

# Intensity-controlled treadmill running in rats: $\dot{V}O_{2\max}$ and cardiac hypertrophy

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**Wisløff, Ulrik, Jan Helgerud, Ole Johan Kemi, and Øyvind Ellingsen.** Intensity-controlled treadmill running in rats:  $\dot{V}O_{2\max}$  and cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* 280: H1301–H1310, 2001.—Physiological studies of long-term cardiovascular adaptation to exercise require training regimens that give robust conditioning effects and adequate testing procedures to quantify the outcome. We developed a valid and reproducible protocol for measuring maximal oxygen uptake ( $\dot{V}O_{2\max}$ ), which was reached at a 25° inclination with a respiratory exchange ratio > 1.05 and blood lactate > 6 mmol/l. The effect of intensity-controlled aerobic endurance training was studied in adult female and male rats that ran 2 h/day, 5 days/wk, in intervals of 8 min at 85–90% of  $\dot{V}O_{2\max}$  and 2 min at 50–60% of  $\dot{V}O_{2\max}$ , with adjustment of exercise level according to  $\dot{V}O_{2\max}$  every week. After 7 wk, the increase in  $\dot{V}O_{2\max}$  plateaued at 60–70% above sedentary controls. Ventricular weights and myocyte length were up 25–30% and 6–12%, respectively. Work economy, oxygen pulse, and heart rate were sufficiently changed to indicate substantial cardiovascular adaptation. The model mimics important human responses to training and could be used in future studies on cellular, molecular, and integrative mechanisms of improved cardiovascular function.

oxygen pulse; heart rate; respiratory exchange ratio; maximal oxygen uptake

RECENT STUDIES SUGGEST that endurance training increases cardiovascular capacity and quality of life and reduces mortality in patients with heart failure (5, 36). The paucity of data on cellular and molecular mechanisms of improved heart function calls for well-defined experimental models of endurance training that evoke adaptations similar to those after aerobic training in humans (7, 21). Endurance training could increase exercise capacity as measured by maximal oxygen uptake ( $\dot{V}O_{2\max}$ ), improve work economy, and enhance anaerobic threshold (15). Cardiac effects should include reduced resting and submaximal heart rates (HR), increased ventricular weights and volumes, and myocyte hypertrophy.

In the adult rat treadmill-running model, the previously published training regimens used are known to elicit minor effects on ventricular mass and/or cardiac

myocyte dimensions (22, 23, 30). Studies have shown a 0–20% increase in left ventricular weight and myocyte length (1, 2, 22, 23, 34). Several studies did not find any significant change in myocardial mass as a result of treadmill training in female rats and concluded that ventricular enlargement in female rats depends critically on the mode of training, with effects observed only with swim training (10, 14, 27, 28, 34). In contrast, treadmill training increases  $\dot{V}O_{2\max}$  and work economy by 10–20% and reduces resting HR by ~5% both in male and female rats (10, 24).

Even though the intensity of the load required to induce physical conditioning increases as the performance improves during the course of training (3), most studies use a fixed exercise intensity throughout the experiment. Our working hypothesis was that adjustment of the training load relative to the level of the fitness of the individual should evoke adaptations similar to those induced by aerobic training in humans.

The aims of the present study were as follows: 1) establish a valid and reliable treadmill protocol for evaluating endurance capacity in rats and 2) determine the effect of intensity-controlled treadmill running on endurance capacity, ventricular weights, and cardiac myocyte dimensions in male and female rats.

## MATERIAL AND METHODS

### Study Population and Design

A total of 46 adult female and 12 adult male (270–300 g) Sprague-Dawley rats (Møllegaards Breeding Center, Lille Skensved, Denmark) were maintained with 4 rats in each cage, with a volume of 46 liters. Light was controlled on a 12:12-h light-dark cycle. The training and test protocols were performed during the rats' dark cycle except for evaluation of basal metabolism and resting HR. Temperature was  $22.5 \pm 1.4^\circ\text{C}$ , and humidity was  $55.6 \pm 4.0\%$ . Animals were fed a pellet rodent diet ad libitum and had free access to water. After each training or test session, each rat was rewarded with 0.5 g of chocolate (Crispo, Nidar Bergene, Norway). Sedentary rats were given the same amount of chocolate. None of the rats were excluded from the study because they avoided running. Stewment was changed every 4 days, and the same person handled the rats throughout the study. The

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experimental procedures conform with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes," and the protocol was approved by the Norwegian Council for Animal Research. The rats were assigned into six groups, as described in Table 1. HR was measured in all animals in groups II–VI, and blood lactate was measured in three animals from group I after establishing the optimal inclination for measuring  $\dot{V}O_{2\max}$ .

#### Test Protocol

From human studies (3), it is known that inclination of the treadmill affects oxygen uptake at maximal aerobic exercise. To establish the optimal protocol for assessing  $\dot{V}O_{2\max}$ , rats (group I) were tested at five different inclinations (0°, 15°, 20°, 25°, and 30°). To avoid bias caused by conditioning, the sequence of testing was randomized, and each test was separated by 1 day. Each rat had a 15-min warmup at 40–50% of  $\dot{V}O_{2\max}$  before the  $\dot{V}O_{2\max}$  protocol. Treadmill speed was increased by 0.03 m/s every 2 min. After the optimal protocol for measuring  $\dot{V}O_{2\max}$  was established, the reproducibility of basal metabolism and submaximal work economy was examined in another set of rats (group II). After a 15-min warmup at 40–50% of  $\dot{V}O_{2\max}$ , the rats exercised for 4 min at 50, 60, and 70% of  $\dot{V}O_{2\max}$ . In test 1, four animals exercised at increasing intensities (50, 60, and 70% of  $\dot{V}O_{2\max}$ ), and four animals exercised at decreasing intensities (70, 60, and 50% of  $\dot{V}O_{2\max}$ ). In test 2, the stages were given in reverse order. At each level, oxygen uptake, HR, and running speed were recorded. From a metabolic point of view, oxygen uptake was expected to be the same at corresponding exercise intensities in different tests, regardless of whether the exercise stages were given in increasing or decreasing order. The reason for choosing 4 min at each submaximal stage was that oxygen uptake leveled off after ~3 min at each exercise stage after changing work load (data not shown), which concurs with what has been shown in human studies (3). A potential disadvantage of starting at 70% of  $\dot{V}O_{2\max}$  was that this intensity might have exceeded the anaerobic threshold for treadmill-running rats. In that case, differences in oxygen uptake between increasing and decreasing exercise stages would have been expected. Thus oxygen uptake at lower exercise levels would have been higher because of oxygen deficit, causing a higher cost of running. The reproducibility of  $\dot{V}O_{2\max}$  and maximal HR (25° inclination of the treadmill) was examined in a third set of rats (group III). A 1-day rest period separated tests.

