# Developmental Plasticity in Sympathetic Nervous System Response to Fasting in Adipose Tissues of Male Rats

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**While fasting suppresses sympathetic nervous system (SNS) activity in most peripheral tissues, the response of sympathetic nerves in white adipose tissue (WAT) is reportedly stimulatory. To confirm this finding and to determine if the fasting response in WAT is susceptible to developmental modification, SNS activity was compared in fed and fasted animals using techniques of [$^3$H]norepinephrine ([$^3$H]NE) turnover. Studies were performed in commercially reared 7-week-old male rats, in male rats reared in the local vivarium, and in male rats reared until 30 days of age at either 18°C or 30°C. In commercially reared animals, [$^3$H]NE turnover was elevated in epididymal fat during the second day of fasting, a response not seen during the preceding or succeeding day of fasting. On the other hand, in rats reared from birth in the local vivarium a 1-day fast led to suppression of SNS activity in epididymal fat. In rats reared at 18°C for the first 30 days of life, fasting suppressed SNS activity in retroperitoneal fat in contrast to the absence of this response noted in 30°C-reared rats. These studies demonstrate that sympathetic responses in WAT to fasting in young adult, male rats are susceptible to modification by the environmental conditions present during the first month of life.**


THE CONTRIBUTION of the sympathetic nervous system (SNS) to metabolic regulation in adipose tissue has been the focus of inquiry for many years. The view is widespread that in the fasting state activation of local sympathetic nerves plays an important role in mediating the metabolic adjustments required for lipid mobilization. Most evidence in support of this contention had been indirect, being based upon alterations in physiological responses to fasting in experimental animals following nerve destruction or pharmacological antagonism of sympathetic effects.[1] In 1997, however, Migliorini et al showed using techniques of norepinephrine (NE) turnover that SNS activity in epididymal and retroperitoneal fat was increased in male Wistar rats fasted for 1 day prior to study.[2] This work provided the first indication that the activity of a portion of the SNS, apart from that of the adrenal medulla, might be increased rather than suppressed in response to fasting.

The following studies were performed in an attempt to apply the findings of Migliorini et al[2] to the phenomenon of increased adiposity in rats exposed to a cool environmental temperature during rearing. Animals reared at 18°C are fatter and gain more weight as adults when fed an enriched diet than rats reared at 30°C.[3] The mechanism(s) responsible for this predisposition to weight gain and fat accretion is(are) unclear since studies in interscapular brown adipose tissue (IBAT) suggest that energy metabolism in BAT is increased, not decreased in the 18°C-reared animals.[4-8] The adrenal medullary response to fasting, on the other hand, is blunted in the cold-reared rats.[8] If similar alterations were evident in other centrally controlled mechanisms for lipid mobilization, such as SNS activation in white adipose tissue (WAT), then the increased adiposity in the 18°C-reared rats might reflect modification in central nervous system (CNS) control of adipose tissue metabolism. Consequently, the studies described here were designed to test the hypothesis that fasting-induced stimulation of SNS activity in WAT is also blunted in rats reared in a cool environment. The findings, however, showed that, rather than suppressing an excitatory response to fasting, early exposure to 18°C magnified a sympatholytic effect of fasting. Moreover, the qualitative nature of sympathetic response itself to fasting in WAT appears to be dependent upon unidentified environmental exposures during development.

## MATERIALS AND METHODS

### *Animals*

Initial studies examined fasting responses in 7-week-old male CD rats (Sprague-Dawley–derived) obtained from Charles River Laboratories (Wilmington, MA). Rats were housed 3 per cage during the week prior to study at a room temperature of 21 ± 2°C with a light:dark cycle of 14:10. A subsequent experiment examined fasting responses in male offspring of multiparous, timed-pregnant rats (Sasco/Sprague-Dawley rats, Charles River Laboratories) reared in the vivarium of the Feinberg School of Medicine. Pregnant rats were obtained on days 7 and 8 of gestation, housed singly, and fed lab chow throughout pregnancy. On the day following delivery, litters were standardized at 10 pups each. Animals were weaned at 21 days; male rats were housed 3 per cage until study at 7 weeks of age.

