Gender differences in human skeletal muscle and subcutaneous tissue gases under ambient and hyperbaric oxygen conditions.

G. B. HART¹, M.B. STRAUSS²

Submitted 6/30/05; accepted 12/1/06

¹Medical Director (Emeritus), Department of Hyperbaric Medicine, Long Beach Memorial Medical Center, Long Beach, CA 90801-1428; Assistant Clinical Professor of Surgery, University of California Irvine, Irvine, CA 92697; ²Medical Director Department of Hyperbaric Medicine, Long Beach Memorial Medical Center, Long Beach, CA 90801-1428, Clinical Professor, Department of Orthopaedics, University of California Irvine, Irvine, CA 92697

Hart GB, Strauss MB. Gender differences in human skeletal muscle and subcutaneous tissue gases under ambient and hyperbaric oxygen conditions. Undersea Hyperb Med 2007;34(3):147-161. Objectives: The purpose of this study is to ascertain if gender differences exist in human skeletal muscle (MM) and subcutaneous (SC) tissue gases using monoplace and multiplace hyperbaric oxygen (HBO₂) treatment protocols. Methods: Gas tensions in resting MM and SQ tissues were recorded at 4-minute intervals using two protocols: The 150 minute monoplace HBO₂ chamber protocol utilized continuous oxygen (O₂) breathing at 202.6 kPa, that is 2 atmospheres absolute (2 ATA). The multiplace HBO₂ chamber protocol had four 5-minute air breaks between five 20 minute O₂ breathing periods at 2 ATA and took 180 minutes to complete. Tissue gas samples were obtained by the vacuum technique through a low permeable Teflon membrane and analyzed using a mass spectrometer. Results: Over 40,000 individual step analyses showed gas tensions changed (repeated measures of variance, p=0.00001) with time as the gas pressures and mixtures breathed were altered. Statistically significant differences between males and females in loading and unloading of SC nitrogen (N₂) (P=0.0001), SC O₂ (P=0.001) and MM O₂ (P=0.003) were observed in the multiplace protocol. Females release SC N₂ more slowly; while increasing their MM and SC O₂ tensions higher than males. Muscle and SC CO₂ levels decrease in both males and females when exposed to HBO₂ and increase when breathing air. Conclusions: Three main gender differences are observed in tissue gas loading and unloading under hyperbaric oxygen exposures: Females release SC N₂ more slowly and saturate MM O₂ and SC O₂ to greater extents. Finally, female MM and SC O₂ rose to higher levels in the multiplace protocol than in the monoplace protocol, which was not observed in the male subjects. This information may help explain why males and females respond differently to diving decompression stresses and the clinical application of HBO₂.

INTRODUCTION

In a previous study of human tissue gas measurements (1), differences were observed between continuous (monoplace chamber protocol) and intermittent multiplace HBO₂ chamber protocol with air breaks hyperbaric oxygen (HBO₂) exposures. In the monoplace protocol muscle (MM) nitrogen (N₂) tensions decreased 9% more than with the multiplace protocol over a shorter (11%) period of time. However, the zenith O₂ tension was 9% higher in the multiplace protocol than with the monoplace protocol at two atmospheres absolute (ATA). This may reflect the longer HBO₂ exposure time with the multiplace chamber protocol. Air breaks in the multiplace chamber protocol were found to be a source of rapid N₂ deposition in
the tissues. This may be a partial explanation why patients with mixed arterial gas embolism and decompression sickness diagnoses have been observed to deteriorate using diving tables with air breaks (2,3). Males may be more sensitive to the central nervous system effects of oxygen toxicity than females. This was observed in mice (4) and later it was reported that seizures during HBO2 treatments were four times higher in males than females (5). An explanation for this may be that there are differences in O2, N2 and carbon dioxide (CO2) rates of loading and off factorial gassing and tissue gas tensions between males and females. From our multi-faceted study of tissue gas dynamics with hyperbaric oxygen exposures we observed that the cohort group of males and females had measurable differences. This report describes the techniques we used for the study and differences between males and females in tissue gas loading and off-loading.

