Relationship between T-wave amplitude and oxygen pulse in guinea pigs in hyperbaric helium and hydrogen

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Kayar, Susan R., Erich C. Parker, and Eugenia O. Aukhert. Relationship between T-wave amplitude and oxygen pulse in guinea pigs in hyperbaric helium and hydrogen. J. Appl. Physiol. 85(3): 798–806, 1998.—Diving is known to induce a change in the amplitude of the T wave (A TW) of electrocardiograms, but it is unknown whether this is linked to a change in cardiovascular performance. We analyzed A TW in guinea pigs at 10–60 atm and 25–36°C, breathing 2% O2 in either helium (heliox; n = 10) or hydrogen (hydrox; n = 9) for 1 h at each pressure. Core temperature and electrocardiograms were detected by using implanted radiotelemeters. O2 consumption rate was measured by using gas chromatography. In a previous study (S. R. Kayar and E. C. Parker, J. Appl. Physiol. 82: 988–997, 1997), we analyzed the O2 pulse, i.e., the O2 consumption rate per heart beat, in the same animals. By multivariate regression analysis, we identified variables that were significant to O2 pulse: body surface area, chamber temperature, core temperature, and pressure. In this study, inclusion of A TW made a significantly better model with fewer variables. After normalizing for chamber temperature and pressure, the O2 pulse increased with increasing A TW in heliox (P = 0.001) but with decreasing A TW in hydrox (P < 0.001). Thus A TW is associated with the differences in O2 pulse for animals breathing heliox vs. hydrox.

diving; gas density; electrocardiogram; heart rate; high pressure; neurological syndrome; metabolic rate; telemetry

The amplitude of the T wave (A TW), which represents the repolarization phase of the electrical activity of the heart, has been shown to change during diving in many studies (20–22, 26, 30). Tall, peaked T waves have been observed in native-diving animals (20, 21), in breathing, non-native-diving animals (30), and in freely breathing animals in dry hyperbaria (22). The reason for this change has not been satisfactorily explained, nor has it been determined whether this change in A TW is associated with a change in cardiovascular performance (22, 26). We studied the electrocardiograms (ECG) of guinea pigs breathing mixtures of helium (He) or hydrogen (H2) and oxygen (O2) (heliox and hydrox, respectively) in a hyperbaric chamber, as well as the O2 consumption rate (VO2) of these animals, to determine whether there was a correlation between A TW and whole-animal VO2 relative to heart rate.

Electrical conduction in the heart occurs in a complex manner in three physical dimensions and over time; only a small part of all the depolarization and repolarization events is recorded in the ECG waveform of a single lead placement (29). Thus cardiologists are wary of overinterpreting ECGs (5). Nevertheless, useful information can be gathered from ECGs by associating characteristic changes in waveform with changes in the cardiovascular health status of the subject (5). The tall, peaked T waves previously reported in diving subjects are not convincingly associated with any particular pathology, because they usually occur without any other obvious ECG anomalies or overt signs of cardiovascular distress, such as ischemia or electrolyte imbalance (6, 22, 26). In recent years, there has been a growing interest in studying subtle changes in electrical activity of the heart, including variation in beat-to-beat time intervals (3, 11) and in A TW (12, 22, 26). These subtle changes do not necessarily reflect a pathological condition, but they are of interest if they can be linked to physiological events. Such a linkage has been shown, for example, for a change in the relative sympathetic vs. parasympathetic nervous system influence (11, 12) and the influence of the renin-angiotensin system on heart function (3).

Changes in heart rate are a classic response in diving animals and are believed to reflect a redistribution of blood flow to conserve O2 (17, 21). Bradycardia has been noted in freely breathing human divers compressed in a dry chamber (4, 26, 32), but the magnitude and duration of bradycardia are apparently highly variable (4), and its cause is unknown. Reductions in electrical conduction velocity and excitability by compression of critical sites on membranes associated with myocardial beating frequency have been proposed (14). Cold, exercise, and general stress are additional factors often present in diving that may have a significant effect on heart rate and metabolism. NarcoSis may counteract some of these effects (15, 19). To differentiate among these factors, it is useful to compute heart rate relative to metabolic rate. The total volume of O2 consumed by an animal per heart beat, known as the O2 pulse, is important, because a change in this ratio implies a change in cardiovascular performance (2, 8, 18, 23, 28, 33). This change in performance must in turn be caused by changes in stroke volume or O2 extraction, or a combination of these, relative to a given heart rate.

