Tissue specificity of SNS response to exercise in mice exposed to low temperatures

DENIS RICHARD, ANTOINE LABRIE, AND SERGE RIVEST
Department of Physiology, Faculty of Medicine, Laval University, Quebec City, Quebec G1K 7P4, Canada

Richard, Denis, Antoine Labrie, and Serge Rivest. Tissue specificity of SNS response to exercise in mice exposed to low temperatures. *Am. J. Physiol.* 262 (*Regulatory Integrative Comp. Physiol.* 31): R921–R925, 1992.—The present study was carried out to investigate the tissue specificity of the sympathetic nervous system (SNS) response to acute exercise in adult mice exposed to four ambient temperatures. SNS activity estimates in heart, pancreas, and brown adipose tissue (BAT) were obtained from the measurement of the dopamine (DA) tissue contents 1 h after the inhibition of the DA-β-hydroxylase with 1-cyclohexyl-2-mercaptoimidazole (CHMI). DA was measured by electrochemical detection after the separation of the monoamine using high-performance liquid chromatography. In both heart and pancreas, temperature and activity influenced DA tissue contents after the CHMI injection. In these tissues, and regardless of whether mice were resting or exercising, the DA contents gradually increased while the ambient temperature was dropped from a thermoneutral temperature of 32 to 5°C. In BAT, however, there was a significant interaction between temperature and activity on the tissue DA content; in contrast to what was observed in resting animals, DA did not uniformly augment in exercising mice when the temperature was decreased from 32 to 5°C. In summary, the present results show that exercise can attenuate the stimulating effect of cold on SNS activity in BAT. This effect is seemingly specific to BAT because, in mice exposed to low ambient temperatures, SNS activity in both heart and pancreas is not lower in exercising than in resting animals.

brown adipose tissue; heart; pancreas; dopamine; cold

THE WELL-ESTABLISHED BELIEF that acute exercise causes an increase in the sympathetic nervous system (SNS) activity has emerged largely from studies describing rises in plasma norepinephrine (NE) levels and urinary NE excretion during and after exercise (5, 24, 26, 32, 36, 37). Unfortunately, there is scant literature detailing the effects of exercise on SNS activity in individual tissues (10, 27, 28, 31). Moreover, the few investigations addressing the effects of exercise on SNS activity in specific tissues have concentrated more on the chronic than on the acute effects of exercise (27, 28, 31). In this regard, the present study was designed to examine the tissue specificity of the SNS response to acute exercise. SNS activity estimates in specific tissues were obtained from the measurement of the tissue dopamine (DA) contents after the inhibition of DA-β-hydroxylase with 1-cyclohexyl-2-mercaptoimidazole (CHMI) (7). DA tissue contents were measured in heart, pancreas, and brown adipose tissue (BAT). BAT is now recognized as a key thermogenic effector in small mammals (6, 14–16), and there is still debate about the effects of exercise on BAT thermogenesis (3, 4, 17–19, 25, 34). Because activity and ambient temperature are known to bring about interactive effects on BAT thermogenesis (1, 2), SNS activity was evaluated at 32, 23, 14, and 5°C. DA tissue content was measured in heart and pancreas because SNS is likely to be stimulated during exercise and cold exposure in both these tissues, which comprise numerous sympathetic nerve endings. Finally, because exercise and cold are liable to affect BAT thermogenesis through effects on whole body heat production, oxygen consumption ($\dot{V}O_2$) measurements were carried out in resting and exercising mice during exposures at the four ambient temperatures enumerated above.

MATERIALS AND METHODS

Animals. Male albino mice weighing ~30 g were purchased from the Canadian Breeding Laboratories (St. Constant, Canada). All mice were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, which is approved by the Natural Sciences and Engineering Research Council of Canada. Mice were housed in pairs in plastic cages and maintained on a 12:12 h light-dark cycle, with the lights on between 0600 and 1800 h. Tap water and a pelleted stock diet (Purina no. 5001) were available ad libitum.