#### Training Protocol

In the training protocols, rats were assigned to either treadmill running or sedentary control. To determine the effect of training (25° inclination) on oxygen uptake, HR, ventricular weights, and left ventricular cellular dimensions, we first studied two groups (groups IV and VI) of sedentary

and exercising female rats. Six trained and six sedentary rats were euthanized after 4 and 13 wk. To study whether male and female rats achieved the similar training responses, we trained a group of male rats (group V) until the training effect on  $\dot{V}O_{2\max}$  leveled off. To assess running economy and HR after the training periods, oxygen uptake and HR were measured at the same absolute exercise intensity before and after the training period. After 2 days of training, all rats avoided the electrical grid with  $2 \pm 1$  touches per training session. After 2 wk of training, the rats would typically jump onto the treadmill when their cages were placed next to it.

#### Oxygen Uptake and HR

The oxygen uptake and respiratory exchange ratio were measured using an oxygen analyzer based on a paramagnetic oxygen transducer (Servomex type 1155, Servomex) and carbon dioxide analyzer (LAIR 12, M&C Instruments). Ambient air was pumped through the metabolic chamber at a flow rate of 4.5 l/min, and samples of extracted air (200 ml/min) were directed to the oxygen and carbon dioxide analyzers. The analyzers were calibrated with known gas mixtures every day and had an accuracy of measurement of  $\pm 2\%$ . The treadmill was placed in a metabolic chamber with a total volume of 11 liters. A custom-made stainless steel grid at the end of the lane supplied an electrical stimulus (0.25 mA, 1 stimulus of 200-ms duration every second) to keep the rats running on the lane. In treadmill construction, care was taken to avoid the rats pinching their feet. Pilot experiments showed that the natural running behavior included periods of stopping and sniffing on the treadmill belt. Therefore, the lane of the treadmill was made 70 cm long to avoid unnecessary contact with the electrical grid.

Each rat had a 15-min warmup at 40–50% of  $\dot{V}O_{2\max}$  before the  $\dot{V}O_{2\max}$  protocol. Treadmill speed was increased by 0.03 m/s every 2 min. The criteria for reaching  $\dot{V}O_{2\max}$  were a leveling off of oxygen uptake despite increased workload, a respiratory exchange ratio above 1.05, and an unhemolyzed blood lactate concentration above 6 mmol/l (3). Lactate measurement was performed using a YSI model 1500 Sport Lactate Analyzer (Yellow Springs Instruments). A blood sample of 35  $\mu$ l was drawn from a polyethylene (PE)-50 catheter in the jugular vein for measurement of blood lactate immediately after  $\dot{V}O_{2\max}$  completion. To measure maximal HR, we used a modified protocol established by Ingjer (19). After completion of the  $\dot{V}O_{2\max}$  test, the rat ran at a work intensity corresponding to 50–60% of  $\dot{V}O_{2\max}$  directly followed by a supramaximal intensity run, which led to exhaustion within ~3 min, that is, when the rat was no longer able to keep running on the lane. The highest HR during the supramaximal run was recorded as maximal HR. To determine the reproducibility of submaximal oxygen uptake ( $\dot{V}O_2$ ), each rat worked at fixed exercise stages, 50, 60, and 70% of  $\dot{V}O_{2\max}$  for 4 min at each intensity.

Table 1. Overview of group assignment and number of rats used in each protocol

Group	Protocol	Female, <i>n</i>	Male, <i>n</i>
I	Optimal inclination for measuring $\dot{V}O_{2\max}$	8	
II	Reproducibility of basal metabolism and submaximal $\dot{V}O_2$	8	
III	Reproducibility of $\dot{V}O_{2\max}$ and maximal heart rate at 25° inclination	6	
IV	Training and control, 4 wk	12	
V	Training and control, 7 wk		12
VI	Training and control, 13 wk	12	

*n*, Number of rats;  $\dot{V}O_{2\max}$ , maximal oxygen uptake;  $\dot{V}O_2$ , submaximal oxygen uptake.

HR was measured by connecting a frequency-modulated acoustical HR transmitter (operating at  $\sim 130$  kHz) to implanted wires as described below (17). Resting metabolism and resting HR were measured as the rat spent 24 h in a Plexiglas metabolic chamber with strewn food, water, and food. In the light cycle, resting metabolism and HR were measured while the rat was sleeping. The total volume of the metabolic chamber was 3.6 liters. Air was pumped through the chamber at a rate of 4.0 l/min, and samples of extracted air were directed to the oxygen and carbon dioxide analyzers. The measurements were performed at least 10 h after training. To indirectly assess ventricular stroke volumes at rest and during exercise, we calculated mean oxygen pulse values ( $\dot{V}O_2/\text{HR}$ ) (33).

#### Training Procedure

Trained rats exercised on the treadmill 2 h/day, 5 days/wk, for 4, 7, or 13 wk. At the start of every week,  $\dot{V}O_{2\text{max}}$  was measured as described, and workloads were adjusted accordingly. In training rats, exercise intervals alternated between 8 min at 85–90% of  $\dot{V}O_{2\text{max}}$  and 2 min at 50–60% of  $\dot{V}O_{2\text{max}}$ . Before the first interval, each rat performed a 20-min warmup at 40–50% of  $\dot{V}O_{2\text{max}}$ . At the day of  $\dot{V}O_{2\text{max}}$  testing, trained rats performed eight intervals after the test. In sedentary rats, treadmill running skill was maintained by a 15-min run at 0° inclination at 0.15 m/s for 3 days/wk.

#### Surgical Procedures

All instrumentation was performed using sterile surgical procedures. Implantation of the HR recorder and positioning of the PE-50 catheter into the right jugular vein were performed under anesthesia by subcutaneous injection of 0.3 ml/100 g midazolam (Dormicum “Roche”) and fentanyl-fluanisone (Hypnorm). A pair of thin enamel-insulated copper wires with tinned ends, connected to the HR transmitter, were subcutaneously advanced from the dorsal aspect of the cervical region of the rat and implanted at midchest to record the HR. The cylindrical transmitter ( $5 \times 15$  mm) was stitched on the rat's neck. The catheter was subcutaneously advanced to the dorsal aspect of the cervical region of the rat, where it was exteriorized. The rats were given 48 h of recovery before any kind of test was performed.

