Pflügers Arch - Eur J Physiol (2000) 440:843–851
DOI 10.1007/s004240000385

ORIGINAL ARTICLE

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Calorimetry and respirometry in guinea pigs in hydrox and heliox at 10–60 atm

Received: 7 April 2000 / Received after revision: 2 June 2000 / Accepted: 14 June 2000 / Published online: 19 July 2000

Abstract We used direct calorimetry and respirometry to measure the total rate of heat loss (Q_Σ) and of oxygen consumption ($\dot{V}O_2$) in guinea pigs in 1-atm (0.1 MPa) air and at 10–60 atm in either heliox (98% He, 2% O_2) or hydrox (98% H_2, 2% O_2). Our objective was to determine if the physiological responses to these two gas mixtures were different and, if so, whether the differences were attributable to the thermal characteristics of the gases alone or were confounded by additional mechanisms. At 10–40 atm, Q_Σ and $\dot{V}O_2$ were not significantly different in the two gas mixtures, whereas at 60 atm, Q_Σ and $\dot{V}O_2$ were significantly higher in heliox than in hydrox. The $\dot{V}O_2/Q_\Sigma$ ratio suggested that the animals were not in thermal equilibrium in hyperbaria. Based solely on the differing thermal properties of the gas mixtures, a mathematical model predicted a Q_Σ that was higher in hydrox than in heliox at all pressures. Two plausible explanations are suggested: one is an adaptive lowering of the surface temperature as a physiological response of the animal to the thermally more stressful hydrox environment, and the other is related to the narcotic suppression of the animal's activity by hydrox.

Key words Hydrogen diving · Hyperbaria · Thermoregulation · Hydrogen narcosis · HPNS

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Introduction

Body heat loss is a serious problem in diving. For simulated human dives in a dry hyperbaric chamber, the major avenues for heat loss are convection and radiation, and the heat loss due to the former increases with the increased density of the gases.

While helium (He) has been used extensively as a major component of a breathing gas for human diving, there is only a limited amount of data on the physiological and thermal effects of using hydrogen (H_2) during hyperbaria [3]. We wanted to determine if the physiological responses to hyperbaric hydrox (98% H_2, 2% O_2) differ from those to heliox (98% He, 2% O_2) and, if so, whether the differences can be explained by the physical characteristics of the gases alone, or whether there are additional factors contributing to the total heat loss rate (Q_Σ) in hyperbaric heliox and hydrox.

Hydrogen was first introduced as a diving gas in 1943 by Arne Zetterström of the Swedish Royal Navy [28]. Hydrogen has the advantage of being lighter than He and thus easier to ventilate with increasing pressure. At elevated pressures, H_2 has a narcotic effect that ameliorates symptoms of high pressure nervous syndrome (HPNS) in some subjects, while inducing psychosis in others [14]. Disadvantages of H_2 as a diving gas include the flammability of some H_2/O_2 mixtures, and its higher thermal conductivity and heat capacity compared with He, suggesting that heat losses in hyperbaric H_2 would be unusually high.

Several studies have measured the oxygen consumption rate ($\dot{V}O_2$) of animals in He mixtures at 1 atm (0.1 MPa). While some have found a reduction in $\dot{V}O_2$ in rats [7], others have found an increase in $\dot{V}O_2$ in mice in a 1-atm He environment [9] compared with air. It has been suggested that the greater heat transfer coefficient (h_c) of He compared with air leads to increased rates of heat loss and thus to an increased metabolic rate in the He environment [26]. Increasing the ambient temperature (T_a) at 1 atm, and thus decreasing the driving force for heat loss, decreases the

difference between $\dot{V}O_2$ of rats in air and that in He [26]. Although there seems to be an effect on the metabolic rate, depending on the gas mixture used, that can be attributed to the thermal properties of the gas mixture, many reports of metabolic disturbances in tissue slices [29], insects [8], microorganisms [36], whole-animal studies in mammals [7, 21, 23], and inhibition of enzyme activity in vitro [35] have suggested other possible mechanisms.

In this study, we measured $\dot{V}O_2$ by respirometry and Q_Σ by direct calorimetry in guinea pigs at 10, 20, 40, and 60 atm in heliox and hydrox. In addition, we derived a mathematical model for partitioning the various paths for heat loss in guinea pigs in 1-atm air and 10- to 60-atm hydrox and heliox.

Materials and methods

Animal protocol

Guinea pigs (male, Hartley strain, *Cavia porcellus*, n=16, mean body mass 822±113 g) were housed in a professionally staffed animal care facility and had ad libitium access to food and water before experiments. The animals were fed a standard guinea pig chow (Prolab, Agway, Syracuse, N.Y., USA) with crude protein (>18%), fat (>4%), and fiber (>15%). All animals were used for calorimetry experiments at 1 atm in air and a subset (n=12) was assigned subsequently at random to either hydrox or heliox groups.

Experimental procedure for direct calorimetry

Calorimeter setup

Direct calorimetry was performed with a Seebeck envelope gradient layer calorimeter (Thermonetics, San Diego, Calif., USA), with internal dimensions of 15×15×30 cm. The calorimeter was equipped with a system of ducts in the walls for holding water to increase the thermal inertia of the walls, but no further temperature regulation of the calorimeter was used. Under ordinary circumstances in 1-atm air, the water would be circulated through a temperature-controlled water bath. However, safety regulations in a hydrogen environment prohibit the use of a heating element or pump in the chamber.