Finally, to determine the impact of rearing temperature on fasting responses, 1-day-old male CD rats with multiparous foster mothers were obtained from Charles River. On the day of arrival, litters were standardized at 10 pups each. Cages containing individual mothers and litters were then placed into 1 of 2 temperature-controlled chambers set at 18°C and 30°C (±0.2°C). Both chambers (Model 66NL, Percival Scientific, Boone, IA; 66 ft$^3$ internal volume) were equipped with double glass doors so that illumination was provided by room lighting. Pups were weaned at 21 days and housed 3 per cage. Pups remained in the chambers until 30 days of age at which time they were housed in the common room with the other animals used in these experiments. Thus, when studied at 8 weeks of age, both groups had been housed at a common environmental temperature for 26 days. Animals used in these studies were maintained in accordance with the guidelines and approval of the Animal Care and Use Committee of the Feinberg School of Medicine of Northwestern University and in accord with the United States National Institutes of Health (NIH) guidelines.

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*Submitted March 26, 2003; accepted June 11, 2003.*

*Supported by USPHS Grant No. DK 20378.*

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*0026-0495/03/5212-0059$30.00/0*

*doi:10.1016/S0026-0495(03)00331-7*

### *Feeding Protocol*

Unless otherwise specified, animals were provided free access to water and standard laboratory chow (NIH-07 Open Formula Mouse/Rat Diet, Harlan Teklad, Madison, WI). In the fasting studies, animals were deprived of food prior to and throughout the turnover study and were given 0.45% NaCl to drink to avoid sodium restriction in addition to caloric deprivation.

### *Assessment of Abdominal Fat*

Epididymal fat on the right side was separated from epididymis, epididymal vessels, and vas deferens. Retroperitoneal fat was removed from the left side from a region bounded caudally by the inguinal region, medially by the medial border of the iliacus muscle, cranially the diaphragm, and laterally as far as fat was apparent. Dissection of adipose tissue depots was performed by the same individual in all studies.

### *[$^3$H]NE Turnover Procedure*

l-[Ring-2,5,6-$^3$H]NE (40-60 Ci/mmol specific activty; Du Pont NEN Research Products, Boston, MA) was diluted with 0.9% NaCl and injected intravenously into the tail veins of unanesthetized animals in a total volume of 1.0 mL, beginning at approximately 9 AM. The dose of [$^3$H]NE used in the current studies was 75 to 95 $\mu$Ci/kg ($\sim$0.30 to 0.38 $\mu$g NE/kg). The rats were killed 2, 6, 12, and 24 hours following tracer injection by $CO_2$ inhalation. For each time point in the NE turnover studies, 4 to 6 animals were killed from each experimental group. The tissues were rapidly removed, frozen on dry ice, and stored at $-20$°C for later processing (usually within 2 weeks).

As indicated above, the measurement of NE turnover by the [$^3$H]NE technique in these studies takes place over 24 hours. To examine sympathetic responses on the first day of fasting, tracer quantitites of [$^3$H]NE were injected into ad libitum–fed animals and only afterward was food removed from the fasted animals; [$^3$H]NE turnover was measured then over the interval from 0 to 24 hours of fasting. To assess responses on the second day of fasting, tracer was administered to 24-hour–fasted rats and the fast continued for an additional 24 hours; [$^3$H]NE turnover was measured over the interval from 24 to 48 hours of fasting. Finally, to measure responses on the third day of fasting, tracer was injected into 48-hour–fasted rats and the fast continued for an additional 24 hours; [$^3$H]NE turnover was measured over the interval from 48 to 72 hours of fasting.

### *Extraction and Analysis of Tissue Catecholamines*

For NE analysis, the organs were weighed and homogenized in iced 0.2N perchloric acid containing 1% $Na_2S_2O_5$ (by weight) and 1 mmol/L EDTA in a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) to extract the catecholamines. After addition of the internal standard, 3,4-dihydroxybenzylamine (DHBA; Sigma, St Louis, MO), catecholamines were isolated from the perchloric acid extract by adsorption onto alumina (Woelm neutral, ICN Biomedicals, Aurora, OH) in the presence of 2 mol/L tris(hydroxymethyl)-aminomethane buffer (pH 8.7; Sigma) containing 2% EDTA. Catecholamines were eluted from the alumina with 0.2N perchloric acid. Aliquots of the alumina eluate were injected onto a liquid chromatographic system for catecholamine analysis following the method of Eriksson and Persson[9] with slight modification. Unless otherwise specified, all chemicals were obtained from Fisher Scientific, Fair Lawn, NJ. Aliquots of the alumina eluates were counted for [$^3$H]NE by scintillation spectrometry in a Packard Tri-Carb 2100TR liquid scintillation analyzer (Packard Instrument, Meriden, CT). Efficiency for $^3$H is $\geq$ 58% in this system.

