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Effects of Energy Restriction on Norepinephrine Turnover and Serum Glucose and Fatty Acids in Lean Mice[1,2]

JANIS E. DAVIS-STREET[3] AND JANICE L. JOHNSTON[4]

Department of Foods and Nutrition, University of Alberta, Edmonton, Alberta, Canada T6G 2M8

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DAVIS-STREET, J. E. AND J. L. JOHNSTON. *Effects of energy restriction on norepinephrine turnover and serum glucose and fatty acids in lean mice*. PHARMACOL BIOCHEM BEHAV **35**(3) 677–683, 1990.—Norepinephrine (NE) turnover rate was determined in several tissues of 5-week-old female mice fed a high carbohydrate diet (58% of energy as carbohydrate, 30% fat) either ad lib or restricted to 34 or 24 kJ/day (36 to 50% restriction) presented as 1 or 2 daily meals. When the restricted intakes were divided into 2 equal meals, daily NE turnover did not differ from that of ad lib-fed mice. When the above restricted amounts were provided as a single daily meal at the beginning of the dark period, NE turnover was 38% and 46% lower, respectively, in the heart only compared to ad lib-fed controls. Serum glucose and total free fatty acids were affected by dietary conditions known to produce sympathetic activation (high carbohydrate and high fat diets) and suppression (high protein diet and energy restriction as a single meal), but the changes were unrelated to fractional NE turnover. Thus, the lower NE turnover seen when food intake is restricted is due to the prolonged overnight fast and not due to the lower energy intake per se, and is not associated with serum concentration of glucose or total free fatty acids.

Energy restriction Norepinephrine turnover Glucose Free fatty acids

DIET-INDUCED changes in sympathetic nervous system activity have been proposed to mediate changes in energy expenditure via diet-induced thermogenesis (19). Thus, it is logical to hypothesize that energy intake per se, regardless of macronutrient source, will affect sympathetic activity. Evidence that energy intake affects sympathetic nervous system activity is most clearly demonstrated by the observations that sucrose overfeeding stimulates (34) and fasting suppresses (35), NE turnover in rats. Feeding isocaloric high fat or high carbohydrate diets, with protein concentration as percent of energy held constant, similarly increases NE turnover in sympathetically innervated organs when compared to controls fed a nonpurified diet (15, 27, 31, 32). These dietary manipulations affect the intake of all nutrients and make it difficult to attribute the effects to any particular nutrient, or energy intake. Moreover, energy intake is almost always affected in the studies of macronutrient manipulation either by the alteration in palatability and, hence, voluntary food intake, or by controlling differences in voluntary food intake by pair feeding or restricting food intake resulting in a prolongation of the overnight fast. Thus, the effects of energy intake, particularly energy restriction, on NE turnover have not been adequately tested.

The mechanisms of diet-induced sympathetic activation and suppression have not been identified. Intracellular glucose utilization has been suggested as a possible mediator of carbohydrate-induced sympathetic activation (26), through involvement of central mechanisms (36). For example, intraperitoneal injection of gold thioglucose, which blocks the uptake of glucose into target cells and purportedly destroys the ventromedial hypothalamus, abolishes changes in cardiac NE turnover in response to fasting and sucrose overfeeding (36). Ingestion of fat alone or acute elevation of plasma fatty acids does not stimulate a significant insulin release and, hence, glucose utilization in humans (16). However, when dietary fat is fed in conjunction with carbohydrate, insulin secretion is stimulated (5) and, hence, high-fat diets containing some carbohydrate may affect sympathetic activity via insulin release. Plasma free fatty acid concentration may also effect sympathetic activity indirectly through impairment of hepatic insulin uptake, hence, prolonging glucose utilization (3). Thus, the first objective of this study was to investigate the effect of restricted energy intake and meal frequency on NE turnover in

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[3]Current address: Children's Nutrition Research Center, Houston, TX 77030.

[4]Requests for reprints should be addressed to Dr. Janice L. Johnson, Department of Foods and Nutrition, University of Alberta, 308 Home Economics Bldg. Edmonton, Alberta, T6G 2M8, Canada.

several sympathetically innervated organs including heart, interscapular brown adipose tissue (IBAT), kidney and pancreas. These organs were selected because of their involvement in blood pressure regulation, thermoregulation, and endocrine function, three processes controlled by the sympathetic nervous system. The second objective was to investigate the effects of diets known to affect cardiac NE turnover on serum glucose and total free fatty acids.