Animals were placed in a wire-mesh cage of sufficient size for the animals to turn and walk (11×13×27 cm). The mesh cage was placed inside the calorimeter with a tray of sand underneath to collect urine and feces. The customary tray of oil, which is used to cover the urine and feces to prevent them from releasing water vapor, is not permitted in the dive chamber due to the combustibility of hydrocarbons.

The calorimeter was equipped with a temperature sensor (ΔT sensor) measuring the temperature difference between the incurrent and excurrent gas. This allowed us to account for heat lost by the animal due to warming of the gas flowing through the calorimeter. Calibration and correction factors for the density and molar heat capacity of the gas mixtures were provided by the manufacturer of the ΔT sensor. A thermistor (No. 402, YSI, Yellow Springs, Ohio, USA) was used to record T_a in the calorimeter.

The calorimeter door was sealed, leaving the only entry and exit for gases via the ports of the ΔT sensor. A constant stream of gas passed through the calorimeter. The gas flow rate was measured with a floating ball-type flow meter that had been calibrated using air, heliox, and hydrox. The animal's $\dot{V}O_2$ was calculated from the difference between O_2 content of the incurrent and excurrent gas, and the gas flow rate, once steady state was attained. All incurrent gases used were dry, and $\dot{V}O_2$ was corrected to standard temperature and pressure dry (STPD). The ratio between the rate of CO_2 production and $\dot{V}O_2$ was assumed to be 1.0, with an error of less than 2% in the predicted $\dot{V}O_2$ estimate if this ratio was as little as 0.7 [23]. For the 1-atm air experiments, the O_2 content was measured using a paramagnetic oxygen analyzer (Servomex 540A, Sybron, Boston, Mass., USA). For the hyperbaric experiments, the chamber gases were analyzed with a gas chromatograph (GC-9A, Shimadzu, Columbia, Md., USA) to monitor the O_2, He, H_2, and N_2 content of the chamber and of the excurrent gas from the calorimeter.

Water vapor was scrubbed from the excurrent gas with an absorbent, anhydrous $CaSO_4$ (Drierite, W.A. Hammond, Xenia, Ohio, USA), and the water vapor loss estimated by weighing the Drierite canister before and after each experiment.

For hyperbaric experiments, the calorimeter was placed in a hyperbaric chamber (Bethlehem, Bethlehem, Pa., USA), and the same chamber was used for all measurements. The facility housing the chamber was designed to comply with the safety regulations for work with H_2, as described in detail elsewhere [21, 23].

Calibration of the calorimeter

The calorimeter was calibrated in 1-atm air by placing a small heater inside the instrument. A regulated direct current power supply (BK Precision Model 1601, Maxtec International, Chicago, Il., USA) was used to generate power of 1–15 W in the heater, with a random sequence of step changes in power input. When the calibration was completed, average power output measured by the calorimeter was within 2% of the power input to the heater, with a time-to-stable calorimeter reading of approximately 30 min. The calibration was repeated with the calorimeter placed in the dive chamber at 10, 20, 40, and 60 atm heliox. Average calorimeter output was within 3% of the power input from the heater, without the need to make corrections for pressure or thermal properties of the gas. However, the time to reach stable calorimeter readings was increased to 45–60 min, due to the increased time needed to stabilize the thermostatically-controlled temperature inside the chamber. Safety regulations did not allow the calibration in the presence of H_2, and we therefore assumed that if no correction was necessary in heliox, this would also be the case for the hydrox mixture.

Calorimeter measurements

Guinea pigs (n=16) in 1-atm air served as controls. The calorimeter was near room temperature, with a range of values of 19.3–34.0 °C. Animals were placed in the calorimeter for 1 h and data were collected during the second half-hour when the calorimeter output was constant. Animals were removed from the calorimeter for at least 1 h or up to a few days, before a replicate measurement was made. The repeated measurements were scheduled such that one was performed in the morning and one in the afternoon to determine any diurnal effects. A subset of five randomly selected animals from the control 1-atm air group (mean body mass 836±59 g) were kept in the calorimeter for 6 h continuously in 1-atm air. The 6-h extended exposure was chosen since this was the approximate length of the hyperbaric experiments. Q_Σ and $\dot{V}O_2$ were measured for each hour to test for systematic temporal changes in heat output during extended confinement without food or water.

At a later date (1–89 days), 12 of the original 16 animals were used for the hyperbaric experiments, and these animals were assigned randomly to either the heliox (n=6, mean body mass 894±65 g) or the hydrox (n=6, mean body mass 898±79 g) group. The animals were placed in the calorimeter, which was put inside the hyperbaric chamber. The chamber was pressurized as described earlier for hydrox and heliox experiments [21, 23] to 10,

20, 40, or 60 atm. The chamber was kept at each pressure for 1 h to measure the heat output and $\dot{V}O_2$. Heat output from the animal was computed from a mean of the values from the last 10–15 min of the 1 h spent at each pressure, when the calorimeter output was stable. If stable measurements had not been achieved in 45 min, the total time under pressure was prolonged for up to 15 min. The temperature of the chamber was the same for the two gases at each pressure (Table 1), and selected such that the animal could maintain a rectal temperature at or near 38.0±0.5 °C, as determined from an earlier experiment [21]. At equilibrium, the temperature difference between the inside and the outside of the calorimeter was usually not more than ±0.5 °C.

At the end of the experiment, animals were returned rapidly to 10 atm and euthanized immediately using CO_2 (2 atm) as previously described [23].