### *Data Analysis*

Data are displayed as means $\pm$ SE. Statistical analyses of variance (ANOVA) and covariance (ANCOVA) were performed using Data Desk 6.1 statistical software (Data Description Inc, Ithaca, NY).[10] Post hoc, pairwise comparisons following ANOVA utilized Scheffé's test. Analyses employed $P < .05$ as the criterion for statistical significance; $P$ values between .05 and .1 are provided in text and tables, while $P$ values $\geq 0.1$ are indicated by "NS" (not significant).

In studies of NE turnover, the data were plotted semilogarithmically. The method of least squares was used to calculate the slope ($k$) of decline in NE specific activity over time after tracer injection.[11] The statistical significance of each computed regression line was assessed by ANOVA, and ANCOVA was used in comparison of fractional turnover rates. In the studies examining differences due to rearing temperature in [$^3$H]NE turnover between fed and fasted animals, indicator variables were used for rearing temperature and diet and the slopes of the regression lines analyzed in a $2 \times 2$ ANCOVA model.[12] Goodness-of-fit for each regression line was evaluated by examination of externally studentized residuals. NE turnover rates were calculated as the product of the fractional turnover rate and the endogenous NE concentration and confidence intervals computed as previously described.[13,14]

**Table 1. [$^3$H]NE Turnover in Commercially Reared Male Rats Fasted for 1 Day Prior to Study**

| | Fed | Fasted | *P* |
|---|---|---|---|
| n | 24 | 21 | |
| Body weight (g) | 224 $\pm$ 2 | 198 $\pm$ 2 | $<$.0001 |
| IBAT | | | |
| Tissue weight (mg) | 213 $\pm$ 5 | 188 $\pm$ 6 | .0031 |
| Tissue NE* (ng) | 382 $\pm$ 13 | 332 $\pm$ 20 | .0433 |
| Fractional NE turnover (%/h) | 4.1 $\pm$ 0.6 | 2.9 $\pm$ 0.9 | NS |
| Total NE turnover (ng NE/h) | 15.8 $\pm$ 3.0 | 9.6 $\pm$ 3.6 | NS |
| Epididymal fat | | | |
| Tissue weight (mg) | 704 $\pm$ 19 | 569 $\pm$ 17 | $<$.0001 |
| Tissue NE (ng) | 25.4 $\pm$ 1.1 | 27.7 $\pm$ 1.4 | NS |
| Fractional NE turnover (%/h) | 2.8 $\pm$ 0.5 | 4.4 $\pm$ 0.8 | NS |
| Total NE turnover (ng NE/h) | 0.70 $\pm$ 0.16 | 1.21 $\pm$ 0.30 | $<$.05 |
| Retroperitoneal fat | | | |
| Tissue weight (mg) | 640 $\pm$ 36 | 419 $\pm$ 29 | $<$.0001 |
| Tissue NE (ng) | 45.8 $\pm$ 1.8 | 38.9 $\pm$ 1.1 | .0028 |
| Fractional NE turnover (%/h) | 5.9 $\pm$ 0.6 | 5.3 $\pm$ 1.0 | NS |
| Total NE turnover (ng NE/h) | 2.71 $\pm$ 0.37 | 2.06 $\pm$ 0.43 | NS |

*NE levels in adipose tissues are not expressed in relation to tissue weight in this or subsequent tables because of marked fasting-induced reductions in tissue wet weight due presumably to changes in tissue lipid content.