METHODS

Justification for the Study and Subject Procurement and Supervision
The present study was designed to ascertain whether or not muscle and subcutaneous gas loading and off-loading measured by mass spectrometry were different between males and females when using two different hyperbaric oxygen treatment protocols. Previous studies of tissue gases using our mass spectrometer have confirmed that this instrument is highly accurate for the intended purpose of measuring loading and off-loading of tissue gases in human subjects (6,7,8).

After Institutional Review Board (IRB) approval of the study, participants where asked to volunteer through announcements in hospital publications and by word of mouth. All subjects were informed of the risks and objectives of the study and especially those associated with probe insertion into the subcutaneous and muscle tissues of the thigh, in accordance with our IRB standards for the use of human subjects and the Helsinki Accords (Figure 1). The study was not designed to randomize, blind, or cross over the subjects. The senior author supervised the probe insertions and hyperbaric treatment protocol of each subject. The multiplace treatment protocol required gas breathing through a SCUBA diving regulator.

Subject Characteristics and Participation
Thirty-six healthy volunteers participated in the study. To achieve statistical significance we ascertained that a minimum of ten subjects of each gender would need to complete the study. The physical characteristics of the subjects are summarized in Table 1. All 36 subjects participated in the monoplace arm of the study. Three males did not return to participate in the multiplace arm of the study for personal reasons. A minimum 30 day interval occurred for each subject between the multiplace and monoplace protocol arms of the study.
Tissue Gas Analyses

Tissue gas analyses were done with a Perkin-Elmer 1100 Mass Spectrometer® Perkin-Elmer Inc, Foster City, California. Four fixed detectors recorded argon (Ar), CO₂, N₂, and O₂ gas tensions in mmHg surrounding the instrument’s two sampling catheters. Separate probes measured gases in the SQ space and in the MM compartment. Measurements were made at two-minute intervals alternating the compartments. Each measurement represents an individual step analysis (ISA). Thus gas tensions for each sampling site were obtained every 4 minutes for the SC space and every 4 minutes for the MM compartment. Tissue gases were obtained by continuous vacuum evacuation of minute gas samples from indwelling tubular catheters fitted with low permeability Teflon membranes (MEDSPEC Catheters, Allied Health Care Products, St. Louis, Missouri.). The 45.72 centimeters (18 inches) long catheters had malleable, tubular stainless steel cores, with an outside diameter of 0.139 centimeter (0.055 inch). The exterior of the catheter was covered with a low permeability Teflon® sheath. The distal tip, approximately one cm in length, had perforations to provide communication with the catheter lumen. Gases entered the catheter lumen by diffusion through the Teflon sheath (the low permeability of the Teflon membrane prevents depletion of the gases at the sampling site). The gases were vacuum evacuated into the mass spectrometer for analysis with a sampling rate of approximately 5 X 10⁻⁶ ml/sec negative pressure (9). Each catheter was connected to the sampling port of the spectrometer with a six-foot malleable stainless steel extension catheter that passed through gas tight fittings in the cephalic bulkhead of the hyperbaric chamber.

Calibration

The mass spectrometer was calibrated with catheters in dry 37°C reference gases prior to probe insertion. Each gas was adjusted to zero while immersed in a reference gas free of Ar, N₂, O₂, or CO₂. The catheters were then exposed to a 37°C, one ATA reference gas as follows: 1) O₂ = 152 mmHg, 2) CO₂ = 38 mmHg, 3) N₂ = 380 mmHg and 4) Ar = 190 mm Hg. The readouts were calibrated to the preceding gas values. Spectrometer stability was confirmed by repeating the testing upon completion of each trial. Dry calibration was used for expedience. Differences between dry and wet calibration are predictable and

