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Effects of hypoxia and hypercapnia on aerobic metabolic processes in northern elephant seals

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Abstract

An hypoxia-induced metabolic down-regulation has been implicated as an important protective mechanism against tissue deoxygenation in mammals. Whether the same response to hypoxia occurs in northern elephant seals was studied. The effects of hypercapnia were also examined to determine whether the reduced ventilatory response of seals to CO_2 is associated with an analogous protective metabolic down-regulation. Thirty three seals (7–300-days-old) were studied using open-flow respirometry with simultaneous monitoring of apnea frequencies and heart rates. Hypoxia (11% O_2) and hypercapnia (7% CO_2) caused increases in metabolism of up to 38% with corresponding decreases in the percent time spent apneic (%AP) and increases in heart rate. The metabolic, breathing and heart rate responses to altered inspired gases were independent of age. Metabolism was strongly negatively correlated with %AP suggesting that elevated metabolism during hypoxia and hypercapnia exposure is attributable to decreases in %AP. In young elephant seals metabolic down-regulation is not an automatic protective response to experimentally-imposed hypoxia or hypercapnia.

Keywords: Diving mammals; Hypoxia; Hypercapnia; Open-flow respirometry; Intermittent breathing; Oxygen consumption; Carbon dioxide production

1. Introduction

During periods of arrested breathing in mammals, sustained cellular function requires energy, first produced aerobically consuming on board oxygen stores, then produced by a switch to anaerobic metabolic processes (Elsner and Gooden, 1983). Most terrestrial mammals tolerate only a few minutes of arrested breathing due mainly to the high sensitivity of neural tissue to oxygen lack. In contrast, marine mammals demonstrate extreme tolerances to arrested breathing as evidenced by their ability to endure extended breath-hold dives and spontaneous apneas. Phocid seals are among the most proficient breath-holders with individual dives of free-ranging northern and southern elephant seals

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(*Mirounga angustirostris* and *M. leonina*, respectively) routinely exceeding 30 min, and with record dives lasting up to 120 min (Le Boeuf et al., 1989; De Long and Stewart, 1991; Hindell et al., 1991).

During breath-hold dives, marine mammals face decreased oxygen supplies coupled with increasing tissue carbon dioxide (Kooyman et al., 1980; Qvist et al., 1986). In terrestrial mammals, the general response to analogous experimental changes in tissue oxygen and CO_2 tensions is an increase in ventilation. As a result, O_2 delivery to hypoxic tissues is improved and CO_2 accumulation is prevented (Piiper, 1986). This response in diving marine mammals would decrease the durations of dives and compromise feeding, travel and social behaviors that are associated with submergence. Accordingly, Irving (1939) suggested that marine mammals may have a blunted response to increasing CO_2 levels which would allow them to prolong their breath-holds. Since Irving's suggestion, a number of researchers have examined the effects of hypercapnia and hypoxia on ventilation and/or diving patterns in marine mammals (for review see Butler and Jones, 1982). Although the results and methodologies differ between studies, the general consensus is that both hypoxia and hypercapnia interfere with the normal diving behavior of marine mammals, usually by stimulation of respiratory drive. However, relative to terrestrial mammals, in marine mammals higher CO_2 levels are necessary to stimulate respiratory drive (Robin et al., 1963; Butler and Jones, 1982; Milsom et al., 1996).

An overall reduction in metabolic rate represents a second strategy widely used in the face of decreases in tissue oxygen availability (Frappell et al., 1992). This response appears to be particularly prevalent in newborn mammals (Mortola et al., 1989) and may contribute to a greater tolerance to asphyxia than observed in adults. A high tolerance to asphyxia in newborns is important during the potentially threatening transition from maternal placental respiration to lung respiration (Lagercrantz, 1996). In contrast to the ventilatory response, a decrease in metabolism in response to hypoxia and/or hypercapnia in marine mammals would be advantageous and perhaps allow extensions of diving times (Hochachka, 1992). Whether marine mammals down-regulate metabolism in response to experimentally imposed hypoxia and/or hypercapnia has only been addressed by two studies (Gallivan, 1980; Mortola et al., 1989). Gallivan (1980) found no significant change in the oxygen uptake of Amazonian manatees older than one year during either hypoxia or hypercapnia. Mortola et al. (1989) report a decrease in $\dot{V}_{O_2}$ in 3-day-old grey seal pups during hypoxia exposure. Due to the limited number of experiments performed, the metabolic responses of marine mammals to hypoxia and hypercapnia are unresolved.

In terrestrial mammals, ventilation is closely matched to metabolic demand over a wide range of activities (Taylor et al., 1987; Cameron, 1989). A limited number of studies demonstrate that the same relationship appears to exist for seals engaged in different levels of swimming activity (Craig and Påsche, 1980; Williams et al., 1991). Furthermore, while breathing intermittently between dives or spontaneous terrestrial apneas, oxygen consumption rates of elephant seals are negatively correlated with the percent time spent in breath-hold (Huntley, 1984; Thorson and Le Boeuf, 1994; Webb et al., 1998). During hypoxia exposure in terrestrial mammals, however, the coupling between ventilation and metabolism breaks down as evidenced by simultaneous increases in ventilation and decreases in metabolic rate (Frappell et al., 1992). Therefore, metabolic responses cannot be reliably deduced from changes in ventilatory patterns during hypoxia.

The present study was conducted to determine the metabolic responses of northern elephant seals to hypoxia and hypercapnia. In a previous study, ventilation frequencies of elephant seal weanlings were shown to increase in response to hypoxia and hypercapnia (Milsom et al., 1996), therefore we examined the relationships between metabolic rate and ventilation frequency under normoxic, hypercapnic and hypoxic conditions in order to assess whether metabolic responses parallel ventilatory responses in this species. Aerobic metabolism, ventilation patterns and heart rate were monitored during hypoxia and/or hypercapnia exposure in seals ranging in age from 7 to 300

days. The northern elephant seal was studied because of its extraordinary diving ability and because of its extended post-weaning developmental phase during which important physiological and behavioral changes take place which enhance diving ability (Thorson and Le Boeuf, 1994). If differences in metabolic responses occur as a function of age, as they do in several terrestrial mammals, then the differences may be most apparent in this species as a consequence of the prolonged developmental period.