## RESULTS

### *Effects of Fasting on [$^3$H]NE Turnover in Male Rats Reared by Commercial Supplier*

[$^3$H]NE turnover was measured in fed and fasted male rats obtained as 7-week-old adults from a commercial supplier (Charles River). In an initial experiment [$^3$H]NE turnover was measured in animals fasted for 1 day prior to study and in fed controls, as in the studies of Migliorini et al,[2] and the results presented in Table 1. The reduction in IBAT weight of fasted animals ($-12$%) was proportional to the fall in body weight ($-12$%), while the decreases in epididymal and retroperitoneal fat weights were slightly greater ($-19$% and $-35$%, respectively). The [$^3$H]NE turnover rate in IBAT was slightly, but not significantly, lower in the fasting rats ($-39$%, from 15.8 $\pm$ 3.0 to 9.6 $\pm$ 3.6). While [$^3$H]NE turnover was also slightly, though not significantly, reduced by fasting in retroperitoneal fat

**Table 2. [$^3$H]NE Turnover in Commercially Reared Male Rats Fasted After Tracer Injection**

| | Fed | Fasted | *P* |
|---|---|---|---|
| n | 9 | 8 | |
| Body weight (g) | 220 ± 2 | 226 ± 2 | NS |
| IBAT | | | |
| Tissue weight (mg) | 209 ± 7 | 182 ± 6 | .0103 |
| Tissue NE (ng) | 370 ± 28 | 314 ± 41 | NS |
| Ln(NE specific activity) | 3.50 ± 0.09 | 3.81 ± 0.13 | .0755 |
| Epididymal fat | | | |
| Tissue weight (mg) | 684 ± 20 | 587 ± 27 | .0098 |
| Tissue NE (ng) | 27.4 ± 2.0 | 25.0 ± 1.6 | NS |
| Ln(NE specific activity) | 4.00 ± 0.07 | 4.00 ± 0.04 | NS |
| Retroperitoneal fat | | | |
| Tissue weight (mg) | 625 ± 58 | 423 ± 41 | .0146 |
| Tissue NE (ng) | 45.0 ± 3.2 | 41.8 ± 2.6 | NS |
| Ln (NE specific activity) | 3.95 ± 0.06 | 3.94 ± 0.07 | NS |

(−24%, from 2.71 ± 0.37 ng NE/h to 2.06 ± 0.43), it was increased 73% in epididymal fat (from 0.70 ± 0.16 ng NE/h to 1.21 ± 0.30, $P < .05$) over the 24 hours of the measurement.

To determine if fasting-induced differences in [$^3$H]NE turnover in epididymal or retroperitoneal fat were greater in animals subjected to shorter or longer periods of fasting, [$^3$H]NE turnover was measured over the first and the third days of fasting and the results presented in Tables 2 and 3, respectively. In the first experiment, animals were injected with tracer before fasting began and only afterward was food removed from the fasting animals. In such NE turnover experiments where animals are divided into treatment groups after tracer administration, tissue content of [$^3$H]NE is assumed to be equivalent in both treatment groups at the start of the measurement and the amount present after 24 hours is then an inverse measure of [$^3$H]NE turnover, the more tracer remaining, the lower the level of SNS activity over the time interval. As can be seen in Table 2, tracer content in IBAT of fasted rats was slightly, though not significantly, increased above that in fed controls consistent with decreased SNS activity. Tracer content in epididymal and retroperitoneal fat did not differ between fed and fasted animals, implying no difference in SNS activity in these tissues during the first 24 hours of fasting.

**Table 3. [$^3$H]NE Turnover in Commercially Reared Male Rats Fasted for 2 Days Prior to Study**

| | Fed | Fasted | *P* |
|---|---|---|---|
| n | 21 | 21 | |
| Body weight (g) | 233 ± 2 | 182 ± 2 | <.0001 |
| IBAT | | | |
| Tissue weight (mg) | 214 ± 7 | 184 ± 9 | .0094 |
| Tissue NE (ng) | 327 ± 28 | 254 ± 24 | .0583 |
| Fractional NE turnover (%/h) | 6.9 ± 1.0 | 3.1 ± 1.5 | .0383 |
| Total NE turnover (ng NE/h) | 22.6 ± 5.1 | 7.8 ± 4.6 | <.05 |
| Epididymal fat | | | |
| Tissue weight (mg) | 771 ± 30 | 372 ± 30 | <.0001 |
| Tissue NE (ng) | 23.4 ± 1.4 | 23.5 ± 1.2 | NS |
| Fractional NE turnover (%/h) | 3.5 ± 0.8 | 4.3 ± 0.7 | NS |
| Total NE turnover (ng NE/h) | 0.83 ± 0.23 | 1.01 ± 0.22 | NS |
| Retroperitoneal fat | | | |
| Tissue weight (mg) | 663 ± 45 | 288 ± 36 | <.0001 |
| Tissue NE (ng) | 32.4 ± 1.8 | 33.0 ± 1.5 | NS |
| Fractional NE turnover (%/h) | 6.8 ± 0.7 | 7.6 ± 1.3 | NS |
| Total NE turnover (ng NE/h) | 2.22 ± 0.36 | 2.52 ± 0.54 | NS |