---

**Table 1** Anthropomorphic characteristics of subjects

<table>
<thead>
<tr>
<th>PROTOCOL A</th>
<th>MALES</th>
<th>FEMALES</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>15(44)</td>
<td>21(56)</td>
<td>36(100)</td>
</tr>
<tr>
<td>Mean Age/Yrs (+/-)</td>
<td>38.9 Yrs (13)</td>
<td>31 Yrs (6.5)</td>
<td>34.5 Yrs (10.9)</td>
</tr>
<tr>
<td>Mean Height/Centimeters (+/-SD)</td>
<td>177.8 (7.11)</td>
<td>163.83 (5.58)</td>
<td>169.67 (9.45)</td>
</tr>
<tr>
<td>Mean Weight/Kilograms (+/-SD)</td>
<td>83.64 (10.84)</td>
<td>59.56 (8.5)</td>
<td>69.58 (15.46)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PROTOCOL B</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>12(36)</td>
<td>21(64)</td>
<td>33(100)</td>
</tr>
<tr>
<td>Mean Age/Yrs (+/-SD)</td>
<td>41.6 Yrs (13.4)</td>
<td>31Yrs (6.5)</td>
<td>35 Yrs (11.2)</td>
</tr>
<tr>
<td>Mean Height/Centimeters (+/-SD)</td>
<td>178.6 (7.11)</td>
<td>163.83 (5.58)</td>
<td>66.8 (3.8)</td>
</tr>
<tr>
<td>Mean Weight/Kilograms (+/-SD)</td>
<td>83.69 (11.75)</td>
<td>59.56 (8.5)</td>
<td>68.04 (14.96)</td>
</tr>
</tbody>
</table>
are amenable to mathematical correction, (Addressed later in this report.) Consequently, the accuracy of our measurements was +/- 0.05 mm Hg.

**Catheter Insertion**

The catheters were inserted into skeletal MM and adjacent SC tissues of the middle third of the lateral aspect of the thigh. This corresponded to the vastus lateralis MM site and the overlying SC tissue. One half ml of one percent Lidocaine® was used to generate skin wheals to provide local anesthesia. An angiocath, 20 cm (7.874 inches) long, was inserted through the wheal and advanced 12.7 cm (5 inches) distally into the chosen site. The angiocath trochar was removed and discarded. Then a 22 gauge thermistor-tipped probe was introduced into the catheter and positioned so the thermistor was at the tip of the angiocath sheath at the intended gas sampling sites. The angiocath sheath was left undisturbed for 30 minutes to facilitate hemostasis along the track of angiocath insertion and to provide time for thermal equilibration. After temperatures were recorded, the temperature probes were removed. Next, the mass spectrometer catheter was inserted through the sheath and advanced the length of the sheath. The catheter was held in place while the angiocath sheath was withdrawn. The catheter was secured to the extremity with adhesive tape so the gas-sampling surface remained positioned at the deepest point of penetration.

**Sampling Site Temperature Effects**

Mass spectrometer gas measurement systems used in this study were, as expected, sensitive to sampling site temperature. The chamber is flushed with the indicated gas at 21.2° Celsius flowing at 400 liters per minute to prevent warming or cooling of the ambient environment due to the volunteer. The gas temperature remains constant using a heat exchanger in line with the gas supply.

Tissue catheters were calibrated to reflect the sampling site temperatures of each subject at the onset of each trial. In vivo tissue gas tension readings were mathematically corrected for temperature in two fashions: First, corrections were made for measurement errors from catheter calibration at one temperature and tissue recordings made at another. Second, corrections were made for dry and for wet catheter calibrations. Gender differences of the temperature recordings were substantial. Consequently, we used gender-specific temperature corrections (Table 2). Compensations for both errors are incorporated into the correction values. Correction constants are obtained by multiplying the mean temperature difference from 37° by the mass spectrometer reading of the measured vs. calibrated gas ratio.

**Mass Spectrometer Response Delays**

Mass spectrometers using low permeability catheters do not respond instantaneously to changes in gas tensions.

Changes in calibration gases are not fully reflected in the mass spectrometer readouts for six minutes. This delay is a reflection of the

<table>
<thead>
<tr>
<th>Table 2. Correction Constants For Tissue Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Temperature</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>O</strong></td>
</tr>
<tr>
<td><strong>N</strong></td>
</tr>
<tr>
<td><strong>CO</strong></td>
</tr>
<tr>
<td><strong>2</strong></td>
</tr>
</tbody>
</table>
time required for gas diffusion through the diffusion-limiting membranes, transport through the catheter tubing and processing in the mass spectroscopy unit itself.

**PROTOCOLS**

Two protocols were used. The first protocol is one of continuous O₂ breathing over a 90 minute period at two ATA, representative of a clinical monoplace HBO₂ treatment. The second represents a multiplace treatment protocol with a two hour exposure at two ATA utilizing five 20 minute O₂ breathing periods interspersed with four 5 minute 2 ATA air breaks (Table 3).

