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Fuel homeostasis in the harbor seal during submerged swimming

R.W. Davis[1,*], M.A. Castellini[2], T.M. Williams[3], and G.L. Kooyman[1]

[1] Physiological Research Laboratory, Scripps Institution of Oceanography, La Jolla, CA 92037, USA
[2] Institute of Marine Science, University of Alaska, Fairbanks, AK 99775, USA
[3] Naval Oceans Systems Center, P.O. Box 997, Kailua, HI 96734, USA

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Summary. 1. The turnover rates and oxidation rates of plasma glucose, lactate, and free fatty acids (FFA) were measured in three harbor seals (average mass = 40 kg) at rest or during voluntary submerged swimming in a water flume at 35% (1.3 $m \cdot s^{-1}$) and 50% (2 $m \cdot s^{-1}$) of maximum oxygen consumption ($\dot{M}O_{2max}$).

2. For seals resting in water, the total turnover rates for glucose, lactate, and FFA were 23.2, 26.2, and 7.5 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, respectively. Direct oxidation of these metabolites accounted for approximately 7%, 27%, and 33% of their turnover and 3%, 7%, and 18% of the total ATP production, respectively.

3. For swimming seals, $\dot{M}O_{2max}$ was achieved at a drag load equivalent to a speed of 3 $m \cdot s^{-1}$ and averaged 1.85 mmol $O_2 \cdot min^{-1} \cdot kg^{-1}$, which is 9-fold greater than resting metabolism in water at 18 °C.

4. At 35% and 50% $\dot{M}O_{2max}$, glucose turnover and oxidation rates did not change from resting levels. Glucose oxidation contributed about 1% of the total ATP production during swimming.

5. At 50% $\dot{M}O_{2max}$, lactate turnover and anaerobic ATP production doubled, but the steady state plasma lactate concentration remained low at 1.1 m*M*. Lactate oxidation increased 63% but still contributed only 4% of the total ATP production. Anaerobic metabolism contributed about 1% of the total ATP production at rest and during swimming.

6. The plasma FFA concentration and turnover rate increased only 24% and 37% over resting levels, respectively, at 50% $\dot{M}O_{2max}$. However, the oxidation rate increased almost 3.5-fold and accounted for 85% of the turnover. The percentage of total ATP produced (21%) from FFA oxidation at 35% and 50% $\dot{M}O_{2max}$ did not increase greatly over that at rest.

7. Dive duration decreased from 78 s while resting in water to 28 s at 50% $\dot{M}O_{2max}$.

8. The RQ ranged from 0.78 at rest to 0.74 at 50% $\dot{M}O_{2max}$, indicating that fat was an important source of energy during submerged swimming.

9. By adjusting breath-hold duration during strenuous underwater swimming, harbor seals are able to maintain an aerobic, fat-based metabolism.

Key words: *Phoca vitulina* – Swimming – Metabolism – Fuel

Abbreviations: ATP adenosine-triphosphate; [*LAC*] lactate concentration; [*GLU*] glucose concentration; [*FFA*] free fatty acid concentration; $\dot{M}O_2$ oxygen consumption; $\dot{M}O_{2max}$ maximum oxygen consumption; $\dot{M}CO_2$ carbon dioxid production; *RQ* respiratory quotient; *TG* triglycerides

* To whom offprint requests should be sent
** *Present address:* Department of Marine Biology, Texas A & M University at Galveston, Galveston, TX 77553, USA

Introduction

When terrestrial vertebrates exercise at a submaximal level, a suite of physiological and metabolic changes occur that establish a new steady state at an increased level of energy metabolism. The principal requirements for the new steady state are an increase in the delivery of oxygen and fuel to the active tissues (primarily skeletal muscle) and the concomitant removal of carbon dioxide, metabolic by-products, and excess heat. To a large extent, these adjustments are made by increasing ventilation and tissue blood flow. However, when seals swim underwater, they stop breathing, decrease their heart rate, and reduce peripheral blood flow as part of a series of physiological changes called the dive response (Elsner and Gooden 1983). Although the bradycardia and peripheral vasoconstriction that occur during voluntary dives and submerged swimming are less pronounced than during forced dives, the delivery of oxygen to active muscles appears to be reduced at a time when their need for oxygen has increased (Kooyman and Campbell 1972; Jones et al. 1973; Guppy et al. 1986; Castellini 1988; Fedak et al. 1988; Williams et al. 1990). Nevertheless, blood lactic acid profiles indicate that muscle metabolism usually remains aerobic (Kooyman et al. 1980; Guppy et al. 1986; Castellini et al. 1988). To accomplish this, the seal must balance the rate of oxygen utilization and the dive duration so as not to exceed the body oxygen stores, which are 3–4 times larger per kilogram of body mass than in a terrestrial mammal. If this bal-

ance is not achieved, then metabolism will become anaerobic.

The metabolism of important metabolic fuels such as free fatty acids (FFA) and glucose will also depend on the distribution of blood flow and the level of tissue oxygenation. When the tissues are well perfused and oxygenated at the surface, seals metabolize fat as their principal source of energy (Davis 1983). This probably results from their fish diet, which is rich in fat and protein but contains little carbohydrate (Roberts et al. 1943; Blazquez et al. 1971; Kettelhut et al. 1980). In contrast, the severe peripheral vasoconstriction that occurs during forced submergence and voluntary dives of very long duration causes many of the seal's tissues to become hypoxic after several minutes. The resulting switch to anaerobic metabolism in these tissues requires glucose as a fuel, which is available in limited quantities from liver and muscle glycogen stores and must be synthesized by gluconeogenesis. As a fuel, glucose can not sustain even basal metabolism for very long under anaerobic conditions. However, the results from physiological studies of Weddell seals *(Leptonychotes weddellii)* suggest that most tissues remain aerobic during voluntary dives, and that fat may continue to be the principal metabolic fuel (Kooyman et al. 1980) In terms of readily available energy, this seems reasonable given the high fat diet and large fat stores that are characteristic of seals. However, fuel homeostasis has yet to be quantified in any marine mammal during voluntary dives.

In this study, we measured the plasma turnover rates and oxidation rates of glucose, lactate, and FFA in harbor seals that were swimming submerged. We used this information to quantify the oxidative energy production from these metabolites as well as the anaerobic contribution to total metabolism. We designed the experiments so that the seals could choose the duration of submergence while they were swimming at two water velocities that required a submaximal effort. The results show that the seals decrease dive duration at increasing levels of exertion and in doing so are able to maintain an aerobic, fat-based metabolism.