2. Materials and methods

2.1. Experimental subjects

Thirty three northern elephant seals were studied over 2 years of experiments. To assess whether physiological responses differ with age, seals close to the ages of 7, 30, 60, 90 or 300 days were selected as experimental subjects. Table 1 summarizes the physical characteristics of the seals within each age class. Experimental protocols differed between the 7-day-old suckling pups and the weaned seals as described below.

2.1.1. Weaned seals

Twenty nine weaned seals ranging in age from 23 to 300 days were captured at Año Nuevo State Reserve, San Mateo County, CA, and transported 30 miles to Long Marine Laboratory, Santa Cruz, CA. With the exception of the two approximately 300-day-old seals, all were born to tagged females who were observed daily as part of ongoing breeding history studies (e.g. Reiter and Le Boeuf, 1991). As a result, time of birth was known to within one half-day. The seals were captured during periods of natural fasting and therefore were not fed during their tenure at Long Marine Lab. They were held in an outdoor pen (5.3×2.7 m) filled with sand to a depth of approx. 12 cm.

On the day following capture, each seal was weighed on a platform scale and lightly anesthetized with 1 mg kg^{-1} Tiletamine HCl and Zolazepam HCl (Telazol, Aveco) intramuscularly. Under sterile conditions a tygon catheter was inserted percutaneously into the extradural intravertebral vein with a 14 gauge guide needle. Two 2.5 cm diameter copper heart rate electrodes mounted on neoprene patches and connected to a heart rate telemeter (model Polar Favor, Polar Electro) were glued along the dorsal midline on either side of the heart. During experiments, a receiver for the VHF heart rate signal combined with a data logger (model MK3, Wildlife Computers) was fastened to the telemeter and stored heart rate information. A T-type thermocouple (Physitemp Instruments) inserted approx. 25 cm into the rectum and connected to a digital thermometer (model Bat-12, Physitemp Instruments) continuously monitored deep rectal temperature. Following placement of the catheter and monitoring equipment, the animal was placed in a metabolic chamber and allowed to recover for at least 2 h prior to commencing metabolic trials. Seals responded normally to visual and tactile stimuli within 30 min of anaesthesia, and within 120 min their behaviors were indistinguishable from those of unanaesthetized seals resting quietly in the lab. At the end of the first day of experiments a prophylactic dose of antibiotic, 1 g of

Table 1
Physical characteristics of the seals used in this study

	Age class (days)				
	7	30	60	90	300
n (m, f)	4 (3, 1)	9 (5, 4)	8 (5, 3)	10 (6, 4)	2 (1, 1)
Age (days)	7 ± 0	33.0 ± 2.2	61.5 ± 1.5	94.7 ± 2.6	300[a]
Mass (kg)	47.9 ± 3.0	114.6 ± 5.0	100.9 ± 6.8	82.9 ± 3.0	116.5 ± 0.1
Lean mass (kg)	41.2 ± 2.6	59.6 ± 2.6	52.5 ± 3.6	43.1 ± 1.5	81.5 ± 0.0

[a] Approximate. Lean mass is estimated from body mass as described in the methods. All values are means $\pm$ SE.

sodium cephazolin (Marsam Pharmaceuticals), was injected i.v. through the catheter.

Twenty one of the 29 weaned seals were studied during 2 days of trials. In these seals, at the end of the first day the catheter was filled with 500 I.U. sodium heparin, knotted, and stowed in a neoprene pocket glued to the seal's back. Each seal was then returned to the outdoor pen for the night. On the second day the seal was placed in the metabolic chamber and brought into the laboratory for further experiments. To minimize handling the seals, deep rectal temperature was not monitored on the second day. Metabolic trials were begun at least 1 h after delivery of the seal to the laboratory on the second day, at a time when the seal was resting quietly within the metabolic chamber. Whenever there were multiple trials conducted on the same day, the order of the trials was varied and the responses of seals during the different trials did not depend on trial order. When all trials were completed, each seal received another prophylactic dose of antibiotic i.v. and was returned to Año Nuevo Reserve.

2.1.2. Suckling pups

Four suckling pups whose birth times were known to within one half-day were studied on Año Nuevo Island located 800 m from the coast in Año Nuevo State Reserve. Each pup was separated from its mother and carried to a laboratory approx. 100 m from the beach. In the lab, each pup was lightly anesthetized with an intramuscular injection of 0.25 ml Telazol (approx. 0.5 mg kg^{-1}). The dose of Telazol was just enough to permit ease in handling pups prior to the metabolic trials, while ensuring rapid recovery. Each pup was weighed in a bag using a hanging spring scale and heart rate electrodes were affixed as previously described. In order to return pups as quickly as possible to their mothers, metabolic trials were begun about 45 min after Telazol administration. At this time the pups' behaviors were indistinguishable from those of unanaesthetized pups. Measurements were made for approx. 90 min after which pups were returned to the beach.

2.2. Measurement of aerobic metabolism

Metabolism was measured using an open-flow respirometry system of a design previously used to measure metabolism of young elephant seals (Rea and Costa, 1992). The metabolic chamber consisted of a rectangular box ($160 \times 58 \times 38$ cm) covered with a shallow (8 cm) Plexiglas dome. During metabolic trials on suckling pups, empty airtight plastic containers were placed in the chamber to reduce the volume by about 30%. Two fans positioned within the chamber near the inlet port ensured complete circulation of gases. Metabolic measurements were made only after the chamber was sealed for at least 15 min to allow for the equilibration of gases. A series of copper coils covered by a thin aluminum plate was laid in the bottom of the chamber and connected to a hose through which cold water circulated to keep the chamber and animal cool. Because experimental sessions were short, the heat exchanger was not used during trials on the suckling pups. Chamber temperature was monitored continuously using a T-type thermocouple and a digital thermometer (model BAT-12, Physitemp) and averaged 19.6 ± 2.8°C (mean $\pm$ SD).