**Table 4. [$^3$H]NE Turnover in Locally Reared Male Rats Fasted for 1 Day Prior to Study**

| | Fed | Fasted | *P* |
|---|---|---|---|
| n | 21 | 21 | |
| Body weight (g) | 305 ± 4 | 277 ± 4 | <.0001 |
| IBAT | | | |
| Tissue weight (mg) | 260 ± 9 | 167 ± 9 | <.0001 |
| Tissue NE (ng) | 648 ± 15 | 740 ± 24 | <.0001 |
| Fractional NE turnover (%/h) | 9.8 ± 0.9 | 7.5 ± 1.2 | NS |
| Total NE turnover (ng NE/h) | 63.3 ± 7.2 | 55.7 ± 10.6 | NS |
| Epididymal fat | | | |
| Tissue weight (mg) | 1,322 ± 74 | 1,080 ± 63 | .0171 |
| Tissue NE (ng) | 27.3 ± 1.1 | 25.9 ± 1.2 | NS |
| Fractional NE turnover (%/h) | 5.4 ± 0.5 | 2.8 ± 0.8 | .0149 |
| Total NE turnover (ng NE/h) | 1.48 ± 0.19 | 0.72 ± 0.25 | <.05 |
| Retroperitoneal fat | | | |
| Tissue weight (mg) | 1,095 ± 94 | 765 ± 79 | .0107 |
| Tissue NE (ng) | 59.7 ± 2.8 | 60.3 ± 2.5 | NS |
| Fractional NE turnover (%/h) | 8.6 ± 0.8 | 8.3 ± 0.9 | NS |
| Total NE turnover (ng NE/h) | 5.12 ± 0.73 | 5.01 ± 0.77 | NS |

During the interval between 48 and 72 hours of fasting (Table 3), [$^3$H]NE turnover in IBAT was 65% lower in the fasting rats ($P < .05$). Here again, however, [$^3$H]NE turnover did not differ between fed and fasted rats in either epididymal or retroperitoneal fat. Thus, SNS activity, as assessed by measurement of [$^3$H]NE turnover, was increased in epididymal fat, but only during the interval between 24 and 48 hours of fasting. [$^3$H]NE turnover was not altered by fasting in retroperitoneal fat and was suppressed significantly in IBAT only during the third day of fasting. Thus, further comparisons of sympathetic responses to fasting were made during the second day (from 24 to 48 hours) of fasting.

### *Effects of Fasting on [$^3$H]NE Turnover in Male Rats Reared in Local Vivarium*

A subsequent experiment was performed examining SNS activity in fasting male rats born and reared in the vivarium at the Feinberg School of Medicine and the results are presented in Table 4. In contrast to the previous comparison on the second day of fasting (Table 1), the reductions in weight of all adipose tissues (−36%, − 30%, and −18% for IBAT, epididymal fat, and retroperitoneal fat, respectively) were greater than the fall in body weight (−9%). [$^3$H]NE turnover in IBAT was again slightly, but not significantly, lower in the fasting rats (−12%, from 63.3 ± 7.2 to 55.7 ± 10.6). While [$^3$H]NE turnover was unaffected by fasting in retroperitoneal fat (−2%, from 5.12 ± 0.73 ng NE/h to 5.01 ± 0.77), it was decreased 51% in epididymal fat (from 1.48 ± 0.19 ng NE/h to 0.72 ± 0.25, $P < .05$) over the 24 hours of the measurement. Thus, sympathetic activity in epididymal fat was decreased by 1 day of fasting in locally reared male rats rather than increased, as in the previous experiment, in commercially reared animals, while sympathetic responses to fasting were qualitatively similar in IBAT and retroperitoneal fat in the 2 experiments.