Materials and methods

Animals. Three male, subadult harbor seals (average body mass = 40 kg) were held in large concrete pools that received a continuous supply of filtered sea water. They were fed twice daily on a diet of fresh frozen herring and mackerel that was supplemented with vitamins. The seals were fasted overnight before each experiment. This study was conducted in accordance with standard guidelines for the care and use of laboratory animals established by the National Institutes of Health (Publication No. 85–23).

Catheterization. Each seal was anesthetized with an intramuscular injection of ketamine chloride (3 mg · kg^{-1}) and two catheters inserted percutaneously into the intravertebral extradural vein. With the aid of fluoroscopy, the tip of the infusion catheter (4F, 30 cm) was positioned near the first thoracic vertebra. The tip of the second catheter (4F, 10 cm), which was used for blood sampling, was positioned about 20 cm caudal from the tip of the first catheter. By injecting radiopaque dye through both catheters, it was confirmed that blood in this region of the intravertebral extradural vein flows in an anterior direction. The relative positions of the two catheters ensured that blood samples were taken upstream from the point of isotope infusion. The catheters were filled with heparinized saline and secured in position by gluing (cyanoacrylic) the ends to the seals' hide. Cephalothin (Keflin, 1 g) in sterile saline was infused daily to prevent infection. In an earlier study (Davis 1983), this catheter arrangement was observed to give the same calculated turnover rates for lactate and glucose as infusing into the pulmonary artery and taking mixed venous blood samples from the right atrium. Surgical catheterization of the heart is difficult in seals compared to percutaneous catheterization of the intravertebral extradural vein.

Radioisotopes. Radioactivity labeled L-(^{14}C(U))-lactate, D-(^{14}C(U))-glucose, and Na$H^{14}CO_3$ were each diluted in 30 ml sterile saline to activities of 17, 33, and 30 μCi · ml^{-1}, respectively. The acidity of the Na$H^{14}CO_3$ was adjusted from pH 6.3 to 8 to prevent the loss of the radioactive label as $^{14}CO_2$ (this was verified by measuring the activity of the infusate before and after each experiment). Radioactively labeled (1-^{14}C)-palmitic acid in toluene was prepared for infusion by evaporating the toluene under a flow of dry nitrogen gas. The palmitic acid was then redissolved in 2 ml 100% ethanol and diluted in 30 ml sterile saline containing 2% bovine serum albumin. For one experiment, ^{3}H-(9,10)-oleic acid was simultaneously infused along with (1-^{14}C)-palmitic acid. The activities of palmitic acid and oleic acid were 33 μCi · ml^{-1}.

Swimming metabolism. The swimming metabolism of each seal was measured in a flow channel using methods described previously (Davis et al. 1985). The channel was 1.1 m square and 16 m long, with the center 12 m equipped with glass walls for viewing. Closed-loop flow from zero to 1.4 m · s^{-1} was generated by two propeller pumps. Water velocity was measured with an electromagnetic flow sensor positioned 30 cm above the floor of the flume. A vertical profile of water velocity varied by less than 0.1 m · s^{-1}. Water temperature in the flow channel ranged from 18 to 23 °C.

Seals swam in a test section 2.5 m long that was covered with a plexiglass metabolic hood (1.1 m long, 0.6 m wide, 0.3 m high). Air was pumped through intake and exhaust ports mounted in the dome. Seals determined their own breathing frequency but could surface only inside the dome. Oxygen consumption ($\dot{M}O_2$, mmol · min^{-1}) and carbon dioxide production ($\dot{M}CO_2$, mmol · min^{-1}) were measured as described by Davis et al. (1985).

Maximum oxygen consumption. Prior to the experiments, the $\dot{M}O_{2max}$ of each seal was estimated while it swam in the flow channel. In order to measure $\dot{M}O_{2max}$, the seals had to swim faster than 1.4 m · s^{-1} which was the maximum water velocity in the flow channel. To increase swimming effort, three drag cups of nylon fabric (12 cm × 12 cm) were attached with velcro to pieces of neoprene rubber that were glued (cyanoacrylic) to the seal's hide, one on each flank behind the fore flippers and one mid-dorsally. The increased drag created by these cups exceeded the ability of the seals to swim against a water velocity of 1.4 m · s^{-1}.

The $\dot{M}O_2$ of each seal was estimated during five swimming sessions. Seals swam at 0.5 m · s^{-1} for 10 min to become orientated in the flume, after which the velocity was increased in increments of 0.2 m · s^{-1} at 2-min intervals to a point where the seal was just able to maintain its position in the flume. The seals used their hind and fore flippers for propulsion during maximum effort, which they could sustain for only a few minutes; the session was then ended for the day. After estimating the $\dot{M}O_{2max}$, each seal was conditioned to swim with the drag cups at 35% and 50% of its $\dot{M}O_{2max}$ for 60 min. Five practice sessions scheduled several weeks prior to an experiment were adequate to train a seal to swim consistently at each speed in order to obtain reproducible values for oxygen consumption.

Equivalent swim speed without drag cups. The metabolic power required for the seals to overcome the additional drag created by the cups was equivalent to that while swimming at a faster speed. To estimate the equivalent speed without drag cups, we used the

methods of Williams and Kooyman (1985) and Williams et al. (1990). Briefly, the drag force produced by the seals with and without the cups (and infusion pump, details below) was measured by towing the animals behind a variable speed, electrically driven cart. We trained the seals to bite onto a soft neoprene mouthpiece on the end of a tow rope that was connected to a strain gauge mounted on the cart. The seals were passively towed around a ring tank that was 4 m deep and whose outer and inner diameters were 21 m and 15 m, respectively. Drag force was measured at speeds from 1 to 4 $m \cdot s^{-1}$ while the seals were submerged at a depth of 1 m. The drag-equivalent speed for an unencumbered seal swimming in the water flume was then estimated from the measured relationship of drag force versus towing speed with and without drag cups. In calculating the drag-equivalent swimming speeds, we assumed that the effect of the drag cups was independent of whether the seal was towed through the water or actively swimming. At 35% $\dot{M}O_{2max}$, the water velocity in the flume was 0.5 $m \cdot s^{-1}$ and the drag-equivalent speed with three drag cups (or two drag cups and an infusion pump) was 1.3 $m \cdot s^{-1}$. At 50% $\dot{M}O_{2max}$, the water velocity was 0.8 $m \cdot s^{-1}$ and the drag-equivalent speed was 2.0 $m \cdot s^{-1}$.