Air was drawn through the chamber at a rate of approx. 70 l min^{-1} using a vacuum pump (model Portapower 2.2, Hoover). The flow rate of the gas leaving the chamber was determined using a rototype dry gas meter (American Meter Division) and was corrected to STDP. O_2 and CO_2 analyzers (models SA-I and CD-3A, respectively, Ametek) continuously sampled gas from the exhaust port of the chamber. Moisture and CO_2 were removed from the gas entering the O_2 analyzer with Drierite and Baralyme, respectively. Gas entering the CO_2 analyzer was dried only. A continuous record of O_2 and CO_2 concentrations was collected using a data acquisition system (Datacan V, Sable Systems) connected to a PC computer. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using equations A5 and A8 of Frappell et al. (1992).

The accuracy of the respirometry system was checked using a gas dilution method (Fedak et al., 1981; Davis et al., 1985). For calibration trials, N_2 was bled into the empty chamber at 3.3 l min^{-1} and CO_2 was bled into the empty chamber at 1.4

l min^{-1}, respectively. Theoretical and measured changes in the gas concentrations within the chamber agreed to within 0.02% during the calibration trials. N_2 dilutions were conducted each day of trials while CO_2 dilutions were conducted between animals. In addition, the CO_2 analyzer was calibrated using two mixtures of research grade calibration gases, one containing 5.00% CO_2 and the second containing 10.00% CO_2.

2.3. Experimental trials

2.3.1. Normoxia

For weaned seals, on both the first and second day, metabolic responses were measured while the seal breathed air during 60 min trials. O_2 and CO_2 were assumed to make up 20.94 and 0.03%, respectively, of the dry inspired gas for all normoxia trials. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated using data collected during the last 35 min of each trial. Determinations of the metabolic effects of hypoxia and hypercapnia were drawn by comparing the experimental trial to the corresponding normoxia trial of the same day. For suckling pups, a single trial was conducted to measure both normoxic metabolic rates and the response to hypoxia as described below.

2.3.2. Hypoxia

For weaned seals, on the first day the inflowing air was diluted with 100% N_2 at a rate of 23.4 ± 0.4 l min^{-1} which brought the oxygen concentration within the chamber to approx. 14%. On the second day, the inflowing air was diluted with 100% N_2 at a rate of 33.4 ± 0.5 l min^{-1} which brought the oxygen concentration within the chamber to approx. 11%. Animals were exposed to each level of hypoxia for 90 min. For the metabolic measurements, $\dot{V}_{CO_2}$ was calculated using the data collected during the last 50 min of hypoxia exposure, and compared to normoxic $\dot{V}_{CO_2}$. Paired data for normoxia and 14% hypoxia trials were collected for 24 weaned seals. Paired data for normoxia and 11% hypoxia trials were collected for 19 weaned seals.

For suckling pups a single trial was conducted which consisted of a 45 min period breathing air followed by a 45 min period during which 100% N_2 was added to the chamber at a rate of 19.9 ± 0.2 l min^{-1} which decreased the inspired O_2 concentration to approximately 15%. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated for the last 30 min breathing air and $\dot{V}_{CO_2}$ was calculated for the last 30 min of hypoxia exposure. For analysis, paired data for normoxia and hypoxia trials on suckling pups were grouped with the results of the 14% hypoxia trials conducted on weaned seals.

2.3.3. Hypercapnia

For weaned seals, on either the first or second day, the inflowing air was diluted with 100% CO_2 at a rate of 4.9 ± 0.1 l min^{-1} which brought the CO_2 concentration within the chamber to approx. 7%. Animals were exposed to hypercapnia for 90 min. For the metabolic measurements, $\dot{V}_{O_2}$ was calculated using data collected during the last 50 min of hypercapnia exposure and was compared to the normoxic $\dot{V}_{O_2}$ from the trial run on the same day. Paired data for normoxia and hypercapnia trials were collected for 28 weaned seals. Hypercapnia trials were not conducted on suckling pups.

2.4. Carbon dioxide production as a determinant of metabolic rate

The delivery of N_2 into the chamber was prone to a small error due to the resolution of the flow meter. Consequently, the resultant O_2 deflection due to the inexact gas manipulation was large relative to the deflection due to the oxygen consumption rates of the animals precluding use of the O_2 trace to precisely calculate $\dot{V}_{O_2}$. When $\dot{V}_{O_2}$ was calculated assuming an inspired O_2 concentration of exactly that targeted during each experiment, however, the resultant relationship between $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ was qualitatively very similar to that measured during normoxia (see Fig. 1). Therefore, we conclude that changes in $\dot{V}_{CO_2}$ are likely indicative of changes in the aerobic metabolic rate and are paralleled by changes in $\dot{V}_{O_2}$. Errors in the delivery of CO_2 similarly precluded the possibility of measuring $\dot{V}_{CO_2}$ during hypercapnia trials.

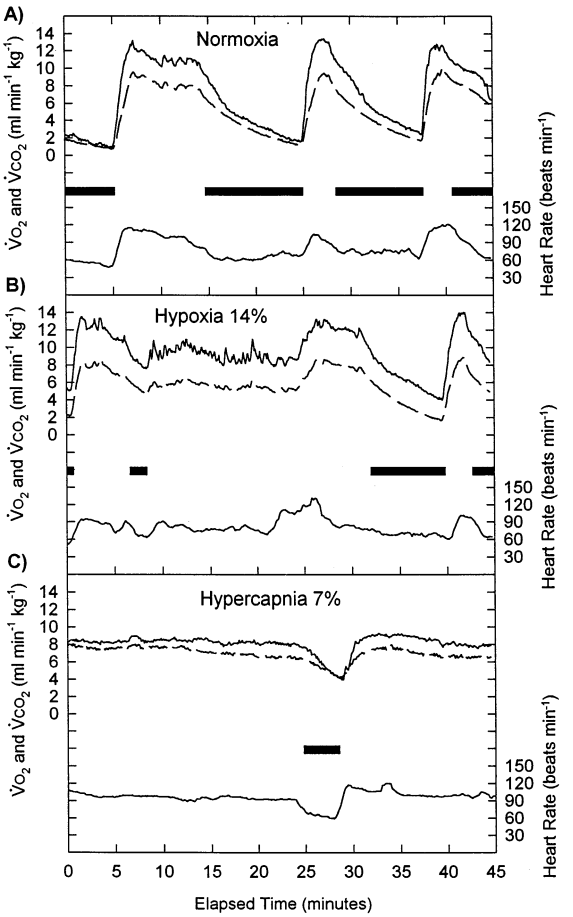

Fig. 1. Representative traces of the simultaneously measured variables during (A) normoxia, (B) hypoxia and (C) hypercapnia. In each of the three graphs, lean mass-specific metabolic rate is shown near the top as $\dot{V}_{O_2}$ (solid line) and $\dot{V}_{CO_2}$ (dashed line), periods of apnea are represented in the middle by solid bars, and heart rate is shown near the bottom. $\dot{V}_{O_2}$ for hypoxia trials and $\dot{V}_{CO_2}$ for hypercapnia trials are estimated variables and may contain errors associated with the measurement procedures (see Section 2).