METHOD

Animals and Diets

Four-week-old lean female mice (C57BL/6J+/+, Jackson Laboratories, Bar Harbor, ME) were used in all experiments (n = 18 mice per treatment). Mice were housed in individual hanging wire-mesh cages in a temperature-controlled room (23 ± 2°C), artificially lit for 12 hr daily. The mice were allowed ad lib access to water throughout all experiments. One week prior to each experiment, animals were adapted to the purified high carbohydrate diet consisting of 22% of metabolizable energy as protein, 58% as carbohydrate and 20% as fat containing in g/100 g diet: casein 21.1 (casein was 87% protein), dextrose 60.6, corn oil 8.2, AIN mineral mix (2) 3.7, AIN vitamin mix (2) 1.9, cellulose 4.2, and choline bitartrate 0.3. Casein, cellulose (Celufil) and AIN vitamin mix were obtained from United States Biochemical, Cleveland, OH; dextrose from Fisher Scientific Canada, Edmonton; corn oil from Best Foods Canada, Montreal; mineral mix and choline bitartrate from ICN Nutritional Biochemicals, Cleveland, OH. The mice were then randomly assigned to the experimental diets for 3 days. In all experiments, food intake, corrected for spillage, was monitored every second day during the adaptation period, and daily when the experimental diets were fed. Body weights were measured on the first day of adaptation and on the first and third days of the experimental period.

Experimental Protocol

Mice were fed the high carbohydrate diet ad lib or restricted to 34 kJ/day fed as two daily meals presented at the beginning and middle of the dark period (Experiment 1) or as a single daily meal presented at the beginning of the dark period (Experiment 2). In Experiment 3, mice were fed the high carbohydrate diet either ad lib, or restricted to 24 kJ fed either as a single daily meal presented at the beginning of the dark period or as two daily meals presented at the beginning and middle of the dark period. In Experiment 4, 6 mice per group were fed one of four diets concurrently: each of the three high carbohydrate diets as in Experiment 3, or a high protein diet (40% protein, 36% carbohydrate, 23% fat) (12) ad lib. In all experiments, mice were killed at the beginning of the light period.

In Experiments 1 to 3, NE turnover rate was measured in heart, kidney, pancreas and IBAT from the decline in NE concentration over 6 hours after synthesis inhibition with α-methyl tyrosine (4,12). The rate of decline in endogenous NE after α-methyl tyrosine is monoexponential in several tissues (4,12) and provides turnover rates similar to those using tritiated NE (4). On day 4 at the beginning of the light period, mice were injected IP with α-methyl tyrosine (α-methyl D,L-tyrosine methyl ester hydrochloride, Sigma Chemical Co., St. Louis, MO) (400 mg/kg, in 0.9% saline) and killed by decapitation at 0, 3, 6 hr after injection. Heart, kidney (capsule removed), pancreas and IBAT were rapidly dissected, blotted with tissue to remove excess blood, weighed, wrapped in preweighed aluminum foil, frozen on dry ice and held at −40°C until analysis for NE content within 1 month.

In Experiment 4, serum concentrations of glucose and total free fatty acids were determined on day 4, in mice fed one of the above 4 diets. Mice were killed by decapitation, commencing at the beginning of the light period. Blood was drained from the thoracic stump into cold, untreated test tubes, centrifuged after clotting, and the serum held at −40°C for subsequent analysis within 2 months.

Analytical Method

NE in all organs was isolated by alumina extraction under alkaline conditions from the tissue homogenate supernatant as previously reported (17). Detection of NE was achieved by high performance liquid chromatography (HPLC) (model 2000, Varian Canada Inc., Georgetown, Ontario) with electrochemical detection (model LC4, Bioanalytical Systems Inc., West Lafayette, IN) (17).

Serum glucose was determined in duplicate by a glucose oxidase analyzer (Beckman Instruments, Inc., Fullerton, CA) (1).

Serum free fatty acids were determined by a standard enzymatic colorimetric method for the quantitative determination of free fatty acids (NEFA C. Wako Chemicals U.S.A., Inc., Dallas, TX) (22).