Experimental design. Four experiments were conducted with each catheterized seal. Labeled lactate, glucose, and palmitic acid were infused on days 1, 3, and 5, respectively. No experiments were performed on days 2, 4, and 6 to allow the seal to rest and feed. A solution of $NaH^{14}CO_3$ was infused on day 7 to measure the retention of $^{14}CO_2$ and derive correction factors for the oxidation of labeled lactate, glucose, and palmitic acid during rest and swimming (Steele et al. 1956; Wolfe and Burke 1977; Davis 1983). Each experiment consisted of an initial 2 h period resting in water in the flow channel followed by 30–60 min of swimming at 35% $\dot{M}O_{2max}$; for 50% $\dot{M}O_{2max}$ only two experiments were conducted because of behavioral problems with one of the seals.

After administering a priming dose (ratio of priming dose to infusion rate was 65:1), isotopically labeled metabolites were continuously infused (0.0511 $ml \cdot min^{-1}$) using a battery powered Porta Cath infusion pump (Pharmacia Nu Tech, MA) mounted in a waterproof plexiglass housing 16 cm in diameter and 7 cm wide. The housing was attached with velcro straps to neoprene patches glued to the seal's hide in the mid-dorsal area. The infusion pump was connected to the infusion catheter through a waterproof connector mounted in the wall of the plexiglass housing. A 2-m catheter extension on the blood sampling catheter trailed behind the seal during swimming. Blood samples were taken by retrieving the end of the catheter through a trap door mounted behind the plexiglass dome. Before taking a blood sample, the catheter was cleared of saline and old blood by withdrawing a pre-sample that was three times the volume of the catheter. After blood samples were taken, the catheter was flushed with saline and sealed with a stopcock.

Lactate, glucose and FFA oxidation rates. In addition to measuring $\dot{M}O_2$ and $\dot{M}CO_2$, oxidation rates for lactate, glucose and FFA were determined by measuring $^{14}CO_2$ production. A portion of the gas exiting the chamber was pumped through a calibrated flow meter using a sealed diaphragm pump and bubbled through a graduated cylinder containing 2 l 5% NaOH. Earlier tests in which known amounts of CO_2 were passed through the NaOH solution showed no detectable escape of CO_2. Duplicate 1.0-ml aliquots of the solution were taken every 10 min during the experiment and their activities later determined by liquid scintillation counting. Quenching was corrected using internal standards. The fractional flow through the CO_2 trap was corrected to give the total rate of $^{14}CO_2$ formation (Davis 1983).

Sample analysis. Serial blood samples taken with heparinized syringes were placed in tubes with sodium fluoride, centrifuged, and the plasma frozen. Plasma lactate and glucose were analyzed enzymatically with Sigma kits No. 826-UV and No. 15-UV, respectively. Separate blood samples in non-heparinized syringes were taken and centrifuged for the determination of free fatty acids (FFA) in plasma by the method of Shimizu et al. (1979). Lactate, glucose, and FFA specific activities were determined by the methods of Reilly (1975), Jones (1965), and Bergmann et al. (1980) as modified by Castellini et al. (1987), respectively. Sample activity was determined with a Beckman LS 8000 Liquid Scintillation Counter and quenching was corrected using internal standards. Crossover activity was corrected for samples doubly labeled with ^3H and ^{14}C.

Calculations. Equations for calculating the steady state rates of turnover, oxidation and ATP production for glucose, lactate and FFA have been described (Davis 1983). The kinetic relationship of lactate and glucose under steady state conditions was determined using the mathematical model of Depocas and DeFreitas (1979) for an open, two-pool system in isotopic and material equilibrium (Fig. 1). This model may be applied to any two interconvertible metabolites (M_1, M_2) and provides the independent rates of formation and removal (R_{10}, R_{01}, R_{20}, R_{02}) and the rates of interconversion (R_{21}, R_{12}). A ^{14}C-tracer for each metabolite is infused into each animal during different experiments, and the results are combined in a single model. In this case, ^{14}C-lactate was infused at a constant rate β and the specific activities of lactate (D) and glucose (C) derived from lactate were measured. Two days later, ^{14}C-glucose was infused at a constant rate (α) and the specific activities of glucose (A) and lactate (B) derived from glucose were measured. At isotopic and material equilibrium, six equations can be derived to yield the six unknown rates:

$$R_{10} = [\alpha(D-C)]/(DA-BC), \quad R_{01} = (\alpha D-\beta B)/(DA-BC)$$
$$R_{20} = [\beta(A-B)]/(DA-BC), \quad R_{02} = (\beta A-\alpha C)/(DA-BC)$$
$$R_{21} = \beta B/(DA-BC), \quad R_{12} = \alpha C/(DA-BC)$$

The steady state turnover rates ($mmol \cdot min^{-1}$) for lactate, glucose, and FFA equal the rate of tracer infused ($\mu C \cdot min^{-1}$) divided by the steady state specific activity ($\mu Ci \cdot mmol^{-1}$). Because ^{14}C-tracers are recycled during metabolism, they measure net rather than total turnover rates. The total turnover rates for glucose and lactate were estimated from the steady state model. The Cori cycle (glucose – lactate – glucose) was calculated from the steady state model as the fraction of glucose metabolized to lactate which was resynthesized to glucose.

Apparent oxidation rates (Ro, $mmol \cdot min^{-1}$) for lactate, glucose, and FFA were calculated as follows:

$$Ro = \frac{^{14}CO_2 \text{ expired} \times R}{^{14}C \text{ infused}}$$

where the expired $^{14}CO_2$ ($\mu Ci \cdot min^{-1}$) has been corrected for bicarbonate pool behavior (Davis 1983); the activity infused

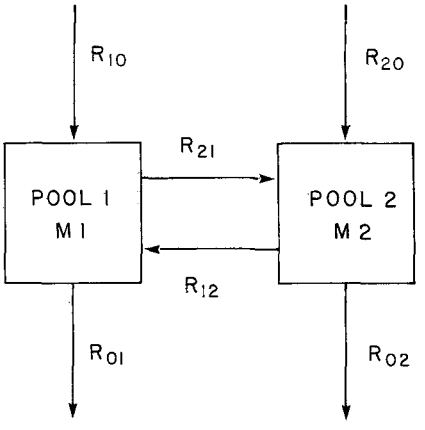

Fig. 1. Two-compartment steady-state model for the independent rates of formation and removal (R_{10}, R_{01}, R_{20}, R_{02}) and rates of interconversion (R_{21}, R_{12}) for any two interconvertible metabolites M_1 and M_2