Mathematical analysis

Model

The model of theoretical heat loss used the following heat balance equation:

$$M=S+Q_{\Sigma} \quad (1)$$

This equation states that the total heat loss rate (Q_{Σ}) plus the rate of storage of body heat (S) is equal to the metabolic heat production rate (M). Unless otherwise stated, the above thermal variables are expressed in watts. A negative value of S indicates net loss of heat from the animal's body (i.e., heat loss exceeds heat production). The Q_{Σ} from the animal's body is due to the sum of heat losses by convection across body surfaces (Q_C), convection by respiration (Q_{RC}), radiation (Q_R), conduction (Q_{CD}), and evaporation (Q_{EVAP}) from the skin (Q_{SE}) and from the respiratory tract (Q_{RE}):

$$Q_{\Sigma}=Q_C+Q_{RC}+Q_R+Q_{EVAP} \quad (2)$$

On the basis of criteria described by Clarkson et al. [7], Q_{CD} (which depends on the heat loss due to direct contact with a surface) was assumed to be negligible in this study and therefore excluded from Eq. 2. Q_{SE} and Q_{RE} were estimated together by measuring the total amount of water in the absorbent canister and converting to its evaporative energy equivalence (Q_{EVAP}, 2.4 kW·s·g^{-1} at 37 °C [27]). Convective heat loss was determined from techniques for estimating heat loss from a horizontal cylinder [25, 30]. Following the criteria of Clark et al. [6], the Q_C is characterized by natural convection, and predicted by:

$$Q_C=h_c \cdot A \cdot (T_s-T_a) \quad (3)$$

where A is the body surface area (in cm^2), T_s the surface temperature (T_a and T_s in degrees Celsius), and h_c the heat transfer coefficient (in W·m^{-2}·C^{-1}) predicted by:

$$h_c=\mathrm{Nu}\cdot\lambda\cdot L^{-1} \quad (4)$$

where Nu is Nusselt's factor, determined by $\mathrm{Nu}=a\cdot(\mathrm{Gr}\cdot\mathrm{Pr})^{0.25}$, in which a is a geometry-dependent constant (approximately 0.53 in this case in which the shape of a small mammal is approximated by a horizontal cylinder [25, 30]), and Gr and Pr are the dimensionless Grashof and Prandtl numbers, respectively [30]. λ, the thermal conductivity (in W·m^{-1}·C^{-1}), was determined for each gas mixture and temperature according to equations for binary gas mixtures [33]. L, the shape factor (in centimeters) was estimated as 1/3 of the animal's volume [31]. The latter was assumed to be equal to the animal's mass divided by the density of mammalian muscle ($1.06\cdot10^{-3}$ g·mm^{-3} [23]).

T_s was estimated using the following [18]:

$$T_s=0.84\cdot T_a+8.64 \quad (5)$$

where T_a was measured by a thermistor located inside the calorimeter.

A was first estimated according to measurements in 1-atm air in guinea pigs [18], in which:

$$A=9\cdot m^{2/3} \quad (6)$$

where m is the animal's mass (in grams). However, the body surface area available for heat loss, expressed as a percentage of total body surface area [15] was adjusted such that the model would fit the measured values for heat loss in 1-atm air. The adjusted value or effective area for heat loss was assumed to be independent of pressure and temperature, and the same value was used to estimate Q_{Σ} for the hyperbaric experiments.

Heat loss due to radiation was determined by the equation:

$$Q_R=A\cdot\varepsilon\cdot\sigma\,(T_s^4-T_a^4) \quad (7)$$

where σ is the Boltzmann constant ($5.67051\cdot10^{-8}$ W·m^{-2}·K^{-4} [27, 39]) and temperatures are in degrees Kelvin. The emissivity (ε), which is 0.95–1.00 for small mammals [5], was set to unity for this study.

The portion of the convective heat exchange from the respiratory tract was determined by:

$$Q_{RC}=V_E\cdot\rho\cdot C_p\cdot(T_{ex}-T_{in}) \quad (8)$$

where V_E is the respiratory minute volume (in cm^3·s^{-1}) for guinea pigs estimated by $V_E=\dot{V}O_2\cdot 0.028$ [1, 39], ρ the density of the gas mixture (in mol·cm^{-3}), C_p the heat capacity of the gas mixture (in J·mol^{-1}·C^{-1}), T_{in} the temperature of the inhaled gas, and assumed to be equal to T_a, and T_{ex} the temperature of the exhaled gas [39]. For estimating T_{ex}, equations for cats in 1-atm air and hyperbaric heliox were used [32], which estimate the temperature of exhaled gas as:

$$T_{ex}=a+b\cdot T_{in} \quad (9)$$

where a is 15.8 and 17.4, and b 0.68 and 0.56 for 1-atm air and hyperbaric heliox, respectively. The calculation for hyperbaric hydrox used the same value as heliox.

The Q_{Σ} was computed using the mathematical model (Eq. 2) assuming that the animals were in thermal equilibrium (S=0). These values were compared with the measured Q_{Σ} from the calorimeter experiments. Other models explored here are various modifications to Eq. 2, as explained below.

Statistical analysis

Estimates of variance are reported as SD, and all comparisons of means are based on two-tailed t-tests with acceptance of significance at P<0.05, unless stated otherwise. Group sample sizes are indicated in Tables 1 and 2 .

