Modulation of decompression sickness risk in pigs with caffeine during H₂ biochemical decompression

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Fahlman, Andreas, Winston C. Lin, William B. Whitman, and Susan R. Kayar. Modulation of decompression sickness risk in pigs with caffeine during H₂ biochemical decompression. J Appl Physiol 93: 1583–1589, 2002—In H₂ biochemical decompression, H₂-metabolizing intestinal microbes remove gas stored in tissues of animals breathing hyperbaric H₂, thereby reducing decompression sickness (DCS) risk. We hypothesized that increasing intestinal perfusion in pigs would increase the activity of intestinal Methanobrevibacter smithii, lowering DCS incidence further. Pigs (Sus scrofa, 17–23 kg, n = 20) that ingested caffeine (5 mg/kg) increased O₂ consumption rate in 1 atm air by ~20% for at least 3 h. Pigs were given caffeine alone or caffeine plus injections of M. smithii. Animals were compressed to 24 atm (20.5–23.1 atm H₂, 0.3–0.5 atm O₂) for 3 h, then decompressed and observed for signs of DCS. In previous studies, DCS incidence in animals without caffeine treatment was significantly (P < 0.05) lower with M. smithii injections (7/16) than in controls (9/10). However, contrary to our hypothesis, DCS incidence was marginally higher (P = 0.057) in animals that received caffeine and M. smithii (9/10) than in animals that received caffeine but no M. smithii (4/10). More information on gas kinetics is needed before extending H₂ biochemical decompression to humans.

HYDROGEN, BY VIRTUE OF BEING the smallest gas molecule and possessing unusual narcotic properties under pressure, is a suitable diluent to O₂ in a breathing gas mixture for deep dives by humans to 100–600 m (10–60 atm) (1). By conventional methods, safe decompression of animal divers after an excursion to 600 m of more than 24 h duration requires on the order of 2 wk. H₂ biochemical decompression is a novel process for reducing the risk of decompression sickness (DCS) and shortening decompression time from deep H₂ dives. This process is based on the active removal of a critical fraction of the H₂ dissolved in the tissues of divers by means of intestinal microbes that metabolize H₂ to CH₄ (6, 10–12). The present study examines a possible approach to increasing the rate of delivery of H₂ to the intestinal microbes, thereby potentially increasing the benefits of H₂ biochemical decompression.

It has been shown that after some compression and decompression sequences, pigs with a higher activity of H₂ metabolism by their native intestinal flora had a lower DCS incidence compared with pigs with a lower activity (10). By injecting additional methanogenic microbes into the intestines, the CH₄ release rate (V˙CH₄) from pigs increased significantly, and the DCS incidence was lower compared with control animals (6, 11). It appeared that injections of increasing methanogenic activity increased the V˙CH₄ up to a certain point, beyond which greater injected activity did not usually elicit further increases in V˙CH₄. A similar result was observed in an earlier study in rats (12). It has been suggested that H₂ metabolic activity within the intestine is limited by the rate of supply of H₂ via vascular perfusion (12). If this hypothesis is correct, then increasing the blood flow to the intestines should increase the V˙CH₄ and reduce the DCS risk even further for a given activity of injected methanogens.

We sought a pharmacological means of increasing vascular perfusion strictly to the intestine but could not identify a drug with this selectivity. As a second choice, we tested our hypothesis by oral administration of caffeine to pigs. Caffeine has been shown to stimulate resting heart rate, cardiac output, or metabolic rate in a number of studies (5, 9, 13, 17) but not all studies (7, 16). After caffeine administration, animals were subjected to the same sequence of pressurization and depressurization in hyperbaric H₂ as used previously to test for V˙CH₄ and DCS incidence (11).