Experimental conditions. Two separate sets of experiments, each with distinct groups of mice, were conducted (Table 1). They were aimed at evaluating the main and interactive effects of activity and temperature on SNS activity and $\dot{V}O_2$, respectively. Specifically, SNS activity and $\dot{V}O_2$ were assessed in resting or exercising mice living at 32, 23, 14 or 5°C (see below for description of techniques). The mice were accustomed for 1 wk to the temperature at which tissue SNS activity was to be analyzed. The acclimation period was warranted by preliminary experiments that highlighted the inability of mice raised at the thermoneutral temperature of 32°C to run at the low temperature of 5°C. Mice in every group were accustomed to the treadmill on two 10-min sessions during the week before the week of experiment. In the SNS activity set of experiments, 25 mice were used at each of the four environmental temperatures to which mice were exposed: a group of 5 mice, which was used to determine the basal tissue content of both DA, was killed at the beginning of the experiment without being injected with CHMI, and two groups of 10 mice were injected with CHMI and then allowed, before being killed, either to rest for 60 min in their respective cages or to exercise on a rodent treadmill also for 60 min at a speed of 20 m/min. All mice were killed by cervical dislocation. In the $\dot{V}O_2$ experiment, 16 mice were used at each of the four environmental temperatures to which mice were exposed. Finally, because DA tissue levels do not readily vary in the absence of a DA-β-hydroxylase inhibitor (11), resting and exercising groups without CHMI injections were not employed for the study.

SNS activity. SNS activity was assessed from the NE synthesis estimates obtained by measuring the tissue content of DA 1 h after an intraperitoneal injection (50 mg/kg) of CHMI, a DA-β-hydroxylase inhibitor, which was generously provided by Lilly Research Laboratories, a division of Eli Lilly (Indianapolis, IN). This method represents a useful technique for indexing NE synthesis over periods of time ranging from 30 to 120 min (7, 11, 39). In fact, tissue DA with time accumulates quickly and linearly after the inhibition of DA-β-hydroxylase(7). Other methods for estimating NE turnover through inhibiting NE

Table 1. *Schematic representation of conditions to which mice were subjected at each of the 4 ambient temperatures (32, 23, 14, and 5°C)*

	SNS Activity			Oxygen Consumption	
Activity	nothing	resting	exercising	resting	exercising
Injection	nothing	CHMI	CHMI	nothing	nothing
n	5	10	10	7	7

SNS, sympathetic nervous system; CHMI, 1-cyclohexyl-2-mercaptoimidazole. See MATERIALS AND METHODS for more details.

synthesis and determining the decline in tissue NE concentrations cannot be applied over brief spans because the decline in NE concentrations become detectable only after a few hours, typically ≥6 h in resting laboratory rodents.

DA contents were measured in heart, pancreas, and BAT. Immediately after the mice were killed, tissues of each mouse, cooled on ice, were quickly dissected out and immediately homogenized in 1 ml of 0.4 perchloric acid (PCA) containing sodium metabisulfite (0.1 M) and EDTA (0.15 M). The homogenate was centrifuged for 10 min. After the centrifugation the supernatant was collected and transferred to a tube containing 20 mg of acid-washed alumina, which was used to extract the catecholamines. Two milliliters of 0.5 M Tris·HCl and 1 ng of 3,4-dihydroxybenzylamine hydrobromide (DHBA), which served as an internal standard, were added to the tube content, which was shaken for 20 min before being centrifuged. The supernatant was discarded, and alumina was washed three times with distilled water. Extracted catecholamines were then freed by adding 100 μl of 0.1 N PCA to the tubes containing alumina. The samples were shaken for 10 min and centrifuged. Finally, the supernatant was collected and stored at −80°C pending the determination of DA tissue contents.

DA was determined using an electrochemical detector (M460, Waters, Missisauga, Ontario, Canada) after a high-performance liquid chromatography (HPLC) separation of the monoamine on a C_{18} reverse-phase HPLC column. The mobile phase that was circulated in the HPLC system included NaH_2PO_4 (0.15 M), EDTA (0.1 mM), 1-octanesulfonic acid, and methanol (8%).