Results

Calorimetry measurement of Q_{Σ}

There was no systematic change in either Q_{Σ} (P=0.40) or $\dot{V}O_2$ (P=0.95) with length of exposure in 1-atm air, as determined by repeated-measures, single-factor ANOVA (Table 2). The $\dot{V}O_2$ and Q_{Σ} measurements for the 1-h exposure in 1-atm air were not significantly different from the 6-h exposure (P>0.62; Tables 1 and 2). Furthermore, there were no diurnal differences in the $\dot{V}O_2$ (P=0.83) and Q_{Σ} (P=0.61) measurements. Thus, any changes in $\dot{V}O_2$ or Q_{Σ} in hyperbaria are not expected to be due to diurnal effects or the experimental duration.

During the hyperbaric exposure, there was an increase in $\dot{V}O_2$ with pressure for animals in heliox, but not in hydrox (Table 1), with a significantly higher $\dot{V}O_2$ in heliox at 60 atm (P<0.03, two-tailed Mann-Whitney test) than in hydrox. Q_{Σ} increased with increasing pressure in heliox and at 10–40 atm in hydrox (Table 1). At 60 atm

Table 1 Mean (±SD) oxygen consumption rate $\dot{V}O_2$, mean total heat loss rate (Q_Σ), and average calorimeter temperature (T_a), for guinea pigs in heliox (n=6) and hydrox (n=6) at 10–60 atm, and in air (n=16) at 1 atm, as measured by respirometry and calorimetry

Gas	Pressure (atm)	$\dot{V}O_2$ (ml $O_2 \cdot s^{-1}$)	Q_Σ (W)	T_a (°C)
Air	1	0.164±0.016	3.31±0.32	26.5±3.1
Heliox	10	0.190±0.025	3.81±0.31	32.7±1.5
20	0.196±0.012	4.43±0.52	33.4±0.7	
40	0.218±0.034	5.08±0.61	34.3±0.2	
60	0.258±0.052*	6.51±0.72*	35.1±0.5	
Hydrox	10	0.178±0.030	4.07±0.48	32.8±1.2
20	0.208±0.052	4.75±0.89	33.2±0.5	
40	0.193±0.018	5.57±0.62	34.5±0.7	
60	0.200±0.021	5.40±0.82	35.3±0.6	

*P<0.05 vs. hydrox

Table 2 Mean (±SD) of Q_Σ, $\dot{V}O_2$, and T_a for guinea pigs (n=5) in air at 1 atm during a 6-h enclosure in a calorimeter

Period (h)	Q_Σ (W)	$\dot{V}O_2$ (ml $O_2 \cdot s^{-1}$)	T_a (°C)
0–1	3.65±0.67	0.177±0.030	23.4±1.0
1–2	3.27±0.54	0.174±0.026	24.2±0.9
2–3	3.07±0.46	0.168±0.025	24.9±0.8
3–4	3.13±0.37	0.169±0.022	25.2±0.8
4–5	3.10±0.38	0.162±0.018	25.4±0.9
5–6	3.15±0.30	0.171±0.022	25.6±0.9
Overall	3.23±0.48	0.170±0.023	24.8±1.1
P	0.40	0.95	

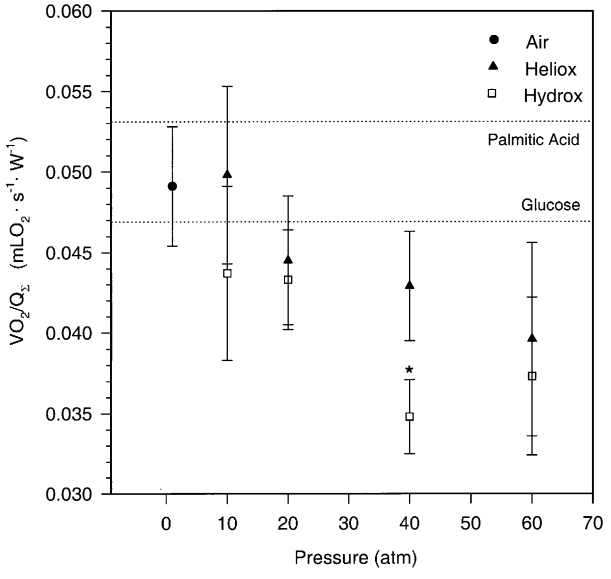

Fig. 1 Relationship between the mean (±SD) ratio of oxygen consumption rate to heat loss rate $\dot{V}O_2/Q_\Sigma$ and pressure for air, 98% H_2/2% O_2 (*hydrox*) and 98% He/2% O_2 (*heliox*), as measured by direct respirometry and direct calorimetry. The *upper* and *lower dotted lines* indicate the ratio for pure palmitic acid and glucose substrates respectively [11]. *P<0.05 between hydrox and heliox at the specified pressure

Q_Σ was significantly higher in heliox than in hydrox (P<0.03). $\dot{V}O_2/Q_\Sigma$ ratio for the hydrox and heliox mixtures at 10 atm was close to that in air, but the ratio decreased with increasing pressure in both gas mixtures (Table 1, Fig. 1). At 40 atm, the ratio was significantly lower in hydrox (Table 1, P<0.03, two-tailed Mann-Whitney test) than in heliox or 1-atm air. At all pressures in hydrox and at 20, 40, and 60 atm in heliox, $\dot{V}O_2/Q_\Sigma$ was out of the range of biological fuel sources (Fig. 1). This suggests that the animals were not in thermal equilibrium and lost stored heat (S≠0). This speculation will be discussed in detail below.