($\mu Ci \cdot min^{-1}$) equals the infusion rate of labeled metabolite; R is the net turnover rate ($mmol \cdot min^{-1}$). Apparent oxidation rates include the oxidation of labeled metabolites derived from the infused tracer and overestimate rates of direct oxidation. The steady state oxidation rates for lactate and glucose can be partially corrected for the simultaneous oxidation of labeled glucose and lactate, respectively. For example, the corrected oxidation rate for lactate equals the apparent rate (8.4 μmol lac · min^{-1} · kg^{-1} or 35% of lactate turnover) minus the fraction of glucose oxidized which was derived from lactate (3.3 μmol glu · $min^{-1} \cdot kg^{-1} \times 0.21 \times 2$ μmol lac · μmol $glu^{-1} = 1.4$ lac · min^{-1} · kg^{-1}) Hence, the direct rate of oxidation equals $8.4 - 1.4 = 7.0$ μmol lac · $min^{-1} \cdot kg^{-1}$ or 30% of total lactate turnover. A similar calculation can be made for glucose. All values used in these calculations were derived from the steady state model for lactate and glucose metabolism.

The percentage of energy metabolism contributed by lactate, glucose, or FFA oxidation was calculated as follows:

$$\%P = \frac{Ro \times A \times 100}{\dot{M}O_2 \times 5.65 \text{ mmol ATP} \cdot \text{mmol } O_2^{-1}}$$

where %P is the percent energy contribution; Ro the oxidation rate in $mmol \cdot min^{-1}$; A the ATP produced during oxidation of lactate (17 mmol ATP · $mmol^{-1}$), glucose (36 mmol ATP · $mmol^{-1}$) or FFA (130 mmol ATP · $mmol^{-1}$); $\dot{M}O_2$ the oxygen consumption in $mmol\ O_2 \cdot min^{-1}$. Assuming fat is the primary metabolic fuel (respiratory quotient about 0.74), 5.65 mmol ATP · mmol O_2^{-1} is the average ATP production per millimole O_2 consumed.

Results

Resting in water

The average $\dot{M}O_2$, $\dot{M}CO_2$, respiratory quotient (RQ), and the concentrations of lactate, glucose, and FFA for the three seals were stable while resting in water, indicating that the animals were in a steady state condition (Fig. 2 and Table 1). The total turnover rates for glucose, lactate, and FFA were 23.2, 26.2, and 7.5 μmol · $min^{-1} \cdot kg^{-1}$, respectively (Table 1; see Appendix 1 for data used in the calculations of turnover and oxidation rates). There was no difference in the turnover rates of palmitic and oleic acids. Direct oxidation accounted for approximately 7%, 27%, and 33% of the turnover for glucose, lactate, and FFA, respectively.

A steady-state model for glucose and lactate metabolism is shown in Fig. 3 (see Appendix 1 for data used in the model). The Cori cycle represented 16% of glucose turnover, or 3.7 μmol · $min^{-1} \cdot kg^{-1}$ (Table 1), and was calculated from the model as follows: 20 μmol · $min^{-1} \cdot kg^{-1}$ of lactate production came from glucose (i.e., 2×10 μmol glucose · $min^{-1} \cdot kg^{-1}$) and 37% of this was converted back into glucose (or 20 μmol lac-

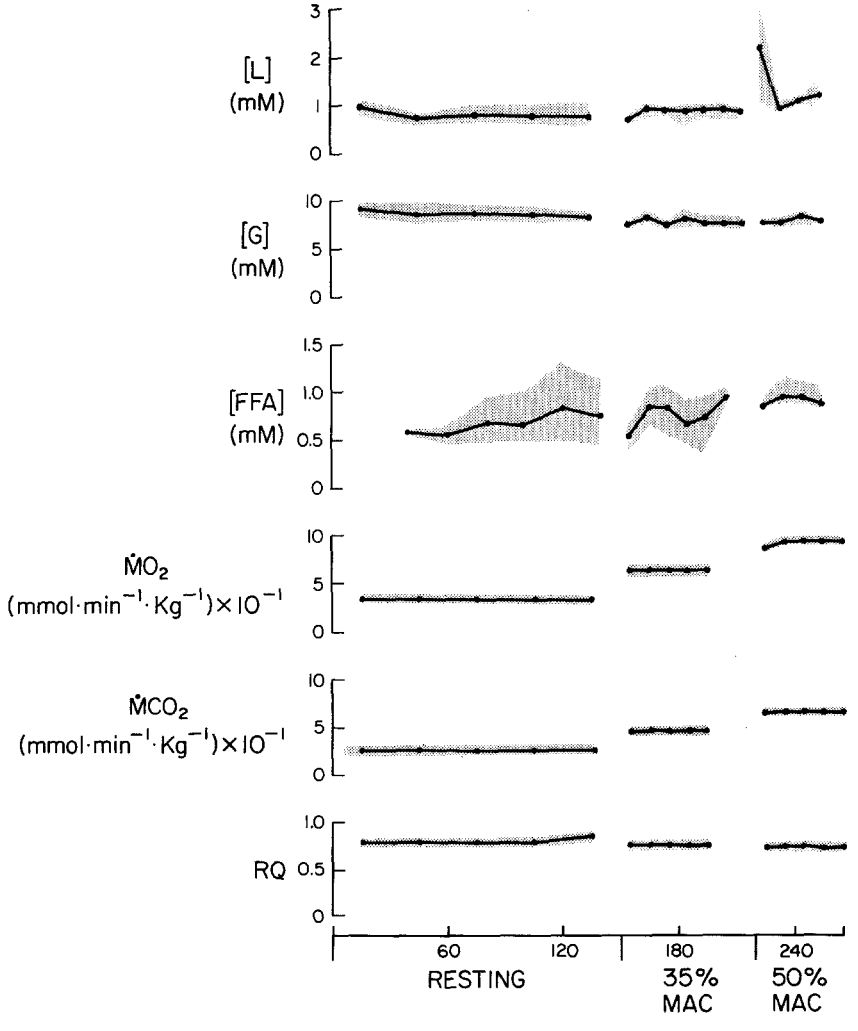

Fig. 2. Average values for lactate, glucose, and FFA concentrations, $\dot{M}O_2$, $\dot{M}CO_2$ and RQ for harbor seals resting in water and swimming at 35% and 50% $\dot{M}O_{2max}$. Shaded areas indicate the range of values

