*J Appl Physiol* 93: 1583–1589, 2002.
First published July 19, 2002; 10.1152/japplphysiol.00349.2002.

# Modulation of decompression sickness risk in pigs with caffeine during $H_2$ biochemical decompression

ANDREAS FAHLMAN,[1] WINSTON C. LIN,[2] WILLIAM B. WHITMAN,[2] AND SUSAN R. KAYAR[1]
[1]*Environmental Physiology Department, Naval Medical Research Center, Silver Spring, Maryland 20910-7500; and* [2]*Department of Microbiology, University of Georgia, Athens, Georgia 30602*

Received 18 April 2002; accepted in final form 2 July 2002

**Fahlman, Andreas, Winston C. Lin, William B. Whitman, and Susan R. Kayar.** Modulation of decompression sickness risk in pigs with caffeine during $H_2$ biochemical decompression. *J Appl Physiol* 93: 1583–1589, 2002. First published July 19, 2002; 10.1152/japplphysiol.00349.2002.— In $H_2$ biochemical decompression, $H_2$-metabolizing intestinal microbes remove gas stored in tissues of animals breathing hyperbaric $H_2$, 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 $O_2$ 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 $H_2$, 0.3–0.5 atm $O_2$) 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 $H_2$ biochemical decompression to humans.

cardiac output; hydrogen diving; hyperbaria; intestine; perfusion

HYDROGEN, BY VIRTUE OF BEING the smallest gas molecule and possessing unusual narcotic properties under pressure, is a suitable diluent to $O_2$ in a breathing gas mixture for deep dives by humans to 100–600 m (10–60 atm) (1). By conventional methods, safe decompression of human divers after an excursion to 600 m of more than 24 h duration requires on the order of 2 wk. $H_2$ biochemical decompression is a novel process for reducing the risk of decompression sickness (DCS) and shortening decompression time from deep $H_2$ dives. This process is based on the active removal of a critical fraction of the $H_2$ dissolved in the tissues of divers by means of intestinal microbes that metabolize $H_2$ to $CH_4$ (6, 10–12). The present study examines a possible approach to increasing the rate of delivery of $H_2$ to the intestinal microbes, thereby potentially increasing the benefits of $H_2$ biochemical decompression.

It has been shown that after some compression and decompression sequences, pigs with a higher activity of $H_2$ 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 $CH_4$ release rate ($\dot{V}_{CH_4}$) 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 $\dot{V}_{CH_4}$ up to a certain point, beyond which greater injected activity did not usually elicit further increases in $\dot{V}_{CH_4}$. A similar result was observed in an earlier study in rats (12). It has been suggested that $H_2$ metabolic activity within the intestine is limited by the rate of supply of $H_2$ via vascular perfusion (12). If this hypothesis is correct, then increasing the blood flow to the intestines should increase the $\dot{V}_{CH_4}$ 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 $H_2$ as used previously to test for $\dot{V}_{CH_4}$ 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 $H_2$-metabolizing microbes (CA+INJ+, $n = 10$, 18.9 ± 1.7 kg; 1,222 ± 439 μmol $CH_4$/min injected activity) before hyperbaric expo-

Address for reprint requests and other correspondence: S. R. Kayar, National Center for Research Resources, National Institutes of Health, 6705 Rockledge Dr., Suite 6030, Bethesda, MD 20892-7965 (E-mail: kayars@ncrr.nih.gov).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1. *Data from pigs at 24 atm*

