic activity in rats (9) and humans (20). Although the physiologic significance of diet-induced changes in SNS activity is not entirely clear, a reasonable case has been made for an important role of the SNS in the regulation of energy expenditure as a function of dietary intake (30), thus contributing to dietary thermogenesis. That fat increases sympathetic activity in rat IBAT, the major thermogenic organ in this species (31), is consistent with participation of dietary fat in the stimulation of diet-induced thermogenesis.

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