Table 1. Gas exchange and rates of turnover, recycling, and oxidation for glucose, lactate, and FFA. The results at rest and 35% $\dot{M}O_{2max}$ are averaged from three seals with the range shown in parentheses. At 50% $\dot{M}O_{2max}$, the results are from two seals

	Rest	35% $\dot{M}O_{2max}$	50% $\dot{M}O_{2max}$
Gas Exchange			
$\dot{M}O_2$ (mmol · min^{-1} · kg^{-1}) × 10^{-1}	3.24 (2.9–3.4)	6.19 (5.7–6.7)	9.10 (9.0–9.2)
$\dot{M}CO_2$ (mmol · min^{-1} · kg^{-1}) × 10^{-1}	2.54 (2.4–2.7)	4.60 (4.3–4.9)	6.51 (6.5–6.6)
RQ	0.78 (0.74–0.82)	0.74 (0.73–0.77)	0.72 (0.72–0.73)
Glucose Metabolism			
[GLU] (m*M*)	8.6 (8.3–9.4)	7.7 (7.3–8.6)	7.8 (7.3–8.3)
Total turnover rate (μmol · min^{-1} · kg^{-1})[a]	23.2 (19–26)	21.4 (16–24)	23.0 (18–28)
Net turnover rate (μmol · min^{-1} · kg^{-1})[b]	19.6 (15–24)	17.2 (13–21)	15.8 (14–18)
Cori cycle (μmol · min^{-1} · kg^{-1})[c]	3.7 (3–5)	4.2 (3–7)	7.2 (4–10)
Oxidation rate (μmol · min^{-1} · kg^{-1})	1.5 (0–4)	1.2 (0–3)	0.8 (1)
% Oxidation (%)	7 (0–13)	6 (0–14)	4 (3–5)
Lactate Metabolism			
[LAC] (m*M*)	0.8 (0.6–1.0)	0.9 (0.7–1.0)	1.1 (0.9–1.3)
Total turnover rate (μmol · min^{-1} · kg^{-1})[a]	26.2 (17–38)	39.7 (25–68)	55.0 (32–78)
Net turnover rate (μmol · min^{-1} · kg^{-1})	21.5 (16–30)	30.6 (20–49)	37.1 (24–50)
Oxidation rate (μmol · min$_{-1}$ · kg^{-1})	7.0 (7)	12.4 (11–14)	11.4 (11–12)
% Oxidation (%)	27 (19–40)	31 (20–47)	21 (14–38)
FFA Metabolism			
[FFA] (m*M*) × 10^{-1}	7.32 (4.91–11.13)	7.83 (4.74–9.70)	9.10 (9.1)
FFA Turnover (μmol · min^{-1} · kg^{-1})	7.5 (5–11)	8.8 (6–13)	10.3 (8–12)
Oxidation rate (μmol · min^{-1} · kg^{-1})	2.5 (2–4)	5.2 (3–7)	8.7 (7–10)
% Oxidation (%)	33 (28–35)	59 (46–75)	85 (90)

[a] Based on the steady state model.
[b] Based on the turnover rate of D-(^{14}C(U))-glucose.
[c] Refer to results and the steady state model for calculations.

tate · min^{-1} · kg^{-1} × 0.37 ÷ 2 μmol lac · μmol glu^{-1} = 3.7 μmol glu · min^{-1} · kg^{-1}

The average dive and surface durations were 78 s (range 69–95) and 39 s (range 19–78), respectively (Fig. 4). On average, resting seals spent 67% of the time submerged and 33% at the surface.

Swimming

An average $\dot{M}O_{2max}$ of 1.85 mmol O_2 · min^{-1} · kg^{-1} for the three seals was attained at a drag equivalent speed of about 3 m · s^{-1}. Although this was the maximum oxygen consumption measured, we were not able to demonstrate that it had reached a plateau. At higher speeds, the seals refused to swim consistently and would fall back on the grate. As a result, the $\dot{M}O_{2max}$ must be considered an estimate, and it may have been limited by the seal's behavior.

Steady-state $\dot{M}O_2$ at 35% and 50% $\dot{M}O_{2max}$ was achieved at drag equivalent speeds of 1.3 and 2.0 m · s^{-1}. During the transition from rest to 35% $\dot{M}O_{2max}$, the concentration of glucose, lactate, and FFA did not change appreciably (Table 1 and Fig. 2). Gas exchange was stable at a new steady state and the RQ remained low at 0.74. The glucose turnover rate, percentage of glucose oxidized, and the Cori cycle did not change. The turnover rate of lactate increased 52% and the percentage oxidized increased to 31%. Most of the increase in lactate production came from sources other than plasma glucose (Fig. 3). The concentration and turnover rate of FFA increased 7% and 17%, respectively, but the percentage oxidized increased to 59% of total turnover (Table 1).

The average dive and surface durations were 50 s (range 19–88) and 22 s (range 10–39), respectively (Fig. 4). Although the average dive duration decreased from resting values, the percent time submerged increased to 69% and the percent time on the surface decreased to 31%.

At 50% $\dot{M}O_{2max}$, the lactate concentration for one of the seals increased briefly to 3.2 m*M*, but then returned to a concentration of about 1.1 m*M* (Fig. 2). The lactate turnover rate increased 2-fold over resting levels, but the amount oxidized (11.4 μmol · min^{-1} · kg^{-1}) was similar to that at 35% $\dot{M}O_{2max}$. In contrast to the changes in lactate metabolism, the glucose concentration, turnover rate and oxidation rate remained almost unchanged from resting levels (Table 1). However, a greater percentage (86%) of the glucose turnover was metabolized to lactate, and the Cori cycle increased almost 2-fold over resting levels (Table 1 and Fig. 3). Although the FFA concentration and turnover rate increased only 24% and 37% over resting levels, respectively, 85% of the turnover was oxidized (Table 1).