2.5. Measurement of ventilatory patterns

For all trials, ventilation was determined to the resolution of apnea versus eupnea. These determinations were made by one or a combination of the following: (1) the flow-through respirometry traces with apneic periods characterized by a exponential fall in percent CO_2 and an exponential increase in percent O_2, (2) a trace of relative thoracic pressure obtained by connecting the intravertebral catheter to a strain gauge manometer (model P1000-B, Narco Bio-Systems), or (3) from actual tallies of respirations which were discernable by placing a microphone within the metabolic chamber attached to a digital audio tape recorder (model DAT 7, Sony). Based on the durations of individual apneas during the measurement period, the percent time seals spent in apnea was calculated. For those trials during which respirations were monitored by audio recordings (7 seals), mean trial and mean eupneic ventilation frequencies were determined.

2.6. Measurement of heart rate

An attempt to monitor heart rate was made during every trial. Heart rate was telemetered as the number of beats per 10 s interval and was converted to a running sum for every six 10 s intervals. Means and standard errors (SE) of the individual one-min running sums were calculated separately for periods of apnea and eupnea, as well as for the entire period during which the metabolic rate determinations were made.

2.7. Analysis

In elephant seal pups, differences in metabolic rates are more highly correlated with lean mass than with total body mass (Rea and Costa, 1992). Elephant seals undergo large changes in body mass and body composition as a consequence of alternating feeding and fasting periods during the first year (Table 1). Thus, in order to eliminate the effects of changes in body composition from the analyses, metabolic rates were divided by the approximate mass of lean tissue for each seal. Lean masses (LBM) of seals of different ages were assumed to be 86% of body mass for the 7-day-old pups (Kretzmann, 1990), 52% of body mass for seals during the post-weaning fast (Rea and Costa, 1992), and 70% of body mass for 300-day-old seals (D. Crocker, personal communication).

Fig. 1 shows representative traces of the data collected during a series of trials. General linear model *F*-tests were used to compare each variable measured during normoxia among sexes and age classes (Neter et al., 1985). All models initially

included sex, age class and an interaction term. In all cases the interaction term was found to be non-significant demonstrating no relationship between the sex of the seals and their ages and thus the interaction term was dropped from the analysis in order to obtain greater power in detecting sex or age class effects. The subsequent analyses demonstrated no significant differences between sexes for any measured variable, and thus sex was dropped from the model in order to obtain greater power in detecting an age class effect. If further analysis revealed significant differences between age classes, a Bonferroni multiple comparison test was performed to determine which age classes differed.

The effects of hypoxia and hypercapnia between sexes and age classes were assessed by examination of the differences between the normoxia and experimental values using general linear model F-tests. As above, in all cases the interaction term and sex were found to be non-significant and thus were dropped from subsequent analyses in order to obtain greater power in determining whether there was an age class effect. Since all responses during the experimental trials did not differ significantly between age classes, the absolute effects of hypoxia and hypercapnia were determined by comparing normoxic with experimental values using paired Student's t-tests on the complete data sets (Neter et al., 1985). Relationships between metabolism and ventilation were determined by Pearson correlations. Parameters describing relationships between metabolism and ventilation or heart rate were determined using linear least-squares regression techniques and compared using F-tests (Wall, 1986). All statistical analyses were performed using SYSTAT (SPSS Inc.). Significance was determined at the $P < 0.05$ level. Values are reported as means $\pm$ SE unless otherwise indicated.

3. Results

3.1. Normoxia

Table 2 lists the values of the measured variables while the seals rested quietly in the metabolic chamber breathing air. During the first 3 months there were decreases in LBM corrected

Table 2
Summary of the whole animal metabolic variables measured during normoxia[a]

	Age class (days)				
	7	30	60	90	300
$\dot{V}_{O_2}$ (ml O_2 min^{-1})	419.3 ± 37.4	519.8 ± 60.7^{d}	390.6 ± 25.4	273.4 ± 14.7^{e}	642.1 ± 65.7
$\dot{V}_{O_2LBM}$(ml O_2 kg^{-1} min^{-1})	10.1 ± 0.3^{d}	8.8 ± 1.0	7.7 ± 0.8	6.3 ± 0.2	7.9 ± 0.8
$\dot{V}_{CO_2}$ (ml CO_2 min^{-1})	338.8 ± 35.2	359.9 ± 49.1^{d} (7)	286.6 ± 18.5 (7)	206.3 ± 11.5^{e}	470.0 ± 45.5
$\dot{V}_{CO_2LBM}$(ml CO_2 kg^{-1} min^{-1})	8.2 ± 0.4^{d}	6.1 ± 0.8 (7)	5.3 ± 0.4 (7)	4.8 ± 0.2	5.8 ± 0.6
Respiratory Quotient	0.805 ± 0.02	0.74 ± 0.02 (7)	0.735 ± 0.02 (7)	0.755 ± 0.01	0.73 ± 0.004
Time spent in apnea (%)	$12.2 \pm 6.9^{b,c,d}$	47.4 ± 6.3	57.1 ± 7.0	55.1 ± 4.4	44.2 ± 10.5
Heart rate (beats min^{-1})					
Overall	$116.2 \pm 7.0^{c,d,e}$ (2)	86.4 ± 6.0^{d} (4)	74.6 ± 5.7	61.0 ± 2.0 (7)	63.6 ± 0.8
Eupneic periods	$116.6 \pm 7.1^{c,d,e}$ (2)	97.5 ± 6.6 (4)	93.4 ± 3.3	84.1 ± 2.1 (7)	73.2 ± 2.0
Apneic periods	$107.4 \pm 10.5^{b,c,d,e}$ (2)	72.5 ± 3.5^{d} (4)	61.2 ± 2.0	47.4 ± 1.1 (7)	51.4 ± 3.8

[a] Numbers in parentheses indicate the number of values used to calculate means when there were incomplete data sets. All other n's are as in Table 1. Values are reported as means $\pm$ SE.