Mathematical model to estimate Q_Σ

For estimating Q_Σ in 1-atm air, the model (Eq. 2) was evaluated in three separate trials, using one randomly chosen result for each animal from either the duplicate 1-h trial at 1 atm, or the mean value of the 6-h trial (Model 0, Table 3). Model 0 used Eqs. 5 and 9 to estimate T_s and T_{ex}, respectively. On the assumption that the animals were in thermal equilibrium with their environment, i.e., M=Q_Σ and S=0 (Eq. 1), the model predicted values for heat loss in 1 atm air that were similar to the measured values (P>0.60 for all three trials, Table 3 , Model 0).

Q_Σ estimated from the hyperbaric experiments was up to 211% higher than the measured values in both gas mixtures when using the same model as in 1-atm air (Model 0; data not shown). Since the deviation in the $\dot{V}O_2/Q_\Sigma$ ratio could be attributed potentially to the loss of stored heat (S≠0, Eq. 1, Fig. 1), we speculated that S≠0 was the reason for the failure of Model 0 to estimate Q_Σ in hyperbaria. On this assumption, we used the measured values of $\dot{V}O_2$ and Q_Σ from the calorimeter to fit the equations to estimate new values for T_s. Since an independent measurement was made on each animal at each pressure, this yielded four independent values for each animal and experiment [12]. These values were regressed against T_a (Fig. 2) and found to be significantly lower in hydrox than in heliox at all pressures (P<0.05, Student's t-test comparing slopes, [40]). We used the regression equations to estimate the Q_Σ in heliox and hydrox at elevated pressures (Models 1 and 2, Table 3).

Q_C and Q_{RC} were predicted to account for 63–71% and 4–15% of the total heat loss, respectively. In both gas mixtures, these contributions tended to increase with pressure at 10–60 atm (Table 3 , Models 1.0, and 2.0).

Table 3 Mean (±SD) Q_Σ measured by direct calorimetry (*Actual*), or estimated by a mathematical model (Eq. 2, *Theoretical*), and the percentages of Q_Σ partitioned into various avenues of heat loss (Eq. 2). *Model 0* (n=16) is specific for air at 1 atm and uses Eqs. 5 and 9 to estimate the surface (T_s) and exhaled (T_{ex}) temperatures. *Models 1* (n=6) and *2* (n=6) are for hyperbaric 98% He/2% O_2 (*heliox*) and 98% H_2/2% O_2 (*hydrox*), respectively, and use the regression equations in Fig. 2 to estimate T_s. *Models 2.1* and *2.2* are modifications of Model 2, using different values for T_{ex}. (Model 2.1: T_{ex} 1 °C lower in hydrox; Model 2.2: T_{ex} 2 °C lower in hydrox) (Q_C convective heat loss across body surfaces, Q_{RC} convective heat loss via respiration, Q_R heat loss by radiation, Q_{CD} heat loss by conduction, Q_{EVAP} evaporative heat loss)

Gas	Pressure (atm)	Actual Q_Σ (W)	Theoretical Q_Σ (W)	Model No.	Q_C (%)	Q_R (%)	Q_{RC} (%)	Q_{EVAP} (%)
Air	1	3.31±0.32	3.30±0.22[a]	0	32.4	55.9	1.5	10.2
Heliox	10	3.81±0.31	3.53±0.48[a]	1.0	63.6	22.8	4.7	8.9
20	4.43±0.52	4.41±0.38[a]		68.4	17.6	7.0	7.0	
40	5.08±0.61	5.66±0.38[a]		70.6	13.1	10.2	6.1	
60	6.51±0.72	6.57±0.60[a]		69.9	10.9	13.0	6.2	
Hydrox	10	4.07±0.48	3.84±0.75[a]	2.0	65.1	18.9	5.5	10.5
20	4.75±0.89	4.98±0.68[a]		66.1	13.8	9.3	10.8	
40	5.57±0.62	5.53±0.89[a]		67.5	10.6	12.4	9.5	
60	5.40±0.82	5.77±0.90[a]		66.5	8.9	15.1	9.5	
Hydrox	10	4.07±0.48	3.87±0.70[a]	2.1	66.8	19.3	3.6	10.3
20	4.75±0.89	4.97±0.63[a]		68.9	14.3	6.0	10.8	
40	5.57±0.62	5.50±0.85[a]		72.4	11.3	6.8	9.5	
60	5.40±0.82	5.70±0.86[a]		73.7	9.7	7.0	9.6	
Hydrox	10	4.07±0.48	3.87±0.66[a]	2.2	68.3	19.6	1.8	10.3
20	4.75±0.89	4.92±0.58[a]		71.6	14.8	2.7	10.9	
40	5.57±0.62	5.43±0.80[a]		77.3	11.9	1.2	9.6	
60	5.40±0.82	5.56±0.81[a]		80.9	10.5	–1.3	9.9	

[a]Not significantly different from Actual (P>0.60)