**Table 5. [$^3$H]NE Turnover in 18°C- and 30°C-Reared Male Rats Fasted for 1 Day Prior to Study**

| | 18°C-Reared | | 30°C-Reared | | *P* Value | | |
|---|---|---|---|---|---|---|---|
| | Fed | Fasted | Fed | Fasted | $T_{rear}$ | Fast | $T_{rear} \times$ Fast |
| n | 21 | 22 | 21 | 22 | | | |
| Body weight (g) | 325 ± 4 | 288 ± 4 | 298 ± 3 | 264 ± 2 | <.0001 | <.0001 | NS |
| IBAT | | | | | | | |
| Tissue weight (mg) | 348 ± 15 | 312 ± 10 | 260 ± 9 | 229 ± 6 | <.0001 | .0017 | NS |
| Tissue NE (ng) | 764 ± 26 | 720 ± 30 | 356 ± 19 | 304 ± 19 | <.0001 | .0486 | NS |
| Fractional NE turnover (%/h) | 6.6 ± 1.1 | 3.0 ± 1.0 | 5.2 ± 0.8 | 3.4 ± 0.9 | NS | .0056 | NS |
| Total NE turnover (ng NE/h) | 50.8 ± 10.3 | 21.5 ± 8.0 | 18.5 ± 3.9 | 10.3 ± 3.3 | | | |
| Epididymal fat | | | | | | | |
| Tissue weight (mg) | 1,386 ± 69 | 1,124 ± 60 | 1,139 ± 51 | 996 ± 36 | .0010 | .0004 | NS |
| Tissue NE (ng) | 29.6 ± 1.7 | 31.3 ± 2.1 | 31.1 ± 1.3 | 32.2 ± 1.4 | NS | NS | NS |
| Fractional NE turnover (%/h) | 4.4 ± 0.6 | 4.0 ± 1.0 | 3.7 ± 0.7 | 4.3 ± 0.9 | NS | NS | NS |
| Total NE turnover (ng NE/h) | 1.32 ± 0.25 | 1.27 ± 0.41 | 1.16 ± 0.27 | 1.40 ± 0.34 | | | |
| Retroperitoneal fat | | | | | | | |
| Tissue weight (mg) | 1,296 ± 76 | 1,018 ± 78 | 1,030 ± 59 | 829 ± 52 | .0011 | .0006 | NS |
| Tissue NE (ng) | 55.9 ± 3.5 | 61.8 ± 3.5 | 46.2 ± 2.3 | 46.8 ± 2.2 | <.0001 | NS | NS |
| Fractional NE turnover (%/h) | 9.9 ± 0.7 | 4.3 ± 1.1 | 7.3 ± 0.7 | 7.0 ± 1.0 | NS | .0021 | .0054 |
| Total NE turnover (ng NE/h) | 5.55 ± 0.75 | 2.67 ± 0.86 | 3.35 ± 0.51 | 3.26 ± 0.61 | | | |

### *Effects of Fasting on [$^3$H]NE Turnover in 18°C- and 30°C-Reared Male Rats*

Since the animals in the previous experiment, though similar in age to those in the initial studies, were heavier and showed higher levels of NE in IBAT, the possibility arose that the differences in sympathetic responses to fasting between studies might be attributable to differences in environmental temperature during rearing.[3,7] Consequently, the impact of rearing temperature on SNS responses to a 1-day fast was examined and the results presented in Table 5. In order to study male rats of approximately the same age as those used in previous experiments (Tables 1 and 4), the animals in this experiment were removed from the temperature chambers at 30, rather than 60 days of age, as in earlier studies.[3,8] Animals were then housed at a common room temperature until [$^3$H]NE turnover was measured simultaneously in both rearing groups at 8 weeks of age.