#### Isolation of Left Ventricular Myocytes

After 4, 7, or 13 wk of the experimental period, the animals were anesthetized with diethyl ether and heparinized (0.2 ml heparin, 1,000 IU/ml iv; Novo Nordisk, Copenhagen, Denmark). Hearts were rapidly removed from the animals, kept for 1 min in ice-cold perfusion buffer, and connected to an aortic cannula of a standard Langendorff retrograde perfusion system. To balance the variation of myocytes isolated, one heart from either group was taken each day. Myocytes were isolated from septal plus left ventricular free wall portions of the myocardium using a modified protocol from Holt and Christensen (18). The heart was retrogradely perfused via the aorta (7.5 ml/min) for 10 min with *medium A*, which consisted of 24 g of Joklik's medium (Life Technologies, Paisley, Scotland) mixed in 2,000 ml of deionized water added with (in mM) 1.2  $\text{MgSO}_4$ , 1.0 DL-carnitine (Sigma, St Louis, MO), and 23.8  $\text{NaHCO}_3$ . *Medium A* was equilibrated with 5%  $\text{CO}_2$ -95%  $\text{O}_2$  for 15 min (at 37°C; pH 7.4). After 10 min, the hearts were perfused for 20 min with *medium B* (7.5 ml/min), which consisted of 300 ml of *medium A* mixed with 150 U/ml collagenase (Worthington, Freehold, NJ) and 0.1% bovine serum albumin (Sigma). After 10 min, *medium B* was

collected for later use. The hearts were cut down into *medium C*, which contained 125 ml of *medium A* supplied with 1% bovine serum albumin and 1.5 mM  $\text{CaCl}_2$ , equilibrated with 5%  $\text{CO}_2$ -95%  $\text{O}_2$ . The atria, great vessels, and right ventricle were removed. The left and right ventricles were weighed. The left ventricular tissue was cut into small pieces, put into *medium C*, and shaken for 10 min (at 37°C; 5%  $\text{CO}_2$ -95%  $\text{O}_2$ , 100 rpm). The supernatant was removed, 20 ml of *medium B* was added, and the tissue was shaken for 30 min (at 37°C; 5%  $\text{CO}_2$ -95%  $\text{O}_2$ , 150 rpm). Thereafter, 10 ml of *medium C* was added to each cell suspension before centrifugation for 20 s at 600 rpm (at 37°C). The supernatant was gently removed, and another 10 ml of *medium C* was added. After centrifugation, the supernatant was gently removed, and 5 ml of *medium C* was added before filtering through a nylon mesh (250  $\mu\text{m}$ ). Coverslips were coated with 10 mg/ml laminin (Life Technologies) in Medium 199. Isolated myocytes on coated coverslips ( $\sim 2 \pm 10^3$  cells/cm<sup>2</sup>) were placed in a cell chamber on an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan) and stimulated electrically by bipolar pulses (5-ms duration, 5 Hz; at 37°C) using platinum electrodes on either side of the chamber. Cells that remained rod shaped, without blebs or other visible morphological alterations, and that responded adequately on electrical stimulation were measured for length and midpoint width. The cells on the coverslips were stored in Medium 199 (M7528, Sigma) mixed with 2 mg/ml serum albumin, 2 mM DL-carnitine, 5 mM creatine, 5 mM taurine, 0.1 mM insulin,  $10^{-10}$  M triiodothyronine ( $\text{T}_3$ ) (all from Sigma), 100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin (both from Life Technologies, Gaithersburg, MD) equilibrated with 5%  $\text{CO}_2$ -95%  $\text{O}_2$  (at 37°C; pH 7.4).

#### Scaling

As demonstrated below, body weight was higher in male rats than female rats and markedly higher in sedentary compared with trained male rats. Changes in ventricular weights and oxygen uptake may not be entirely due to training regimens. Some could be due to growth-related changes in body weight. For example, it may be that training-induced ventricular hypertrophy in rats could be overestimated due to less body fat compared with control rats. It was, therefore, necessary to normalize oxygen uptake and ventricular weights according to correct scaling procedure, which involves the correct normalization of a physiological variable to a body dimension (3). Usually, this is done by dividing, for example, ventricular weights by body weight. It has previously been shown that this is valid only if body weight is expressed as lean body mass (4). However, if lean body mass is not defined, left ventricular mass should be expressed in relation to body mass raised to the power of 0.78 (4). According to the theoretical models and empirical studies, oxygen uptake should be expressed in relation to body mass raised to the power of 0.75, over a wide range of body weights, when individuals with different body weights are compared (29). Because no significant differences in body weights were observed in female rats, oxygen uptake was expressed as milliliters per kilogram per minute when the aim was to compare trained and sedentary female rats.

#### Statistical Analysis

Data are expressed as means  $\pm$  SD. Friedman tests applying appropriate procedures for multiple comparisons (8) were used to determine changes in oxygen uptakes, respiratory exchange ratios, and body weights throughout the experimental period as well as differences in oxygen uptake using different inclinations of the treadmill. A Mann-Whitney *U*-



Table 2. Peak oxygen uptake at different treadmill inclinations

Degrees of Inclination	$\dot{V}O_{2\text{peak}}$ , $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	RER, $\dot{V}CO_2/\dot{V}O_2$	Blood Lactate, $\text{mmol/l}$
0	$64.5 \pm 1.9$	$1.04 \pm 0.05$	$6.5 \pm 0.5$
15	$67.8 \pm 0.8$	$1.14 \pm 0.03$	$6.5 \pm 0.5$
20	$70.7 \pm 0.8$	$1.14 \pm 0.04$	$6.6 \pm 0.6$
25	$79.9 \pm 3.1^\dagger$	$1.15 \pm 0.02$	$7.1 \pm 1.0$
30	$74.7 \pm 1.0$	$1.18 \pm 0.01$	$9.1 \pm 0.9^*$

Values are means  $\pm$  SD.  $\dot{V}O_{2\text{peak}}$ , highest oxygen uptake measured at each inclination; RER, respiratory exchange ratio;  $\dot{V}CO_2$ , carbon dioxide production. Differences from all other inclinations:  $^*P < 0.05$  and  $^\dagger P < 0.001$ .

test was used to evaluate differences among groups.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Test Protocols

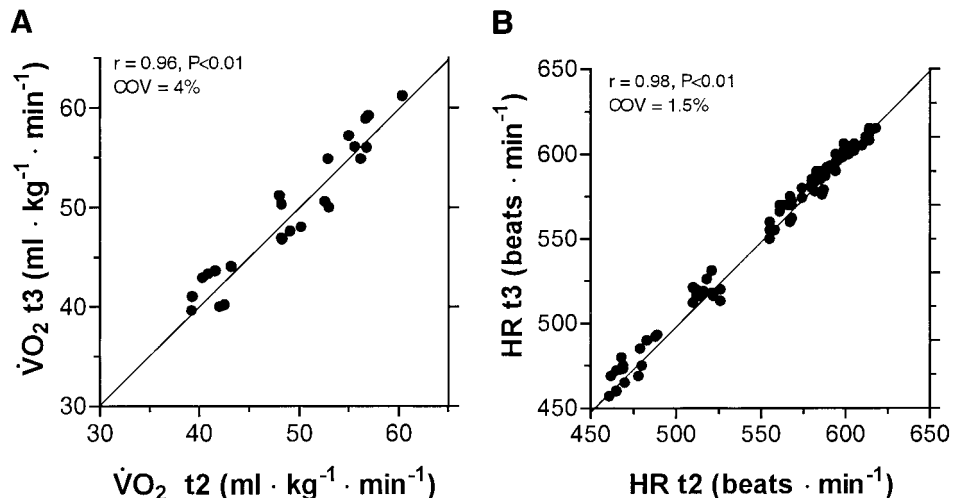
Inclination of the treadmill significantly affected the highest oxygen uptake measured;  $\dot{V}O_{2\text{max}}$  was reached at 25° inclination of the treadmill (Table 2). The procedures were valid and reproducible for evaluating oxygen uptake and HR at rest and during exercise. As shown in Fig. 1, results were similar in three tests, excluding potential learning effects caused by unfamiliarity with the apparatus and discomfort in the test situation. The coefficients of variation for basal metabolism and resting HR were 2.8% and 3.2%, respectively. The  $\dot{V}O_{2\text{max}}$  for tests 1, 2, and 3 were  $79.9 \pm 3.2$ ,  $80.2 \pm 2.9$ , and  $80.3 \pm 3.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , respectively. Results were equivalent in rats with the catheter ( $n = 3$ ) or HR recorder (group II–VI). As shown in Fig. 2, A and B, there were linear increases in oxygen uptake and HR with increased power output. Figure 2A also shows that the oxygen uptake reached a plateau despite increased power output. The oxygen pulse increased up to the intensity corresponding to  $\dot{V}O_{2\text{max}}$ , where it leveled off and declined (Fig. 2C). Figure 3 demonstrates that there was a linear relationship between oxygen uptake and HR. However, maximal HR