[b] Significantly different from 30,

[c] 60,

[d] 90

[e] and 300-day-old seals, respectively.

metabolic rates ($\dot{V}_{O_2LBM}$ and $\dot{V}_{CO_2LBM}$) with age. Similarly, heart rate decreased with age during the first 3 months, and the percent time that seals spent in apnea while breathing air increased during the first month. There was a significant negative correlation between metabolic rate and the percent time seals spent in apnea ($r = -0.748$ for $\dot{V}_{O_2LBM}$; $r = -0.795$ for $\dot{V}_{CO_2LBM}$; $P < 0.001$ for both relationships). Deep rectal temperature of seals during normoxia was 37.1 ± 0.2°C and did not change significantly during any of the trials.

3.2. Hypoxia 14%

The results of exposure to 14% O_2 were examined in 28 seals (Fig. 2). There were no differences in the metabolic, heart rate and ventilatory responses to 14% O_2, either between sexes or between age classes. $\dot{V}_{CO_2LBM}$ of seals ranging in age from 7 to 300 days increased significantly by 0.75 ± 0.22 ml kg^{-1} min^{-1} in response to 14% O_2 ($P = 0.002$) over normoxia. Heart rate was measured successfully throughout 17 of the 28 trials. In 7 day old pups, only one heart rate record was usable, therefore the general linear model based *F*-tests were carried out using data from the remaining 16 trials. Heart rate increased significantly by 5.6 ± 2.2 beats min^{-1} ($P = 0.019$) during 14% O_2 exposure, but neither eupneic nor apneic heart rate changed significantly as compared with normoxia. There was no change in the percent time spent in apnea in response to 14% hypoxia.

3.3. Hypoxia 11%

The effects of exposure to 11% O_2 were examined in 19 seals (Fig. 3). As with 14% O_2 exposure, neither sex nor age class had an effect on the metabolic, heart rate or ventilatory responses to 11% O_2. $\dot{V}_{CO_2LBM}$ increased significantly during 11% O_2 exposure by 1.79 ± 0.27 ml kg^{-1} min^{-1} ($P < 0.001$) over normoxia. Heart rate was successfully measured throughout 11 of the 19 trials. There was a significant increase in heart rate of 13.7 ± 4.0 beats min^{-1} ($P = 0.006$) over normoxia, however eupneic and apneic heart rates did not change significantly. During 11% O_2 exposure, the percent time spent in apnea decreased significantly from 57.4 ± 3.0% in normoxia to 17.6 ± 3.2% ($P < 0.001$). Those trials during which breaths were monitored by audio recording (7 seals), breathing frequency increased significantly from 3.8 ± 0.4 breaths min^{-1} during normoxia to 7.0 ± 0.8 breaths min^{-1} during 11% hypoxia ($P = 0.003$). The increase in breathing

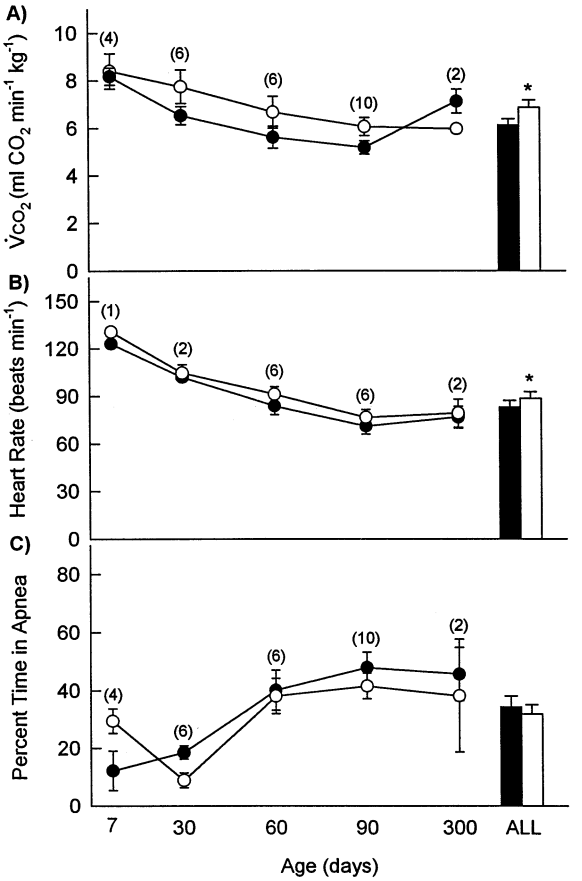

Fig. 2. (A) Metabolic rate, (B) heart rate and (C) percent time spent in apnea for the different age classes of seals during normoxia (solid symbols) and 14% O_2 exposure (open symbols). Data are shown as means ± SE. Numbers in parentheses indicate the number of animals contributing to the data for each age class. Statistical analyses revealed no differences in the responses between pups of different ages. Means for all ages combined are shown in bars on the right side of each graph: normoxia (solid bars) and hypoxia (open bars). CO_2 production is corrected for lean body mass as explained in the methods. * Indicates a significant difference from normoxia measurements.

frequency was attributable to the decreased time spent in apnea; breathing frequency during periods of eupnea remained unchanged (Fig. 4).