In a previous study, Kayar and Parker (24) measured O2 pulse in guinea pigs breathing either heliox or hydrox at 10–60 atm and over a chamber temperature range of 26–36°C. The O2 pulse was found to be correlated with body surface area (S), core body temperature (T core), and chamber temperature (T cham) and pressure (P), and these correlations were different for animals in heliox vs. hydrox. This earlier study concluded that, in order to analyze O2 pulse, multiple factors had to be taken into consideration simultaneously. The present study builds on this earlier work. Using the same animals as in the previous study (24), but including A TW as an additional variable, we used stepwise multivariate regression analysis to determine whether A TW also correlates significantly with O2 pulse.

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This correlation would demonstrate that changes in ATw in hyperbaria are predictive of a change in cardiovascular performance, as expressed by O2 pulse.

Guinea pigs were instrumented with radiotelemeters to transmit electrical signals from the heart. The animals were placed in a dry chamber that was pressurized to generate hyperbaric conditions of up to 60 atm (atmospheres absolute pressure, equivalent to a dive to 590 m) of either heliox or hydrox. The VO2 of the animals was measured by gas chromatographic analysis of the chamber gas throughout the dive. Tcham was varied from 25 to 36°C as a means of generating a range of VO2 values and heart rate at each pressure. The radiotelemeters also transmitted body temperature from their implant site in the abdomen.

Measured values of O2 pulse were mathematically modeled as a function of ATw, body S, Tcore, Tcham, and chamber P. Multivariate regression analysis allowed us to clearly demonstrate the relationship between O2 pulse and ATw, by separating the simultaneous effects of the other measured variables.

**MATERIALS AND METHODS**

Animal preparations. Male guinea pigs (Cavia porcellus, Hartley strain; n = 19) were housed in an accredited, professionally staffed animal care facility and had ad libitum access to food and water before experiments.

Radiotelemeters (model TA11 ETA F40-L20; Data Sciences International, St. Paul, MN) were used to sense and transmit Tcore and electrical signals from the heart. With the use of anesthesia, aseptic surgery was performed to implant a telemeter in the peritoneal cavity of each animal, as described in detail previously (24). The telemeter was equipped with two electrodes for sensing the electrical activity of the heart. These electrodes were exteriorized from the peritoneal cavity and were implanted subdermally in the chest. The tip of the negative electrode was near the right shoulder, and the tip of the positive electrode was below the left axilla. This simulated a conventional lead II configuration.

To monitor the output from the telemeter, we placed an animal inside a box that was wrapped externally with two electrical wires to form the antenna for the telemetry system. The telemetry system transmitted a stream of digital radio-frequency signals encoding heart rate (sampled 10 times/min) and Tcore (sampled 250 times/min). These data were averaged and recorded each minute for the duration of an experiment by using a CTR86 receiver, a BCM-100 Consolidation Matrix, and a Dataquest III analysis system (Data Sciences International). Digital signals from the heart encoding ECG waveform (500 samples) were also transmitted by this system and converted to an analog voltage for recording. Waveforms of 2-s duration were recorded every 5 min throughout the dive.

**Dive protocol.** Animals were selected randomly for diving in the He [n = 10, 761 ± 43 (SE) g mean body mass] or H2 [n = 9, 766 ± 29 g] gas mixtures. The animals in the heliox group were identical to those described previously by Kayar and Parker (24). Seven of the nine animals in the hydrox group were included in the earlier study (24). However, in three of the ten animals in the hydrox group from the original study, the ECG was not sufficiently clear to measure T waves. Two new hydrox animals with clearer ECGs were added to the present study. The new animals were in the same weight range and exposed to temperatures similar to the animals they replaced.

For each dive, an animal was placed inside the antenna box, which was set inside a hyperbaric chamber (Bethlehem, Bethlehem, PA). The box was continuously ventilated with chamber gas. Temperature inside the box was monitored and regulated to within 1.5°C.