MATERIALS AND METHODS

Animals and treadmill training. Male Yorkshire pigs (Sus scrofa; n = 20; body mass range = 16.9–23.4 kg; mean body mass (±SD) = 19.5 ± 1.9 kg; Table 1) were used for all experiments. The animals were randomly assigned to one of two groups: caffeine alone (CA+INJ−, n = 10, 20.0 ± 2.0 kg) or animals given caffeine and also injected with H₂-metabolizing microbes (CA+INJ+, n = 10, 18.9 ± 1.7 kg; 1,222 ± 439 μmol CH₄/min injected activity) before hyperbaric expo-

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methanogenic activity (SD) injected into the intestines of pigs (INJ), and mean (SD) the rate of methane production ranged from 7 to 16 mol/min between animal groups (two-tailed t-test). *Significant difference between animal groups (two-tailed t-test). † Marginally significant difference between animal groups (Fisher's exact test).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mass, kg</th>
<th>% H2</th>
<th>INJ, μmol/min</th>
<th>Vchl, μmol CH4/min</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA+INJ−</td>
<td>19.8</td>
<td>88.6±0.1</td>
<td>0</td>
<td>22.4±25.8</td>
<td>0</td>
</tr>
<tr>
<td>CA+INJ−</td>
<td>23.4</td>
<td>86.1±0.1</td>
<td>0</td>
<td>58.6±27.7</td>
<td>0</td>
</tr>
<tr>
<td>CA+INJ−</td>
<td>19.3</td>
<td>86.6±0.2</td>
<td>0</td>
<td>82.7±41.7</td>
<td>0</td>
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<td>CA+INJ−</td>
<td>21.0</td>
<td>90.4±0.1</td>
<td>0</td>
<td>115.4±5.4</td>
<td>0</td>
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<td>CA+INJ−</td>
<td>19.4</td>
<td>84.9±0.5</td>
<td>0</td>
<td>43.7±8.8</td>
<td>0</td>
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<tr>
<td>CA+INJ−</td>
<td>17.5</td>
<td>87.0±0.2</td>
<td>0</td>
<td>58.7±6.0</td>
<td>0</td>
</tr>
<tr>
<td>CA+INJ−</td>
<td>20.0</td>
<td>94.8±0.2</td>
<td>0</td>
<td>57.5±9.8</td>
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<tr>
<td>CA+INJ−</td>
<td>23.1</td>
<td>85.8±0.2</td>
<td>0</td>
<td>88.6±16.8</td>
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<tr>
<td>CA+INJ−</td>
<td>18.6</td>
<td>85.7±0.2</td>
<td>0</td>
<td>102.0±8.6</td>
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<td>CA+INJ−</td>
<td>17.8</td>
<td>89.7±0.3</td>
<td>0</td>
<td>57.1±12.9</td>
<td>1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>20.0±2.0</td>
<td>88.0±3.0</td>
<td>0</td>
<td>66.7±30.0</td>
<td>4/10</td>
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<tr>
<td>CA+INJ+</td>
<td>18.5</td>
<td>86.8±0.2</td>
<td>1.672±574</td>
<td>145.6±18.3</td>
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<tr>
<td>CA+INJ+</td>
<td>17.4</td>
<td>87.0±0.2</td>
<td>1.056±93</td>
<td>187.4±23.5</td>
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</tr>
<tr>
<td>CA+INJ+</td>
<td>16.9</td>
<td>88.2±0.2</td>
<td>1.337±338</td>
<td>72.1±29.9</td>
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<tr>
<td>CA+INJ+</td>
<td>19.1</td>
<td>88.7±0.3</td>
<td>1.320±12</td>
<td>79.7±26.5</td>
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</tr>
<tr>
<td>CA+INJ+</td>
<td>18.3</td>
<td>88.9±0.2</td>
<td>743±99</td>
<td>46.8±7.0</td>
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<tr>
<td>CA+INJ+</td>
<td>22.7</td>
<td>89.2±0.2</td>
<td>1.767±194</td>
<td>148.5±22.6</td>
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</tr>
<tr>
<td>CA+INJ+</td>
<td>18.4</td>
<td>92.4±0.1</td>
<td>1.148±46</td>
<td>107.1±17.5</td>
<td>1</td>
</tr>
<tr>
<td>CA+INJ+</td>
<td>18.9</td>
<td>92.2±0.1</td>
<td>496±35</td>
<td>70.8±67.5</td>
<td>1</td>
</tr>
<tr>
<td>CA+INJ+</td>
<td>21.0</td>
<td>88.7±0.7</td>
<td>898±179</td>
<td>104.8±15.3</td>
<td>1</td>
</tr>
<tr>
<td>CA+INJ+</td>
<td>18.1</td>
<td>96.0±0.5</td>
<td>1.787±146</td>
<td>80.9±23.0</td>
<td>1</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>18.9±1.7</td>
<td>90.0±2.8</td>
<td>1.222±439</td>
<td>104.4±43.7</td>
<td>9/10</td>
</tr>
<tr>
<td>P value</td>
<td>&gt;0.2†</td>
<td>&gt;0.1†</td>
<td>&lt;0.05*</td>
<td>0.057‡</td>
<td></td>
</tr>
</tbody>
</table>