was not reached at  $\dot{V}O_{2\text{max}}$  but at exercise intensities above those corresponding to  $\dot{V}O_{2\text{max}}$ .

### Training Effects

After 7 wk of training,  $\dot{V}O_{2\text{max}}$  reached a plateau 60% and 70% above controls in female and male rats, respectively. As seen from Fig. 4A, male rats had higher  $\dot{V}O_{2\text{max}}$  (in  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) than female rats except at wk 7. The running speed at  $\dot{V}O_{2\text{max}}$  increased from  $0.36 \pm 0.03$  to  $0.60 \pm 0.04 \text{ m/s}$  after 13 wk of training in female rats. The corresponding values for male rats trained for 7 wk were  $0.40 \pm 0.02$  and  $0.65 \pm 0.02 \text{ m/s}$ . No gender or group differences in body mass were observed at pretest, and body mass was  $277 \pm 10$  and  $287 \pm 11 \text{ g}$  for female and male rats, respectively. However, after 7 wk, only male rats had increased body weight, by 28% and 60% in the trained and sedentary groups, respectively (Table 3). When properly scaled for body mass,  $\dot{V}O_{2\text{max}}$  (in  $\text{ml}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$ ) in trained male rats was ~15% higher than in trained females throughout the study (Fig. 4B). No training or gender effects were observed in maximal HR or resting oxygen uptake. Average values were  $647 \pm 10$  beats/min and  $14 \pm 2 \text{ ml}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$ . In trained female rats, resting HR decreased by 24 and 69 beats/min at 4 and 13 wk, respectively, whereas trained male rats decreased resting HR by 56 beats/min at 7 wk (Table 3). Work economy, measured as oxygen uptake (in  $\text{ml}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$ ) at a given running speed, did not differ between trained groups. The average improvements at 4, 7, and 13 wk of training were  $16 \pm 4.7\%$ . Figure 5A presents work economy for female rats after 13 wk of endurance training compared with sedentary controls. Because there were no differences in body mass in female rats, the relationship is expressed as milliliters per kilogram per minute. In rats with identical  $\dot{V}O_{2\text{max}}$ , work economy differed by as much as 15% ( $P < 0.05$ ; data not shown). The submaximal respiratory exchange ratio was significantly reduced by the training regimen (Table 4) and did not differ between trained female (13 wk) and male rats (data not shown).

Fig. 1. Test-retest of submaximal oxygen uptake ( $\dot{V}O_2$ ) at 50, 60 and 70% of the maximal oxygen uptake ( $\dot{V}O_{2\text{max}}$ ) (A) and heart rate (HR) (B) at progressing intensities from 50 to 80% of  $\dot{V}O_2$  in female rats ( $n = 8$ ). Because no differences were observed among tests, only data from test 2 (t2) and test 3 (t3) are presented. COV, coefficient of variation. Individual data are shown.