3.4. Hypercapnia

The effects of exposure to 7% CO_2 were examined in 28 seals (Fig. 5). As during hypoxia exposure, there were no differences in the metabolic, heart rate, or ventilatory responses of seals to hypercapnia, either between sexes or between age classes. $\dot{V}_{O_2LBM}$ increased significantly during 7% CO_2 exposure by 1.88 ± 0.33 ml kg^{-1} min^{-1} ($P < 0.001$) over normoxia. Heart rate was measured successfully throughout 18 of the 28 trials. Of all the experimental trials, heart rate showed the greatest increase during hypercapnia exposure with a mean increase of 22.3 ± 3.2 beats min^{-1} ($P < 0.001$). Eupneic heart rate increased significantly during hypercapnia exposure by 11.2 ± 1.9 beats min^{-1} ($P < 0.001$), while apneic heart rate remained unchanged. The greatest ventilatory changes occurred during hypercapnia exposure.

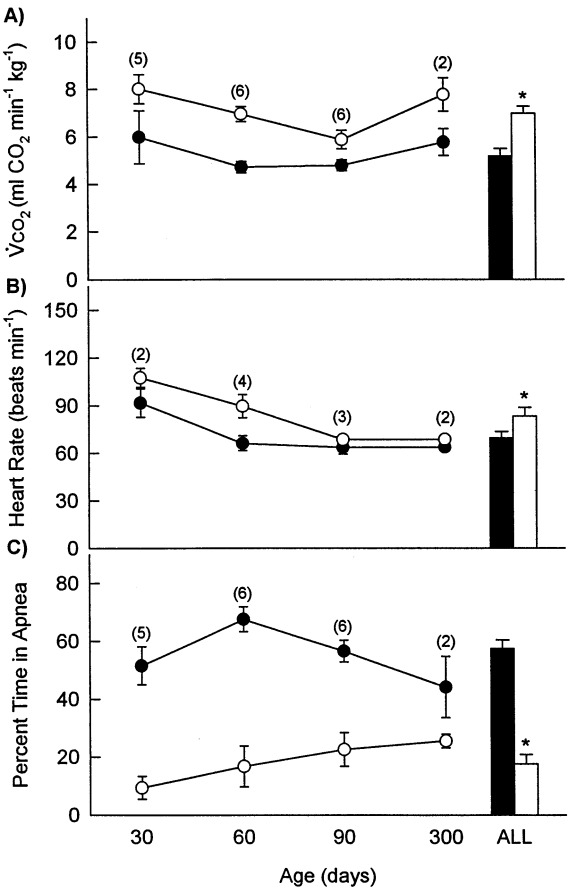

Fig. 3. (A) Metabolic rate, (B) heart rate and (C) percent time spent in apnea for the different age classes of seals during normoxia (solid symbols) and 11% O_2 exposure (open symbols). Data are shown as means $\pm$ SE. Numbers in parentheses indicate the number of animals contributing to the data for each age class. Statistical analyses revealed no differences in the responses between pups of different ages. Means for all ages combined are shown in bars on the right side of each graph: normoxia (solid bars) and hypoxia (open bars). CO_2 production is corrected for lean body mass as explained in the methods. * Indicates a significant difference from normoxia measurements.

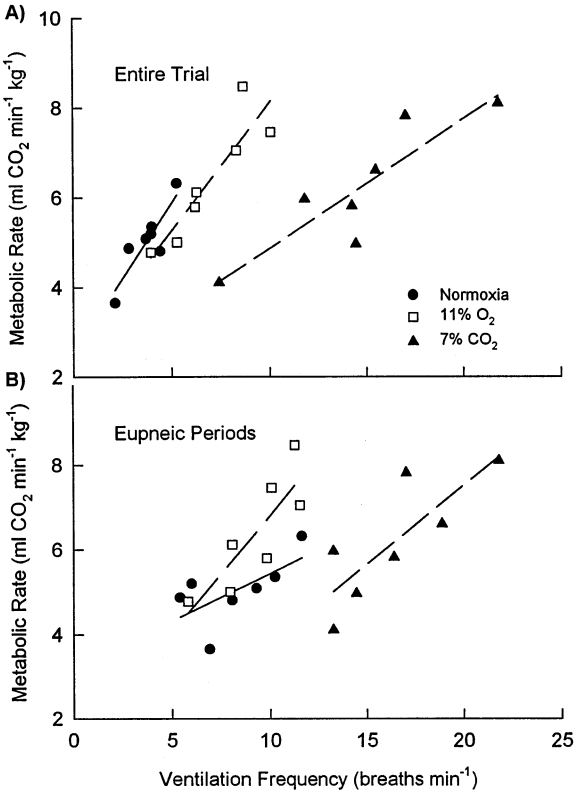

Fig. 4. Relationships between metabolic rate for the entire measurement period and (A) ventilation frequency for the entire measurement period and (B) ventilation frequency during eupneic periods only. In both (A) and (B) the linear least-squares regression equations describing the relationships for CO_2 exposure differed significantly from the relationships for normoxia and hypoxia exposure.

The percent time spent in apnea decreased from $52.4 \pm 3.3\%$ during normoxia to $10.1 \pm 2.2\%$ ($P < 0.001$). In many seals spontaneous apneas disappeared altogether. Those trials for which breathing was monitored by audio recordings, breathing frequency increased significantly from 3.8 ± 0.4 breaths min^{-1} during normoxia to 14.6 ± 1.7 breaths min^{-1} during hypercapnia ($P < 0.001$). Unlike the response observed during 11% O_2 exposure, breathing frequency during eupneic periods increased significantly during hypercapnia exposure ($P < 0.001$; Fig. 4).