The average dive and surface durations decreased at the highest swimming speed to 28 s (range 9–48) and 8 s

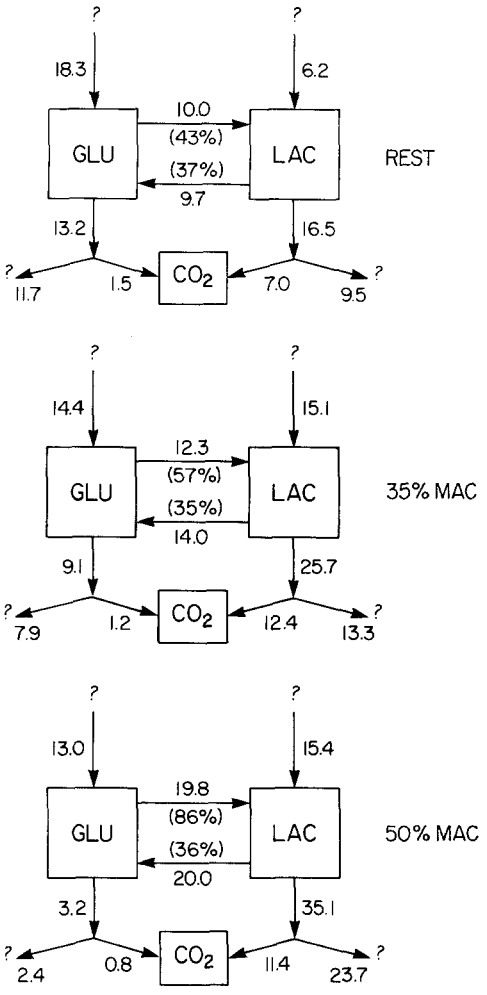

Fig. 3. Model for the interconversion and oxidation of glucose and lactate at rest, 35%, and 50% $\dot{M}O_{2max}$. Values are μmol · min^{-1} · kg^{-1}. Percentages of total turnover are shown in parentheses. Question marks indicate that the origin or fate is undetermined

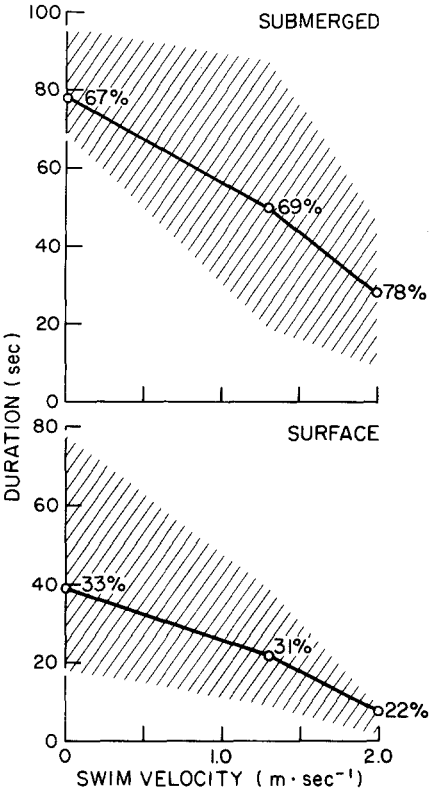

Fig. 4. Submerged and surface durations during swimming at three drag-equivalent velocities. The percentage of time spent submerged or at the surface is shown for each velocity. Values at rest or 1.3 m · s^{-1} are averaged for three seals with the range shown in cross hatching. At 2.0 m · s^{-1} the values are averaged from two seals

(range 2–9), respectively (Fig. 4). The average percent time submerged increased to 78%, and the percent time at the surface decreased to 22%.

Discussion

Resting metabolism

While resting in the water flume, the seals were free to move about in the test section. Although activity levels were low, the average $\dot{M}O_2$ was about 40% higher than the minimum levels (i.e., 0.23 mmol O_2 · min^{-1} · kg^{-1} measured for harbor seals of a similar size and under similar conditions; Davis et al. 1985). Although the seals spent 67% of their time submerged, average dive duration was short (79 s). Metabolism was primarily aerobic with anaerobic ATP production representing only 2% of the total (Table 2).

The plasma concentrations and turnover rates of glucose, lactate, and FFA are similar to previous measurements for harbor seals (Davis 1983), grey seals (Castellini et al. 1985) and terrestrial mammals when scaled using a weight-specific allometric coefficient of 0.75 for standard metabolism (Schmidt-Nielsen 1975). However, as pointed out previously (Davis 1983), the oxidation rate of glucose and lactate is lower than that for terrestrial, omnivorous mammals. In seals, much of the carbohydrate carbon is conserved through recycling rather than entering oxidative pathways for ATP production. Glucose and lactate oxidation contributed only 3% and 7% of total ATP production, respectively (Table 2). In humans, about 50% of glucose turnover is oxidized to produce 35% of total ATP synthesis (Malmendier et al. 1974).

The model for glucose-lactate interconversion and oxidation does not account for 50–70% of production and removal; possible sources and fates have been discussed in detail (Davis 1983). In addition to resynthesis from lactate (Cori cycle), possible sources of glucose include liver glycogen and gluconeogenesis from glycerol and amino acids. Glucose may be removed by deposition in tissue glycogen or serve as a precursor for glycerol 3-phosphate used in triglyceride synthesis in the liver and adipose tissue. Lactate may arise from the anaerobic metabolism of tissue glycogen or be converted and stored as glycogen in the liver.

There was no difference in the turnover rates of ^{14}C-palmitate and ^{3}H-oleate. Similar results have been

Table 2. Rates of ATP production from aerobic and anaerobic metabolism and from the oxidation of glucose, lactate, and FFA. The percent contribution to total ATP production is shown parentheses

	ATP Production (mmol · min^{-1})		
	Rest	35% $\dot{M}O_{2max}$	50% $\dot{M}O_{2max}$
Aerobic metabolism[a]	72.0 (98)	138.0 (99)	206.0 (99)
Anaerobic metabolism[b]	1.1 (2)	1.6 (1)	2.2 (1)
Total	73.1 (100)	139.6 (100)	208.2 (100)
Glucose oxidation[c]	2.2 (3)	1.7 (1)	1.2 (1)
Lactate oxidation[c]	4.8 (7)	8.4 (6)	7.8 (4)
FFA oxidation[c]	13.0 (18)	27.2 (20)	45.2 (22)
Total	20.0 (28)	37.1 (27)	53.2 (26)

[a] Assumes fat is the primary metabolic fuel (RQ≈0.74) so that 5.65 mmol ATP are produced per millimole O_2 consumed.
[b] Assumes 1.5 mmol ATP are produced per millimole lactate produced.
[c] Refer to methods for the equation to calculate the percentage of ATP production contributed by glucose, lactate, and FFA oxidation

reported for humans (Havel et al. 1964). The most common fatty acids found in harbor seals are palmitoleate (16:1, 18%), oleate (18:1, 30%), and gadoleate (20:1, 14%); the remainder consist primarily of longer-chain fatty acids (Jangaard et al. 1968). Although turnover rates have not been measured for most of these fatty acids, it is assumed that they are similar to that for palmitate and oleate.