Subjects in Protocol A and B were pressurized in step two to 2 ATA air for 30 minutes after baseline recordings (step one) were obtained prior to the subjects entering the chamber. In Protocol A the flushing gas was changed to O₂ flowing at 400 liters per minute at a pressure of 2 ATA O₂ during step three. Consequently, the subjects breathed O₂ directly from the chamber atmosphere during the exposure. After four minutes O₂ wash-in the Sechrist 2500 B monoplace chamber, the chambers O₂ concentration at the volunteer’s head (10) is 98 percent. No corrections were attempted for this O₂ loading time or for two minutes delay from muscle to subcutaneous recordings. During step four of Protocol A, the subjects breathed air at 400 liters per minute for 30 minutes at one ATA air. For Protocol B, the O₂ percentage approached 100% almost immediately after beginning to breathe with the regulator as was done in steps 5, 7, 9 and 11. In step 12 of Protocol B, as in step four of Protocol A, the subjects breathed air directly from the chamber atmosphere at a flow rate of 400 liters a minute for a 30 minute period at one ATA while post-HBO₂ exposure measurements were obtained.

**Statistical Assessments**

The F-Test was used for analysis of variance while Student’s paired t-test and the non-parametric Wilcoxon Rank Sum revealed the probability of differences between the variables. Further evaluation used the p values for repeated measures Analysis of Variance. Individual Step Analyses (ISA) distinguished the differences between the SC and MM
RESULTS

The loading of O_2 and off-gassing of N_2 in the subcutaneous tissue and in the muscle are graphically displayed in Figures 2 and 3 for Protocol (monoplace chamber) and Figures 4 and 5 for Protocol B (multiplace chamber). (See pages 154-157 for Figures 2-9). In both Protocols during the O_2 exposure, N_2 off-loads more slowly in the female SC tissues than in the male SC tissues. At the end points of the O_2 HBO_2 exposures, the differences were not statistically significant in Protocol A. Oxygen loading at the end points of the HBO_2 exposures was significantly greater in the females SC (p = 0.03) and MM (p = 0.005) in Protocol B than in the males. During the air breathing periods in Protocol B barely perceptible increases in SC N_2 tensions and decreases in O_2 tensions were observed. None of these changes were statistically significant.

For the MM tissues, the off-gassing of N_2 for both protocols and in both genders was far more dramatic (Figures 3 and 5). The movement of N_2 into and out of the muscle is greater than twice that of the SC compartment. The zenith O_2 tensions varied between the two protocols, but were consistently higher in females than males. The mean differences ranged from a low of 25 mmHg to a high of 151 mmHg in males in Protocol B. In females they ranged from a low of 33 mmHg to a high of 273 mmHg in Protocol B.

During the 5 minute air breaks in Protocol B there were significant decreases in O_2 tensions (F-Test = 0.009, T-Test = 0.001, and Wilcoxon’s Rank Sum = 0.001) as recorded in Table 4. The effects of air breaks resulted in “saw tooth” appearances of the N_2 and O_2 plots for Protocol B (Figures 4 and 5).
During the 30 minute air breathing sequences at two ATA (Step 2 of the protocols) surprising findings were noted. There were significant differences in the female between the monoplace and multiplace protocols in muscle N2 on-gassing (paired t-test = 0.001, Wilcoxon Rank Sum = 0.002) and in muscle O2 loading (paired t-test = 0.013, Wilcoxon Rank Sum = 0.007) as recorded in Table 5. In both protocols air was breathed directly from the monoplace atmosphere, without regulators (which were later used in Protocol B for the O2 breathing periods). Although these observations are surprising (see discussion), it is important to remember that Protocol B was performed at a minimum of 30 days after Protocol A had been completed.

When the data are examined by repeated measures Analysis of Variance (Table 6), the Grouping Variable effect (S) is significant only in Protocol B’s MM O2 (p = 0.026). The “Time Effect” from the changes of pressure and type of inhaled gas (R) is highly significant, (p=0001) for the measured gases in the tissues. Further, Protocol B’s analyses of variance showed significant differences of SC O2, (p = 0.001), SC N2, (p = 0.0001), and MM O2 (p = 0.003).