Data from pigs at 24 atm for animals with caffeine (5 mg/kg po) but without microbial injections (CA+INJ−) and for animals with caffeine and microbial injections (CA+INJ+). Data include body mass, mean (±SD) chamber H2 content (% H2) in the final 36 min at 24 atm, total methanogenic activity (±SD) injected into the intestines of pigs (INJ), and mean (±SD) CH4 release rate (Vchl) from pigs. Decompression sickness (DCS) outcome was either 0 (no DCS) or 1 (DCS).* Significant difference between animal groups (two-tailed t-test). † No difference between animal groups (two-tailed t-test). ‡ Marginally significant difference between animal groups (Fisher's exact test).

Sure. There was no difference in mass between the two groups of animals (P > 0.2, two-tailed Student’s t-test; Table 1). The animals were housed in an accredited animal care facility. They were fed once daily in the morning with laboratory animal chow (Harlan Teklad, Madison, WI; 2% by body weight) and had an unlimited supply of water. Animals were used singly in experiments. On the day before each experiment, the animal was fed a second time in the afternoon (Table 2). On the day of the experiment, no food was made available to the animal until entry into the chamber.

All experimental procedures were approved by an Animal Care and Use Committee, and the experiments reported were conducted according to the principles presented in the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animal Resources, National Research Council, 1996.

Each animal was trained to walk on a treadmill in the chamber as described earlier (Ref. 11; Table 2). This allowed the animal to be acclimated to the treadmill and the chamber and to assist with subsequent evaluation of the animal for symptoms of DCS.

Culturing of the methanogen and activity assay. A sample culture of *Methanobrevibacter smithii* (strain PS) was obtained from Dr. Terry Miller (Wadsworth Center for Laboratories and Research, Albany, NY) and was grown in an atmosphere of H2/CO2 (80:20 vol/vol; 3 atm) at 37°C at the University of Georgia. Stock cultures were maintained in a modified medium 1 (3, 11). Growth of *M. smithii* took place in a 14-liter fermentor using 11 liters of the modified medium 1. The fermentor was sparged with H2/CO2. At least 1 h before inoculation, the temperature was set to 37°C, and 10 ml of Na2S·9 H2O (20% wt/vol) were added. The inoculum size was ~1% vol/vol of the total medium volume. Details of growth phase conditions appear elsewhere (11). Before harvesting, the rate of methane production ranged from 7 to 16 μmol CH4·min−1·ml culture−1, and cell absorbance ranged from 2.0 to 3.1 OD600.

Cells were harvested by centrifugation at 23,000 rpm. The cell paste was transferred to bottles that were flushed with N2, and the cells were resuspended with 2.3 ml of a buffer of 5 mM dithiothreitol and 5% wt/vol NaHCO3 per gram of cells.