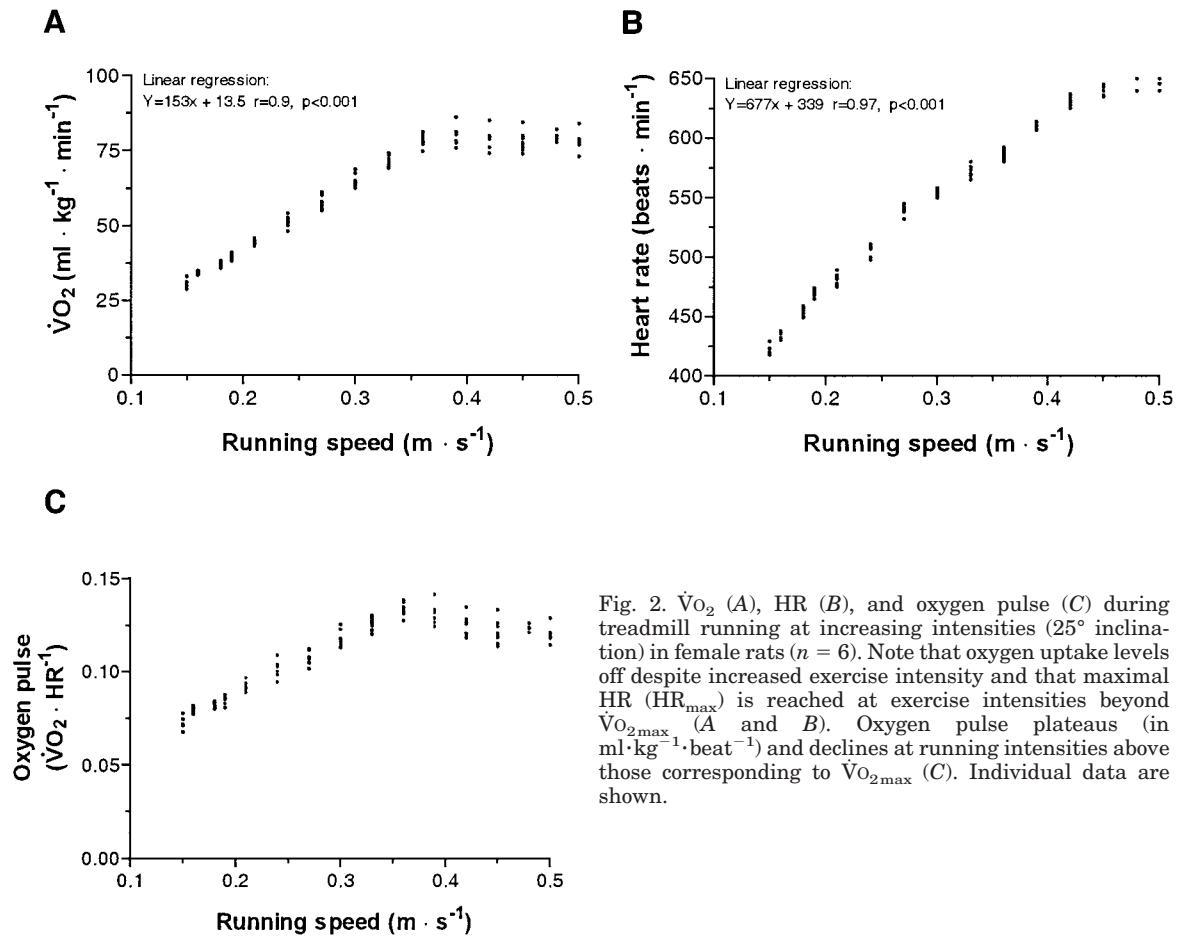


Fig. 2.  $\dot{V}O_2$  (A), HR (B), and oxygen pulse (C) during treadmill running at increasing intensities (25° inclination) in female rats ( $n = 6$ ). Note that oxygen uptake levels off despite increased exercise intensity and that maximal HR ( $\text{HR}_{\text{max}}$ ) is reached at exercise intensities beyond  $\dot{V}O_{2\text{max}}$  (A and B). Oxygen pulse plateaus (in  $\text{ml} \cdot \text{kg}^{-1} \cdot \text{beat}^{-1}$ ) and declines at running intensities above those corresponding to  $\dot{V}O_{2\text{max}}$  (C). Individual data are shown.

At all submaximal exercise intensities ( $< 70\%$  of  $\dot{V}O_{2\text{max}}$ ), HR was reduced by  $13 \pm 2.1\%$  ( $P < 0.01$ ) and  $11 \pm 1.7\%$  ( $P < 0.01$ ) after 13 and 7 wk of training in female (Fig. 5B) and male rats, respectively. In female rats trained for 4 wk, the corresponding reduction was  $7 \pm 2.2\%$  ( $P < 0.01$ ).

No gender effect was observed in the resting and submaximal oxygen pulse. Training increased the resting oxygen pulse by  $\sim 25\%$  in both male and female rats (13 wk). The maximal oxygen pulse (in  $\text{ml} \cdot \text{kg}^{-0.75} \cdot \text{beat}^{-1}$ ) in trained male rats was  $\sim 17 \pm 3.1\%$  higher compared with trained female rats (13 wk). The oxygen

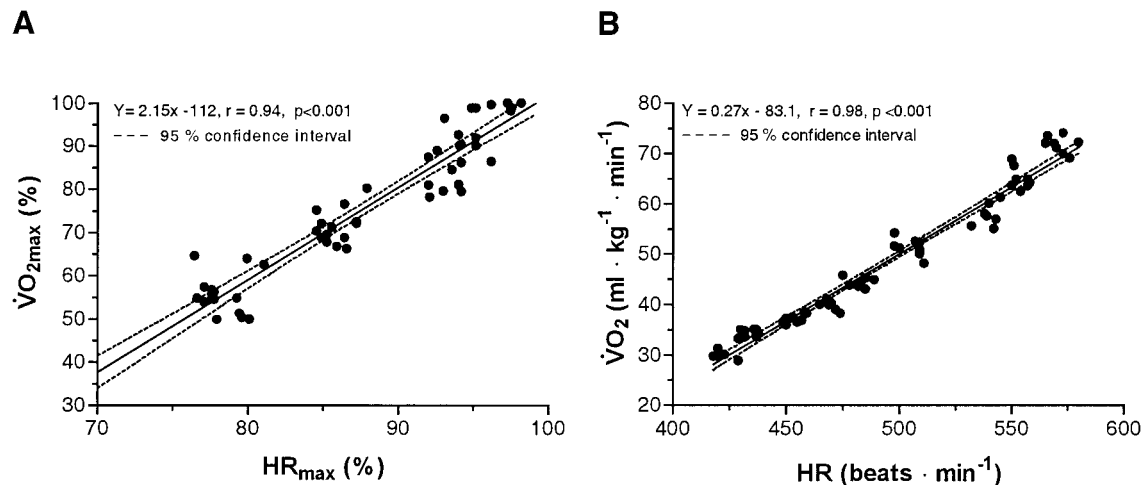


Fig. 3. Relationship between oxygen uptake and HR during treadmill running at 25° inclination in female rats ( $n = 6$ ). Note that the linear relationship is not strictly on a percent basis; when HR is 90% of the maximum, the oxygen uptake is  $\sim 80\%$  of  $\dot{V}O_{2\text{max}}$ . Individual data are shown.

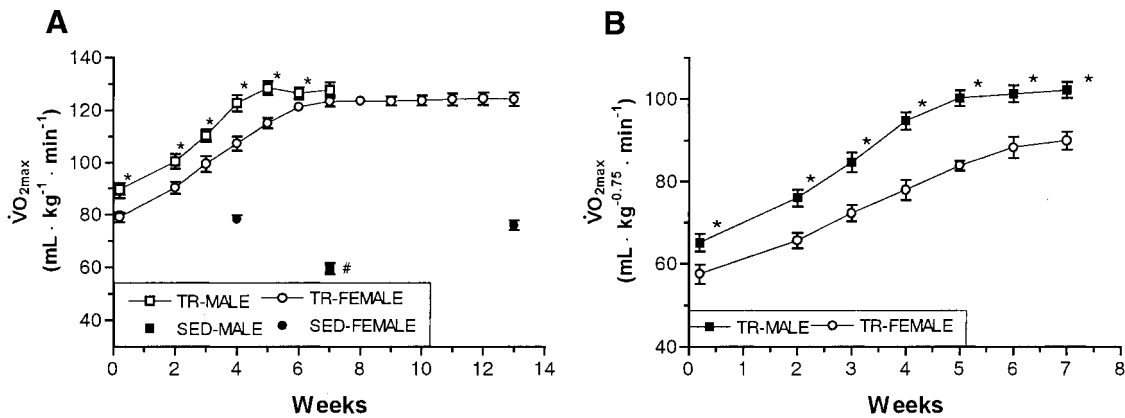


Fig. 4.  $\dot{V}O_{2\max}$  during the training period. Sedentary animals only performed pre- and posttests. Note that  $\dot{V}O_{2\max}$  levels off after 7 wk in both male and female rats. Differences between trained (TR) male vs. female rats,  $^*P < 0.001$ ; differences in sedentary (SED) males compared with pretest,  $\#P < 0.01$ . Data are means  $\pm$  SD.

pulse at increasing intensities for female rats is presented in Fig. 5C.

#### Training-Induced Cardiac Hypertrophy

In female rats, 4 wk of endurance training increased left and right ventricular weights by 10% and 12%, and, at 13 wk, the increases were 34% and 30%, respectively. The corresponding increases for trained male rats were 25% and 23% at 7 wk (Table 3). Table 3 further shows that, in sedentary males, ventricular weights were  $\sim 45\%$  and 10% higher than in sedentary and trained female rats (13 wk), respectively. When normalized to body weight (Table 5), there were no gender differences in ventricular weights in training or control rats. However, in relation to controls, and due to the substantial increase in body weight in sedentary males, trained males showed a more pronounced ven-

tricular hypertrophy than female rats, expressed in relation to body mass.

