Dietary Restriction Modulates the Norepinephrine Content and Uptake of the Heart and Cardiac Synaptosomes (43789)

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Abstract. **The present study was designed to examine the effects of long-term dietary restriction on cardiac sympathetic nerves and neurotransmitter. The food intake of male, 6-week-old Fischer 344 rats was reduced to 60% of the intake of control rats fed *ad libitum*. The body and heart weights of rats diet restricted for 4.5 months were less than those of the *ad libitum* fed animals, while the heart weight to body weight ratios were higher. The norepinephrine (NE) content of hearts from restricted rats (1073 ± 84 ng/g wet wt) was higher than controls (774 ± 38 ng/g wet wt), although the total amount of NE per heart was unchanged. Similarly, the cardiac synaptosomal P_2 fraction from restricted rats possessed a higher NE content (24.1 ± 2.4 ng/mg protein) than the P_2 fraction of *ad libitum* fed controls (13.7 ± 1.3 ng/mg protein). The desmethylimipramine-sensitive norepinephrine uptake of the P_2 fraction from restricted rats was significantly higher than that of control rats (9.44 ± 1.33 vs 4.75 ± 0.35 ng/mg protein/hr). The NE uptakes of the two groups were similar when uptake was normalized to endogenous NE levels. These results demonstrate that long-term dietary restriction affects cardiac sympathetic nerve endings and suggest that part of the beneficial action of life-long dietary restriction on the age-related decline in cardiovascular regulation may be related to changes in cardiac sympathetic nerves.**

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Restriction of caloric intake is the only known means of retarding the aging processes. It extends maximum and median life span, delays and ameliorates the appearance and severity of age-related diseases, and retards much of the physiological decline associated with aging (1), including deterioration of cardiovascular function (2). Recent work from our laboratory (3, 4) has shown that dietary restriction inhibits the age-related loss in the sensitivity of the cardiac component of the arterial baroreflex. The mechanism responsible for this antiaging action is not known. Since the sympathetic nervous system (SNS) is a major component of the efferent limb of the baroreflex, it is possible that changes in cardiac sympathetic nerve function could contribute to the enhanced reflex responsiveness seen with dietary restriction. The SNS is known to be responsive to changes in dietary intake. Landsberg's group (5), for example, has shown that short-term fasting and hypocaloric feeding decreases the norepinephrine turnover in the heart and other organs, suggesting that SNS activity is attenuated. Conversely, consumption of a diet enriched with sucrose leads to hyperactivity of the SNS as indicated by an increase in transmitter turnover (6).

Dietary restriction may also enhance SNS competence at the cardiac neuroeffector junction. Although very little work has been done on this aspect of dietary intervention, the aging heart exhibits substantial alterations in neurotransmitter content and neuronal function (7–10). Dietary restriction may be acting at this level to inhibit the age-related decline in baroreflex

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function. The present work explores the effects of dietary restriction on cardiac norepinephrine content and synaptosomal neurotransmitter uptake.

Materials and Methods

Animals. For the dietary restriction experiments, male, specific pathogen-free Fischer 344 rats were obtained from Charles River at 4 weeks of age and treated in the same manner as described by Yu *et al.* (11). At 6 weeks of age the rats were divided into two groups: Group A, consisting of rats allowed to eat *ad libitum,* and Group R, consisting of rats fed only 60% of the chow consumed by Group A. At 6 months of age, rats were anesthetized with methoxyflurane followed by decapitation. The hearts were removed and either frozen for analysis of NE content (Table I) or immediately utilized for preparation of cardiac synaptosomes. For the characterization of the cardiac synaptosomal preparation (Table II, Study 1), Fischer 344 rats were maintained in nonbarrier facilities and allowed free access to rat chow.

Cardiac NE Content. Frozen hearts were homogenized via polytron (setting 5 for three 10-sec bursts) in 10 volumes of 0.2 *N* perchloric acid (PCA), 1.0 m*M* $NaHSO_3$. The homogenization solution also contained 1.0 μ*M* 3,4-dihydroxybenzylamine (DHBA) which was used as an internal standard for recoveries which averaged 71% ± 1%. One milliliter of the homogenate was centrifuged for 15 min at 2500*g*. The catecholamines were alumina extracted from the supernatant and resuspended in a PCA solution containing 0.2 *N* PCA, 1.0 m*M* $NaHSO_3$ and 0.05 m*M* EDTA. The catecholamines were separated by HPLC utilizing a C_{18} reverse phase column (Microsorb; Rainen, Woburn, MA) and 1.0 ml/min flow. Their concentrations were measured via electrochemical detection (0.6 volts; BAS, West Lafayette, IN). The output of the electrochemical detector was fed into a Hewlett Packard signal conditioner and the area under the peaks obtained by integration. Catecholamine standards were prepared daily from frozen, concentrated stock solutions.