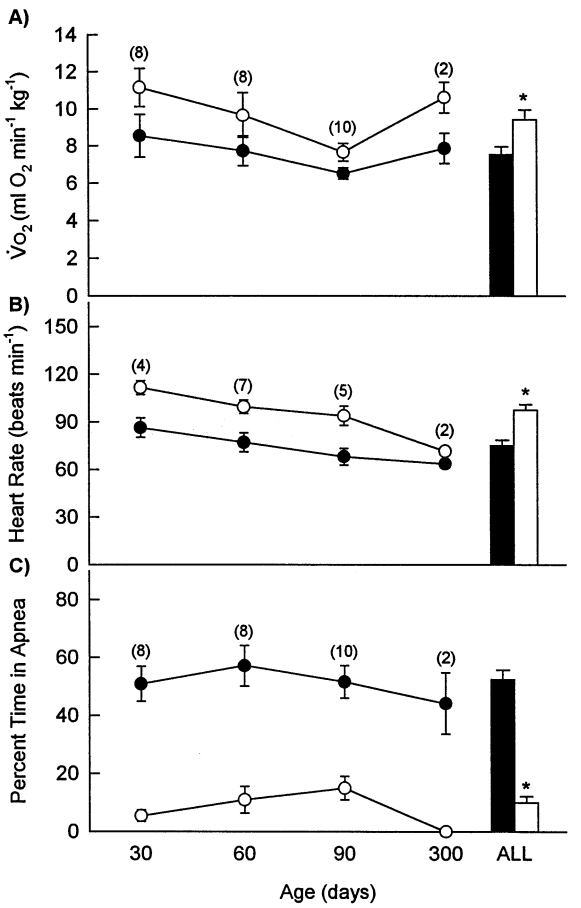

Fig. 5. (A) Metabolic rate, (B) heart rate and (C) percent time spent in apnea for the different age classes of seals during normoxia (solid symbols) and 7% CO_2 exposure (open symbols). Data are shown as means $\pm$ SE. Numbers in parentheses indicate the number of animals contributing to the data for each age class. Statistical analyses revealed no differences in the responses between pups of different ages. Mean responses for all ages combined are shown in bars on the right side of each graph: normoxia (solid bars) and hypercapnia (open bars). O_2 consumption is corrected for lean body mass as explained in the methods. * Indicates a significant difference from normoxia measurements.

4. Discussion

4.1. Metabolic responses

These data clearly demonstrate that elephant seals do not decrease their metabolic rates during hypoxia and hypercapnia exposure. Conversely, metabolic rates increased a mean of approx. 38 and 26% while breathing 11% O_2 and 7% CO_2, respectively, above their normoxic levels. However, these changes were associated with mean increases of greater than 2-fold in the percent time spent breathing. In the extreme, breathing was continuous. The metabolic responses did not differ with age as they do in many terrestrial mammals (Mortola et al., 1989), suggesting that if a reduction in metabolism serves as a protective mechanism, there is no greater protection against hypoxia in newborn seals relative to in older seals. Furthermore, there is no evidence that increases in breath-hold capacities and diving durations with age are attributable to changes in metabolic responses to hypoxia and hypercapnia.

During normoxia there was a significant negative correlation between the percent time seals spent in apnea and their metabolic rates. These findings are consistent with previous studies on elephant seal pups and yearlings which demonstrate reductions in metabolic rates with increasing time spent in breath-hold (Huntley, 1984; Thorson and Le Boeuf, 1994; Webb et al., 1998). Given this relationship, it is not surprising that metabolic rates were higher during hypoxia and hypercapnia when apneas were greatly reduced.

Long duration apneas are known to be associated with sleep in elephant seals (Castellini et al., 1994). It is conceivable that if sleep patterns were interrupted by hypoxia or hypercapnia exposure, then metabolism would increase. However, hypoxia (approximately 13% O_2) and hypercapnia (approx. 6% CO_2) do not affect sleep patterns in

elephant seal weanlings despite changes in ventilatory patterns (Milsom et al., 1996), thus we do not expect that sleep patterns were altered significantly in the present study.

Studying a wide range of mammals, Frappell et al. (1992) found that the effect of hypoxia on $\dot{V}_{O_2}$ is inversely related to the normoxic mass-specific $\dot{V}_{O_2}$. Of the animals they studied, those with the highest mass-specific metabolic rates showed the greatest drop in $\dot{V}_{O_2}$ during hypoxia exposure. Equivalently, considering the inverse relationship between mass-specific $\dot{V}_{O_2}$ and body mass, the smallest animals had the greatest drops $\dot{V}_{O_2}$. They suggest that at a threshold mass of about 50 kg, the change in $\dot{V}_{O_2}$ under hypoxic conditions actually swings from being negative to positive. Thus, extrapolating their relationship to an animal the size of an elephant seal weanling with the observed mean mass-specific $\dot{V}_{O_2}$of 4.0 ml kg^{-1} min^{-1}, it may be expected that there be no decrease in metabolism in this species. An examination of the effects of hypoxia on metabolic rate in ponies of masses comparable to the seals used in the present study also demonstrates increases in $\dot{V}_{O_2}$ (Korducki et al., 1994) supporting the idea that metabolic down-regulation is limited to mammals of small body masses.

To our knowledge, this is the first study examining the effects of hypoxia and hypercapnia on metabolism in an intermittently breathing mammal. Milsom (1991) proposed that intermittent breathing may serve to reduce metabolic rates significantly by limiting ventilation costs. His suggestion is based on ventilatory costs of 10–35% of total metabolic costs in a species of gecko and an aquatic turtle. While we were unable to determine specifically the costs associated with ventilation in elephant seals, the increases in ventilation frequencies during hypoxia and hypercapnia and the associated increases in metabolic rates indicate that these costs may be quite high. However, changes in the ventilation frequencies alone cannot explain the observed increases in metabolism. Breathing 11% O_2 and 7% CO_2 had similar effects on metabolic rate yet ventilation frequency was approximately twice as high during CO_2 inhalation as during hypoxia (Fig. 4A).

Our measurements of resting metabolic rates are in close agreement with two other studies during which metabolic rates of elephant seal pups were measured under similar conditions, i.e. during rest out of water and averaged over long periods which may have included bouts of apnea and/or sleep (Huntley, 1984; Rea, 1990). After converting their data to common units, the average $\dot{V}_{O_2}$ of weaned pups as reported by Huntley (1984) and Rea (1990) are 4.0 and 4.5 ml kg^{-1} min^{-1}, respectively, compared to 4.0 ml kg^{-1} min^{-1} for the weaned pups in the present study. Furthermore, the observed decrease in mass-specific metabolic rate during the first 3 months of life is of the same pattern and magnitude as reported by Rea and Costa (1992), and Thorson and Le Boeuf (1994). There is a discrepancy in the respiratory quotient (R) of 0.81 reported here for nursing pups (Table 2) and that reported previously. Based on the composition of elephant seal milk (approx. 12% protein and 26% fat; Kretzmann, 1990) the R of nursing pups is expected to be about 0.73. It is unlikely that either the oxygen consumption or carbon dioxide production measurements contain a uniform error during our studies since R's of fasted seals were close to those theoretically expected. Whether these high R's are a consequence of differential metabolism of consumed or stored substrates, hyperventilation, or differences in the CO_2 buffering capacities of the younger pups requires further study.