In both protocols CO2 tensions decreased significantly with each time interval (p=0.0001) during HBO2 exposures (Figures 7 and 8). Three observations are noteworthy: First, during the 30 minute air breathing period at 2 ATA the CO2 tensions increased, as they did during the air breathing periods of Protocol B and at the end of each exposure while breathing air for 30 minutes at 1 ATA. Second, CO2 decreases are almost linear during the HBO2 exposures. Finally, SC CO2 tensions are consistently lower than MM CO2 tensions by a factor of approximately 18 percent.

In Protocol B, females off-gas SC N2 significantly more slowly (P=0.001) while SC O2 and MM O2 load to significantly higher levels (P=0.001 and P=0.003) than in males. The SC CO2 and MM CO2 are graphically displayed in Figure 6 (Protocol A) and Figure 7 (Protocol B). The p-values for repeated measures Analysis of Variance confirm the significant differences seen in Protocol B and are recorded in Table 6. The change of pressure and/or inspired gas with time (R) dynamically changes the concentration of the measured gases in the tissues (p=0.0001) in both protocols.

Statistical analysis of the initial 2 ATA air exposures in both protocols (pooled data), were compared in the males using p1=paired t-Test and p2=Wilcoxon’s Rank Sum and in the females using p3=paired t-Test and p4=Wilcoxon’s Rank Sum. The following measurements were also used to confirm...
differences in tissue partial pressures between the protocols: 1) \( p^5 = \text{F-test for homogeneity of variances} \), 2) \( p^6 = \text{t-Test comparing the male and female groups (The pooled variance estimate is used when } p^5 > 0.05 \text{ and the separate variance} \) when \( p^5 \leq 0.05 \) and 3) \( p^7 = \text{Wilcoxon’s Rank Sum test} \). The female cohort was noted to have statistically significant differences in oxygen and nitrogen loading comparing Protocol B to Protocol A 30 minute 2 ATA air exposure, Table 5, Figures 8 and 9.

Legend for Muscle and Subcutaneous Nitrogen (N\(_2\)) and Oxygen (O\(_2\)):

Figure Key: Figures 2,3,4,5:
- Female N\(_2\) = □
- Male N\(_2\) = ♦
- Female O\(_2\) = ○
- Male O\(_2\) = ▲

Fig. 2

Fig. 3
Fig. 4

Loading of O₂ & Unloading of N₂ in SUBCUTANEOUS TISSUE with Protocol A in Males (N=12) and Females (N=21).

Fig. 5

Loading of O₂ & Unloading of N₂ in MUSCLE TISSUE with Protocol B in Males (12) vs Females (21).
Legend Keys For Muscle and Subcutaneous CO₂:
Figure Key: Figures 6 and 7:
Female Muscle CO₂ = ○
Male Muscle CO₂ = ▲
Female Subcutaneous CO₂ = □
Male Subcutaneous CO₂ = ♦

Fig. 6

SUBCUTANEOUS AND MUSCLE CO₂ Tensions with Protocol A in Males (15) and Females (21)

Fig. 7

SUBCUTANEOUS & MUSCLE CO₂ Tensions with Protocol B in Males (12) Vs Females (21)
Legend Keys For Muscle Nitrogen and Muscle Oxygen:

Figure Key: Figures 8 and 9:
Female Protocol A = ♦
Female Protocol B = ■

Fig. 8

MUSCLE NITROGEN TENSIONS in Females During Air Breathing at Two Atmospheres Absolute. Protocol A Versus Protocol B:

Fig. 9

MUSCLE OXYGEN TENSIONS in Females During Air Breathing at Two Atmospheres Absolute. Protocol A Versus Protocol B:
variances, 2) \( p^6 = t\)-Test comparing the male and female groups (The pooled variance estimate is used when \( p^3 > 0.05 \) and the separate variance when \( p^3 \leq 0.05 \)) and 3) \( p^7 = \) Wilcoxon's Rank Sum test. The female cohort was noted to have statistically significant differences in oxygen and nitrogen loading comparing Protocol B to Protocol A 30 minute ATA air exposure, Table 5, Figures 8 and 9.

**DISCUSSION**

This study is an analysis of the male-female cohort of our comprehensive tissue gas study using a mass spectrometer for two different HBO\(_2\) treatment protocols (1). With 36 subjects, over 26,000 measurements (Individual Step Analyses - ISA) and accuracy, with the mass spectrometer of +/- 0.5 mmHg, for each ISA, we feel that the data we have collected are reliable and results arising from it are valid. The results, themselves, require explanations which we provide in the following paragraphs.