Statistical Analysis

NE data were plotted semilogarithmically with NE turnover being calculated by linear regression of the logarithm of NE concentration over 3 time points (0, 3, 6 hr). The slopes (b) of the regression lines were calculated by the least squares method. NE turnover rate (K) was calculated as the product of the fractional turnover rate (k) (k = b/0.434) and the estimated endogenous NE concentration at time 0 [NE_0] of each organ (4). Fractional turnover (k × 100%) was expressed as %/hr: standard error (SE) of k (SE_k) equalled the SE for b (SE_b) divided by 0.434 expressed as %. Turnover rate per organ can be calculated from K × organ weight. Comparison of the slopes of the regression lines was made using the variance estimated for the difference between slopes of the regression equations (8).

The effect of diet on endogenous NE was compared by one-way analysis of variance followed by Duncan's Multiple Range Tests (Experiment 3) and on food intake and weight change by Student's *t*-test for unpaired variables in two-tailed tests of the null hypothesis (Experiments 1 and 2) (29). In Experiment 4, the effects of diet on serum glucose and free fatty acid concentrations were compared by one-way analysis of variance followed by Duncan's Multiple Range Tests (29). Correlation coefficients were determined in a post hoc correlation of serum parameters with fractional NE tunover rates determined from the experiments indicated in the tables. We have noted consistency in turnover values within the same dietary treatments from experiment to experiment within our laboratory (12). Partial correlation coefficients were also determined between energy intake, body weight and fractional NE turnover rates. All values are expressed as means ± SEM.

RESULTS

In Experiment 1, the ad lib-fed mice consumed 46 ± 2 kJ/day and gained 0.36 ± 0.07 g/3 days. The restricted mice fed 2 × 17 kJ/day lost 1.24 ± 0.11 g/3 days. Endogenous NE concentration and fractional and total NE turnover did not differ between mice fed ad lib or restricted to 34 kJ/day presented as 2 equal meals in any organ (Table 1). However, the pancreas and IBAT of the ad lib-fed mice were 12% and 19% heavier, respectively, than the restricted meal fed mice (Table 1).

In Experiment 2, the ad lib-fed mice consumed 53 ± 2 kJ/day

TABLE 1

NORPINEPHRINE (NE) TURNOVER IN AD LIB-FED MICE OR RESTRICTED MICE FED 2 OR 1 MEALS PER DAY (EXPERIMENTS 1 AND 2)

Group	Experiment	Weight (mg)	NE (nmol/g)	Fractional NE (k) Turnover (%/hr)	Calculated Turnover Rate (nmol/g/hr)
		Heart			
High CHO (ad lib)	1	86 ± 2‡	4.52 ± 0.16	29.5 ± 1.6	1.400
High CHO (2 × 17 kJ)*	1	80 ± 2	4.82 ± 0.17	25.2 ± 0.8	1.286
High CHO (ad lib)	2	88 ± 2[a]	4.38 ± 0.14	38.5 ± 2.7[a]	1.930[a]
High CHO (34 kJ)†	2	81 ± 2[b]	4.64 ± 0.20	23.7 ± 1.1[b]	1.180[b]
		Kidney			
High CHO (ad lib)	1	95 ± 2	4.55 ± 0.10	21.9 ± 1.1	1.018
High CHO (2 × 17 kJ)	1	90 ± 2	4.42 ± 0.16	17.6 ± 1.1	0.754
High CHO (ad lib)	2	94 ± 1	4.00 ± 0.02	25.1 ± 1.7	1.068
High CHO (34 kJ)	2	89 ± 2	4.12 ± 0.15	19.6 ± 0.9	0.805
		Pancreas			
High CHO (ad lib)	1	107 ± 4[a]	2.19 ± 0.07	29.4 ± 1.1	0.663
High CHO (2 × 17 kJ)	1	96 ± 3[b]	2.45 ± 0.13	25.8 ± 0.9	0.612
High CHO (ad lib)	2	104 ± 3[a]	1.86 ± 0.06[a]	27.2 ± 1.0	0.526
High CHO (34 kJ)	2	85 ± 3[b]	2.24 ± 0.11[b]	20.9 ± 1.0	0.447
		IBAT			
High CHO (ad lib)	1	168 ± 11[a]	3.05 ± 0.20	23.2 ± 1.7	0.567
High CHO (2 × 17 kJ)	1	142 ± 6[b]	3.46 ± 0.32	27.3 ± 1.3	0.778
High CHO (ad lib)	2	178 ± 7[a]	2.61 ± 0.12	29.6 ± 1.0	0.695
High CHO (34 kJ)	2	135 ± 7[b]	2.98 ± 0.19	22.1 ± 1.3	0.526