| Group | Mass, kg | % $H_2$ | INJ, μmol/min | $\dot{V}_{CH_4}$, μmol $CH_4$/min | Outcome |
|---|---|---|---|---|---|
| CA+INJ− | 19.8 | 88.6 ± 0.1 | 0 | 22.4 ± 25.8 | 0 |
| CA+INJ− | 23.4 | 86.1 ± 0.1 | 0 | 58.6 ± 27.7 | 0 |
| CA+INJ− | 19.3 | 86.6 ± 0.2 | 0 | 82.7 ± 41.7 | 0 |
| CA+INJ− | 21.0 | 90.4 ± 0.1 | 0 | 115.4 ± 5.4 | 0 |
| CA+INJ− | 19.4 | 84.9 ± 0.5 | 0 | 43.7 ± 8.8 | 0 |
| CA+INJ− | 17.5 | 87.0 ± 0.2 | 0 | 58.7 ± 6.0 | 0 |
| CA+INJ− | 20.0 | 94.8 ± 0.2 | 0 | 57.5 ± 9.8 | 1 |
| CA+INJ− | 23.1 | 85.8 ± 0.2 | 0 | 88.6 ± 16.8 | 1 |
| CA+INJ− | 18.6 | 85.7 ± 0.2 | 0 | 102.0 ± 8.6 | 1 |
| CA+INJ− | 17.8 | 89.7 ± 0.1 | 0 | 37.1 ± 12.9 | 1 |
| Mean ± SD | 20.0 ± 2.0 | 88.0 ± 3.0 | 0 | 66.7 ± 30.0 | 4/10 |
| CA+INJ+ | 18.5 | 86.8 ± 0.2 | 1,672 ± 574 | 145.6 ± 18.3 | 0 |
| CA+INJ+ | 17.4 | 87.0 ± 0.2 | 1,056 ± 93 | 187.4 ± 23.5 | 1 |
| CA+INJ+ | 16.9 | 88.2 ± 0.2 | 1,337 ± 338 | 72.1 ± 29.9 | 1 |
| CA+INJ+ | 19.1 | 88.7 ± 0.3 | 1,320 ± 12 | 79.7 ± 26.5 | 1 |
| CA+INJ+ | 18.3 | 88.9 ± 0.2 | 743 ± 99 | 46.8 ± 7.0 | 1 |
| CA+INJ+ | 22.7 | 89.9 ± 0.2 | 1,767 ± 194 | 148.5 ± 22.6 | 1 |
| CA+INJ+ | 18.4 | 92.4 ± 0.1 | 1,148 ± 46 | 107.1 ± 17.5 | 1 |
| CA+INJ+ | 18.9 | 92.3 ± 0.1 | 496 ± 35 | 70.8 ± 67.5 | 1 |
| CA+INJ+ | 21.0 | 89.8 ± 0.7 | 898 ± 179 | 104.8 ± 15.3 | 1 |
| CA+INJ+ | 18.1 | 96.0 ± 0.5 | 1,787 ± 146 | 80.9 ± 23.0 | 1 |
| Mean ± SD | 18.9 ± 1.7 | 90.0 ± 2.8 | 1,222 ± 439 | 104.4 ± 43.7 | 9/10 |
| *P* value | >0.2† | >0.1† | | <0.05* | 0.057‡ |

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 $H_2$ content (% $H_2$) in the final 36 min at 24 atm, total methanogenic activity (±SD) injected into the intestines of pigs (INJ), and mean (±SD) $CH_4$ release rate ($\dot{V}_{CH_4}$) 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 $H_2/CO_2$ (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 $H_2/CO_2$. At least 1 h before inoculation, the temperature was set to 37°C, and 10 ml of $Na_2S \cdot 9\ H_2O$ (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 $CH_4 \cdot min^{-1} \cdot ml\ culture^{-1}$, and cell absorbance ranged from 2.0 to 3.1 $OD_{600}$.

Cells were harvested by centrifugation at 23,000 rpm. The cell paste was transferred to bottles that were flushed with $N_2$, and the cells were resuspended with 2.3 ml of a buffer of 5 mM dithiothreitol and 5% wt/vol $NaHCO_3$ per gram of cells.

Table 2. *Chronology of experimental events*

| Day | Procedure(s) |
|---|---|
| 0 | Animal trained on treadmill and acclimated to chamber<br>Animal given late afternoon supplemental meal<br>*Methanobrevibacter smithii* culture received, assayed, flushed with $H_2/CO_2$ and refrigerated |
| 1 | Surgery performed on INJ+ animal to inject *M. smithii* culture into cecum; animal given 1–2 h recovery from anesthesia; no procedure on INJ− animal<br>Animal fed 100 mg of caffeine<br>Animal placed in chamber alert, with access to food and water<br>Chamber pressurized to 11 atm with He and $O_2$ (0.15–0.45 atm/min)<br>Chamber flushed for 30 min with $H_2$ and $O_2$ at constant 11 atm until gas had a composition of 6.6–8.3 atm $H_2$, 0.2–0.4 atm $O_2$, balance He and $N_2$<br>Chamber pressurized (0.45 atm/min) with $H_2$ and $O_2$ to 24 atm and maintained at 24 atm for 3 h; final gas composition of 20.5–23.1 atm $H_2$, 0.3–0.5 atm $O_2$, balance He and $N_2$; at 2.5 h, animal observed while walking on treadmill for 5 min<br>Chamber depressurized at 0.9 atm/min to 11 atm<br>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 $H_2$ |