For the animals to be dived while they breathed the H2 gas mixture, the chamber was pressurized at 1–2 atm/min with pure He to 10 atm (absolute pressure used throughout). The O2 concentration thus fell to 2%, but the PO2 remained near 0.2 atm from the 1 atm air initially enclosed in the chamber. This initial pressurization with He was needed to dilute the O2 in the chamber to avoid an explosive gas mixture when introducing H2: nonexplosivity limits for mixtures of H2 and O2 are 0–4% O2 in H2 and 0–4% H2 in O2 (10). The chamber was then flushed with a mixture of 2% O2 in H2 (hydrox) until the N2 content of the chamber gas dropped to <0.5% and the He content was <4%, as measured by a gas chromatograph (Shimadzu GC-9A, Columbia, MD). The animal was maintained at 10 atm in hydrox (0.2 atm PO2) for ~1 h at a selected temperature between 25 and 36°C. A constant stream of gas flowed through the animal's box to the gas chromatograph. The VO2 of the animal was computed from the gas flow rate and the O2 content difference between the chamber gas and the gas stream leaving the animal’s box, as described in detail elsewhere (24). The VO2 was computed during the second half of the hour, when three consecutive concurrent O2 content readings appeared to be stable. Water vapor was removed from the gas stream before analysis, but CO2 was not removed or corrected for. The precision of the VO2 measurements was estimated to be ±2% (24).

The chamber was subsequently pressurized with hydrox at 1–2 atm/min to 20, 40, and 60 atm. Because the hydrox always contained 2% O2, PO2 increased throughout the experiments to 0.4, 0.8, and 1.2 atm at 20, 40, and 60 atm chamber pressure, respectively. Each pressure was maintained for ~1 h, with VO2 measured during the second half of the hour. A different temperature between 25 and 36°C was randomly selected for each pressure. At the higher pressures, the lower end of the temperature range had to be limited to prevent the animals from becoming severely hypothermic at 60 atm, 30°C was the coldest chamber temperature sampled. Tcore was reported as the final value measured at the end of the hour at each pressure; this value was known with a precision of ±0.1°C.

Mean heart rate values were also computed near the end of the hour at each pressure. The heart rates reported here were from 10 to 20 recorded values, each representing the mean heart rate for 1 min, with SE from individual animals of 1–5% of the mean values. O2 pulse was computed as VO2 divided by heart rate for each animal at each pressure and temperature combination tested. Precision was set primarily by the heart rate (SE within 5% of the mean value).

One ECG waveform of 2-s duration at each pressure was selected for analysis. This ECG was chosen to be relatively free of motion artifact and electrical interference, but randomly with respect to the length of time spent at that pressure. Within the 2 s of the ECG waveform, there were typically 5–10 clear T waves; these T waves were uniform in height (~2 mm, roughly 20 µV). All ATw values were measured by two scorers, neither of whom knew the corresponding O2 pulse. If the two scorers reported values from one animal that deviated from each other by >10 µV, another ECG from that depth was chosen and rescorded. Because the mean ATw for the study was 110 µV, this places our precision at ~9% (10/110).

The dive profile for animals in the H2 gas mixture (2% O2 in He, heliox) was identical to that used for the animals in...
RESULTS

The $A_{Tw}$ values for all animals appear in Fig. 1. While the animals were breathing 1 atm air before the start of the experiments, 12 of the animals had T waves that were positive, and the remaining 7 animals had T waves that were negative. In one animal in the heliox group, the polarity of its T wave reversed between 1 atm air and 10 atm heliox (Fig. 1). In one animal (animal 19) in the hydrox group, the T wave reversed from a moderately sized positive value at 40 atm to a negative value at 60 atm that was >10 SD from the mean value of all other $A_{Tw}$ values in the study. Data from animal 19 at 60 atm were consequently deleted from all further analyses but will be discussed in greater detail later. The $A_{Tw}$ values from animal 19 at 10, 20, and 40 atm were within 3 SD of the mean of all $A_{Tw}$ values, and, therefore, they were not discarded. However, all statistical tests were performed with and without the data from this animal at 10–40 atm as a test of the influence of these data points; including these data did not change any conclusions.

The polarity of the T wave in each animal included in the study remained the same from 10 to 60 atm. To rectify the polarity differences among animals, we used the absolute value of $A_{Tw}$ measurements in all subsequent calculations. All P waves and R waves (depolarization of the atria and ventricles, respectively) were positive.

Mean $A_{Tw}$ (absolute value) in the animals in the heliox group was the same as that of animals in the hydrox group measured in 1 atm air before the start of the experiments (93 ± 15 and 93 ± 19 µV, respectively). The $A_{Tw}$ did not change significantly with increasing pressure for animals breathing either heliox ($P = 0.21$) or hydrox ($P = 0.34$; Table 1). In heliox, the mean $A_{Tw}$ increased with increasing pressure, but the between-animal variability was too great for this trend to be statistically significant (Fig. 1; Table 1). The nonsignificant trend for animals in hydrox was for $A_{Tw}$ to decrease with increasing pressure (Fig. 1; Table 1). Some sample ECGs from animals in the two gas mixtures at 10 and 60 atm are given in Fig. 2.