$\dot{V}O_2$. Both resting and exercise $\dot{V}O_2$s were measured during the morning between 0800 and 1000 h. An automatic O_2 analyzer (Applied Electrochemistry S3A1) coupled to an O_2 sensor (Applied Electrochemistry n22M) was used for the $\dot{V}O_2$ measurements. Resting $\dot{V}O_2$ values were determined in mice housed in 1-liter Plexiglas metabolic chambers. The air was pumped at a flow rate of 1 l/min through the chamber, and the mice were acclimated to their metabolic chambers for 48 h. Resting $\dot{V}O_2$ values were monitored alternately in 12 separate metabolic chambers (33). Exercise $\dot{V}O_2$ values were monitored from mice placed in metabolic chambers that were installed on the treadmill. During the test the speed of the treadmill was adjusted at 20 m/min. The volume of each chamber was 1.5 liters, and the air was drawn through the ward at an air flow rate of 2.5 l/min. Exercise $\dot{V}O_2$ values were monitored alternately in three metabolic chambers.

Statistics. Two-way analysis of variance (ANOVA) was used to evaluate the main and interaction effect of the factors activity, which comprised two levels (rest and exercise), and temperature, which comprised four levels (32, 23, 14, and 5°C), for the DA content in the three tissues studied. Similarly, ANOVA was used to assess the $\dot{V}O_2$ data. A posteriori comparisons between means were carried out by using the Dunn-Sidak procedure (20). A difference between means was considered to be statistically significant at a probability level of 0.05.

RESULTS

Figure 1 expresses the DA tissue contents of resting and exercising mice 1 h after a CHMI injection. In heart

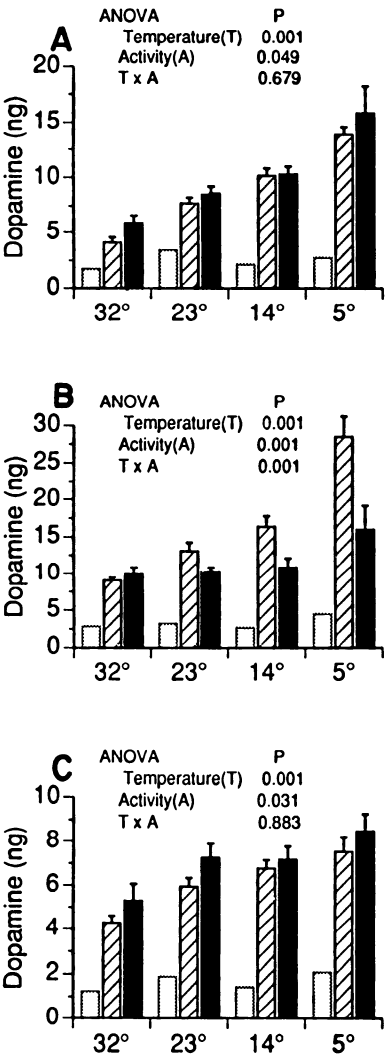

Fig. 1. Tissue dopamine contents of resting (hatched bars) and exercising mice (solid bars) 1 h after a 1-cyclohexyl-2-mercaptoimidazole (CHMI) injection. Open bars, basal tissue contents. Values are means ± SE. Two-way analysis of variance (ANOVA) was used to evaluate main and interaction effects of factors activity and temperature, which comprised 2 (rest and exercise) and 4 levels (32, 23, 14, and 5°C), respectively, on DA tissue contents. Basal means were not included in analysis. *A*, heart; *B*, brown adipose tissue; *C*, pancreas.

as well as in pancreas, both temperature and exercise influenced DA tissue contents after the CHMI injection. In both tissues, and regardless of whether mice were resting or exercising, the DA contents gradually increased as the ambient temperature was dropped from 32 to 5°C. In BAT, ANOVA revealed a significant interaction between temperature and activity on DA tissue contents. Unlike what was observed in resting animals, the DA contents did not uniformly augment in exercising mice as the temperature was decreased from 32 to 5°C; only at 5°C was

BAT DA content of exercising mice significantly higher than that of the other exercising groups of animals. It is noteworthy that at 14 and 5°C, the DA content of BAT in exercising mice was significantly lower than that of resting mice.