About one-third of the FFA turnover was oxidized, which is similar to that observed in humans and dogs; 67% of the resting FFA turnover rate does not undergo direct oxidation (Havel et al. 1963; Issekutz et al. 1964). The remainder may be re-esterified into triglycerides in the liver, adipose tissue, or muscle.

Swimming metabolism

Measuring the $\dot{M}O_{2max}$ of seals is difficult because they often refuse to swim at drag-equivalent speeds faster than 3 m · s^{-1}. In addition, the drag force increases exponentially at these speeds, so that a seal can only swim for short periods in this narrow range of its capacity; this makes it difficult to demonstrate a plateau in oxygen consumption (Ponganis et al. 1990). In an earlier study in which harbor seals were trained to swim upright in the water while wearing a weighted jacket, oxygen consumption reached a plateau at 1.5 mmol $O_2 \cdot min^{-1} \cdot kg^{-1}$ (Elsner 1986). The estimated $\dot{M}O_{2max}$ in this study (1.85 mmol $O_2 \cdot min^{-1} \cdot kg^{-1}$) is higher than the latter value, but similar to that measured for harbor seals swimming in a water flume while pulling against a weighted line [1.7 mmol $O_2 \cdot min^{-1} \cdot kg^{-1}$ (Ponganis et al. 1990)]. Our value is also within the theoretical $\dot{M}O_{2max}$ based on measurements of oxygen consumption and heart rate (Williams et al. 1990). Based on a resting metabolism of 0.2 mmol $O_2 \cdot min^{-1} \cdot kg^{-1}$ (Davis et al. 1985; Ponganis et al. 1990), harbor seals appear to have a metabolic scope of about 9-fold. This is comparable to that observed for many terrestrial mammals, but less than half of the metabolic scope of elite animal athletes such as dogs and race horses (Taylor et al. 1987).

While swimming at 35% and 50% of $\dot{M}O_{2max}$, dive duration decreased to 50 s and 28 s, respectively (Fig. 4). However, surface duration also decreased, so that the percentage of total time spent submerged did not change or increased slightly (i.e., 69% for 35% $\dot{M}O_{2max}$ and 78% for 50% $\dot{M}O_{2max}$). At both speeds metabolism remained primarily aerobic; anaerobic metabolism contributed only 1% of total ATP production (Table 2).

Remarkably, glucose turnover and direct oxidation rates changed little from resting levels at either speed. As a result, the percentage of total ATP synthesis produced from the oxidation of glucose, which was 3% at rest, actually decreased during swimming (Table 2). The conversion of glucose to lactate increased to 57% and 86% of turnover at the two swimming speeds, respectively (Fig. 3). Hence, most of the glucose turnover entered anaerobic pathways as the work load increased to 50% $\dot{M}O_{2max}$. Nevertheless, the total anaerobic ATP production was insignificant compared to that from aerobic metabolism (Table 2). These measurements emphasize the minor role that glucose plays in the aerobic and anaerobic energy metabolism of seals during voluntary submerged swimming. In comparison, the plasma glucose concentration in dogs does not change from resting levels during moderate exercise, but turnover increases about 2-fold; the percentage of total energy from glucose oxidation remains low at about 13% (Issekutz et al. 1967).

Although lactate turnover increased 50–100% during swimming, the plasma concentration remained low at about 1 m*M* (Table 1). Similar results have been observed in humans during exercise at 50% $\dot{M}O_{2max}$ (Mazzeo et al. 1986). Most (62–72%) of the lactate production in the seals came from plasma glucose, while the remainder probably came from muscle glycogen (Fig. 3). The percent of lactate turnover oxidized increased to 31% (12.4 μmol · min^{-1} · kg^{-1}) at 35% $\dot{M}O_{2max}$, then showed no further increase at 50% $\dot{M}O_{2max}$. This level of oxidation is less than observed in exercising humans (i.e., 80–90%; Mazzeo et al. 1986) and reflects the greater tendency in seals to conserve carbohydrate carbon through recycling rather than oxidizing it as a

Appendix 1. Isotope infusion rates, steady state metabolite specific activities (GSA, LSA, FFA SA), and rates of expired $^{14}CO_2$ in three seals at rest, 35% $\dot{M}O_{2max}$ and 50% $\dot{M}O_{2max}$. The rates of expired $^{14}CO_2$ have been corrected for the bicarbonate pool behavior (Davis 1983). To compute the six rate constants for the steady state model in Fig. 1, use equations on p. 629, then correct for (1) the millimoles of carbon per millimole of glucose and lactate, and (2) body mass. The results from the three seals were averaged to make single a model for each level of exertion (Fig. 3) and to calculate the results in Tables 1 and 2

Seal 1: Body mass = 33 kg	Rest	35% $\dot{M}O_{2max}$	50% $\dot{M}O_{2max}$
D-(^{14}C–U)-glucose infusion			
Infusion rate (α) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	7.341	7.341	
GSA (A) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	1.524	1.732	
LSA (B) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	0.689	0.761	
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	1.590	2.270	
L-(^{14}C–(U))-lactate infusion			
Infusion rate (β) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	7.364	7.364	
GSA (C) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	0.716	0.844	
LSA (D) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	4.680	3.230	
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	3.870	4.796	
(1-^{14}C)-palmitate infusion			
Infusion rate ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	6.800	6.800	
FFA SA ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	2.044	2.044	
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	1.900	3.115	

Seal 2: Body mass = 42 kg	Rest	35% $\dot{M}O_{2max}$	50% $\dot{M}O_{2max}$
D-(^{14}C–U)-glucose infusion			
Infusion rate (α) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	8.880	8.880	8.880
GSA (A) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	2.349	2.692	2.552
LSA (B) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	2.119	2.100	2.013
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	1.930	3.386	4.603
L-(^{14}C–(U))-lactate infusion			
Infusion rate (β) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	6.361	6.361	6.361
GSA (C) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	0.564	0.583	0.602
LSA (D) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	2.641	2.503	2.073
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	2.792	4.395	4.167
(1-^{14}C)-palmitate infusion			
Infusion rate ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	6.933	6.933	6.933
FFA SA ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	2.059	1.359	1.201
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	2.416	5.192	5.739