Synaptosomal Preparation. A synaptosomal preparation was obtained from hearts according to the method of Aloyo *et al.* (12) with some modification. Fresh hearts were rinsed of blood with saline (4°C), weighed, and minced in 1 ml of 0.32 *M* sucrose plus 1.0 m*M* EGTA. The minced tissue was transferred to 10 ml of HEPES-buffered Krebs-Ringer solution (KRH) with the following composition: HEPES (50 m*M*), NaCl (144 m*M*), $MgCl_2$ (1.2 m*M*), $CaCl_2$ (1.2 m*M*), KCl (5.0 m*M*), glucose (10 m*M*), ascorbic acid (1.0 m*M*) and pargyline (0.1 m*M*). Collagenase (Class II, Worthington) was added to give a final concentration of 12 units/mg wet wt. Digestion proceeded for 30 min at 37°C under O_2 bubbling. The tissue was collected by centrifugation (10 min at 120*g*) and homogenized in 10 volumes of 0.32 *M* sucrose (pH = 7.0) with a teflon pestle. The homogenate was centrifuged for 10 min at 650*g* and the supernatant recentrifuged for 20 min at 21,000*g*. The resulting pellet (P_2 fraction) was resuspended in 2–3 ml of ice-cold KRH and the protein content determined by the Bradford method, utilizing a commercial kit (BioRad, Melville, NY).

[^{3}H]NE Uptake. The NE uptake by the P_2 fraction was determined according to the method of Aloyo *et al.* (12). Type 1 NE uptake by the P_2 fraction was determined as the difference between the uptakes that were obtained in the presence and absence of 1.0 μ*M* desmethylimipramine (DMI), a specific Type 1 uptake inhibitor. All assays were performed with a final volume of 1.0 ml. In preliminary experiments the dependency of NE uptake by the P_2 fraction was characterized with regard to time as well as protein and substrate concentrations. Uptake rates for P_2 fractions obtained from Group A (*ad libitum* fed) and Group R (restricted) rats were determined for 10 min under optimal conditions (300 μg P_2 protein/ml and 5×10^{-7} *M* total NE including trace amounts of [^{3}H] NE [10–30 Ci/mmol, New England Nuclear]).

Table I. Effect of Dietary Restriction on Body Weight, Heart Weight, and Cardiac NE Content

	Group A	Group R
Body weight (g)	332 ± 12	198 ± 2*
Heart weight (mg)	792 ± 37	520 ± 18*
Heart wt/body wt (mg/g)	2.4 ± 0.03	2.6 ± 0.06*
NE content (ng/heart)	617 ± 54	554 ± 35
NE content (ng/g wet wt)	774 ± 38	1073 ± 84**

Note. Ad libitum versus restricted: $^*P < 0.01$, $^{**}P < 0.02$. $n = 5$ in each group.

Results

Cardiac NE Content. As shown in Table I, both the body weight and heart weight of Group R were lower than those of Group A as expected. In contrast, the heart weight/body weight ratio of Group R was significantly higher than that of Group A. The NE content of hearts from Group R rats was significantly higher than that of the Group A rats while the total amount of NE per heart was not different between the two groups (Table I).

Synaptosomal Uptake Characterization. Table II (Study 1) lists the characteristics of the cardiac synaptosomal fractions prepared in our laboratory. This isolation procedure yielded about 8 mg of P_2 protein per gram of heart and resulted in an approximate 2-fold NE enrichment in the P_2 fraction over that in the homogenate (Table II, Study 1). The time dependence of NE uptake is shown in Figure 1A. Total NE uptake

Table II. Characteristics of Cardiac Synaptosomes and Modulation by Dietary Restriction