### Table 2. Chronology of experimental events

<table>
<thead>
<tr>
<th>Day</th>
<th>Procedure(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Animal trained on treadmill and acclimated to chamber</td>
</tr>
<tr>
<td></td>
<td>Animal given late afternoon supplemental meal</td>
</tr>
<tr>
<td></td>
<td><em>Methanobrevibacter smithii</em> culture received, assayed, flushed with H2/CO2 and refrigerated</td>
</tr>
<tr>
<td>1</td>
<td>Surgery performed on INJ+ animal to inject <em>M. smithii</em> culture into cecum; animal given 1–2 h recovery from anesthesia; no procedure on INJ− animal</td>
</tr>
<tr>
<td></td>
<td>Animal fed 100 mg of caffeine</td>
</tr>
<tr>
<td></td>
<td>Animal placed in chamber alert, with access to food and water</td>
</tr>
<tr>
<td></td>
<td>Chamber pressurized to 11 atm with He and O2 (0.15–0.45 atm/min)</td>
</tr>
<tr>
<td></td>
<td>Chamber flushed for 30 min with H2 and O2 at constant 11 atm until gas had a composition of 6.6–8.3 atm H2, 0.2–0.4 atm O2, balance He and N2</td>
</tr>
<tr>
<td></td>
<td>Chamber pressurized (0.45 atm/min) with H2 and O2 to 24 atm and maintained at 24 atm for 3 h; final gas composition of 20.5–33.1 atm H2, 0.3–0.5 atm O2, balance He and N2; at 2.5 h, animal observed while walking on treadmill for 5 min</td>
</tr>
<tr>
<td></td>
<td>Chamber depressurized at 0.9 atm/min to 11 atm</td>
</tr>
<tr>
<td></td>
<td>Animal observed at 11 atm for 1 h for signs of DCS; euthanized at 11 atm either after confirmation of DCS or at end of 1 h without DCS; chamber returned to 1 atm and flushed with He to eliminate H2</td>
</tr>
</tbody>
</table>

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The cell suspension bottles were flushed with H2/CO2, pressurized to 3 atm, and stored at 0°C. The bottles were shipped with gel refrigerant to the Naval Medical Research Center within 12–24 h of harvesting. On arrival, the cell suspension bottles were flushed again with H2/CO2 and stored in a refrigerator for use within the next 24–48 h (Table 2).

Before use, the cultures were assayed for their methanogenic activity by placing 0.2 ml culture and 0.2 ml of resuspension buffer in a 20-ml bottle with 3 atm H2/CO2 (Table 2). The bottle was incubated in a 37°C water bath, with agitation at 200 rpm. Samples (100 μl) of the gas in the headspace were taken every 12 min for 1–1.5 h and analyzed by gas chromatography for CH4 concentration ((CH4)). These assays were performed on duplicate samples to obtain an estimate of the mean (±SD) for methanogenic activity injected into each animal (Table 1).

**Caffeine administration.** To treat animals with caffeine, the pigs were fed a 100-μg tablet of caffeine (5 mg/kg po; CVS Pharmacy, Woonsocket, RI; Table 2). This tablet was apparently sufficiently palatable to animals, when offered with a few food pellets, so that special efforts to induce the pigs to swallow a tablet was seldom needed.

**Surgery for injection of M. smithii.** The surgical procedure has been described in detail elsewhere (Ref. 11; Table 2). Animals were prepared for surgery by preanesthetizing them with injections of ketamine HCl (20 mg/kg im; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (Rompun, 2 mg/kg im; Bayer, Shawnee Mission, KS). Animals were then kept at a surgical plane of anesthesia with inhaled isoflurane (Abbott Laboratories, Chicago, IL) and O2. With the use of an aseptic technique, a midline incision of 10 cm was made in the abdomen, and the cecum and spiral colon were exteriorized. Injections of M. smithii culture were made into the cecum and spiral colon, with total injectate volumes ranging from 22 to 121 ml and total activities injected ranging from 500 to 1,800 μmol CH4/min (Table 1). All needle puncture sites were sealed with a drop of surgical cement (Vetbond, 3M, St. Paul, MN). The intestines were moistened externally with saline and returned to the abdomen, and the incision was closed with sutures. As the animal recovered from the anesthesia, yohimbine (2 mg iv; Lloyd Laboratories, Shenandoah, IA) was injected into an ear vein to act as an antagonist to the xylazine. Animals appeared to be fully recovered from the anesthesia in 1–2 h. They were then placed in the compression chamber to commence the experiment. Food and water were freely available in the chamber.

**O2 consumption and heart rate measurements in 1 atm air.** The animal was placed in a clear plastic box with internal dimensions of 90 cm × 60 cm × 55 cm (~300 liters). A vacuum pump (wet/dry industrial shop-vac model 700M, Shop-Vac, Williamsport, PA) was attached to the box to create a flow of air through the box of 14 l/min. A second vacuum pump extracted an additional 0.8 l/min from the box. The excurrent gas from the second pump was used to supply samples for analysis of O2 (model 755A, Beckman Industrial, Fullerton, CA) and CO2 (medical gas analyzer, Beckman Industrial). The gas analyzers were calibrated by using purchased gas mixtures of known composition (12.3% O2, 4.97% CO2, balance N2; 21.8% O2, 0% CO2, balance N2; or pure N2). A canister with anhydrous CaSO4 (WA Hammond Drierite, Xenia, OH) was placed downstream of the box to remove water vapor. Consequently, all gases were assumed to be dry when correcting the flow rate from the box. During an experiment, gas analysis data were automatically logged each minute, corrected to STPD, and saved to a file with a routine in Lab VIEW 5.0 (National Instruments, Austin, TX).