First, the nitrogen (N\(_2\)) off-gassing rates and extents are roughly three times greater for MM tissues than SC tissues. In both situations (rates and extents), the washout curves derived from the individual step analyses are essentially congruent between females and males although at the endpoints of the hyperbaric oxygen (HBO\(_2\)) exposures, the SC N\(_2\) tensions are significantly higher (\( p = 0.0001 \), Interaction of Grouping Variable and Time) in females. These differences are best explained by differences in perfusion (much greater in muscles) between these two tissues and differences in gender.

A clinical correlation from the N\(_2\) observations helps to explain the propensity for overweight divers to incur decompression sickness from compressed gas diving. This is usually attributed to the affinity of fat tissues for N\(_2\). The better explanation, based on our observations, are perfusion rate differences between subcutaneous tissues (which are largely fat) and muscle tissues. A second clinical correlation is seen in the differences between the durations of convalescence periods in patients with necrotizing panniculitis and fasciitis versus clostridia myonecrosis. Once the acute fulminating course is arrested for these conditions, the time for healing is typically two to three times longer for the conditions where necrosis of SC tissues are involved as compared to MM tissue necrosis.

An unexpected observation, however, was noted in the 2 atmosphere absolute (ATA) pressurizations using air before the HBO\(_2\) breathing periods were started. These portions of the study were identical for both protocols. Females had higher N\(_2\) on-gassing and O\(_2\) loading in SC and MM compartments during this step of the protocols than males. Furthermore, the differences were statistical significant between monoplace (Protocol A) and multiplace (Protocol B) studies. We offer the explanation that the initial (monoplace) exposure to HBO\(_2\) in someway altered gas uptake in the females, but did not affect it in males and this effect persisted during the 30 day interval between the two protocols. This suggests that if these compartments are the source of enucleated gas, there may be an increased susceptibility for decompression sickness in females. The literature, however, does not support this supposition since the non-pregnant female has a lower propensity for DCS than the male (11,12,13). Height/weight ratios in this study showed that female subjects had 23 percent less weight per centimeter of height than the male (male = 0.47 kilograms/centimeter versus female=0.3635 kilograms/centimeter). The height/weight distribution of the volunteers was similar to prior gender comparisons in a normal population (14,15). There is a slower rate of N\(_2\) off-gassing from the SC tissues into the circulation in females than males during HBO\(_2\) exposures. This may
be a physiological response protecting the female from developing intravascular bubbles in association with decompression sickness.

A second finding that requires explanation is that females consistently have higher O₂ tensions for each individual step analysis during HBO₂ exposures regardless of the protocol used. By the endpoints of the HBO₂ exposures, the ISA differences between O₂ uptakes for males and females were significant (F-Test p = 0.009, T-Test p = 0.001 and Wilcoxon’s Rank Sum p = 0.001 for protocol B). Furthermore, the calculation by repeated measures of variance for Protocol B as functions of the interaction of grouping variables and time demonstrated differences, not only in the SC tissues (p = 0.001), but also in the MM tissues (p = 0.003). We believe these data are reliable since the MM O₂ tensions we recorded in normal lower limb muscle, at rest, are comparable to those recorded in an animal model (16) which increased by a 6-fold factor from room air to two ATA. This supports our hypothesis that there are gender differences in O₂ uptake during HBO₂ conditions with females taking-up significantly more O₂ than males. The higher MM O₂ in the female during HBO₂ breathing may have prognostic considerations. It may account for improved survivability when HBO₂ is used in acute disease states such as clostridial myonecrosis and carbon monoxide poisoning. However, this effect was only minimally observed in our gas gangrene experience where female survival rates were 82 percent versus 80 percent in males (17). A review of our Type 2 decompression sickness cases and their responses to HBO₂, when started within 10 hours of onset, showed that three (7%) of 42 males patients had minimal or no responses to HBO₂ while 3 (10%) of 31 female patients had similarly poor responses to HBO₂ treatments. Finally our survival and recovery rates for females presenting comatose secondary to carbon monoxide poisoning are better in this gender than for males. These differences for all three of these clinical conditions are, however, not statistically significant.