There were no gender differences in myocyte dimensions in sedentary animals. Endurance training increased left ventricular myocyte size by longitudinal growth, whereas width remained unchanged (Table 3).

#### DISCUSSION

The present experiments demonstrate that intensi-ty-controlled treadmill running induces substantial endurance conditioning and myocardial hypertrophy in rats. The results suggest that the present training protocol could be used in future studies of the cellular, molecular, and integrative mechanisms of improved cardiac function in both healthy animals and models of cardiovascular disease.

Table 3. *In vivo* and postmortem data

	Female Rats, 4 wk	Female Rats, 13 wk	Male Rats, 7 wk
Resting HR, beats/min			
Control	389 $\pm$ 2.5	389 $\pm$ 2.5	393 $\pm$ 3.7
Training	365 $\pm$ 4.6 $\dagger$	320 $\pm$ 5.7 $\dagger$	337 $\pm$ 5.1 $\dagger$
Resting oxygen pulse, mL $\cdot$ kg $^{-0.75}$ $\cdot$ beat $^{-1}$			
Control	0.12 $\pm$ 0.007	0.12 $\pm$ 0.008	0.11 $\pm$ 0.007
Training	0.13 $\pm$ 0.009	0.15 $\pm$ 0.003*	0.14 $\pm$ 0.008*
Right ventricular mass, mg			
Control	315.7 $\pm$ 10.0	314.3 $\pm$ 10.4	461.6 $\pm$ 18.2§
Training	366.5 $\pm$ 20.0*	409.8 $\pm$ 21.5 $\dagger$	577.0 $\pm$ 23.9 $\dagger$
Left ventricular mass, mg			
Control	787.4 $\pm$ 32.4	794.7 $\pm$ 14.0	1,155.1 $\pm$ 31.2§
Training	865.9 $\pm$ 21.1*	1,070.2 $\pm$ 18.9 $\dagger$	1,420.7 $\pm$ 27.1 $\dagger$
Left ventricular cell length, $\mu$ m			
Control	119.7 $\pm$ 2.9	119.4 $\pm$ 2.7	118.9 $\pm$ 3.2
Training	127.2 $\pm$ 3.3*	133.7 $\pm$ 2.5 $\dagger$	129.6 $\pm$ 3.1 $\dagger$
Left ventricular cell width, $\mu$ m			
Control	25.4 $\pm$ 1.4	26.4 $\pm$ 1.6	27.4 $\pm$ 1.7
Training	25.9 $\pm$ 1.9	25.3 $\pm$ 1.8	28.2 $\pm$ 2.1
Body wt, g			
Control	279 $\pm$ 6.5	280 $\pm$ 5.9	458 $\pm$ 10.8 $\dagger$
Training	284 $\pm$ 5.3	287 $\pm$ 6.2	367 $\pm$ 13.2

Values are means  $\pm$  SD. HR, heart rate. Resting oxygen pulse was  $\dot{V}O_2$ /HR normalized to body wt. Differences from control:  $^*P < 0.05$ ,  $\dagger P < 0.001$ , and  $\S P < 0.001$ ; higher than comparable values in female rats. Calculations of cardiomyocyte dimensions in female and male rats were performed in a total of 9,744 myocytes, with  $183 \pm 30$  myocytes from each animal.

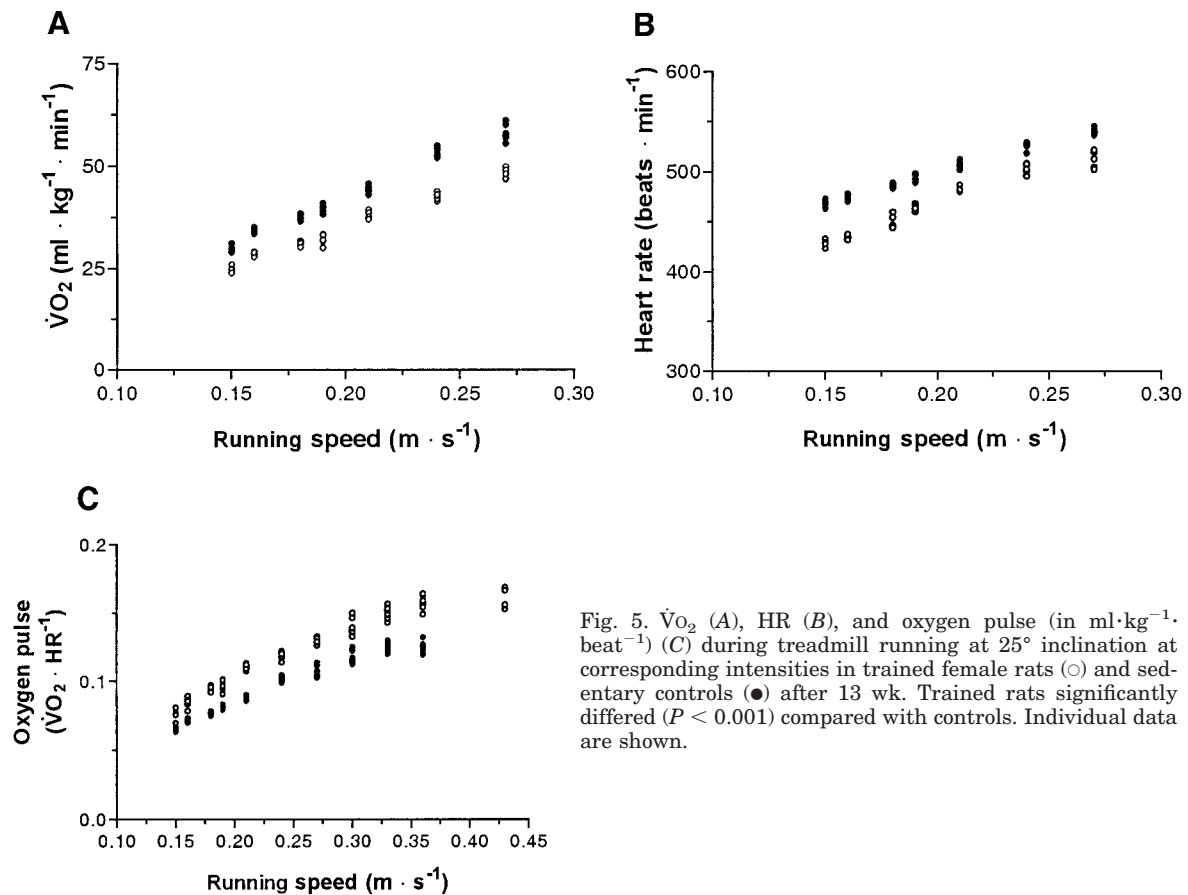


Fig. 5.  $\dot{V}O_2$  (A), HR (B), and oxygen pulse (in  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{beat}^{-1}$ ) (C) during treadmill running at  $25^\circ$  inclination at corresponding intensities in trained female rats ( $\circ$ ) and sedentary controls ( $\bullet$ ) after 13 wk. Trained rats significantly differed ( $P < 0.001$ ) compared with controls. Individual data are shown.