	Study 1	Study 2	
		Group A	Group R
Body weight (g)	—	345 ± 9	205 ± 5**
Heart weight (mg)	865 ± 21	835 ± 27	592 ± 37**
Heart wt/body wt (mg/g)	—	2.4 ± 0.05	2.8 ± 0.10**
Total protein (μg)	7148 ± 389	8074 ± 379	4409 ± 885**
Yield (μg/mg)	8.37 ± 0.52	9.72 ± 0.66	7.45 ± 1.31**
Homogenate [NE] (ng/mg protein)	8.5 ± 0.5*	—	—
P_2 [NE] (ng/mg protein)	14.6 ± 0.8	13.7 ± 1.3	24.1 ± 2.4**
Homogenate [NE]/P_2 [NE] ratio	1.8 ± 0.1	—	—
NE uptake (ng/ng NE/hr)	—	0.36 ± 0.04	0.39 ± 0.04
NE uptake (ng/mg protein/hr)	—	4.75 ± 0.35	9.44 ± 1.33**

* Different from P_2 fraction: $P < 0.0001$, $n = 20$ rats for Study 1.
** Different from Group A: $P < 0.01$, $n = 5$ rats in each group for Study 2.

increased over the 40-min period and was paralleled by an increase in the DMI sensitive NE uptake. Nonspecific uptake ceased to increase after 10 min. The DMI sensitive NE uptake was proportional to the synaptosomal protein concentration in the assay tube (Fig. 1B). Finally, uptake increased as a function of substrate concentration (Fig. 1C). Saturation of the specific NE uptake process occurred at a concentration of about 500 n*M* NE.

Dietary Restriction and Cardiac Synaptosomes. The body weights, heart weights, and heart weight/body weight ratios of both Group A and Group R rats from these studies (Table II, Study 2) were comparable to the earlier studies on total NE content (Table I). Both the total P_2 protein amounts and yields of Group A rats (Table II, Study 2) were similar to those obtained in the characterization studies (Table II, Study 1). The NE content of the P_2 fraction for Group R was approximately 76% greater than that from Group A and dietary restriction caused a nearly 2-fold increase in the specific NE uptake of the P_2 fraction (Table II, Study 2). The NE uptakes of the two groups were similar when uptake was expressed relative to the amount of endogenous NE in the P_2 fraction (Table II, Study 2).

Discussion

The major finding of the present study is that dietary restriction modulates the NE content and uptake of cardiac sympathetic nerves. This dietary manipulation increased the cardiac transmitter content per wet weight of heart (Table I) as well as the NE content per mg protein of the synaptosomal fraction (Table II). Moreover, it elevated the NE uptake by the P_2 fraction when uptake was expressed per mg protein (Table II). Several factors may contribute to the diet-induced elevation in transmitter content seen in the present study. First, the elevated transmitter content in the diet-restricted rats may be indicative of a greater density of nerve endings in the myocardium and, consequently, an increase in the relative number of synaptosomes in the P_2 fraction. Second, since reuptake into nerve endings is a major means of maintaining NE content, the enhanced uptake by the cardiac synaptosomes seen in the present study (Table II) would directly contribute to the higher NE content seen in hearts and synaptosomes of diet restricted rats. *In situ,* this enhanced uptake would minimize the loss of transmitter to the plasma or to metabolism by effector cells. Finally, since transmitter release from nerve endings represents a major site of NE loss, the reported decrease in SNS activity seen with dietary restriction (5) would help to maintain NE content at higher levels. The lower nerve activity would decrease NE release rate and, consequently, would lead to a higher NE content in the nerve endings. The contributions of the various possibilities outlined above to the changes observed here with dietary restriction have not been assessed. However, the observation that the total amount of NE in the heart remains unchanged while the heart weight declines by 30%–35% strongly suggests that the major effect of dietary restriction is to increase sympathetic nerve terminal density.

Changes in nerve terminal density seen with dietary restriction may also arise from processes other than the simple loss of cardiac muscle mass. Roberts' group (7) estimated that the number of cardiac NE varicosities decreased by 13% from 3 to 12 months of age. In the present study, dietary restriction may have retarded the age-related loss in these varicosities, thereby increasing nerve density. Previous studies (13–20) have proposed that free radicals affect the integrity of catecholaminergic nerves. In Parkinsonism, for example, the density of dopaminergic nerves of the substantia nigra declines substantially with age, and part of that decline is associated with the oxidation of dopamine and subsequent production of free radicals

