Maximal Aerobic Metabolism of Mice During Swimming[1] (36431)

ROGER M. GLASER, PAUL M. GROSS, AND HAROLD S. WEISS
Department of Physiology, The Ohio State University College of Medicine, Columbus, Ohio 43210

Maximal oxygen consumption ($\dot{V}_{O_2\,max}$) is generally considered the best single laboratory measurement of endurance (cardiorespiratory) type fitness (1–3), but because of difficulties in measuring $\dot{V}_{O_2\,max}$ in rats and mice, swim time to exhaustion is often substituted. Endurance swimming, however, represents a complexity of stresses—both physiological and psychological (4), and performance may be dependent upon several intrinsic factors besides $\dot{V}_{O_2\,max}$. Therefore, swim time to exhaustion may not be a repeatable measurement or a valid indicator of aerobic fitness.

Oxygen uptake of rats during swimming has been measured by several investigators (5–7), but it has not been reported for mice. It was the purpose of this study to determine the maximal oxygen uptake of swimming mice, and to evaluate its reliability and its relationship to swim time to exhaustion.

Methods. Oxygen uptake during swimming was continuously monitored by means of open-circuit spirometry. The swim chamber consisted of a Plexiglas cylinder (38.1 cm deep × 8.9 cm i.d.), sealed at the top except for gas fittings, which submerged into a 36° temperature controlled water bath. Water level in this chamber was maintained at 2.6 cm from its top. Air input to the chamber was from a compressed gas tank. Gas, pumped from the chamber at 1 liter/min, was dried (calcium chloride) and directed into a Servomex paramagnetic O_2 analyzer. This analyzer was modified by the addition of a dc output to drive a Grass polygraph. One percent O_2 (20.00–21.00) covered full scale on the polygraph. Air (20.93% O_2) and a reference gas mixture (20.03% O_2) were used for calibration. Ninety percent response time of this system (chamber clearance and analyzer lag) was about 30 sec as determined by injection of gas mixtures. Haldane analysis of these mixtures indicated an accuracy of 1%.

Absolute $\dot{V}_{O_2\,max}$ was a function of the maximal difference between the milliliters of O_2 entering the chamber and the milliliters of O_2 leaving the chamber per minute, corrected to standard conditions. This value was divided by the animal's body weight (kg) to give maximal relative oxygen consumption ($\dot{V}_{O_2\,max}$ ml/kg/min, STPD).

Prior to testing, the mouse was supported above the water level for 2 min while gaseous equilibrium took place. A movable floatation platform within the Plexiglas cylinder was used for this purpose. The platform was then lowered, allowing swimming to occur. To help prevent the mice from floating, bubble free (double distilled) water with the addition of 5 ml of liquid detergent was used in the bath. In addition, an eccentric flywheel agitator shook the chamber gently, creating water turbulence which appeared to make the mice swim more vigorously. To terminate a test, the platform was returned to its uppermost position.

Male albino mice (Swiss/Cox) 8–10 weeks old (av 35 g) were used. They were housed 6 in a cage (29.2 × 19.1 × 14.0 cm) and fed Purina Laboratory Chow and water *ad libitum*. Room temperature varied between 20–25°. All testing took place in the early evening.

To determine the tail load weight which elicited the highest $\dot{V}_{O_2}$, and the reliability of this measurement, 36 mice were used. Twelve mice were tested at each of three load intensities — 0 (no weight added), 5 or 7% of body weight. Swimming time was limited

[1] This project was supported in part by NASA Research Grant NGR 36-008-004.