Table 2 summarizes the tissue weights of mice when they were killed. For each temperature, the tissue weights of the mice injected with CHMI and those of the mice killed without injection were added and averaged because injections and acute exercise did not affect tissue weights. Because mice were adapted for 1 wk to the four ambient temperatures studied, all the tissue weights were affected by the factor of temperature, as expected: the tissue weights were higher in mice adapted to 14 and 5°C than in animals at 32 and 23°C.

The results expressed in Fig. 2 show the $\dot{V}O_2$ values measured in exercising and resting mice at various ambient temperatures. ANOVA revealed a significant interaction between the factors of activity and temperature on $\dot{V}O_2$ values. At variance with the values of resting mice, the $\dot{V}O_2$ values of exercising animals did not progressively increase each time the temperature was declined. In fact, at 32, 23, and 14°C, exercise raised $\dot{V}O_2$ to a level that corresponded to a twofold increase above resting levels measured at 32°C; only in mice at 5°C was the exercise $\dot{V}O_2$ significantly higher than that of mice at ambient temperatures of 32, 23, and 14°C.

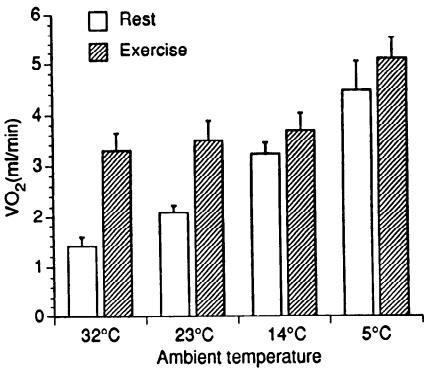

Fig. 2. Oxygen consumption ($\dot{V}O_2$) values measured in exercising and resting rats at various ambient temperatures. Values are means ± SE. Two-way ANOVA was used to evaluate main and interaction effects of factors activity and temperature, which comprised 2 (rest and exercise) and 4 levels, (32, 23, 14, and 5°C), respectively, on $\dot{V}O_2$ values.

DISCUSSION

The present investigation was designed to assess SNS activity in specific tissues during exercise. SNS activity was determined by measuring the tissue DA contents subsequent to the inhibition of the DA β-hydroxylase activity (7). This method appeared appropriate for the conditions of the present study, in which SNS activity had to be assessed over a short bout of strenuous exercise. Most techniques used to assess SNS activity in tissues are based on the measurement of NE disappearance rate, and they cannot, therefore, be applied over a short interval of time. The results of the present study establish a certain degree of tissue specificity in the SNS response to exercise. In fact, whereas it led to a post-CHMI increase in the DA contents in heart and pancreas of mice exposed to various ambient temperatures, exercise prevented the post-CHMI increase in BAT DA induced by the exposure to low ambient temperatures.