*Mice received 2 equal meals daily of 17 kJ each presented at the beginning and middle of the dark period.

†Mice received one meal daily (34 kJ) presented at the beginning of the dark period.

‡Mean ± SEM for n = 18 mice per treatment. Comparisons were made between treatments within each experiment. Means with different superscripts are significantly different within each experiment ($p<0.05$).

and gained 0.82 ± 0.12 g/3 days, whereas the restricted mice fed 1 × 34 kJ/day lost 1.04 ± 0.09 g/3 days. Fractional and total NE turnover was 38% lower in the heart alone of the restricted mice fed one meal only compared to their ad lib-fed controls (Fig. 1, heart and IBAT only shown; Table 1). Cardiac NE concentration was unaffected, but the hearts of the ad lib-fed mice were 10% heavier than those of the restricted mice fed one meal only (Table 1). When NE turnover was expressed per organ, it was similarly 45% lower in the heart of these restricted mice than in controls. The higher pancreatic NE concentration in these restricted mice was attributable to the lower organ weight.

In Experiment 3, the ad lib-fed mice had an intake (2.91 ± 0.06 g/day) almost twice that of either group of restricted mice (1.50 g/day). The weight loss of the restricted mice fed one meal/day (1.76 ± 0.07 g/3 days) was 33% greater than that of the restricted mice fed 2 meals/day (1.32 ± 0.14 g/3 days). The ad lib-fed group gained weight (0.62 ± 0.12 g/3 days). Gut fill may account in part for these differences in body weight. Mice fed ad lib presumably ate steadily throughout the dark period (10,11), whereas the restricted mice fed one meal at the beginning of the dark period normally had empty food cups when checked at the middle of the dark period. Restricted mice fed at the beginning and middle of the dark period normally had empty food cups when checked at the middle and end of the dark period. Thus, the degree of fasting prior to the measurement of NE turnover was approximately 0 hr for the ad lib-fed mice, at least 6 hr for the restricted mice fed one meal/day only, and 0–4 hr for the restricted mice fed 2 meals/day.

Fractional NE turnover was again lower, by 46% and 31% in the heart only, of the restricted mice fed one meal/day compared to their ad lib-fed counterparts or to their restricted counterparts fed 2 meals/day, respectively (Table 2). When the restricted intake was presented as two daily meals, fractional NE turnover did not differ from that of ad lib-fed counterparts in any organ (Table 2).

Endogenous NE concentration was affected by food restriction in heart and pancreas where NE concentration was higher when either type of restricted intake was fed, compared to the ad lib-fed controls (Table 2). The weight of all organs was lower in both groups of mice fed restricted intakes, compared to the ad lib-fed mice.

The lower cardiac NE turnover in the restricted mice fed one meal/day in Experiments 2 and 3 provided a new dietary situation to investigate possible mechanisms for diet-induced changes in sympathetic activity. The effects of 4 dietary treatments in Experiment 4 (a high carbohydrate diet, fed ad lib or restricted to 24 kJ provided as one or two daily meals; or a high protein diet fed ad lib on serum glucose and total free fatty acids) are shown (Table 3).