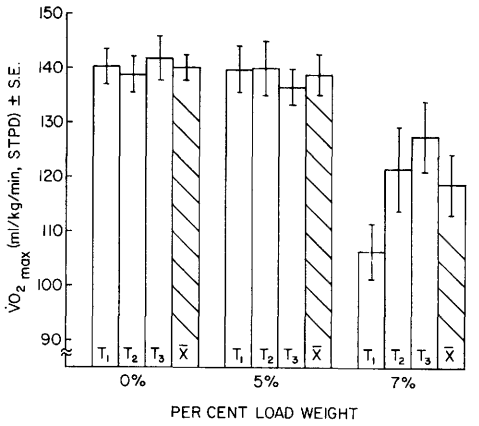

FIG. 1. Mean maximal oxygen uptake of mice swimming at 3 load intensities (% of body weight attached to tail). 12 mice swam at each load, and trials were spaced 3 days apart.

to 5 min. These groups were tested 3 times with a 3-day rest between each swim. Load weights were constructed from miniature alligator clips which were wrapped with solder (lead–tin alloy). Mean specific gravity was about 6.5 g/ml. Weights were clipped 1 in. from the end of the animals' tails.

Another 10 mice were used to examine the relationship between, and the reliability of swim time to exhaustion and $\dot{V}_{O_2\,max}$. These mice swam with a 7% load weight, and were tested twice with a 4-day rest between exposures. Exhaustion was considered the point at which the animal sank to the bottom of the chamber.

Results. A similar swimming pattern was seen for almost all animals regardless of their experience in this activity. Following initial attempts to float, they swam vertically at the surface with a minimum of bobbing. When exhaustion approached, bobbing increased followed by loss of coordinated movements and a marked decrease in $\dot{V}_{O_2}$. All 12 mice swimming without a load weight, and 11 of those with a 5% load, completed the three 5-min swims. At 7% load, 3 mice completed the swim for the first trial, 5 for the second and 7 for the third.

Mean maximal $\dot{V}_{O_2}$ at each load intensity for each trial is shown in Fig. 1. As indicated by the standard errors, essentially no differences in $\dot{V}_{O_2}$ was seen between trials within the 0 and 5% loads. In contrast, at 7% load, $\dot{V}_{O_2}$ tended to increase with each subsequent swim—106.3 ml/kg/min for T_1 compared to 127.4 ml/kg/min for T_3 ($p < .01$). The three $\dot{V}_{O_2}$ values for each subject were averaged, and in turn, load weight group means were established. A one-way analysis of variance applied to the three group means was highly significant ($p < .01$). The multiple range test showed that the significant difference lay between the 7% load (118.67 ml/kg/min) and either the 0% (140.24 ml/kg/min) or 5% (138.68 ml/kg/min) loads. No difference was seen between the 0 and 5% loads.

The $\dot{V}_{O_2\,max}$, recorded during each 1-min interval of the 5 min swim for naive mice at 0 and 5% loads, is given in Fig. 2. Even though the 5% group had a higher response during the first minute, both groups reached peak values during the second minute of exposure. Thereafter, $\dot{V}_{O_2}$ declined sharply for both groups, leveling off at 12.3% below peak response for the 0% group. The 5% group maintained this downward trend and was 23.1% below peak response during the fifth minute. The $\dot{V}_{O_2}$ for 7% load was not indicated graphically because only 3 mice completed the swim. It was, however, consider-

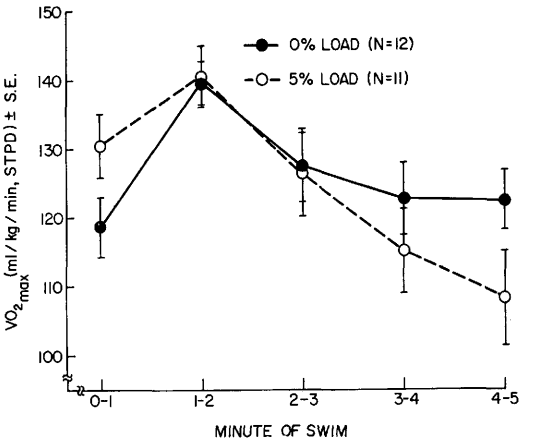

FIG. 2. Mean maximal oxygen uptake for the 0 and 5% load groups during each minute of their first swimming exposure.

TABLE I. Maximal Oxygen Uptake vs Swim Time for 10 Mice Swimming with 7% Load Weight Attached to Tail.