The cell suspension bottles were flushed with $H_2/CO_2$, 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 $H_2/CO_2$ 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 $H_2/CO_2$ (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 $CH_4$ concentration ($[CH_4]$). 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-mg 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 effort 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 $O_2$. 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 $CH_4$/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.

*$O_2$ 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 $O_2$ (model 755A, Beckman Industrial, Fullerton, CA) and $CO_2$ (medical gas analyzer, Beckman Industrial). The gas analyzers were calibrated by using purchased gas mixtures of known composition (12.3% $O_2$, 4.97% $CO_2$, balance $N_2$; 21.8% $O_2$, 0% $CO_2$, balance $N_2$; or pure $N_2$ Air Products and Chemicals, Allentown, PA) before and after each experiment. The temperature inside the box was measured by using a thermistor (no. 402 Yellow Springs Instruments, Yellow Springs, OH). A canister with anhydrous $CaSO_4$ (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 $O_2$ consumption rate ($\dot{V}O_2$). The measurement of $\dot{V}O_2$ for several hours was used to test for systematic temporal change of $\dot{V}O_2$ during extended confinement. Next, the animal was fed a tablet of caffeine, and the $\dot{V}O_2$ was measured again for either 60 min ($n$ = 10 pigs) or 210 min ($n$ = 5 pigs).

$\dot{V}O_2$ 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 $O_2$ saturation for 1 h before and after caffeine administration.

*$\dot{V}CH_4$ in 1 atm air.* $\dot{V}CH_4$ was measured in animals at 1 atm for 30 min, before and after oral administration of 100 mg of caffeine. After caffeine administration, the animal was allowed to walk around freely for 30 min before measurement of $\dot{V}CH_4$ 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 $\dot{V}O_2$ 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 $[CH_4]$ (ppm). $[CH_4]$ was converted to micromoles of $CH_4$ 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 $\dot{V}CH_4$ (μmol $CH_4$/min) was calculated as the change in $[CH_4]$ over time. To avoid build-up of $CO_2$ 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 $\dot{V}CH_4$ was taken as the average of the two independent measurements.

$\dot{V}CH_4$ 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, WSF 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 $O_2$, $N_2$, He, $H_2$, and $CH_4$ occurred every 12 min throughout the experiment. The hyperbaric chamber was initially pressurized to 11 atm (absolute pressure) with He, with concomitant addition of $O_2$ to keep the chamber atmosphere normoxic to slightly hyperoxic ($PO_2$ = 0.2–0.4 atm). After 11 atm was reached, the chamber was flushed with $H_2$ and small volumes of $O_2$ for ~30 min. The initial compression with He followed by replacement

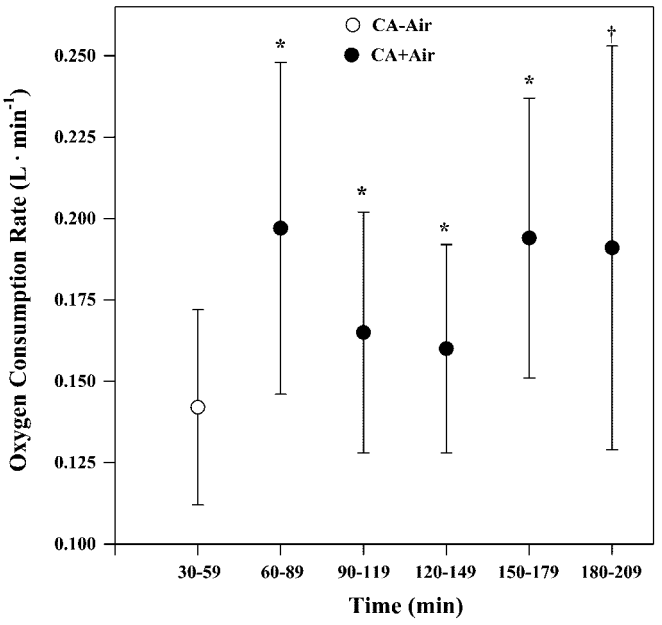


Fig. 1. Elapsed time vs. mean (±SD) oxygen consumption rate before (CA−Air) and after administration of caffeine (CA+Air). Significantly different from CA−Air: *$P < 0.05$; †$P = 0.10$ (paired $t$-test).

with $H_2$ was performed to maintain a noncombustible and breathable mixture of $H_2$ and $O_2$ within the chamber (11).