Seal 3: Body mass = 46 kg	Rest	35% $\dot{M}O_{2max}$	50% $\dot{M}O_{2max}$
D-(^{14}C–U)-glucose infusion			
Infusion rate (α) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	8.665	8.665	8.665
GSA (A) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	1.620	1.830	1.760
LSA (B) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	1.326	1.151	1.218
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	2.267	3.349	5.074
L-(^{14}C–(U))-lactate infusion			
Infusion rate (β) ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	6.290	6.290	6.290
GSA (C) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	0.406	0.427	0.481
LSA (D) ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	1.539	0.935	0.913
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	2.111	2.716	3.596
(1-^{14}C)-palmitate infusion			
Infusion rate ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	12.290	12.290	12.290
FFA SA ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	1.506	1.334	1.402
$^{14}CO_2$ expired ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	4.374	6.901	10.643
^{3}H-(9, 10)-oleate infusion			
Infusion rate ($\mu Ci \cdot min^{-1}) \times 10^{-1}$	4.745	4.745	4.745
FFA ($\mu Ci \cdot mmol\ C^{-1}) \times 10^{-1}$	0.415	0.379	0.412

fuel. As a result, the oxidation of lactate contributed 4–6% of the total ATP production during swimming exercise at 35–50% $\dot{M}O_{2max}$ (Table 2).

Although the FFA concentration and turnover rates increased only 24% and 37% at 50% $\dot{M}O_{2max}$, respectively, the oxidation rate increased almost 3.5-fold. As a result, 85% of the FFA turnover was directly oxidized. A similar percent oxidation rate has been observed in dogs (Issekutz et al. 1967) and humans (Havel et al. 1964) during moderate exercise. Despite this increase, the percentage of total ATP produced from FFA oxidation remained about the same (i.e., 21%) at the two swimming speeds (Table 2).

At rest and during submerged swimming, we could account for only 26–28% of the total ATP production from the oxidation of plasma glucose, lactate, and FFA (Table 2). However, the low RQ indicates that fatty acids comprise most of the fuel for metabolism. Because only 18–22% of the energy is derived from plasma FFA, the question of alternate sources of fatty acids arises. Plasma triglycerides (TG) are a possible source of fatty acids that could be used by muscle and other tissues. Although the postabsorptive plasma concentration of TG is about 0.3 m*M* in seals, the rates of turnover and oxidation have not been measured (Puppione and Nichols 1970; Davis, unpublished observation).

Another important source of fatty acids, at least for muscle metabolism, may be in the muscle itself. Endogenous TG in heart and skeletal muscle have been shown to be a major source of fuel for metabolism (Neptune et al. 1959; Issekutz and Paul 1968). Exercise decreases the concentration of intramuscular TG, which may constitute 50–75% of the total fatty acids oxidized (Oscai et al. 1982). Histochemical studies on the muscles of seals have shown lipid droplets between the myofilaments (Mathieu-Costello, pers. comm.) and a high lipase activity (George et al. 1971). The TG stored in the muscle could be an important energy source for oxidative metabolism in the seal which are not modeled by isotopically labeled plasma FFA. Reduced blood flow to muscle during the dive response may require a greater reliance on endogenous muscle TG when plasma substrate availability is restricted (Issekutz and Paul 1968). Consequently, muscle TG may provide much of the ATP production not accounted for from the oxidation of plasma FFA. Verification of this hypothesis must await further studies.

How are these results related to the metabolism of harbor seals making natural dives in the wild? Unfortunately, there is no information on the routine swim speed or metabolic rate for this species during natural dives. However, it is known that the oxygen stores for a 40-kg seal are about 100 mmol O_2 (see Appendix 2). In order for a harbor seal of this size to maintain aerobic metabolism, it could remain submerged for about 3 min at 50% $\dot{M}O_{2max}$, 4 min at 35% $\dot{M}O_{2max}$, and 8 min at 18% $\dot{M}O_{2max}$ [about 4-, 2.8-, and 1.5-fold greater than resting metabolism, respectively; Davis et al. (1985)]. Routine dive durations of 3–8 min have been measured for free-ranging harbor seals off the coast of England and California (Fedak et al. 1988; Stewart, pers. comm.). If it is assumed, as has been shown for Weddell seals (Kooyman et al. 1980), that most of the dives made by a harbor seal are aerobic, then the seal's metabolic rate is probably less than 50% $\dot{M}O_{2max}$ during most dives. By decreasing dive duration when higher swimming speeds and greater metabolic power are required, when pursuing prey for example, the seal can maintain an aerobic, fat-based metabolism and minimize changes in blood chemistry. This allows the recovery time at the surface to be kept to a minimum (body oxygen stores can be recharged in a couple of minutes) and increases the amount of time that can be spent diving. It also enables the seal to use fat stored in the blubber and muscle as the primary fuel during diving.

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Appendix 2. Calculation of blood and tissue oxygen stores for a 40-kg harbor seal.

1. Blood

a. Blood volume is 0.15 l·kg body weight^{-1} (Elsner and Gooden 1983).
b. Hemoglobin (Hb) concentration is 250 g·l blood^{-1} (Elsner and Gooden 1983).
c. 1.34 ml O_2 can combine with 1 g Hb.
d. Assume one-third of blood volume is arterial and that it can only be desaturated 50% during a dive.
e. Assume two-thirds of the blood is venous and that it can be desaturated 85% during a dive.

Blood O_2 (ml) = (40 kg × 0.15 l blood·kg^{-1} × 250 g Hb·l blood^{-1} × 1.34 ml O_2·g Hb^{-1}) × [(0.33 arterial blood × 0.5 desaturation) + (0.67 arterial blood × 0.85 desaturation)] = 1476 ml O_2.

2. Muscle

a. Assume skeletal muscle mass equals one-third of body mass.
b. Muscle myoglobin concentration is 45 g myoglobin · kg muscle^{-1} (Elsner and Gorden 1983).
c. 1.34 ml O_2 can combine with 1 g myoglobin.

Muscle O_2 (ml) = 40 kg × 0.33 kg muscle·kg body weight^{-1} × 45 g myoglobin · kg muscle^{-1} × 1.34 ml O_2 · g myoglobin^{-1} = 796 ml O_2

3. Total O_2 stores equal 1476 + 796 = 2272 ml O_2 or 100 mmol O_2.

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