The animal was kept in the box for 60 min (n = 14 pigs) or 210 min (n = 5 pigs) to measure its O2 consumption rate (VO2). The measurement of VO2 for several hours was used to test for systematic temporal change of VO2 during extended confinement. Next, the animal was fed a tablet of caffeine, and the VO2 was measured again for either 60 min (n = 10 pigs) or 210 min (n = 5 pigs).

VO2 was computed from the readings of the last 30 or 180 min of confinement by using the Z transformation (4) to correct the gas analysis data to equilibrium conditions. Data from the initial 30 min in the box were not used to allow the animal to acclimate to confinement and for the caffeine to take effect (17). An oximeter (Vet/Ox 4404, Heska, Waukesha, WI) was attached to the tail in a subset of animals (n = 6 pigs). The oximeter allowed measurement of the heart rate and blood O2 saturation for 1 h before and after caffeine administration. The initial compression with He followed by replacement of CH4 commenced. This period of time allowed the caffeine to take effect (17).

An animal was placed individually in the airtight clear plastic box described above for VO2 measurement. Gas samples (~500 μl) were taken with a gas-tight syringe from a sample hole covered with a rubber membrane. A 100-μl sample of the gas was injected into a gas chromatograph (HP 5890 series II, Hewlett-Packard, Wilmington, DE) and analyzed for [CH4] (ppm). [CH4] was converted to micromoles of CH4 by correcting for the volume of the box, assuming that the animal displaced a volume (liters) equal to its body mass (kilograms), and converting the values to STPD. The VCH4 (μmol CH4/min) was calculated as the change in [CH4] over time. To avoid build-up of CO2 in the box, the experiment was limited to 30 min, after which the lid of the box was taken off, and the air in the box was exchanged by using a fan. Each experiment was made in duplicate and the VCH4 was taken as the average of the two independent measurements.

VCH4 was not calculated in 1 atm air for animals that had received injections of M. smithii. This was due to the need for haste in placing injected animals in the chamber and commencing the hyperbaric exposure while the M. smithii cultures were likely to be retained within the intestines.

**Dive protocol.** Immediately before the hyperbaric experiment, each animal was given caffeine and placed in a dry hyperbaric chamber (5,665-liter internal volume, WSP Industries, Buffalo, NY). Subsequently, the animal was compressed as described in detail elsewhere (11). The chronological events of the compression and decompression sequence are summarized in Table 2.

In brief, one pig was placed in the compression chamber for each experiment. A stream of gas flowed continuously from the chamber to a gas chromatograph (model 5890A series II, Hewlett-Packard) that was calibrated before and after each experiment. Automated analysis of O2, N2, He, H2, and CH4 occurred every 12 min throughout the experiment. The hyperbaric chamber was initially pressurized to 11 atm (absolute pressure) with He, with concomitant addition of O2 to keep the chamber atmosphere normoxic to slightly hyperoxic (PO2 = 0.2–0.4 atm). After 11 atm was reached, the chamber was flushed with H2 and small volumes of O2 for ~30 min. The initial compression with He followed by replacement

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**Note:** The text appears to be a scientific description of an experiment involving the use of caffeine and an anaerobic process to measure CO2 and CH4 metabolism in pigs. The methodology includes detailed descriptions of the experimental setup, surgical procedures, and gas analysis techniques. The text mentions the use of caffeine to induce hyperbaric conditions and the measurement of metabolic gases before and after caffeine administration.
CAFFEINE DURING H₂ BIOCHEMICAL DECOMPRESSION

with H₂ was performed to maintain a noncombustible and breathable mixture of H₂ and O₂ within the chamber (11).