Serum glucose was highest in mice fed the high carbohydrate

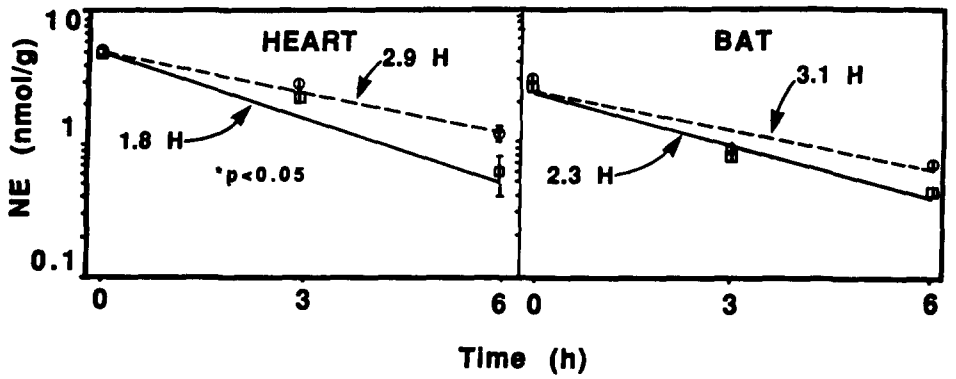

FIG. 1. Disappearance of NE in mouse heart and IBAT at 0, 3 and 6 hr after administration of α-methyl tyrosine in mice fed a high carbohydrate diet ad lib (solid line) or restricted to 34 kJ (dashed line) fed as a single daily meal. Each point represents the mean ± SEM of 5–6 mice. The numbers shown are the half-times of disappearance of NE (in hr).*Slope of the regression line is significantly different from ad lib-fed mice ($p<0.05$).

diet ad lib and lowest in mice fed the restricted intakes, regardless of number of daily meals (Table 3). Mice fed the high protein diet ad lib had serum glucose concentration intermediate to that of the restricted mice and of those fed the high carbohydrate diet ad lib.

FFA concentration was highest in the mice fed restricted intakes independent of number of daily meals (Table 3). Serum FFA concentrations were lowest in mice fed the high protein or the high carbohydrate diets ad lib. The differences in sub-

TABLE 2

NOREPINEPHRINE (NE) TURNOVER IN AD LIB-FED MICE OR RESTRICTED MICE FED 2 OR 1 MEALS/DAY (EXPERIMENT 3)

Group	Weight (mg)	NE (nmol/g)	Fractional NE (k) Turnover (%/hr)	Calculated Turnover Rate (nmol/g/hr)
		Heart		
High CHO (ad lib)	80 ± 2[a]‡	5.02 ± 0.19[a]	28.5 ± 2.0[a]	1.565[a]
High CHO (2 × 12 kJ)*	74 ± 1[b]	5.63 ± 0.10[b]	22.6 ± 1.1[a]	1.437[a]
High CHO (24 kJ)†	77 ± 1[b]	5.70 ± 0.15	15.5 ± 0.7[b]	0.890[b]
		Kidney		
High CHO (ad lib)	100 ± 2[a]	4.63 ± 0.10	19.0 ± 1.2	0.886
High CHo (2 × 12 kJ)	87 ± 2[b]	4.78 ± 0.15	15.4 ± 0.8	0.768
High CHo (24 kJ)	93 ± 2[a.b]	5.14 ± 0.15	15.4 ± 0.8	0.764
		Pancreas		
High CHO (ad lib)	95 ± 3[a]	2.38 ± 0.08[a]	20.8 ± 0.8	0.468
High CHO (2 × 12 kJ)	82 ± 3[b]	2.91 ± 0.15[b]	19.4 ± 1.0	0.563
High CHO (24 kJ)	82 ± 3[b]	2.88 ± 0.13[b]	20.4 ± 0.9	0.565
		IBAT		
High CHO (ad lib)	137 ± 8[a]	3.51 ± 0.21	25.4 ± 1.4	0.745
High CHO (2 × 12 kJ)	106 ± 7[b]	3.74 ± 0.35	21.3 ± 1.3	0.702
High CHO (24 kJ)	103 ± 6[b]	4.38 ± 0.28	23.8 ± 1.2	0.897

*Mice received 2 meals daily, each containing 12 kJ, at the beginning and middle of the dark period.

†Mice received one meal daily containing 24 kJ at the beginning of the dark period.

‡Means ± SEM; for n = 18 mice per treatment; values within columns with a different superscript are significantly different ($p<0.01$).