The dominant mechanism underlying the suppression by exercise of the stimulating effects of cold on BAT SNS activity has not been thoroughly studied, but it seems plausible that exercise blunts SNS activity by reducing the need for BAT thermogenesis. BAT is acknowledged as the main thermogenesis effector in cold-acclimated laboratory rodents (6) when they are exposed to cold. This tissue embodies a unique mitochondrial protein that allows the oxidation of substrates when BAT is stimulated. These substrates are essentially free fatty acids, which are oxidized with minimal respiratory constraints in BAT and, therefore, at an accelerated rate. This rapid oxidation allows the liberation of large quantities of heat (29, 30). The increase in BAT metabolic activity, which occurs in cold environments, requires the SNS activation (8, 21), which is triggered by the cold stimulus. BAT is abundantly innervated by sympathetic fibers, and the norepinephrine released at the nerve endings stimulates thermogenesis through activating the "loose-coupled" respiration of this tissue. Previous data have provided evidence that BAT thermogenic activity is considerably reduced in exercising cold-exposed rats (1, 2). In cold-exposed laboratory rodents, exercise-derived heat appears to largely substitute for BAT thermogenesis (1). This phenomenon seems to be mediated by SNS as revealed by the results of the present study. Additionally, the results of the present investigation indicate that SNS activity of exercising mice exposed at 5°C is higher than that of each of the other groups of mice exposed at 32, 23, or 14°C. It seems that at 5°C, the heat stemming from this level of exercise is not sufficient to totally replace the sympathetically mediated BAT thermogenesis. As corroborated by the indirect calorimetry results, which showed that energy expenditure was higher in mice exercising at 5°C than in mice exercising at 32, 23, or 14°C, it appears that for mice, the effects of cold on energy balance are additive to those of exercise at 5°C. It is worth pointing out that mice have to be previously acclimated to cold to sustain a run at 5°C (Richard and Rivest, unpublished observations), thus supplying evidence that heat loss is larger in exercising than in resting mice.

The present study, by demonstrating that SNS activity is not enhanced in BAT during severe exercise, strongly supports the view that exercise is not a good stimulator of BAT thermogenesis (4, 9, 22, 23, 34, 35, 38). In fact, exercise, especially running exercise, has repeatedly failed to stimulate BAT thermogenesis in rats. On the other hand, some authors have observed that exercise may

Table 2. *Weights of heart, BAT, and pancreas of mice housed at 32, 23, 14, and 5°C*

Temperature, °C	Heart, mg	BAT, mg	Pancreas, mg
32	131 ± 2^{ab}	94 ± 3^{ab}	146 ± 4^{ab}
23	130 ± 2^{cd}	90 ± 3^{cd}	146 ± 4^{cd}
14	151 ± 3^{ace}	138 ± 7^{ace}	174 ± 4^{ac}
5	174 ± 6^{bde}	142 ± 6^{bde}	205 ± 4^{bd}

Values are means ± SE. BAT, brown adipose tissue. Within a tissue type, values with similar superscripts are significantly different ($P < 0.05$).

enhance BAT thermogenesis (17–19). It must be mentioned that most if not all of the studies relating those stimulating effects of exercise on BAT thermogenesis have been carried out in swimming laboratory rodents. There is nothing accidental in the fact that swimming may stimulate thermogenesis in small laboratory rodents. In some of the studies in which mice or rats were trained by swimming, the animals were trained in water kept below 38°C, a temperature under which cold-specific adaptations appear evident (12, 13). Failure to observe any increases in BAT thermogenesis in response to exercise has also been sparsely reported with swimming (4, 23).

In summary, the present results show that exercise can prevent the stimulating effect of cold on SNS activity in BAT. This effect is specific to BAT because, in mice exposed at low ambient temperatures, SNS activity in both heart and pancreas is not lower in exercising than in resting animals. Although the present results do not propose any central mechanisms underlying the suppressing effects of exercise on cold-induced thermogenesis in BAT, they nonetheless emphasize, in animals exercising in the cold, the relationship between the suppression of SNS activity in BAT and the reduction in the thermogenic state of this tissue.

Address reprint requests to D. Richard.

Received 1 August 1991; accepted in final form 25 October 1991.