To examine the influence of the chosen variables on the relationship between O2 pulse and $A_{Tw}$, and to consider the differences in this relationship between heliox and hydrox. Initial pressurization to 10 atm was with pure He. The chamber was then flushed with heliox until the N2 content fell below 0.5%. Animals spent ~1 h at 10, 20, 40, and 60 atm in heliox (0.2, 0.4, 0.8, and 1.2 atm PO2, respectively). All measurements of physiological variables were as described for animals in hydrox.

At the end of all experiments, animals were rapidly decompressed within 1–2 min to 10 atm and then killed by adding 1.5 atm CO2 to the chamber (24).

O2-pulse data are missing from one animal in heliox at 60 atm (weak telemeter signal; no heart rate) and from one animal in hydrox at 60 atm (gas shortage prevented completion of the dive profile; no Vo2).

Statistical analysis. Data were analyzed by stepwise least squares regression (9). Regressions were compared with each other by F-tests, with significant difference assigned at the $P = 0.05$ level. For consistency with our previous study (24), all data from each gas mixture were analyzed separately to generate a multivariable model of O2 pulse, using $A_{Tw}$, chamber P, T core, and body S as the variables. For least squares regressions of the model, these terms were analyzed sequentially, starting from only the y-intercept term and adding each successive variable in order of greatest statistical significance (forward step). After the addition of each variable, an F-test was performed to determine whether there had been a significant improvement. To test the robustness of the fit, we then started with all variables and eliminated any that were not significant (backward step). There was no difference in the best-fit parameter estimates in the forward vs. backward step analyses. The functions fitted in the analysis were of the form $y = a + bx$.

More complex terms in x, including squared terms and cross terms, were tested as described previously (24) and were not found to contribute significantly to the analysis, as confirmed by an examination of residuals (9). Accounting for variability between individual animals was also tested, as described previously (24), and was found not to change the results of the analysis.

The animals in heliox in the present study were identical to those described in the earlier study (24), but three hydrox animals from the earlier study were deleted from the present study and two other animals substituted to obtain clearer ECG waveforms. We confirmed that these substitutions of animals did not alter the results of the previous hydrox model analysis. Using Eq. 1 of the previous study on the data obtained with the new hydrox group of this study, we obtained parameter fits that were not significantly different from those reported previously (t-tests of each pair of parameters, $P > 0.50$). Omitting the new hydrox animals from the current study was also tested and found not to alter any conclusions.
animals in heliox vs. hydrox, we constructed a multivariate regression model. As in the previous study (24), we reasoned that the heat drain of a small animal in hyperbaria should have a major impact on metabolism, heart rate, and potentially on O₂ pulse. The most complex model with statistically significant parameters that we identified was the following

\[
\dot{V}_O_2 = \beta_0 + \beta_{\text{surf}} \cdot S + \beta_{\text{cham}} \cdot T_{\text{cham}} + \beta_{\text{core}} \cdot T_{\text{core}} + \beta_p \cdot P + \beta_{A_{T_W}} \cdot A_{T_W}
\]

(1)

where S (in m²) is estimated body surface area of an animal (computed from body mass \(M_b\), in g) as \(9 \times 10^{-4} M_b^{2/3}\) (16), \(T_{\text{cham}}\) is chamber temperature (°C) measured inside the animal’s box, \(T_{\text{core}}\) is the temperature (°C) registered by the telemeter in the peritoneal cavity, \(P\) is chamber pressure (in atm), and \(A_{T_W}\) is the absolute value of the amplitude of the T wave (in µV). The \(\beta\)-parameters were estimated by fitting Eq. 1 to data from animals in the two gases separately (Table 2).

For animals breathing heliox, O₂ pulse increased with decreasing \(T_{\text{cham}}\), decreasing \(P\), and increasing \(A_{T_W}\) \([P < 0.0001; R^2 = 0.70; \text{residual sum of squared errors (RSSE) = 0.398 \times 10^{-8}}; \text{Table 2}\)\). Neither \(T_{\text{core}}\) nor body \(S\) were significant variables \((P = 0.39\) and 0.17, respectively; Table 2). For animals in hydrox, O₂ pulse increased with decreasing \(T_{\text{cham}}\) and decreasing \(A_{T_W}\) \((P < 0.0001; R^2 = 0.57; \text{RSSE = 0.228 \times 10^{-8}}; \text{Table 2}\)\). There was also a trend for O₂ pulse to increase with decreasing pressure \((P = 0.06)\) in hydrox, but \(T_{\text{core}}\) \((P = 0.16)\) and body \(S\) \((P = 0.14)\) were not significant variables (Table 2).