After the flush, the chamber was further pressurized to 24 atm with $H_2$ and $O_2$ 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 $H_2$ and $O_2$ to make up for the gas exhausted to the gas chromatograph. Final $H_2$ concentration in the chamber was 85–96% (Table 1). Reported values (Table 1) for $H_2$ concentration are the means of the final three gas chromatograph readings at 24 atm. There was no difference in $H_2$ 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 at 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 $\dot{V}_{CH_4}$.* The change in the chamber [$CH_4$] during the time at constant pressure was corrected by using the Z transformation (4) to estimate the $\dot{V}_{CH_4}$ 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 $\dot{V}_{O_2}$ ($P > 0.4$; repeated-measures single-factor ANOVA) over a 1–3.5 h period before caffeine administration in 1 atm air. Thus baseline $\dot{V}_{O_2}$ was represented by a single value in air (CA−Air; Fig. 1). During the first hour after caffeine administration (CA+Air), $\dot{V}_{O_2}$ was 24% higher than baseline (Fig. 1). $\dot{V}_{O_2}$ 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 $\dot{V}_{CH_4}$ for animals in 1 atm air was 14.2 ± 13.2 μmol $CH_4$/min before caffeine administration and 13.8 ± 11.1 μmol $CH_4$/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). $\dot{V}_{CH_4}$ 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*

| Pig | Body Mass, kg | CA−Air Heart Rate, beats/min | CA+Air Heart Rate, beats/min | *$P$ | % Change, (post−pre)/pre |
|---|---|---|---|---|---|
| *1* | 17.6 | 130.4 ± 6.1 | 128.2 ± 6.7 | | −1.7 |
| *2* | 17.9 | 110.2 ± 8.7 | 152.0 ± 11.1 | <0.01 | 37.9 |
| *3* | 18.4 | 128.3 ± 6.8 | 136.6 ± 9.7 | <0.01 | 6.5 |
| *4* | 19.9 | 98.3 ± 7.2 | 93.0 ± 7.7 | | −5.4 |
| *5* | 21.0 | 118.5 ± 5.7 | 125.7 ± 6.9 | <0.01 | 6.1 |
| *6* | 21.5 | 147.7 ± 9.6 | 153.1 ± 9.1 | <0.10 | 3.7 |
| Mean ± SD | 19.4 ± 1.7 | 122.2 ± 17.2 | 131.4 ± 22.1 | >0.2† | 7.8 ± 15.5 |

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 ($n = 6$).

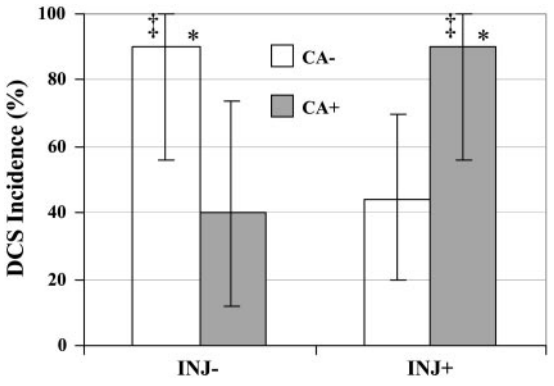


Fig. 2. Incidence of decompression sickness (%DCS with 95% confidence intervals in a binomial distribution) from pigs with normal intestinal flora (INJ−) or with intestinal injections of *Methanobrevibacter smithii* (INJ+), and with (CA+; solid bars) or without (CA−; open bars) administration of 5 mg/kg caffeine. All animals were exposed to 24 atm $H_2$-$O_2$ mixture for 3 h, followed by decompression at 0.9 atm/min. *Significantly different from CA−INJ+ group ($P < 0.05$, Fisher's exact test). ‡Marginally different from the CA+INJ− group ($P = 0.057$, Fisher's exact test). CA−INJ−, $n = 10$; CA+INJ−, $n = 10$; CA−INJ+, $n = 16$; CA+INJ+, $n = 10$. Data from animals without caffeine are from Kayar et al. (11).