Rats reared at 18°C were 9% heavier ($P < .0001$) than 30°C-reared males, and both groups experienced an 11% loss of body weight with fasting ($P < .0001$). In IBAT, tissue weight and NE content were both significantly greater in the 18°C-reared rats ($P < .0001$ for both), as noted previously.[8] Fractional [$^3$H]NE turnover rates were reduced by fasting overall ($P = .0056$); in pairwise comparisons this effect was observed in 18°C-reared males ($P = .0185$), but not in 30°C-reared animals ($P$ = NS). While total [$^3$H]NE turnover in IBAT was 58% lower in fasting 18°C-reared rats and 44% lower in fasting 30°C-reared animals than in fed controls, the markedly elevated IBAT NE levels in the 18°C-reared rats resulted in higher rates of [$^3$H]NE turnover in both diet groups than in the corresponding groups of 30°C-reared male rats. Moreover, although proportionally similar, the magnitude of the drop in [$^3$H]NE turnover in relation to body weight was considerably greater in the 18°C-reared males, from 15.7 ng NE/100g body wt/h in fed rats to 7.4 with fasting and in the 30°C-reared rats from 6.2 to 3.9. Thus, fasting led to a substantially greater overall reduction in SNS activity in IBAT of 18°C-reared rats than that seen in 30°C-reared animals.

The weight of epididymal fat was, likewise, 18% greater overall in the 18°C-reared rats ($P = .0010$) and fell 19% with fasting in 18°C-reared and 13% in 30°C-reared rats ($P = .0004$). NE content and fractional [$^3$H]NE turnover rates did not differ between 18°C- and 30°C-reared rats nor between fed and fasted animals. Total [$^3$H]NE rates were 4% lower with fasting in 18°C-reared males and were 20% higher with fasting in 30°C-reared rats, though neither of these differences was statistically significant. Retroperitoneal fat pads were 24% heavier in 18°C-reared rats ($P = .0011$) and fell 21% with fasting in 18°C-reared and 20% in 30°C-reared rats ($P = .0006$). NE content of retroperitoneal fat was 27% greater in 18°C-reared rats, both fed and fasted ($P < .0001$), than in 30°C-reared animals. Moreover, while fasting lowered fractional [$^3$H]NE turnover rates in both 18°C- and 30°C-reared rats ($P = .0021$), it did so to a significantly greater extent in 18°C-reared than in 30°C-reared animals ($P = .0054$). Fractional [$^3$H]NE turnover rates in retroperitoneal fat were reduced by fasting overall ($P = .0021$); in pairwise comparisons this effect was observed in 18°C-reared males ($P = .0002$), but not in 30°C-reared animals ($P$ = NS). Consequently, on the second day of fasting total [$^3$H]NE turnover was suppressed by 52% in 18°C-reared rats (from 5.55 ± 0.75 ng NE/h to 2.67 ± 0.86, $P < .05$), but by only 3% in 30°C-reared animals (from 3.35 ± 0.51 to 3.26 ± 0.61, $P$ = NS). Thus, the environmental temperature to which a male rat was exposed during the first 30 days of life influenced the SNS response to fasting in IBAT and retroperitoneal fat.

## DISCUSSION

A starting point for the current studies was the observation by Migliorini et al[2] that sympathetic activity in WAT, as assessed by NE turnover techniques, was increased in male rats fasted for 1 day prior to measurement. In the 25 years since fasting was shown initially to suppress cardiac SNS activity,[15] this inhibitory response has been seen in a number of other peripheral tissues, including pancreas, liver, brown fat, and kidney.[16-18] While a sympatholytic effect of fasting was not

seen in all tissues,[19] the observation of Migliorini et al that a portion of the peripheral SNS, apart from the adrenal medulla, might be activated by fasting was without precedent. The current studies were undertaken, therefore, to confirm the finding of Migliorini et al and to determine whether the effect of fasting might be susceptible to developmental modification. In their studies, Migliorini et al noted increases in total [$^3$H]NE turnover in epididymal and retroperitoneal fat (+93% and +72%, respectively) and a concomitant 27% decrease in turnover in IBAT in male Wistar rats fasted for 1 day prior to turnover measurement.[2] In the first experiment of the current series (Table 1), total NE turnover was increased in epididymal (+73%), but not retroperitoneal (−24%), fat and slightly, but not significantly decreased in IBAT (−39%), responses which in epididymal fat and IBAT appeared to agree quite well with the previous publication.[2]

The physiological importance of this observation is uncertain, however. Sympathetic activity in WAT is not increased in male rats during the first 24 hours of fasting (Table 2) nor during the interval from 48 to 72 hours of fasting (Table 3) and is also not seen in female CD rats fasted for 1 day prior to study (data not shown). Moreover, in male CD rats reared from birth in the vivarium of the Feinberg School of Medicine, the [$^3$H]NE turnover response in epididymal fat on the second day of fasting is inhibitory, not excitatory (Table 4). Consequently, sympathetic responses to fasting in epididymal and retroperitoneal fat appear to vary depending upon the duration of the fast, the gender of the animal and the environmental temperature and other unspecified factors during development.