We tested whether two separate parameter estimates of \(\beta_{A_{T_W}}\) in heliox and hydrox were justified within the model, in comparison to a single parameter estimate for \(\beta_{A_{T_W}}\) in both gases. Separate values provided a significantly better fit to the data \([F(1,66) = 27.15; P < 0.0001]\). When data in heliox and hydrox were combined, no significant value \((P = 0.55)\) for a parameter estimate for \(\beta_{A_{T_W}}\) was obtained. We also tested whether the data were better fit by a model with separate \(\beta_{A_{T_W}}\) parameters for each of the 19 animals, to account for differences among individuals. Inclusion of 19 additional parameter estimates was not justified \([F(17,49) = 1.63; P > 0.09]\). Of the ten individual \(\beta_{A_{T_W}}\) parameter estimates computed for heliox animals, nine were of positive slope. Of the nine individual \(\beta_{A_{T_W}}\) parameter estimates computed for hydrox animals, eight were of negative slope. Thus the correlations described in Table 2

### Table 1. Amplitude of T wave (absolute value) of the electrocardiograms of guinea pigs breathing either heliox or hydrox at various pressures and a range of temperatures from 26 to 36°C at each pressure

<table>
<thead>
<tr>
<th>Pressure, atm</th>
<th>Breathing Heliox ((n = 10))</th>
<th>Breathing Hydrox ((n = 9))</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>98 ± 14</td>
<td>116 ± 24</td>
</tr>
<tr>
<td>20</td>
<td>115 ± 22</td>
<td>92 ± 18</td>
</tr>
<tr>
<td>40</td>
<td>125 ± 29</td>
<td>86 ± 9</td>
</tr>
<tr>
<td>60</td>
<td>140 ± 27</td>
<td>90 ± 16</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), no. of guinea pigs. Heliox, 2% O₂ in He; Hydrox, 2% O₂ in H₂.

Fig. 2. Sample electrocardiograms (lead II configuration) from 2 selected guinea pigs used in the present study. A and B: animal breathing heliox at 10 and 60 atm, respectively; C and D: animal breathing hydrox at 10 and 60 atm, respectively.
between O₂ pulse and A Tw are supported by data from individual animals as well as by a fit to all animals in each gas mixture.

**DISCUSSION**

The volume of O₂ consumed per heart beat was found to correlate with A Tw in guinea pigs in a hyperbaric chamber at various ambient temperatures and pressures and in two different gas mixtures (Table 2). This suggests that a change in A Tw is not merely an artifact of diving (26) but is related to physiological events associated with cardiovascular function. The correlation between O₂ pulse and A Tw was positive for animals breathing heliox but negative for animals breathing hydrox (Table 2). That is, taller T waves were correlated with higher O₂ pulse in heliox and with lower O₂ pulse in hydrox. This indicates that repolarization events in the heart are affected by the choice of breathing gas mixture, in a manner that ultimately is connected to whole body V O₂ relative to heart contraction rate.

The most accurate way to present the relationships between O₂ pulse and A Tw is as shown in Table 2; i.e., as multidimensional relationships, explicitly listing the other variables that have been shown to be significantly correlated, and their numerical values in the regression. There is also a way to illustrate some of the information in Table 2 graphically. We plotted O₂ pulse vs. A Tw for animals in each gas mix and separated by pressure (Fig. 3). At each of the four pressures sampled, there was a positive correlation between these variables in heliox but a negative correlation in hydrox, just as shown by the multidimensional analysis (Table 2). Note that the slopes of these regressions decrease systematically with increasing pressure in heliox, whereas there is no consistent trend between slope and pressure in hydrox. This is illustrative of the interactions of pressure and O₂ pulse shown in Table 2. With the use of data collected at all temperatures and pressures together, the positive correlation between O₂ pulse and A Tw was significant in heliox (P = 0.02), and the negative correlation between these variables was significant in hydrox (P < 0.001), even when the other significant variables in the model were not included.