TABLE 3

EFFECT OF DIET ON SERUM GLUCOSE AND FREE FATTY ACIDS (FFA) AND RELATIONSHIP TO CARDIAC FRACTIONAL NE TURNOVER*

Group	Serum Glucose (mmol/dl)	Serum FFA (μmol/dl)	Fractional NE Turnover (%/hr)
High CHO (ad lib)	10.4 ± 0.4†[a,b]	41.5 ± 4.4[b]	28.5
High CHO (2 × 12 kJ)	8.4 ± 0.2[c]	58.2 ± 5.8[a]	22.6
High CHO (24 kJ)	8.5 ± 0.3[c]	58.6 ± 2.8[a]	15.5
High Protein (ad lib)	9.6 ± 0.2[b,c]	39.4 ± 3.8[b]	14.7
Correlation Coefficient	+.45	−.16	

*Turnover values determined from Experiment 3 and (12) for high protein diet.

†Values are means ± SEM; n = 6; values within columns with a different superscript are significantly different ($p<0.01$).

TABLE 4

RELATIONSHIPS AMONG AD LIB ENERGY INTAKE, BODY WEIGHT AND CARDIAC FRACTIONAL NE TURNOVER

Group	Exp	Energy Intake[1] (kJ/day)	Body Weight[2] (g)	Fractional NE Turnover[3] (%/hr)
High CHO (ad lib)	1	46.16	16.25	29.5
High CHO (2 × 17 kJ)	1	33.75	14.46	25.2
High CHO (ad lib)	2	53.01	16.52	38.5
High CHO (ad lib)	3	46.32	16.66	28.5
High CHO (2 × 12 kJ)	3	23.88	14.73	22.6

r^{12} (correlation of energy intake with body weight) = .89, $p<0.05$.

r^{13} (correlation of energy intake with fractional NE turnover) = +.89, $p<0.05$.

r^{23} (correlation of body weight with NE turnover) = +.73, $p>0.05$.

$r^{13.2}$ (correlation of energy intake with fractional NE turnover, controlling for body weight) = +.77, $p>0.05$.

strate concentrations were unrelated to differences in cardiac NE turnover.

Post hoc comparisons of fractional NE turnover rate with energy intake and body weight were made in experiments where mice were fed the high carbohydrate diet ad lib because it remained possible that NE turnover was related to either or both of these variables in unconstrained animals on the same diet, i.e., larger animals have larger organ size, greater innervation and hence greater whole body sympathetic activity but also a larger food intake. Indeed, an association of lean body mass but not energy intake with sympathetic activity has been demonstrated in humans (13).

Fractional NE turnover was correlated with energy intake (Table 4), but when the partial correlation coefficient was determined, eliminating the effect of body weight, the relationship was no longer significant. Gut fill was very unlikely to affect the correlation between body weight and NE turnover because within each feeding paradigm all mice would have the same approximate gut fill. The lack of relationship between body weight and cardiac fractional NE turnover was apparent, even when data was examined across the ad lib-fed mice only (Table 4).

DISCUSSION

The results indicate that the fasting effect of food restriction and not energy intake per se, is the predominant factor in the organ-specific suppression of NE turnover associated with low energy intake. Specifically, when energy intake of a high carbohydrate diet was restricted to 50–64% of ad lib intake, NE turnover in the heart alone was reduced when mice were fed the restricted intake as a single daily meal presented at the beginning of the dark period. When an identical restricted intake was fed as two daily meals presented at the beginning and the middle of the dark period, NE turnover was unaffected in all organs studied. Secondly, NE turnover was not related to serum concentration of glucose or FFA.

These results separate the effects of fasting from those of caloric restriction on NE turnover for the first time. The role of 24–48 hours fasting in suppressing sympathetic activity has been well established (25,34). However, an effect of modest fasting (6 hr), an event that occurs in many controlled feeding paradigms as an independent variable in relation to restriction of energy intake, has not previously been identified. An effect of modest fasting on lowering sympathetic activity has been previously suggested by the observation of reduced cardiac NE turnover in mice restricted to 35% of their ad lib intake (24) presumably presented as a single meal, but the effect was not attributed at that time to fasting, but rather to reduced energy intake. Although an association between energy intake and NE turnover was shown in the present study, the relationship was due to the covariant effect of body weight. When body weight was controlled, there was no association between ad lib energy intake and NE turnover.