Since the focus of this effort was on the impact of rearing temperature on the regulation of SNS activity in WAT, SNS responses to fasting for 24 to 48 hours were compared within the same experiment in 18°C- and 30°C-reared male rats (Table 5). Although fasting did not affect [$^3$H]NE turnover in epididymal fat in either rearing group, fasting suppressed [$^3$H]NE turnover in retroperitoneal fat and did so to a far greater extent in the 18°C-reared rats than in the 30°C-reared males. In addition, tissue NE levels in retroperitoneal fat were 27% higher in the 18°C-reared rats than in the 30°C-reared animals. Since elevations in NE levels in IBAT of 18°C-reared rats correspond with evidence of greater sympathetic innervation in the tissue,[7] it is likely that the postganglionic sympathetic innervation in retroperitoneal fat is increased by rearing in a cool environment as well. Thus, environmental temperature during rearing appears to alter the sympathetic innervation and its regulation in WAT, especially retroperitoneal fat, just as it does sympathetic function in BAT.[7,8]

The role of sympathetic nerves in the regulation of lipolysis in adipose tissue has been the subject of debate for many years,[1] one which the current studies do not resolve. Much of the evidence in support of neural stimulation of lipolysis during fasting is indirect and relies upon changes in lipolysis in the presence of adrenergic antagonists or after sympathetic denervation.[1] The present study attempted to measure SNS activity in several adipose tissue pads (IBAT, epididymal and retroperitoneal) using the technique of [$^3$H]NE turnover which permits simultaneous assessment of sympathetic function in multiple tissues of unanesthetized animals. Although this work found little evidence of sympathetic activation by fasting in WAT, given the heterogeneity in responsiveness among adipose tissue regions, it is entirely possible that a stimulatory response to fasting may occur in sympathetic nerves of other WAT pads in which [$^3$H]NE turnover was not measured. On the other hand, suppression of WAT SNS activity with fasting might promote lipolysis either via an increase in local blood flow due to a reduction in neurally mediated vasoconstriction or via disinhibition of lipolysis due to a decrease in $\alpha_2$-adrenoceptor–mediated antilipolytic effects.[20-22] The variability in sympathetic responses in different WAT sites to fasting suggests that circulating catecholamines from the adrenal medulla may play a more direct role in stimulating lipolysis than local release of NE from sympathetic nerves. While the role of adrenergic mechanisms in the regulation of adipose tissue metabolism is far from clear, the current observations indicate that assessment of SNS activity in specific adipose tissue depots must be taken into account in the construction of models describing the regulation of lipolysis.

Finally, in conjunction with a recent report comparing sympathetic and adrenal medullary activity in 18°C- and 30°C-reared rats,[8] the current findings provide additional insight into the mechanisms responsible for the enhanced weight gain in the 18°C-reared animals.[3] Since rats reared at 18°C exhibit increased innervation, higher rates of [$^3$H]NE turnover and greater expression of sympathetically-related genes in IBAT than do 30°C-reared animals,[7,8] energy metabolism is unlikely to be deficient, and may even be enhanced, in the obesity-prone, 18°C-reared rats. With fasting sympathetic outflow to BAT falls in both 18°C- and 30°C-reared rats, though the magnitude of this effect is greater in the 18°C-reared animals. Consequently, the blunting of the adrenal medullary response to fasting coupled with the marked suppression of SNS activity with fasting in retroperitoneal fat (Table 5) in 18°C-reared male rats suggests that the greater weight gain in these animals is due to diminished fuel mobilization rather than to differences in energy metabolism.[8]

## ACKNOWLEDGMENT

The skillful technical assistance of Yiu-Kuen Chow and Siddique A. Mahmoud and the helpful analytical advice provided by Professor Isis C. Kettelhut (Universidade de São Paulo, São Paulo, Brazil) are gratefully acknowledged.

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