REFERENCES

1. **Arnold, J., J. Leblanc, J. Coté, J. Lalonde, and D. Richard.** Exercise suppression of thermoregulatory thermogenesis in warm- and cold-acclimated rats. *Can. J. Physiol. Pharmacol.* 64: 922–926, 1986.
2. **Arnold, J., and D. Richard.** Exercise during intermittent cold exposure prevents acclimation to cold in rats. *J. Physiol. Lond.* 390: 45–54, 1987.
3. **Bell, R. R., and T. J. McGill.** Body composition and brown adipose tissue in sedentary and active mice. *Nutr. Res.* 11: 633–642, 1991.
4. **Bell, R. R., T. J. McGill, P. W. Digby, and S. A. Bennett.** Effects of dietary protein and exercise on brown adipose tissue and energy balance in experimental animals. *J. Nutr.* 114: 1900–1908, 1984.
5. **Conlee, R. K., R. C. Hickson, W. W. Winder, J. M. Hagberg, and J. O. Holloszy.** Regulation of glycogen resynthesis in muscles of rats following exercise. *Am. J. Physiol.* 235 (*Regulatory Integrative Comp. Physiol.* 4): R145–R150, 1978.
6. **Foster, D. O.** Quantitative contribution of brown adipose tissue thermogenesis to overall metabolism. *Can. J. Biochem. Cell Biol.* 62: 618–622, 1984.
7. **Fuller, R. W., H. D. Snoddy, and K. W. Perry.** Dopamine accumulation after dopamine β-hydroxylase inhibition in rat heart as an index of norepinephrine turnover. *Life Sci.* 31: 563–570, 1982.
8. **Girardier, L., and J. Seydoux.** Neural control of brown adipose tissue. In: *Brown Adipose Tissue*, edited by P. Trayhurn and D. G. Nicholls. London: Arnold, 1986, p. 122–151.
9. **Gohil, K., S. Henderson, S. E. Terblanche, G. A. Brooks, and L. Packer.** Effects of training and exhaustive exercise on the mitochondrial oxidative capacity of brown adipose tissue. *Biosci. Rep.* 4: 987–993, 1984.
10. **Gordon, R., S. Spector, A. Sjoerdsma, and S. Udenfriend.** Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. *J. Pharmacol. Exp. Ther.* 153: 440–447, 1966.
11. **Griggio, M. A., J. Leblanc, and D. Richard.** The role of brown adipose tissue on postprandial thermogenesis in mice. *Int. J. Obes.* 15: 711–715, 1991.
12. **Harri, M., T. Dannenberg, R. Oksanen-Rossi, E. Hohtola, and U. Sundin.** Related and unrelated changes in response to exercise and cold in rats: a reevaluation. *J. Appl. Physiol.* 57: 1489–1497, 1984.
13. **Harri, M., and P. Kuusela.** Is swimming exercise or cold exposure for rats? *Acta Physiol. Scand.* 126: 189–197, 1986.
14. **Himms-Hagen, J.** Thermogenesis in brown adipose tissue as an energy buffer. *N. Engl. J. Med.* 311: 1549–1558, 1984.
15. **Himms-Hagen, J.** Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28: 67–115, 1989.
16. **Himms-Hagen, J.** Brown adipose tissue thermogenesis: interdisciplicnary studies. *FASEB J.* 4: 2890–2898, 1990.
17. **Hirata, K.** Blood flow to brown adipose tissue and norepinephrine-induced calorigenesis in physically trained rats. *Jpn. J. Physiol.* 32: 279–291, 1982.
18. **Hirata, K.** Enhanced calorigenesis in brown adipose tissue in physically trained rats. *Jpn. J. Physiol.* 32: 647–653, 1982.
19. **Hirata, K., and T. Nagasaka.** Enhancement of calorigenic response to cold and to norepinephrine in physically trained rats. *Jpn. J. Physiol.* 31: 657–665, 1981.
20. **Kirk, R. E.** *Experimental Design: Procedures for the Behavioral Sciences.* Pacific Grove, CA: Brooks-Cole, 1982.
21. **Landsberg, L., and J. B. Young.** The role of the sympathetic nervous system and catecholamines in the regulation of energy metabolism. *Am. J. Clin. Nutr.* 38: 1018–1024, 1983.
22. **Leblanc, J., and P. Diamond.** Interaction between exercise training and cold acclimation in rats. *Eur. J. Appl. Physiol. Occup. Physiol.* 57: 89–92, 1988.
23. **Leblanc, J., J. Dussault, D. Lupien, and D. Richard.** Effect of diet and exercise on norepinephrine-induced thermogenesis in male and female rats. *J. Appl. Physiol.* 52: 556–561, 1982.
24. **Leblanc, J., A. Labrie, D. Lupien, and D. Richard.** Catecholamines and triiodothyronine variations and the calorigenic response to norepinephrine in cold-adapted and exercise-trained rats. *Can. J. Physiol. Pharmacol.* 60: 783–787, 1982.
25. **Leblanc, J., A. Labrie, D. Lupien, and D. Richard.** The role of cathecolamines and triiodothyronine on the calorigenic response to norepinephrine in cold-adapted and exercise-trained rats. In: *Biochemistry of Exercise V*, edited by H. G. Knuttgen, J. A. Vogel, and J. R. Poortmans. Champaign, IL: Human Kinetics, 1983, p. 733–737.
26. **Lucas, A., A. Therminarias, and M. Tanche.** Catécholamines plasmatiques chez le chien soumis au froid et a l'exercice musculaire a dépense énergétique équivalente. *J. Physiol. Paris* 78: 231–234, 1982.
27. **Mazzeo, R. S., and P. A. Grantham.** Norepinephrine turnover in various tissues at rest and during exercise: evidence for a training effect. *Metabolism* 38: 479–483, 1989.
28. **Nadeau, A., G. Tancrede, M. H. Nguyen, and S. Rousseau-Migneron.** Effect of physical training on norepinephrine turnover in tissues of normal and diabetic rats. *Metabolism* 35: 25–29, 1986.
29. **Nicholls, D., S. Cunningham, and H. Wiesinger.** Mechanisms of thermogenesis in brown adipose tissue. *Biochem. Soc. Trans.* 14: 223–226, 1986.
30. **Nicholls, D. G., and R. M. Locke.** Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64: 1–64, 1984.
31. **Ostman, I., N. O. Sjostrand, and G. Swedin.** Cardiac noradrenaline turnover and urinary catecholamine excretion in trained and untrained rats during rest and exercise. *Acta Physiol. Scand.* 86: 299–308, 1972.
32. **Peronnet, F., J. Cleroux, H. Perrault, D. Cousineau, J. de Champlain, and R. Nadeau.** Plasma norepinephrine response to exercise before and after training in humans. *J. Appl. Physiol.* 51: 812–815, 1981.
33. **Richard, D., P. Boily, M. C. Dufresne, and M. Lecompte.** Energy balance and facultative diet-induced thermogenesis in mice fed a high-fat diet. *Can. J. Physiol. Pharmacol.* 66: 1297–1302, 1988.