After the flush, the chamber was further pressurized to 24 atm with H₂ and O₂ at a rate of 0.45 atm/min. When 24 atm was reached, the pressure was maintained constant (±0.3 atm) for 3 h by continuous addition of H₂ and O₂ to make up for the gas exhausted to the gas chromatograph. Final H₂ concentration in the chamber was 85–96% (Table 1). Reported values (Table 1) for H₂ concentration are the means of the final three gas chromatograph readings at 24 atm. There was no difference in H₂ concentration between the CA⁻INJ⁻ and CA⁺INJ⁺ groups (P > 0.1, two-tailed Student’s t-test; Table 1).

Throughout most of the time at 24 atm, the animal was free to rest or move about its space within the chamber at will. The chamber temperature at 24 atm was maintained at 32°C, a seemingly comfortable level for the animals, as judged by absence of shivering with blanched skin or rapid breathing with flushed skin. After 2.5 h at 24 atm, the animal was made to walk on the treadmill within the chamber for 5 min to observe its gait before decompression.

After 3 h (±30 s) at 24 atm, the chamber was depressurized to 0.90 atm/min to 11 atm. The animal was observed for severe symptoms of DCS for 1 h at 11 atm as it walked intermittently on the chamber treadmill. These symptoms were primarily neurological and included falling, difficulty standing or righting after falling, and seizures. Some animals had labored breathing, which may have indicated cardiopulmonary DCS in addition to neurological DCS. Many animals were also observed to have signs of skin DCS (conspicuous lavender to dark purple mottling of the skin, with or without itching), but these signs alone did not warrant a diagnosis of severe DCS. Mild, transient behavioral changes (agitation or lethargy) were also not considered sufficient for a diagnosis of severe DCS. Once the diagnosis was made or the hour had passed without evidence of DCS, the animal was quickly killed by asphyxiation with He. The chamber was returned to 1 atm after the animal was dead.

Corrected V̇CH₄. The change in the chamber [CH₄] during the time at constant pressure was corrected by using the Z transformation (4) to estimate the V̇CH₄ under equilibrium conditions, as described earlier (11). Values are reported as means ± SD of five pairs of chromatographic readings at 24 atm, with a time change of 120 min (Table 1). The mean flow rate through the chamber was 115 l/min.

RESULTS

There was no systematic temporal change in V̇O₂ (P > 0.4; repeated-measures single-factor ANOVA) over a 1–3.5 h period before caffeine administration in 1 atm air. Thus baseline V̇O₂ was represented by a single value in air (CA⁻Air; Fig. 1). During the first hour after caffeine administration (CA⁺Air), V̇O₂ was 24% higher than baseline (Fig. 1). V̇O₂ remained significantly higher after caffeine administration for at least 180 min (P < 0.05, two-tailed paired t-test; Fig. 1). Heart rate was significantly higher by 7–40 beats/min after caffeine administration in three animals, whereas it was unchanged in three animals (Table 3).

Mean V̇CH₄ for animals in 1 atm air was 14.2 ± 13.2 μmol CH₄/min before caffeine administration and 13.8 ± 11.1 μmol CH₄/min after caffeine administration. These values are not different from each other (P > 0.50, two-tailed Student’s t-test).

During the hyperbaric experiments, DCS incidence in CA⁺INJ⁺ animals (9/10) was marginally higher than in CA⁻INJ⁻ animals (4/10; P = 0.057, Fisher exact test; Table 1, Fig. 2). V̇CH₄ in animals in the CA⁺INJ⁺ group was significantly higher (P < 0.05, two-tailed Student’s t-test) by >50% compared with animals in the CA⁻INJ⁻ group (Table 1, Fig. 3).