We also measured the amplitude of the R wave (A Rw) in these animals (data not shown) and found that A Rw did not fit into the model as a substitute for A Tw for animals in either heliox (P = 0.41) or hydrox (P = 0.47). This indicates that changes in electrical depolarization events in the heart, as illustrated by lead II configuration A Rw, are not associated with changes in O₂ pulse in a manner analogous to lead II configuration A Tw changes under the conditions of these experiments. That is, the phenomena we are observing (Table 2) are specific to electrical repolarization events as viewed from this orientation of electrodes.

Our model can now be used to predict differences in guinea pigs under the conditions of our experiments. Consider a guinea pig at a selected temperature and pressure in each of these two gas mixtures. From the raw data, we find that 34°C is a Tcham that will allow a guinea pig in both heliox and hydrox at 60 atm to maintain a stable Tcore near its normal value of 38.8°C. At 34°C and 60 atm, the mean A Tw of two guinea pigs measured in heliox (80 and 250 µV) was 165 µV, and the A Tw in one guinea pig in hydrox was 80 µV. From the parameters in Table 2, we compute the O₂ pulse for animals at this temperature, pressure, and respective A Tw values to be 64.8 and 76.8 ml O₂·g⁻¹·beat⁻¹ in heliox and hydrox, respectively. Thus we predict that normothermic animals breathing hydrox at 60 atm have an O₂ pulse ~20% higher than those breathing heliox [(76.8 – 64.8)/64.8]. Because A Tw is predictive of O₂ pulse (Table 2), some of the difference in O₂ delivery may lie in the heart itself, i.e., may reflect a subtle difference in stroke volume between animals in heliox vs. hydrox at 60 atm.

The O₂-pulse-model analysis we performed previously (24) was identical to the present analysis minus the A Tw term (Eq. 1). In that earlier analysis, we demonstrated that there were significant differences in O₂ pulse for animals breathing heliox vs. hydrox, with these differences attributable to heat loss and pressure effects. In heliox, O₂ pulse increased with decreasing body S (b surf = −0.52 × 10⁻³ ± 0.22 × 10⁻³), decreasing Tcham (b cham = −0.23 × 10⁻⁵ ± 0.84 × 10⁻⁶), decreasing Tcore (b core = −0.46 × 10⁻⁵ ± 0.17 × 10⁻⁵), and decreasing P (b p = −0.42 × 10⁻⁶ ± 0.12 × 10⁻⁶). In

Table 2. Estimated values for parameters of Eq. 1, standard errors, and level of significance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Helox</th>
<th>Standard error</th>
<th>P</th>
<th>Hydrox</th>
<th>Standard error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>b₀</td>
<td>0.189×10⁻³</td>
<td>0.19×10⁻⁴</td>
<td>&lt;0.001</td>
<td>0.175×10⁻³</td>
<td>0.19×10⁻⁴</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>b_cham</td>
<td>−0.382×10⁻³</td>
<td>0.21×10⁻²</td>
<td>0.17</td>
<td>−0.357×10⁻³</td>
<td>0.31×10⁻³</td>
<td>0.13</td>
</tr>
<tr>
<td>b_p</td>
<td>−0.262×10⁻⁵</td>
<td>0.19×10⁻⁵</td>
<td>0.39</td>
<td>−0.193×10⁻⁵</td>
<td>0.14×10⁻⁵</td>
<td>0.16</td>
</tr>
<tr>
<td>A_rw</td>
<td>0.851×10⁻⁷</td>
<td>0.24×10⁻⁷</td>
<td>0.001</td>
<td>−0.123×10⁻⁶</td>
<td>0.29×10⁻⁷</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

b₀, constant; b_cham, parameter for chamber temperature; b_p, parameter for pressure; b_rw, parameter for T-wave amplitude; b_surf, parameter for body surface area; b_core, parameter for core body temperature. *Significantly different in heliox vs. hydrox, P < 0.01.
hydrox, $O_2$ pulse increased with decreasing body $S$ ($\beta_{\text{surf}} = -0.68 \times 10^{-3} \pm 0.27 \times 10^{-3}$) and decreasing $T_{\text{cham}}$ ($\beta_{\text{cham}} = -0.31 \times 10^{-5} \pm 0.56 \times 10^{-6}$), with parameter values for these two variables not significantly different from those of heliox animals. Neither $T_{\text{core}}$ nor pressure was a significant variable in hydrox, using the analysis for which we had the strongest statistical support (Eq. 1, Ref. 24). We speculated that animals in heliox were experiencing a decline in cardiovascular performance with increasing pressure, as a symptom of high pressure neurological syndrome (HPNS), whereas animals in hydrox were not experiencing this decline because of narcotic suppression of HPNS by $H_2$.