### Test Protocols

**Maximal oxygen uptake.**  $\dot{V}O_{2\max}$  was significantly affected by the inclination of the treadmill. Maximum values were 7–25% higher at  $25^\circ$  compared with the other inclinations. Thus use of inappropriate treadmill inclination might hide training-induced adaptations if the true  $\dot{V}O_{2\max}$  is not reached. An inclined (vs. level) treadmill offers the same advantage when testing rats as it does when testing humans; it recruits a larger

muscle mass and a slower cadence. At  $25^\circ$  inclination, there was no further increase in oxygen uptake despite increased running speed when testing  $\dot{V}O_{2\max}$  (Fig. 2A); the blood lactate concentration was above 6 mmol/l, and the respiratory exchange ratio was above 1.05 (Table 2). We therefore suggest that these criteria should be met in testing  $\dot{V}O_{2\max}$  in rats running on a treadmill, as in human subjects (3). In a similar study by Fitzsimons et al. (11), no leveling off of oxygen

Table 4. Respiratory exchange ratio at submaximal exercise levels in trained and sedentary female rats at pretest and at 4 and 13 wk of experimental period

Respiratory Exchange Ratio, $\dot{V}CO_2/\dot{V}O_2$	Female Rats		
	Pretest	4 Wk	13 Wk
<i>Exercise stage I (0.15 m/s)</i>			
Control	$0.87 \pm 0.02$	$0.86 \pm 0.01$	$0.87 \pm 0.02$
Training	$0.86 \pm 0.01$	$0.83 \pm 0.01^*$	$0.82 \pm 0.02^*$
<i>Exercise stage II (0.20 m/s)</i>			
Control	$0.93 \pm 0.02$	$0.93 \pm 0.01$	$0.92 \pm 0.01$
Training	$0.93 \pm 0.01$	$0.89 \pm 0.01^*$	$0.89 \pm 0.01^*$
<i>Exercise stage III (0.25 m/s)</i>			
Control	$0.96 \pm 0.01$	$0.97 \pm 0.02$	$0.97 \pm 0.01$
Training	$0.97 \pm 0.02$	$0.93 \pm 0.01^*$	$0.93 \pm 0.02^*$

Values are means  $\pm$  SD. Difference from control group at the same exercise stage: \* $P < 0.05$ .

Table 5. Ventricular dimension in relation to body mass

	Female Rats	Male Rats
<i>Left ventricular weight</i>		
<i>Relative expression I, mg/g</i>		
Control	$2.8 \pm 0.2$	$2.5 \pm 0.3$
Training	$3.7 \pm 0.3^*$	$3.8 \pm 0.2^*$
<i>Relative expression II, mg/g<sup>0.78</sup></i>		
Control	$9.8 \pm 0.6$	$9.7 \pm 0.5$
Training	$12.9 \pm 0.7^*$	$14.2 \pm 0.6^*$
<i>Right ventricular mass</i>		
<i>Relative expression I, mg/g</i>		
Control	$1.1 \pm 0.2$	$1.0 \pm 0.2$
Training	$1.4 \pm 0.1^*$	$1.6 \pm 0.3^*$
<i>Relative expression II, mg/g<sup>0.78</sup></i>		
Control	$3.9 \pm 0.2$	$3.9 \pm 0.2$
Training	$5.0 \pm 0.3^*$	$5.8 \pm 0.3^*$

Values are means  $\pm$  SD. Difference from controls: \* $P < 0.001$ .

uptake was found in female rats running at 20% ( $\sim 11^\circ$ ) inclination. These differences are probably due to differences in treadmill inclination, because we did not find any true leveling off at 0, 15, 20, or  $30^\circ$  inclination of the treadmill. It is conceivable that working at  $30^\circ$  or higher inclination results in a longer muscle contraction-relaxation duty cycle and sufficient intramuscular compression to obstruct local blood flow, thereby limiting peripheral oxygen transport and peak oxygen consumption. This view is supported by the fact that, compared with other inclinations evaluated, a significantly higher lactate concentration was observed during exercise at  $30^\circ$  inclination of the treadmill (Table 2).

**Relationship among oxygen uptake, HR, and power output.** HR was observed to increase linearly with power output (Fig. 2B), a typical feature of dynamic exercise with large muscle groups in rats (11) and humans (3). In many situations, the most practical way to control the training intensity is by monitoring HR. Obviously, the usefulness of such intensity scales depends on HR obtained from a reliable test of maximal HR. From regression analysis (Fig. 3A), it is apparent that maximal HR is not reached at  $\dot{V}O_{2\max}$ . This is a normal observation (3), because the relationship between HR and oxygen uptake becomes nonlinear at high exercise intensities. It is thus not necessary to reach maximal HR to reach  $\dot{V}O_{2\max}$ . This probably explains why we observed higher values of maximal HR compared with previous studies (11, 24). There exists no established protocol for measurement of maximal HR, but to achieve it usually requires exercise beyond the intensity at  $\dot{V}O_{2\max}$  (19). For practical reasons, we have chosen to modify a model established by Ingjer (19). Figure 3A shows that, with the present protocol, it is possible to estimate the relative exercise intensity (in percentage of  $\dot{V}O_{2\max}$ ) from HR measurements (measured as percentage of maximal HR) with an accuracy of about  $\pm 5\%$ . Furthermore, the estimate of oxygen uptake from HR is possible with an accuracy of  $\pm 6 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (Fig. 3B) using the present protocol. The usefulness of the former is obvious to control the relative exercise intensity by HR, whereas the latter could be used to estimate work economy. In the present study, a lowering of the HR by 25 beats/min at submaximal exercise intensities corresponds to a reduced oxygen uptake by  $\sim 5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ .

### Cardiovascular Effects of Aerobic Endurance Training

**$\dot{V}O_{2\max}$  and HR response.** The present study demonstrates that intensity-controlled interval running induces significantly larger training effects than previously reported (11, 13, 20, 24, 34). It is conceivable that these effects resulted from a carefully controlled level of exercise intensity throughout the study. Differences in training response reported in the literature are probably due to different training regimens used, insufficient control of exercise intensity, or different protocols for measuring  $\dot{V}O_{2\max}$ . The load required to

produce a training effect increases as the performance is improved in the course of training (3). The training load should, therefore, be set relative to the level of fitness of the individual. This principle may explain why we observed a leveling off after  $\sim 7$  wk of endurance training (Fig. 4). It is possible that a higher volume of training (e.g., 2 sessions per day) might have led to further improvements in  $\dot{V}O_{2\max}$  beyond 7 wk. Christensen (6) demonstrated, in humans, the need for a gradual increase in training load with improved performance, in the case of the effect on HR, as early as in 1931. He observed that regular endurance training at a given exercise rate gradually lowered the HR, in line with results from the present study (Fig. 5B). Further training did not modify this HR response. After a period of training at a higher load, a standard workload could then be performed with even lower HR. The following general principle of training is apparent in a number of parameters, among them  $\dot{V}O_{2\max}$ : an adaptation to a given load takes place and, to achieve further improvement, the absolute exercise intensity has to be increased (3). In a study by Fitzsimons et al. (11), 10 wk of treadmill training in female rats reduced resting HR similarly to that observed after 4 wk in the current study but less than after 7 and 13 wk. In the last 5 wk of exercise, Fitzsimons et al. (11) used a constant absolute exercise intensity, which probably explains the similarities and discrepancy in training response in these two studies. No change was found in the maximal HR over the experimental period. This finding is in agreement with a previous study (16) and confirms that training does not affect maximal HR. The lower resting HR observed in the endurance-trained rats may result from a reduced intrinsic HR, enhanced vagal tone in response to a higher stroke volume, or both. Reduced resting rate and submaximal HR are good indicators of the trained state (10, 16). No changes were observed in basal metabolism, which is in accordance with previous studies on training (16, 24).