Table 3. Body mass and mean heart rate of pigs in 1 atm air before and 30 min after administration of caffeine

<table>
<thead>
<tr>
<th>Pig</th>
<th>Body Mass, kg</th>
<th>CA⁻Air Heart Rate, beats/min</th>
<th>CA⁺Air Heart Rate, beats/min</th>
<th>% Change, (post – pre)/pre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.6</td>
<td>130.4 ± 6.1</td>
<td>128.2 ± 6.7</td>
<td>−1.7</td>
</tr>
<tr>
<td>2</td>
<td>17.9</td>
<td>110.2 ± 8.7</td>
<td>152.0 ± 11.1</td>
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</tr>
<tr>
<td>3</td>
<td>18.4</td>
<td>128.3 ± 6.8</td>
<td>136.6 ± 9.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>4</td>
<td>19.9</td>
<td>98.3 ± 7.2</td>
<td>93.0 ± 7.7</td>
<td>−5.4</td>
</tr>
<tr>
<td>5</td>
<td>21.0</td>
<td>118.5 ± 5.7</td>
<td>125.7 ± 6.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>6</td>
<td>21.5</td>
<td>147.7 ± 9.6</td>
<td>153.1 ± 9.1</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>19.4 ± 1.7</td>
<td>122.2 ± 17.2</td>
<td>131.4 ± 22.1</td>
<td>7.8 ± 15.5</td>
</tr>
</tbody>
</table>

Values are means ± SD of n = 20 one-minute reading of pigs. CA⁻Air, before caffeine administration; CA⁺Air, after caffeine administration (5 mg/kg). *Based on a two-tailed paired t-test comparing mean heart rate pre- (pre) and postcaffeine (post) administration within each animal. †Based on paired t-test of all animals (n = 6).
DISCUSSION

H2 biochemical decompression has been demonstrated to reduce DCS risk in two different animal models after a number of different exposures to hyperbaric H2 (6, 10–12). Mathematical modeling has supported our concept that H2 biochemical decompression reduces DCS risk by lowering tissue H2 content via microbial H2 metabolism, with significant correlations between microbial activity injected, VCH4 from animals, and DCS outcome (6). Even the native intestinal flora in pigs can significantly reduce DCS risk if the methanogenic activity is sufficiently high (10).

The caffeine treatments of the present study led to a result even more disappointing than a failure to support our hypothesis of lowering DCS risk further for a given activity of methanogens injected. The caffeine treatments were actually associated with an increased risk of DCS for animals receiving injections of methanogens (Fig. 2). This unexpected result deserves careful consideration since we had been hoping to offer biochemical decompression, first for H2 diving and eventually for N2 diving, to human divers in the foreseeable future.

Caffeine, a methylxanthine, exerts a number of physiological effects (5, 7–9, 16, 17), including the stimulation of gastric acid and digestive enzyme secretion (9). Caffeine does not selectively increase intestinal perfusion but can increase cardiac output and, therefore, perfusion to many vascular beds (9). VO2 values measured before caffeine administration for the pigs in this study are within the normal range for these animals, when normalized for body mass and age effects (15, 18). The higher VO2 in air (which is the only option we had for measuring VO2) given the technical difficulties of working with large volumes of hyperbaric H2 after caffeine treatment (Fig. 1) supports our assumption that the caffeine was increasing cardiac output in these animals. This elevation in VO2 lasted at least for a time span corresponding to the length of the chosen hyperbaric exposure. The variable effect on heart rate of individual animals (Table 3) may reasonably reflect changes in stroke volume as well as heart rate after caffeine administration.

Consequently, the treatment of these animals with caffeine is likely to offer mixed effects bearing on DCS outcome. Increasing the cardiac output may increase the rate of H2 uptake throughout the body during the hyperbaric exposure for the period of hours needed before attaining H2 saturation (Fahlman, unpublished observation). However, if some fraction of the higher cardiac output increases the perfusion of the intestines, this should potentially increase the rate of H2 elimination across the intestine by the metabolism of the methanogens. The net effect on subsequent DCS risk is likely to be determined by whether the increased H2 uptake or the increased H2 elimination is greater.

Under normal atmospheric conditions, intestinal CH4 production in mammals is almost exclusively attributable to metabolism of H2 generated by other intestinal microbes (14). The lack of change in VCH4 after caffeine administration in 1 atm air was expected because increased cardiac output, and potentially increased perfusion to the intestines, would not alter the supply of H2 within the intestines of air-breathing animals.