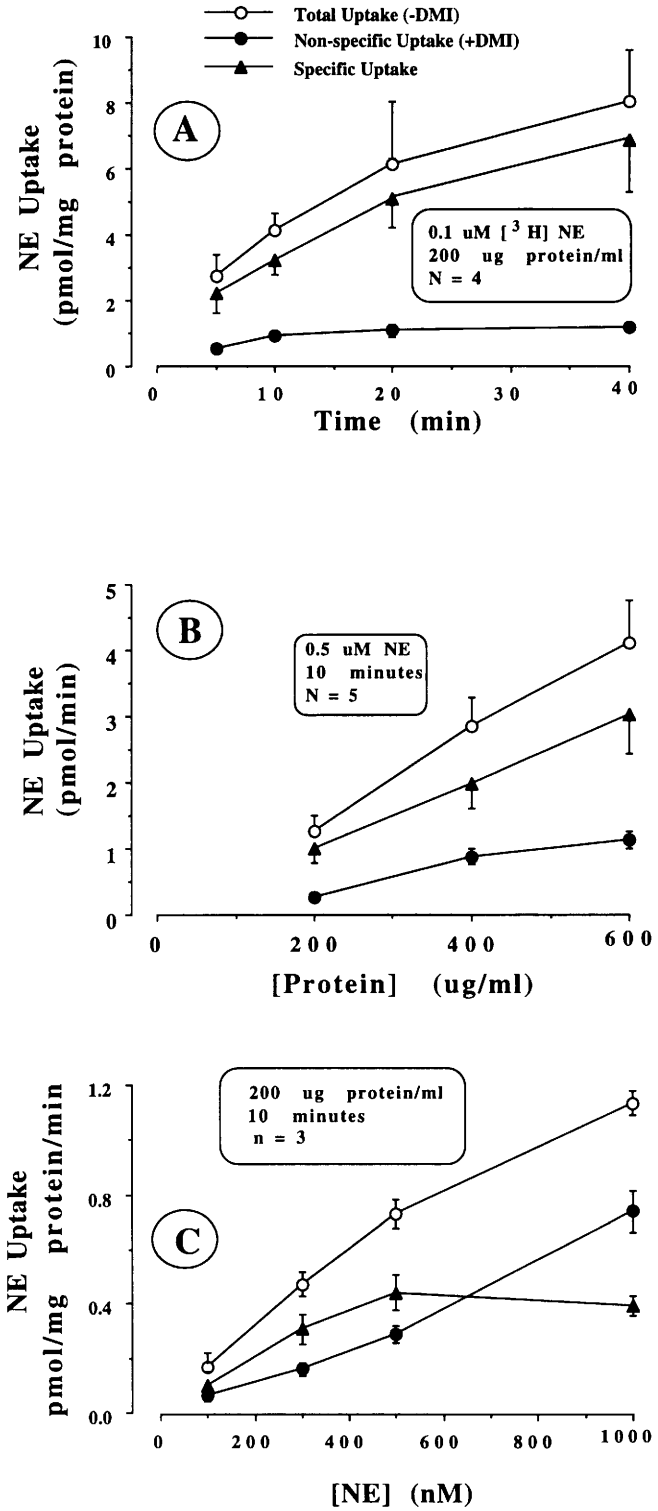

Figure 1. NE uptake characteristics of the cardiac synaptosomal fraction. In all panels, the assay conditions and number of synaptosomal fractions utilized are enclosed within the box. Open circles represent total NE uptakes. Filled circles represent nonspecific NE uptakes measured in the presence of desmethylimipramine, an Uptake 1 inhibitor. Filled triangles represent specific NE uptakes calculated as the difference between total and nonspecific uptakes. Panel A, uptake plotted as a function of time. Panel B, uptake plotted as a function of the P_2 protein content within the assay mixture. Panel C, uptake plotted as a function of substrate concentration within the assay mixture.

(13–16). Free radicals are known to cause the release of NE from the sympathetic terminals in the heart (17) and blood vessels (18, 19) and the catecholamine itself is thought to oxidize and become cytotoxic (19, 20). This mechanism has been proposed (19) to explain the loss in vascular sympathetic nerve endings during elevated NE infusions. It is interesting to speculate that the age-related decline in the number of cardiac sympathetic nerve terminals (7) may result from the oxidation of NE and consequent free radical production. Dietary restriction would then protect against that damage and impede the loss in synaptosomal number and function.

The aging rat heart exhibits a decline in NE content and a loss in sympathetic nerve endings (7). In addition, the cardiac component of the arterial baroreflex deteriorates with age (3, 4), and the basis for this latter deterioration may reside in the age-related changes in cardiac NE content and sympathetic nerve endings. Given the well known antiaging action of dietary restriction generally (1), and in the cardiovascular system in particular (2), the results from the present experiments suggest that the beneficial effects of food restriction on the age-related decline in baroreflex sensitivity may reside in the ability of dietary restriction to maintain cardiac neurotransmitter content and reuptake.

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