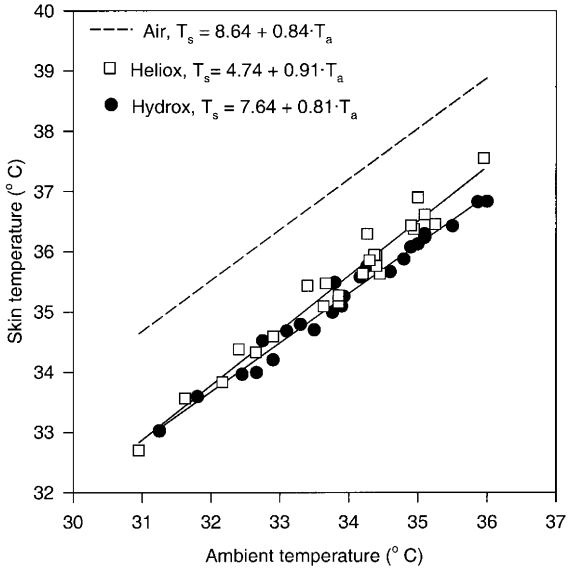

Fig. 2 Relationship between ambient temperature (T_a) and estimated skin temperature (T_s) for guinea pigs in 1 atm air [18], and 10–60 atm heliox and hydrox. The *regression equations* are for temperatures measured in degrees Celsius

The contribution to Q_Σ from Q_R and Q_{EVAP}, on the other hand, tended to decrease with increasing pressure and were approximately 23–9% and 10–6%, respectively, at 10–60 atm (Table 3 , Models 1.0, and 2.0).

Discussion

The average $\dot{V}O_2$ measured in air at 1 atm was similar to values reported in other studies with guinea pigs [2, 18, 23]. For the hyperbaric experiments, we compared $\dot{V}O_2$ measured in this study with values measured by Kayar et al. [23] at 10–60 atm in guinea pigs in hydrox and heliox. The $\dot{V}O_2$ values in the earlier study were higher by 28–46% and 30–73% in heliox and hydrox, respectively. This disparity can be attributed to methodological differences between the experiments. The lower $\dot{V}O_2$ of the animals in the present study might be due to their calmer state during confinement in a dark calorimeter, and without the use of an invasive rectal probe as in the earlier study.

When animals are in thermal equilibrium (S=0, Eq. 1), the $\dot{V}O_2/Q_\Sigma$ (in ml $O_2 \cdot s^{-1} \cdot W^{-1}$ STPD) ratio has been shown to vary within narrow limits depending on the fuel source utilized [11]. Thus, by referring to the energetic equivalents of O_2 for a pure glucose substrate (0.0469 ml $O_2 \cdot s^{-1} \cdot W^{-1}$) and a pure palmitic acid substrate (0.0531 ml $O_2 \cdot s^{-1} \cdot W^{-1}$) we can obtain an estimate of the fuel source used during the calorimeter experiment [11]. The stable $\dot{V}O_2/Q_\Sigma$ ratio in 1-atm air of 0.0495±0.0037 ml $O_2 \cdot s^{-1} \cdot W^{-1}$ (Fig. 1) suggested aerobic metabolism [17], using a fuel source with an energetic equivalent between protein and glucose [11]. An earlier study [18] has reported similar fuel sources utilized when measuring the respiratory quotient (RQ) of guinea pigs during a 6-h confinement, suggesting that our

Table 4 Pressure, gas mixture, and percentage difference in Q_Σ (hydrox/heliox) for a test to compare the difference in Q_Σ between heliox and hydrox based solely on their differing thermal properties (*TP Q_Σ difference*), and their measured values (*Measured Q_Σ difference*). The following variables were used for both gases at 10–60 atm: T_a=32.4 °C in Eq. 5, a=17.4 and b=0.56 in Eq. 9, W=863 g, $\dot{V}O_2$=0.184 ml·s^{-1}

Pressure (atm)	Gas	TP Q_Σ (W)	TP Q_Σ difference (%)	Measured Q_Σ difference (%)
10	He	7.0		
	H_2	8.5	21.4	6.8
20	He	9.4		
	H_2	11.5	22.3	7.2
40	He	12.8		
	H_2	15.8	23.4	9.6
60	He	15.4		
	H_2	19.2	24.7	–17.1

method of using the $\dot{V}O_2/Q_\Sigma$ ratio gave reliable results. However, in the hyperbaric experiments the measured $\dot{V}O_2/Q_\Sigma$ ratio was much lower than could be accounted for by changing fuel sources (Fig. 1). The most likely reason for the deviation is that the animals were not in thermal equilibrium (S≠0, Eq. 1). This explanation would also account for the failure of Model 0 (data not shown) to estimate the Q_Σ in animals in hyperbaria. Following this speculation, we adjusted T_s for animals in hyperbaric heliox and hydrox (Models 1 and 2, Table 3).

When computing the theoretical heat loss in 1-atm air using our mathematical model, we used Herrington's method of estimating T_s (Eq. 5 [18]) to calculate Q_C and Q_R. The theoretical values of Q_Σ for the hyperbaric experiments were considerably larger than the measured values, suggesting that Eq. 5 was not valid for the hyperbaric experiments. Consequently, we used the actual Q_Σ and $\dot{V}O_2$ to fit the model, which made it possible to estimate T_s for each animal. The estimate assumes that all factors except T_s remain constant at elevated pressures and temperatures. This is probably an oversimplification, since a change in the body posture, and thus the area available for heat loss (Eqs. 3 and 7), or a change in T_{ex} (Eq. 8) might alter this value. Nevertheless, our estimated values for T_s were similar to the measured values at 1–60 atm in cats in heliox [32]. Since T_s has been shown to vary linearly with T_a in small mammals [16], we regressed T_s on T_a and used the resulting equations to estimate T_s (Fig. 2) for estimation of Q_Σ in hyperbaria (Model 1 and 2, Table 3).