Surprisingly, the greatest O₂ elevations in all of our measurements where observed in females at the endpoint of the HBO₂ exposures in Protocol B. This was statistically significant in the MM compartment (F-Test = 0.009, T-Test = 0.0001, Wilcoxon’s Rank Sum = 0.001). In Protocol A the O₂ elevations in muscles between males and females were not statistically significant at the endpoint of the HBO₂ exposure. Why these differences in the two protocols occurred is unclear. Perhaps, the air breathing periods open an “oxygen window” to the muscle and subcutaneous tissues. Another explanation is that air breaks increase tissue carbon dioxide tensions which cause a vasodilatation, carrying through each hyperbaric air breathing period in Protocol B. Another question is why CNS O₂ toxicity is not higher in the female than the male? The O₂ toxicity rate in the male has been reported to be four times higher than in the female (5). These observations when considered in the context of clinical experiences do not suggest that the multiplace HBO₂ treatment protocol has advantages over the monoplace protocol for the therapeutic applications of HBO₂ or O₂ toxicity rates are lower in the monoplace chamber than the multiplace chamber.

We are not aware of clinical correlations for these observations, other than those mentioned above for gas gangrene, decompression sickness and carbon monoxide poisoning. To our knowledge, there are no reports of improved limb salvage rates in female as compared to male patients with similar limb-threatening wounds who receive HBO₂ as an adjunct to their management or whether a monoplace or multiplace chamber protocol is used. A possible explanation for this is that wound healing in the problem foot wound is more a function of perfusion to the skin and
SC tissues than to MM tissues where females exhibit the greatest increments in $O_2$ uptake.

The third finding that requires discussion pertains to the changes in carbon dioxide ($CO_2$) associated with the two HBO$_2$ treatment protocols. First, why did $CO_2$ decrease with time while breathing $O_2$ in both protocols? In a previous study this was attributed to hyperventilation secondary to pain and anxiety with insertion of a large bore cannula into SC and MM tissues without anesthesia (8). Since $CO_2$ levels were unchanged for the first 12 minutes of the above reference study, the authors concluded that catheter insertion did not provide an adequate explanation. During pressurization, imperceptible hyperventilation with a secondary respiratory alkalosis has been offered as a reason blood $CO_2$ tensions decrease with hyperbaric exposures. During the $O_2$ exposures at 2 ATA pressures, hyperventilation was not observed, yet $CO_2$ tensions progressively declined in MM and SC tissues. Since $CO_2$ levels were unchanged in our present study, during the first 12 minutes and pain was nearly totally eliminated with catheter insertion using local anesthesia, we conclude that hyperventilation does not provide an adequate explanation. Consequently, we postulate that $CO_2$ changes in MM and SC tissues are entirely accountable from changes in breathing gases. This is further supported by the finding that when breathing air under hyperbaric pressures in Protocol B, the $CO_2$ levels increased and conversely when breathing $O_2$ they declined. Although we are unable to offer any clinical correlations to these $CO_2$ finding, in critical care problems where acidosis is a feature, HBO$_2$ may contribute to re-establishing normal acid-base balances from this effect.

In conclusion, our findings indicate that $N_2$, $O_2$ and $CO_2$ tensions change with pressurization in air and with HBO$_2$ exposures. The changes are different between males and females and between using a monoplace chamber protocol (no air breaks during HBO$_2$) and the multiphase chamber protocol (with air breaks interspersed between HBO$_2$ breathing periods). Our findings are validated by sample size, consistency of methodology (the same HBO$_2$-trained personnel recorded all measurements), adherence to protocols and the principal investigator supervising all studies and probe insertions. Our data warrant further analyses to ascertain whether or not the state of parturition (nulliparous versus parous), smoking, fitness (athlete versus non-athlete) and/or age alter tissue gas deposition and release under HBO$_2$ and air breathing conditions as this reports has shown for genders.

ACKNOWLEDGMENTS

The statistical assessments were performed in the Department of Biostatistics and Epidemiology, University of Massachusetts, Amherst, under the supervision of Stanley Lemeshaw Ph.D.

The protocol design was established with the aid of Charles H. Wells, Ph.D. Physiologist, The Woodlands, Texas

REFERENCES

5. Hart GB, Strauss MB: CNS Oxygen Toxicity in