	Trial 1	Trial 2 (4 days later)	Increase (%) for trial 2
$\dot{V}_{O_2 max}$ (ml/kg/min) ± SE	116.6 ± 4.75	120.5 ± 4.38	3.3
Swim time (min) ± SE	4.92 ± 0.97	10.70 ± 1.44[a]	117.5
Correlation coef (r) ($\dot{V}_{O_2 max}$ vs swim time)	.27	.33	—

[a] Significant at the .01 level.

ably reduced at the termination of the test.

Results for the 10 mice who swam to exhaustion with 7% load weight are given in Table I. There was a nonsignificant 3.3% increase in $\dot{V}_{O_2}$ in the second trial, whereas the 117.5% increase in swim time was highly significant ($p < .01$). The low and nonsignificant correlation coefficients (av $r = .30$) indicated a rather poor relationship between $\dot{V}_{O_2 max}$ and swim time.

Discussion. The addition of load weights has been shown to greatly decrease swim time to exhaustion in rats and mice (8, 9). This had led to the logical belief that the increased energy demand due to loading is met by higher aerobic metabolism. Our findings do not support this. Oxygen uptakes at 0 and 5% load weights were almost identical, and increasing the load to 7% significantly decreased $\dot{V}_{O_2}$ (Fig. 1). Similar results were reported by McArdle (7) who titrated the load weight of swimming rats. As the load weight was increased, he found a slight increase in $\dot{V}_{O_2}$ in rats who swam at the surface, but for those who had difficulty remaining at the surface, $\dot{V}_{O_2}$ decreased. With 0 and 5% loads, our mice appeared to breathe through their mouths. As they swam lower in the water with 7% load, nose breathing was prevalent, possibly causing difficulty in gas exchange which could lower $\dot{V}_{O_2}$. At least for mice, and possibly for rats, it appears that the highest $\dot{V}_{O_2}$ during swimming may be achieved without loading providing the animal swims vigorously at the surface. Agitation of the water seems to elicit this vigorous swimming pattern in mice.

Because of the short time lag in this metabolism system (30 sec for 90% response), changes in $\dot{V}_{O_2}$ were detected quite rapidly. This damping would not effect $\dot{V}_{O_2}$ accuracy during steady state work of longer duration than the system's lag time. However, momentary peak responses may slightly underestimate actual $\dot{V}_{O_2}$.

About 140 ml/kg/min was the mean maximal $\dot{V}_{O_2}$ we obtained for swimming mice. Using a previously reported resting value of 40 ml/kg/min (10), swimming increased aerobic metabolism 3.5-fold in mice. Rats swimming in thermal neutral water similarly have been shown to increase $\dot{V}_{O_2}$ 3 to 3.5 times resting levels (5, 7). The maximal relative $\dot{V}_{O_2}$ of rats adept at swimming, however, was about 80 ml/kg/min (7). This lower value might be expected as there is an inverse relationship between relative $\dot{V}_{O_2}$ and body weight (10).

Peak $\dot{V}_{O_2}$ response occurred during the second minute of swimming for both the 0 and 5% groups (Fig. 2) followed by a decline in aerobic metabolism. This pattern was independent of the animal's experience, and indicated that a test of 5-min duration was adequate to detect maximal response. For the 0% group, we believe that the plateauing of $\dot{V}_{O_2}$ around 12% below peak response reflected an increase in swimming efficiency as unweighted mice can swim well over 1 hr (11, 12). In contrast, the continuous drop in $\dot{V}_{O_2}$ with the 5% load was probably due to the exhaustive effect of weighting. Seven percent was considered an overload and $\dot{V}_{O_2}$ fell off rapidly as exhaustion was approached.

Oxygen uptake increased with subsequent

swims at 7% load. This was possibly due to a training effect which enabled the animals to remain at the surface for longer periods. No training effect was seen with 0 and 5% loads, and mean $\dot{V}_{O_2\,max}$ for the 3 trials was reproducible. However, in spite of the consistency in mean responses, individual responses varied somewhat with each trial. By combining the 0 and 5% groups ($N = 24$), correlation coefficients for $\dot{V}_{O_2\,max}$ between each trial were found to be just significant ($p < .05$, $r = .43$ for T_1 vs T_2, and $r = .49$ for T_2 vs T_3).