An earlier study has shown that T_{ex} in humans is approximately 2 °C lower in hydrox than in heliox at 21 atm, at a T_a range of 10–31 °C [4]. To estimate this effect on our model, we recalculated the Q_Σ and Q_{RC} for hydrox by lowering T_{ex} by 1 °C (Model 2.1) or 2 °C (Model 2.2) compared with heliox (Table 3). Animals with a 2 °C lower T_{ex} in hydrox than in heliox (Table 3, Model 2.2) had a predicted heat loss from Q_{RC} that contributed only 1.3–2.7% to Q_Σ at 10–40 atm, and a predicted heat gain at 60 atm (negative Q_{RC}, Model 2.2, Table 3). It is unlikely that animals will lose peripheral heat while gaining heat from respiration (Table 3 , Model 2.2). Humans have been reported to have a loss due to Q_{RC} of 10% at 30.8 atm in heliox [38], which is better approximated by our hydrox models 2.0 or 2.1 (Table 3). Thus, it appears from these calculations that the T_{ex} is probably the same or only 1 °C lower in hydrox than for animals breathing heliox (i.e., Models 2.0 and 2.1 are more realistic than Model 2.2).

Upon appropriate adjustment, our model was able to estimate Q_Σ values in both gas mixtures that were not significantly different from the values measured by direct calorimetry. Furthermore, the predictions made by our model for the various sources of heat loss in guinea pigs (Table 3) corresponded relatively well with the predictions made for humans in heliox mixtures at 0–150 atm [13]. Additionally, it appears that our model (Eq. 2) is relatively stable when using a range of realistic values for *a* (Eq. 4), ε (Eq. 7), and the percentage of total surface area available for heat loss (Eqs. 3 and 7; see Appendix for sensitivity test). The value for Q_{EVAP} was probably overestimated since some of the water vapor could have been derived from evaporation of the urine and feces collected in the sand. The only variable with a large impact on the model was T_s; using a plausible range of values for T_s (Eq. 5) caused a large change in the Q_Σ.

Q_Σ at 10, 20, and 40 atm was 7–9% higher in hydrox than in heliox (Table 1), although the difference was not significant (P>0.2). At 60 atm the heat loss was significantly higher in heliox than in hydrox (P<0.03). To investigate this, we evaluated the model (Eq. 2) to determine the expected difference in Q_Σ between hydrox and heliox, based strictly on the thermal properties (TP) of the two gas mixtures (Table 4). This approach indicated that Q_Σ in hydrox should be 21–25% higher than in heliox at all pressures (TP Q_Σ, Table 4). Thus, the thermoregulatory responses to hydrox, compared with those to heliox, at elevated pressures appear to be different from those predicted solely on the basis of the thermal properties of the gases.

Earlier studies have shown that there are different physiological responses in guinea pigs to hyperbaric heliox and hydrox [21, 23, 24]. It has been suggested that the differences could be due partly to HPNS in animals breathing hyperbaric heliox, while the hydrox animals could be narcotized. In hyperbaric heliox, there was a positive correlation between $\dot{V}O_2$ and Q_Σ with pressure (Table 1, P<0.03). From 40–60 atm there was a large increase in Q_Σ that might indicate that the animals in heliox were suffering from HPNS, with increased

Table 5 Sensitivity test of model to estimate total heat loss rate (Q_Σ) using Models 0, 1.0, and 2.0 (see Table 3) to estimate Q_Σ for 1-atm air, heliox and hydrox, respectively. Values are expressed as the percentage change compared with the original model estimate (a a constant used to derive a component of Eq. 4, A body surface area available for heat loss, ε emissivity)

Gas	Pressure (atm)	Q_Σ (W)	Model No.	a=0.60	a=0.45	A=65%	A=100%	ε=0.9	T_s=38.4	T_s=T_a–0.5	T_s=0.84·T_a+8.64
Air	1	3.30±0.22	0	3.0	–6.1	–21.2	15.2	–6.1	79	–30	
Heliox	10	3.53±0.48	1.0	8.5	–9.6	–21.0	14.2	–2.3	284	–66	111
	20	4.41±0.38		9.1	–10.2	–20.9	14.1	–1.6	248	–65	114
	40	5.66±0.38		9.4	–10.6	–20.3	13.6	–1.2	191	–62	114
	60	6.57±0.60		9.1	–10.5	–19.6	13.2	–1.1	135	–59	112
Hydrox	10	3.84±0.75	2.0	8.6	–9.9	–20.6	13.8	–1.8	320	–62	133
	20	4.98±0.68		8.8	–9.8	–19.5	13.1	–1.4	295	–58	138
	40	5.53±0.89		8.9	–10.3	–19.2	12.7	–1.1	251	–52	167
	60	5.77±0.90		8.8	–10.1	–18.4	12.3	–0.9	206	–46	191

muscular activity leading to increased convective heat loss. In these experiments, the animals were confined to a calorimeter and could therefore not be observed. However, during earlier experiments with guinea pigs at 40–60 atm in heliox, animals were seen clearly to be agitated and had tremors consistent with a diagnosis of HPNS [23]. In hydrox, on the other hand, there was no simple correlation between $\dot{V}O_2$ and Q_Σ with pressure (Table 1). Hydrogen has anesthetic and narcotic properties at elevated pressures [14] and anesthesia and narcosis are known to cause a lower body temperature (T_b), loss of perception of cold, and a lower T_b before the onset of thermogenesis [10]. Hydrogen narcosis could explain why the animals in hydrox did not defend the increasing Q_Σ by an increase in their $\dot{V}O_2$ at 60 atm and therefore lost stored heat.