To help understand VCH4 and DCS incidence in these experiments, we compared the data from this study with those of an earlier study that did not include caffeine treatment (11). In that study, animals were exposed to the same compression and decompression sequence in H2 and the same range of H2 concentrations as in the present study; some animals had only their own native intestinal flora (CA−INJ−; n = 10)
and others received intestinal injections of *M. smithii* (CA−INJ+; *n* = 16). Before making any pairwise statistical comparisons between the animal groups in the two studies, we first performed statistical procedures on the data from the four groups together. Body masses did not differ among the four groups (ANOVA, *P* > 0.10); the null hypothesis that VCH4 was the same in all four groups was rejected (ANOVA, *P* < 0.001); and the null hypothesis that the DCS outcome for the two groups with caffeine was the same as the outcome for the two groups without caffeine was rejected (*χ²* test, *P* < 0.01).

Mean VCH4 in hyperbaric H2 was nearly twofold higher (*P* < 0.01, two-tailed Student’s *t*-test) in CA+INJ− animals compared with CA−INJ− animals (Fig. 3). This supports the hypothesis that caffeine ingestion increased intestinal perfusion and that intestinal methanogenesis (using only a native population of microbes) is to some extent perfusion limited when there is an ample source of H2 supplied from the blood.

Pigs from the CA+INJ− group had a marginally lower incidence of DCS (4/10) than those from the CA−INJ− group (9/10; *P* = 0.057, Fisher exact test; Fig. 2). This result is also as expected if the caffeine treatment increased the rate of supply of blood-borne H2 to the microbes, thereby increasing the H2 elimination process. Any changes in H2 uptake in the CA+INJ− group would be undetectable in these experiments because we had no assay for H2 uptake.

In designing this study, we predicted that the CA+INJ+ group would have the highest VCH4 and lowest DCS risk of any animal group. Instead, the CA+INJ+ animals released CH4 at a mean rate that was similar to that of the CA−INJ+ pigs (*P* > 0.40, two-tailed Student’s *t*-test; Fig. 3). When we compared methanogenic activity injected into animals to VCH4 from animals in the CA−INJ+ and CA+INJ+ groups, no significant differences were found (Fig. 4). The CA+INJ+ animals had a higher incidence of DCS (9/10) than the CA−INJ+ animals (7/16; *P* < 0.05, Fisher exact test; Fig. 2). One explanation for this observation, which we explore below, is that a higher cardiac output after caffeine administration and surgery may have had a greater effect on H2 uptake than on H2 elimination, thereby increasing DCS risk.

In the prior study (11), we included a group of pigs that underwent the same surgical procedure as the methanogen-treated animals except that their intestinal injections were of deoxygenated saline. The pigs in this surgical control group had a similarly high incidence of DCS (7/10) and a VCH4 (34 ± 16 µmol CH4/min) that was nearly identical to that of the controls without surgery (CA−INJ−; Figs. 2 and 3). Thus we did not expect to need a surgical control group for the animals with caffeine treatment. We now suspect that such a caffeine plus surgical control group may have revealed that the caffeine treatment coupled with surgery had the effect of increasing cardiac output without allowing an increase in intestinal perfusion for pharmacological reasons we presently do not understand. This combination of caffeine and surgery may have augmented H2 uptake but kept H2 elimination from rising, leading to an elevated risk of DCS in this hypothetical caffeine plus surgical control group.

The issue of cardiac output in relation to intestinal perfusion is a very realistic concern for bringing biochemical decompression from laboratory animal studies to human field use. Exercise is known to increase cardiac output, with selectively elevated perfusion of the muscles in demand, and decreased perfusion of less critical tissues such as intestine (2). Thus future laboratory studies of biochemical decompression should be careful to include exercise and its effects on blood flow distribution, along with analyses of gas uptake and elimination rates with and without intestinal microbes.

We conclude that that the administration of caffeine to pigs increased their cardiac output as suggested by their increased VO2. Caffeine administration also increased VCH4 during hyperbaric H2 exposure in animals with a native intestinal flora, which led to a decreased DCS incidence. The anomalous effect of increased DCS incidence in CA+INJ+ can only be speculatively attributed to an increased H2 uptake without a matching H2 elimination by intestinal methanogens. Thus there is much that we do not understand about gas fluxes during biochemical decompression, and this missing information will be critical to offering this process to facilitate diving in humans.

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REFERENCES


18. Wieser W. A distinction must be made between the ontogeny and the phylogeny of metabolism in order to understand the mass exponent of energy metabolism. Respir Physiol 55: 1–9, 1984.