**Work economy.** Comparing steady-state  $\dot{V}O_2$  at fixed absolute submaximal work intensities provides evidence of a different aspect of physical conditioning than a standard  $\dot{V}O_{2\max}$  test. Our experiments demonstrated that running economy remained stable from 4 to 13 wk, whereas  $\dot{V}O_{2\max}$  increased substantially. The amount of increase was similar to the 17% reported by Fitzsimons et al. (11) after 10 wk of endurance training, which was probably carried out at lower absolute intensities than those in the present study (12). It is conceivable that training at lower intensities may substantially improve work economy without changing  $\dot{V}O_{2\max}$  and that work economy, in many cases, could be a more relevant measure to assess the effect of specific training regimens. In some cases, improved work economy would, in fact, imply increased work capacity in the face of unchanged  $\dot{V}O_{2\max}$ .

**Oxygen pulse.** Oxygen pulse, or oxygen uptake divided by HR, is the volume of oxygen transported by the blood and extracted by peripheral tissues for each heartbeat. This variable is useful to assess changes in



stroke volume in response to training because it equals the product of stroke volume and the arterial-mixed venous oxygen difference. It has been shown that the upward displacement of the curve in response to training over time depends primarily on the stroke volume (31). In agreement with previous studies (31, 35), we have shown that training increased the oxygen pulse both at rest and during exercise (Fig. 5C and Table 3). Several factors may contribute to increased stroke volume in trained rats, including increased ventricular volumes because of hypertrophy, intrinsic or reflex bradycardia, and increased intrinsic myocardial contractility. Unpublished results from our laboratory indicate that cardiomyocytes isolated from trained rats have increased contractility and calcium sensitivity.

**Respiratory exchange ratio.** In line with Musch et al. (24), we observed that training lowers the respiratory exchange ratio at submaximal workloads in rats. These findings are in agreement with observations in human subjects. The greater the  $\dot{V}O_{2\max}$ , the greater the percent contribution of fat to the energy metabolism at a given work rate (9). As muscle glycogen stores are reduced, an increasing percentage of substrate utilization must be taken from fat. Individuals with better endurance capacity would be expected to "spare" glycogen during moderate intensities, providing greater reserves for fueling more intense exercise.

**Ventricular weights and left ventricular cardiac myocyte dimensions.** The increases in left ventricular weight in the present study are the largest ever reported in female rats after treadmill training. Our data also provide evidence that aerobic endurance training with sufficient training intensity leads to longitudinal myocyte growth. The cardiomyocyte elongation observed at 4 wk was similar to that observed after 20–30 wk of endurance training in previous studies (22, 23). After 7 and 13 wk, it was larger than in some studies (22, 23) but smaller than the 20% increase observed after 20 wk of treadmill running or swim training in young male and female rats, respectively (34). The differences could be age related or be due to the duration and/or intensity of the training regimen used.

**Gender differences.** In both genders, the increase in  $\dot{V}O_{2\max}$  plateaued after 5–7 wk of training. When properly normalized to body weight (in  $\text{ml}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$ ),  $\dot{V}O_{2\max}$  and oxygen pulse were 15% higher in males, which concurs with observations in humans (3, 32). [In sedentary rats,  $\dot{V}O_{2\max}$  appeared to drop when expressed as  $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  but remained unchanged when expressed as  $\text{ml}\cdot\text{kg}^{-0.75}\cdot\text{min}^{-1}$  (Fig. 4).] The discrepancy is probably because a large proportion of the substantial increase of body weight in sedentary rats resulted from increased fat content. Hence, the latter expression should be used when oxygen uptake and derived measures are compared between individuals with different body masses. Otherwise, the capacity of light individuals will be overestimated at  $\dot{V}O_{2\max}$  and underestimated at submaximal workloads, whereas the opposite will be the case for heavier individuals.

The present experiments demonstrated a comparable amount of ventricular hypertrophy after 7 and 13

wk of training in male and female rats, respectively. Unpublished data from our laboratory show that the degree of hypertrophy in females is similar at 8 and 13 wk. Although ventricular mass was larger in male rats, we found no gender differences in myocyte dimensions, suggesting that males have higher myocyte numbers. In contrast, White et al. (34) reported that male rats had larger cross-sectional areas of their endocardial myocytes than female rats and that this could partly explain the differences in ventricular mass. They found no enlargement of endocardial myocytes, only enlargement of epicardial myocytes. In their study, cell dimensions were measured by morphometry of tissue samples, whereas we quantified size in isolated myocytes, which probably originated from all myocardial layers. Also, differences in the age at start of training and the duration of training might account for the apparent discrepancies between the studies.

In contrast to previous studies (11, 14, 27, 28, 34), the present experiments do not support the hypothesis that exercise-induced cardiac hypertrophy requires swim training in female rats. It is more likely that the training intensity, not the mode used, determines the stimulus for cardiac hypertrophy. In swim training, it is very hard to control the relative and absolute training intensities, and it has been suggested that swimming involves a significant learning component in rats (12). Swimming is potentially stressful to the rat and may induce substantial sympathetic stimulation, high catecholamine levels, and increased HR and blood pressure, which may confound the interpretation of observed effects. It may be that the mode, intensity, and amount of physical activity should mimic and permit the normal behavior of the species to optimize the experimental model. It has been estimated that a wild rat runs ~8 km per night (25, 26). Distances run in the present study were ~1.8 km/day before and 2.5 km/day after 13 wk of training. Russell et al. (26) showed that it was easier to have a rat run voluntarily if it received a reward after running sessions. The fact that all rats in the present study were willing to run supports this view.

**Conclusions.** The present training model mimics important human responses to training with increased  $\dot{V}O_{2\max}$ , improved work economy, reduced HR, and myocardial hypertrophy. It could be used in future studies on cellular, molecular, and integrative mechanisms of cardiovascular adaptation to exercise, e.g., contractile characteristics and calcium regulation in cardiac myocytes and differences in signaling pathways between training-induced adaptive myocyte enlargement and hypertrophy observed in heart failure. Understanding the cellular mechanisms of training-induced amelioration of myocardial function may help identify molecular targets for the treatment and prevention of cardiovascular disease.

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