H_2 has been shown to be biologically inert in isolated tissues at 1–50 atm [22], and in intact multicellular animals at 1 atm [37]. However, whether gaseous H_2 is biologically inert in intact animals in hyperbaria is unknown. If H_2 were to be metabolized in the mammalian body at elevated pressures, $\dot{V}O_2/Q_\Sigma$ should deviate from the value in 1-atm air such that more heat is released than could be attributed to aerobic metabolism. In heliox, on the other hand, we would not expect a change in the $\dot{V}O_2/Q_\Sigma$ ratio since helium is believed to be completely biologically inert [26]. The substantial decrease in the $\dot{V}O_2/Q_\Sigma$ ratio in both gases made it impossible to draw a firm conclusion regarding H_2 metabolism. The simplest explanation for the decrease in the $\dot{V}O_2/Q_\Sigma$ ratio in both gas mixtures is that this ratio can be used to suggest metabolic pathways only when an animal is in thermal equilibrium, with no loss or gain of stored heat, a condition not attained in our hyperbaric animals. However, we cannot exclude the possibility that animals in hydrox received a contribution of heat from H_2 metabolism by microbes resident in the intestine [20].

Non-shivering thermogenesis has been reported in neonatal guinea pigs as a mechanism for defending core temperatures during absence of maternal heat, but in the adult animal, shivering thermogenesis replaces non-shivering thermogenesis [19]. In this study we used adult guinea pigs, and we therefore did not consider further heat production from brown adipose tissue.

Not surprisingly, we found that the major avenue for Q_Σ in hyperbaric heliox and hydrox is through convection (Q_C and Q_{RC}, Eq. 2). Since Q_C and Q_{RC} are exceedingly dependent on the difference between T_a and T_s (Table 5), one would expect that animals in hyperbaria would regulate the latter to minimize the density-induced increase in Q_Σ at elevated pressures. This could be done by the commonly found regulatory vasoconstriction of appendages, reducing heat loss from exposed areas and with an overall lowering of T_s [34]. This was supported by our model, which predicted that animals in hyperbaria regulated their T_s more strictly to minimize the gradient for heat loss than did animals in 1-atm air (Fig. 2). The finding that animals in hyperbaria had increasing difficulty in maintaining a normal core temperature (i.e., S<0), as suggested by the decreasing the $\dot{V}O_2/Q_\Sigma$ ratio, supports the conclusion of others, that thermal failure in human divers is most likely due to insufficient heating of the chamber [13]. Thus, we predict that human divers in hydrox will find that the usual strategies of adjusting environmental temperature and layers of clothing will suffice to maintain stable core temperature. Indeed, given the additional problems of HPNS and narcosis at high pressures, human divers at 60 atm may find that hydrox is not as stressful as heliox.

In this study, we wanted to determine if physiological responses to hyperbaric heliox and hydrox could be explained on the basis of their thermal properties alone. We created a model that predicted Q_Σ at similar T_a to be approximately 20% higher in hydrox than in heliox at 10–60 atm, based solely on the thermal properties of the gas mixtures. Q_Σ was measured by direct calorimetry and was no more than 9% higher at 10–40 atm, and was 17% lower at 60 atm in hydrox than in heliox. We suggest that the elevated Q_Σ of animals in hyperbaric heliox is in part due to HPNS, whereas animals in hyperbaric hydrox allow T_s to fall to reduce Q_Σ. We conclude that the physiological responses in hyperbaric heliox and hydrox cannot be explained solely by the thermal properties of the two gas mixtures.

Acknowledgements We gratefully acknowledge the technical support of Mr. Walter Long Jr., Mr. William Mints, Mr. Jerry Morris, Mr. William Porter, Mr. David Schoenhauer, and Dr. Robert Weinberg. Dr. Jeff Himm provided programming expertise. Mr. John Braisted and Ms. Tracy Cope provided many hours of assistance with the care of the animals. Ms. Susan Mannix provided editorial services. This work was funded by the Naval Medical Research and Development Command Work Unit No. 61153 N MR04101.00D-1103. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department and the naval service at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources National Research Council, National Academy Press, 1996.

Appendix

We tested the sensitivity of the model (Eq. 2) by changing the assumed constants and variables one at a time, and comparing the estimated heat loss rates with the Q_Σ from the original model (Table 5).

The constant *a*, used to calculate Q_C (a=0.53 in original model; Eq. 3), was changed to 0.60 and 0.45, which is the range of values most commonly used for this constant [5, 6, 25, 30]. The body surface area available for heat loss (A, 85% in original model) was changed to 65 and 100% which is the range reported for humans [15], and ε (1.0 in original model; Eq. 7) was changed to 0.9 as an extreme example of the values reported [5, 15]. Three values of T_s were tested: 38.4 °C (the assumed value of T_b), 0.5 °C greater than T_a, and measured T_s values from guinea pigs in 1 atm air [18]. The results are reported as the percentage change compared with the estimated Q_Σ for the initial model (Model 0). Model 0 uses the constants for a, A, ε, T_{ex} and T_s as described in the Results.

It can be seen that Q_C (Eq. 3) is directly affected by a change in the constant a (Table 5). The surface area available for heat loss affects both Q_C and Q_R. A change in ε has negligible effect on the Q_Σ (Table 5). T_s, however, affects Q_Σ substantially, as indicated by the large percentage changes in Q_Σ for the various estimates of T_s (Table 5).

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