It is conceivable that the feeding paradigm, providing one meal at the beginning of the dark period, induced a shift in the circadian rhythm of NE turnover and, thus, accounted for the reduction in cardiac NE turnover measured 12 hr later commencing at the beginning of the light period. However, Yoshida *et al.* (33) indicate that in an obesity-restricted strain of rats when the availability of a low fat diet is shifted from the dark period to the light period, a more extreme shift in feeding pattern than ours, and when NE turnover is measured at an undefined time during the respective dark and light periods, the fractional and total NE turnover in the heart and pancreas are not affected, but in IBAT, NE turnover is lower by about half. However, the effect of changing feeding pattern on NE turnover was affected by diet composition and rat strain in their study (33). The differences between their results and ours may, thus, arise from differences in feeding patterns, the time of measurement of NE turnover, the diets and strains of animals.

Nevertheless, in Yoshida's treatment group most comparable to ours, i.e., their obesity-resistant rats fed the low fat diet, there was no effect of changing feeding from the dark to the light period on the circadian rhythm of NE turnover in heart or pancreas. Moreover, the lack of effect of the meal feeding paradigm in our study on pancreatic, IBAT or kidney turnover, suggests that the heart was uniquely affected, independent of a generalized shift in circadian rhythm.

The reduced NE turnover in the heart only, of the restricted mice fed one meal per day, suggests that the heart is particularly sensitive to dietary manipulation. This is consistent with earlier observations with fasting and sucrose overfeeding (31, 35, 36) in which NE turnover in the heart was also more affected than that in IBAT. The lower cardiac NE turnover in restricted mice may reflect an adaptive response to food restriction resulting in a prolonged fast, to reduce heat production via reduction in heart rate, myocardial contractility and peripheral vasodilation (28) and, hence, blood flow to IBAT and other tissues (9). The lack of effect in the other organs was somewhat surprising in light of the fact that manipulation of dietary protein concentration in ad lib-fed mice affects NE turnover over the same time periods in IBAT and kidney as well as heart (12); cafeteria feeding (19) or 48 hr fasting

(37) of rats affects both heart and IBAT NE turnover. It is possible that the dietary restriction in the present study was of insufficient duration or severity to suppress NE turnover in IBAT or the other organs.

The present findings are relevant to the human situation in which, during severe energy restriction for weight reduction, sympathetic activity is also reduced (6,23). Cardiac complications have also been observed in humans refed after a modified fast (7, 14, 18), a consequence attributed to sympathetic stimulation during refeeding after suppression during caloric restriction or fasting (20,21). The results of the present study suggest that one way of minimizing this fall in sympathetic activity and minimizing the risk of subsequent cardiovascular complications would be to increase the frequency of feeding.

The peripheral signal that coordinates dietary intake and central sympathetic function is not evident from the results of this study. Dietary factors known to stimulate or suppress NE turnover should be accompanied by simultaneous changes in turnover and in the peripheral signal. However, the lower NE turnover seen in mice deprived or fed a high protein diet was unrelated to changes in serum concentrations of glucose or FFA. The inability of blood glucose levels per se to determine the sympathetic response has been previously suggested by the observation of sympathetic suppression in spite of hyperglycemia produced by 2-deoxyglucose, a glucose analogue that impairs intracellular glucose metabolism (24). In addition, fructose produces greater increments in energy expenditure than glucose, despite significantly lower plasma insulin and glucose and similar FFA levels (30). As well, a pure fat diet maintains NE turnover as well as a pure glucose diet, although serum glucose and insulin are lower in blood sampled concurrently with NE turnover over 3 days of feeding (32). These observations suggest that one or more intracellular, particularly intraneuronal, events such as glucose or energy utilization, independent of serum insulin, glucose or FFA are the signals that link changes in diet to those in sympathetic activity.

In conclusion, this study demonstrates that duration of fasting, rather than energy intake, per se, is the major factor in lowering cardiac NE turnover in response to a caloric restriction to 50–64% of ad lib intake. Specifically, the amount of food eaten in the previous 6 hr is the determining factor for cardiac NE turnover, and not the amount of food eaten in the previous 12 hr to 3 days. The low cardiac NE turnover observed with the modest fasting of food restriction in this study and with high protein diets (12) is not associated with changes in serum glucose or FFA. The results have implications for interpreting the sympathetic nervous system effects of any controlled feeding paradigm.

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