Seven percent load was used to determine the reproducibility of, and the relationship between swim time to exhaustion and $\dot{V}_{O_2\,max}$ in mice. A reasonable swim time of about 5 min for naive mice was achieved with this weight (Table I), and because it was a supramaximal load, we hypothesized that the animal's swim time to exhaustion would be directly related to his $\dot{V}_{O_2\,max}$. With an r of about .3 (Table I) this hypothesis could not be substantiated. However, the consistency of $\dot{V}_{O_2\,max}$ was again shown in spite of the fact that swim time more than doubled for the second exposure. Training has been shown to improve swim time reliability in rats (8), and is probably necessary for mice as well.

Pacing and swimming efficiency could possibly account for the fact that there was almost no relationship between swim time and $\dot{V}_{O_2\,max}$. Some animals appeared to work hard in the initial stage of the swim, and even though relatively high aerobic power was achieved, swim time was short possibly due to blood lactate accumulation. Other subjects maintained a relatively low $\dot{V}_{O_2}$ for a considerable period of time. In addition, swim time may also be influenced by the availability of glycogen and fats for fuel, anaerobic capacity, and the animal's surrendering to the task before reaching exhaustion.

Summary. Using swimming stress to evaluate the effects of experimental conditions, the maximal oxygen consumption seems to be a preferable measurement to swim time to exhaustion. Mice swimming to exhaustion with 7% weight load in 36° water exhibited similar $\dot{V}_{O_2\,max}$ values for their first and second exposures. However, swim time more than doubled for the second exposure. Almost no correlation existed between $\dot{V}_{O_2\,max}$ and swim time. This suggests that swim time is dependent on several factors, both physiological and psychological, other than aerobic power. Mean maximal $\dot{V}_{O_2}$ for mice with 0 and 5% loads was about 140 ml/kg/min. This response was achieved well within the 5-min duration of the test and was consistent for three trials. At 7% load, $\dot{V}_{O_2\,max}$ was lower as difficulty was encountered remaining at the surface. This $\dot{V}_{O_2}$ test appears suited for experimental designs which require groups of mice to be tested several times. The duration of the test is relatively short, and no previous training is necessary. In addition, because of the high relative $\dot{V}_{O_2}$ of mice, they seem to be well suited for experiments which may alter the ability to pick up, deliver and consume oxygen.

The authors express their appreciation to Mr. Joseph F. Pitt for his assistance.

1. Åstrand, P-O., and Rodahl, K., "Textbook of Work Physiology," p. 314. McGraw-Hill, New York (1970).

2. Mitchell, J. H., Sproule, B. J., and Chapman, C. B., J. Clin. Invest. **37**, 538 (1958).

3. Taylor, H. L., Buskirk, E., and Henschel, A., J. Appl. Physiol. **8**, 73 (1955).

4. Dawson, C. A., and Horvath, S. M., Med. Sci. Sport **2**, 51 (1970).

5. Baker, M. A., and Horvath, S. M., J. Appl. Physiol. **19**, 1215 (1964).

6. Dawson, C. A., Roemer, R. B., and Horvath, S. M., J. Appl. Physiol. **29**, 150 (1970).

7. McArdle, W. D., J. Appl. Physiol. **22**, 50 (1967).

8. McArdle, W. D., and Montoye, H. J., J. Appl. Physiol. **21**, 1431 (1966).

9. Werboff, J., Haggett, B. N., and Anderson, A., Physiol. Behav. **2**, 39 (1967).

10. Krogh, A., "The Comparative Physiology of Respiratory Mechanisms," p. 3. Univ. of Pennsylvania Press, Philadelphia (1959).

11. Foss, C. R., and Horvath, S. M., Proc. Soc. Exp. Biol. Med. **120**, 588 (1965).

12. Wilber, C. G., and Hunn, J. B., J. Appl. Physiol. **15**, 704 (1960).

Received Dec. 27, 1971. P.S.E.B.M., 1972, Vol. 140.