Including $A_{\text{Tw}}$ in Eq. 1 provided a significantly better fit to the data, as assessed by smaller RSSE of the regressions (Eq. 1, Ref. 24; 2% smaller RSSE in heliox with 1 less parameter, and 17% smaller RSSE in hydrox with the same number of parameters). In addition, more of the variance in $O_2$ pulse was explained for animals in each gas mixture when changes in $O_2$ pulse were correlated with changes in $A_{\text{Tw}}$ in addition to the other variables considered. The regression $R^2$ was closer to 1 for the new model with $A_{\text{Tw}}$ ($R^2 = 0.701$ in heliox and 0.570 in hydrox) compared with the old model ($R^2 = 0.693$ in heliox and 0.480 in hydrox).

Including $A_{\text{Tw}}$ in the model allowed a trend for a negative effect of pressure on $O_2$ pulse to be apparent for animals in hydrox (Table 2). The magnitude of this pressure effect was less than that for animals in heliox ($\beta_p = -0.181 \times 10^{-6}$ vs. $-0.303 \times 10^{-6}$, respectively; Table 2). In our earlier study (24), we found some statistical support for a second $O_2$-pulse model for animals in hydrox. In this model, $O_2$ pulse increased with increasing pressure at 10–40 atm but decreased with increasing pressure at 40–60 atm. In the present study, in which we substituted two new animals in hydrox, this alternative “pressure maximum” model was no longer found to have statistical support ($P = 0.08$). However, it remains possible that there are more complex terms involving pressure that explain more of the variance in $O_2$ pulse or in $A_{\text{Tw}}$ than we have found thus far.

In heliox, the addition of $A_{\text{Tw}}$ made both $T_{\text{core}}$ and $S$ insignificant as variables, and in hydrox, the addition of $A_{\text{Tw}}$ made $S$ insignificant (Table 2). Body $S$ was included as a model variable because we wanted to normalize for differences in body size among animals by using a variable that could be related logically to the heat-exchange $S$ of an animal. Because $A_{\text{Tw}}$ substitutes for $T_{\text{core}}$ and/or $S$ in this model, this suggests that there is a connection between changes in $A_{\text{Tw}}$ and temperature effects. There was an animal in hydrox (animal 19; see Fig. 1) with a $T$ wave that changed both polarity and amplitude dramatically between 40 and 60 atm, to the extent that the $A_{\text{Tw}}$ for this animal at 60 atm (Fig. 4) was discarded to avoid bias in the analysis. A depression of the S-T segment was also noted in this waveform (Fig. 4). At 60 atm, this animal had an unusually low $T_{\text{core}}$ of 34.1°C. Hypothermia is known to be associated with a variety of changes in ECG waveforms and with inconsistent changes in $A_{\text{Tw}}$ (7). We speculate that more
Dramatic changes in T wave, either in amplitude or in polarity, might have been found if animals had been permitted to become more severely hypothermic.

Thus the interconnections between $A_{Tw}$ and $O_2$ pulse are likely to be multifaceted, with temperature and pressure exerting pivotal influences. Although our mathematical approach is appropriate for identifying correlated variables and calculating relative values, it cannot explain the underlying physiological mechanisms that cause the interconnections. The roles of such critical factors as myocardial contractility, stroke volume, arteriovenous $O_2$ extraction, and blood flow distribution are unknown. Further experimentation is needed to determine causality, now that it is clear that changes in $A_{Tw}$ during diving are indeed likely to have a physiological significance to cardiovascular function. We can, however, speculate that changes within the heart, such as myocardial contractility or stroke volume, are likely, given that a change in an electrical component of the cardiac cycle is predictive of a change in $O_2$ pulse.

Gennser and Örnhagen (15) demonstrated that the beating frequency of isolated atria of rats declined in response to environmental pressure and that beating frequency increased after superfusion with solutions containing $H_2$. Superfusing with $He$ caused only a slight increase in beating frequency (15). This partial reversal of bradycardia by $H_2$ was attributed to its narcotic properties, which in turn are generally attributed to the relative solubility of $H_2$ in the lipid component of nerve tissues (15). $H_2$ is more soluble in lipids than is $He$, and one of the perceived benefits of diving with $H_2$ is the observed reduction in symptoms of HPNS (1, 13, 24). This suggests that anatomic differences between human and guinea pig hearts, or minor variation in electrode placements among individuals, may affect the amplitude or polarity of the T wave under any circumstances. In the present experiment, the deliberate inclusion of cold as well as two different gas mixtures appears to have added further confounding influences on T-wave appearance.