34. **Richard, D., and S. Rivest.** The role of exercise in thermogenesis and energy balance. *Can. J. Physiol. Pharmacol.* 67: 402–409, 1989.
35. **Richard, D., and P. Trayhurn.** Fatty acid synthesis in exercise-trained mice: effects of exercise on synthesis in liver, white adipose tissue, and brown adipose tissue. *Can. J. Physiol. Pharmacol.* 62: 695–700, 1984.
36. **Scheurink, A. J. W., A. B. Steffens, H. Bouritius, G. H. Dreteler, R. Bruntink, R. Remie, and J. Zaagsma.** Adrenal and sympathetic catecholamines in exercising rats. *Am. J. Physiol.* 256 (*Regulatory Integrative Comp. Physiol.* 25): R155–R160, 1989.
37. **Von Euler, U. S.** Sympatho-adrenal activity in physical exercise. *Med. Sci. Sports Exercise* 6: 165–173, 1974.
38. **Wickler, S. J., J. S. Stern, Z. Glick, and B. A. Horwitz.** Thermogenic capacity and brown fat in rats exercise-trained by running. *Metabolism* 36: 76–81, 1987.
39. **Yen, T. T., R. W. Fuller, S. K. Hemrick-Luecke, and N. B. Dininger.** Effects of LY104119, a thermogenic weight-reducing compound, on norepinephrine concentrations and turnover in obese and lean mice. *Int. J. Obes.* 12: 59–67, 1988.