4.2. Ventilatory patterns

We have shown that in elephant seals both hypoxia and hypercapnia cause increases in ventilation frequencies and decreases in the percent time spent in apnea. As with the metabolic response, ventilatory responses appear to be independent of age. Based on our data, the hypoxia response is dose dependent. None of the ventilatory variables measured changed significantly during exposure to 14% O_2, however we observed a decrease in the percent time spent in apnea, in the average apnea duration, and in the frequency of apneas during 11% oxygen exposure. Milsom et al. (1996), also studying weaned elephant seal pups, report that at moderate levels of hypoxia

and hypercapnia (approx. 13% O_2 and 6% CO_2, respectively) apnea length was not affected despite increases in the instantaneous breathing frequency. At lower oxygen and higher CO_2 levels they were able to elicit a decrease in apnea duration and eventually to eliminate apneas altogether. Our data vary qualitatively from theirs in that, for the seven animals from which we recorded individual breaths, the eupneic breathing frequency did not change significantly during hypoxia (11% O_2) but doubled during hypercapnia (Fig. 4B). Mean breathing frequencies during periods of eupnea were 8.2 ± 0.9, 9.2 ± 0.8, and 16.4 ± 1.2 breaths min^{-1} for normoxia, hypoxia and hypercapnia, respectively. The increases in overall breathing frequencies (Fig. 4A) were due to decreases in the percent time spent in apnea as a result of both shorter and fewer apneas. Hypercapnia (7% CO_2) eliminated apneas in 10 of our 28 trials while hypoxia (11% O_2) eliminated apneas in four of 19 trials.

While changes in ventilatory patterns appear to be dose dependent during hypoxia exposure, we evaluated the effects of high CO_2 on ventilatory patterns for only one level of CO_2. Milsom et al. (1996) suggest that increases in ventilation frequencies of elephant seal weanlings in response to high CO_2 (approx. 6%) do not necessarily counter the earlier reports of a decreased responsiveness to CO_2 in marine mammals. They proposed that the ventilatory response to CO_2 in elephant seals in similar to that in other mammals once the inspired CO_2 is above a threshold level. Our data support their conclusion that elephant seals are quite responsive to CO_2, and show clearly that 7% CO_2 is beyond the threshold at which an increase in respiratory drive occurs.

Since we did not measure ventilation rate per se, but rather ventilation and apnea frequencies, it is possible that the observed responses included a decrease in tidal volume. During hypercapnia exposure, for example, the increases in ventilation frequencies were 2-fold greater than during hypoxia exposure despite similar increases in metabolic rate. Hyperventilation during hypercapnia exposure may have been associated with decreases in tidal volume. This was the case for hooded and harp seals in which ventilation rates, as measured by a combination of respiratory frequency and tidal volume, increased during hypoxia and hypercapnia exposure, but the increases were not as great as those in ventilation frequency alone (Påsche, 1976a,b).

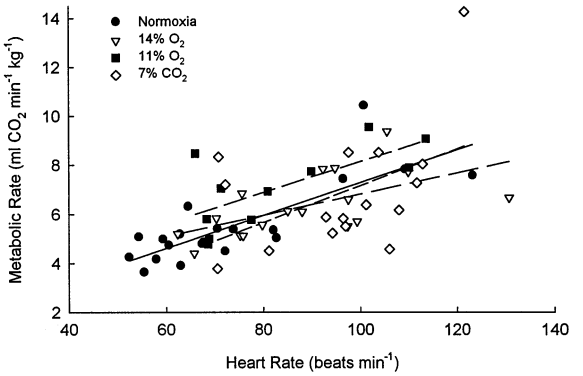

Fig. 6. The relationships between the heart rate and metabolic rate during the experimental trials. Linear least-squares regression equations describing the relationships did not differ significantly from one another. Metabolic rates are corrected for lean body mass as explained in the methods.

4.2.1. Heart rate response

In diving marine mammals, heart rate, when averaged over entire dive cycles, has been shown to be a good indirect measure of metabolic rate (Fedak et al., 1988; Williams et al., 1991; Butler et al., 1992). In agreement, we found a significant positive correlation between heart rate and metabolic rate during normoxia. Although heart rates increased during hypoxia and hypercapnia exposure, quantitatively the relationships between mean trial heart rate and metabolic rate did not depend on the inhaled gas (Fig. 6). Thus, the heart rate response may be more indicative of the metabolic status of the animal than the ventilatory response.

4.2.2. Relevance to diving

The rate of oxygen utilization in elephant seals during diving is likely quite low by virtue of their large body sizes (Kooyman and Ponganis, 1998), slow, low-effort swimming modes (Williams et al., 1996), and an energy-conserving dive response (Butler and Jones, 1982). Thus, changes in tissue O_2 and CO_2 levels will occur gradually during a

dive. Whether there is a further suppression in metabolism once oxygen supplies are reduced and CO_2 levels increase is still uncertain. The fact that both experimentally-imposed hypoxia and hypercapnia serve to increase ventilation frequencies in these seals indicates that either there may be inhibition of the respiratory drive during diving which annuls the ventilatory response, or that the ventilatory stimulus is not routinely present.

In our experiments, the marked increases in ventilation frequencies necessarily prevented any breath-hold associated energy conservation. It is possible that during diving, energy conservation is elicited by a combination of factors. Hypoxia or hypercapnia alone will not elicit metabolic suppression or a dive response, but perhaps if the ventilatory response were prevented, a reduction in metabolism would occur. Alternatively, combined hypoxia and hypercapnia, changes in pressure, circulation, circulating metabolites, pH, or any number of factors affected by breath-hold diving, may be necessary to elicit the response. It would be insightful to alter some of the other factors and determine the effect on physiological processes associated with diving.

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