Joulia et al. (22) reported that changes in $A_{Tw}$ were dependent on the density of the breathing mixture, but they found no specific effects that were attributable to whether the inert gas portion of the breathing mixture contained $He$, $H_2$, or $N_2$. This is in contrast with the finding in the present study that the relationship between $A_{Tw}$ and $O_2$ pulse was dependent on whether the breathing mixture was heliox or hydrox (Table 2; Fig. 3).

The guinea pigs in the present study were exposed to more stresses than hyperbaria, cold, and differing inert gas mixtures that may have had additional effects on the heart. Increasing $O_2$ from 0.2 to 1.2 atm with increasing chamber pressure may also have led to an alteration in electrical conduction. Hyperoxia is known to affect cardiac output and blood flow distribution to the heart and many other tissues (19). Changes in T-wave configuration have been seen in human divers as $P_{O_2}$ changed from 0.2 to 0.3 atm (34). Because we used a constant 2% $O_2$ in our exposures, dependence of $O_2$ pulse on $P_{O_2}$ would be 100% correlated with ambient
pressure \(P\) in Eq. 1) and thus would not be distinguishable from pressure effects. The work of Hordnes and Tyssebotn (19) showed that both heart rate and cardiac output are reduced under the combined effects of high \(P_O_2\) and high ambient total pressure. This makes it difficult to speculate on how \(O_2\) pulse should be affected by hyperbaric hyperoxia.

We have no evidence that the changes we measured in \(A_{Tw}\) are an indication of myocardial pathology in diving. Changes in \(A_{Tw}\) in two or more leads are considered to reflect nonspecific changes, primarily in oxygensecretion or electrolyte balance of the endocardium of the left ventricle (6). These changes are of clinical concern only when they are coupled with a number of other ECG abnormalities, such as S-T segment depression, elevation, or prolongation (5, 27). We did not systematically measure these other features of the ECG waveforms, but such changes were not overtly present in the animals included in our analysis (Fig. 2; also note the S-T segment depression in the excluded animal 19 in 60 atm hydrox, Fig. 4). Similarly, the increases in \(A_{Tw}\) reported by Joulia et al. (22) were not regarded by them as consistent with a pathological state. We have some measurements of heart rate (25) and \(V\dot{O}_2\) (24) taken from normothermic guinea pigs breathing 1 atm air, although these two variables were not measured in the same individuals. From these data, we compute that \(O_2\) pulse in a resting 750-g guinea pig at 25°C breathing 1 atm air is on the order of 50 ml \(O_2\) \(g^{-1}\) \(beat^{-1}\) \((V\dot{O}_2 = 0.73 ml O_2 \cdot g^{-1} \cdot h^{-1} \cdot f_H = 243 beats/min). All of the \(O_2\)-pulse values in this study were higher than this 1-atm air estimate (Fig. 3), suggesting that cardiovascular function, as assessed by whole body \(V\dot{O}_2\) per heart beat, is not impaired by breathing either \(He\) or \(H_2\) gas mixtures in hyperbaria, despite the associated changes in \(A_{Tw}\).

We conclude that there is a difference in the amount of \(O_2\) consumed per heart beat in guinea pigs in a hyperbaric \(H_2\) vs. \(He\) environment and that \(A_{Tw}\) and ambient temperature and pressure are associated with this difference. Taller \(T\) waves were correlated with higher \(O_2\) pulse for animals breathing helium, but shorter \(T\) waves were correlated with higher \(O_2\) pulse for animals in hydrox. Inclusion of \(A_{Tw}\) in our model explained more of the observed variance in \(O_2\) pulse than did inclusion of \(T_{core}\) and body \(S\) for animals in helium or body \(S\) alone for animals in hydrox. This suggests that body heat loss is involved in changing \(A_{Tw}\). These results demonstrate that the changes in \(A_{Tw}\) found in these experiments in hyperbaric \(He\) and \(H_2\) are correlated with events that are significant to cardiovascular function. The underlying physiological mechanisms for these correlations remain to be elucidated.

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