## DISCUSSION

$H_2$ biochemical decompression has been demonstrated to reduce DCS risk in two different animal models after a number of different exposures to hyperbaric $H_2$ (6, 10–12). Mathematical modeling has supported our concept that $H_2$ biochemical decompression reduces DCS risk by lowering tissue $H_2$ content via microbial $H_2$ metabolism, with significant correlations between microbial activity injected, $\dot{V}_{CH_4}$ 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 $H_2$ diving and eventually for $N_2$ 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). $\dot{V}_{O_2}$ 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 $\dot{V}_{O_2}$ in air (which is the only option we had for measuring $\dot{V}_{O_2}$, given the technical difficulties of working with large volumes of hyperbaric $H_2$) after caffeine treatment (Fig. 1) supports our assumption that the caffeine was increasing cardiac output in these animals. This elevation in $\dot{V}_{O_2}$ 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 $H_2$ uptake throughout the body during the hyperbaric exposure for the period of hours needed before attaining $H_2$ 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 $H_2$ 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 $H_2$ uptake or the increased $H_2$ elimination is greater.

Under normal atmospheric conditions, intestinal $CH_4$ production in mammals is almost exclusively attributable to metabolism of $H_2$ generated by other intestinal microbes (14). The lack of change in $\dot{V}_{CH_4}$ 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 $H_2$ within the intestines of air-breathing animals.

To help understand $\dot{V}_{CH_4}$ 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 $H_2$ and the same range of $H_2$ concentrations as in the present study; some animals had only their own native intestinal flora (CA−INJ−; $n = 10$)

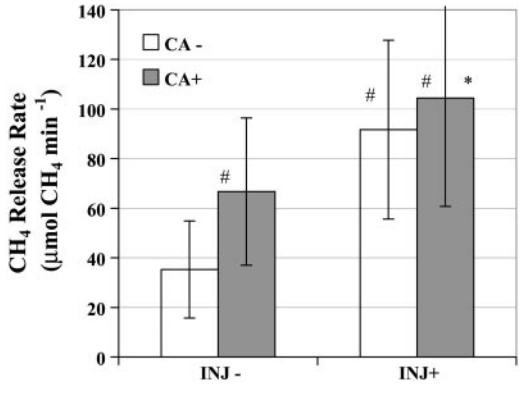


Fig. 3. Methane release rate (μmol $CH_4$/min) from pigs with INJ− or INJ+ and with CA− (open bars) or CA+ (solid bars). All animals were exposed to 24 atm $H_2$ and $O_2$ mixture for 3 h, followed by decompression at 0.9 atm/min to 11 atm. Values are means ± SD. *Significant difference between CA+INJ+ and CA+INJ− groups ($P < 0.05$, two-tailed $t$-test). #Significantly different from CA−INJ− group ($P < 0.05$, two-tailed $t$-test). CA−INJ−, $n = 10$; CA+INJ−, $n = 10$; CA−INJ+, $n = 16$; CA+INJ+, $n = 10$. Data from animals without caffeine are from Kayar et al. (11).

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 $\dot{V}_{CH_4}$ 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 ($\chi^2$ test, $P < 0.01$).

Mean $\dot{V}_{CH_4}$ in hyperbaric $H_2$ 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 $H_2$ 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 $H_2$ to the microbes, thereby increasing the $H_2$ elimination process. Any changes in $H_2$ uptake in the CA+INJ− group would be undetectable in these experiments because we had no assay for $H_2$ uptake.

In designing this study, we predicted that the CA+INJ+ group would have the highest $\dot{V}_{CH_4}$ and lowest DCS risk of any animal group. Instead, the CA+INJ+ animals released $CH_4$ 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 $\dot{V}_{CH_4}$ 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 $H_2$ uptake than on $H_2$ 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 $\dot{V}_{CH_4}$ (34 ± 16 μmol $CH_4$/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

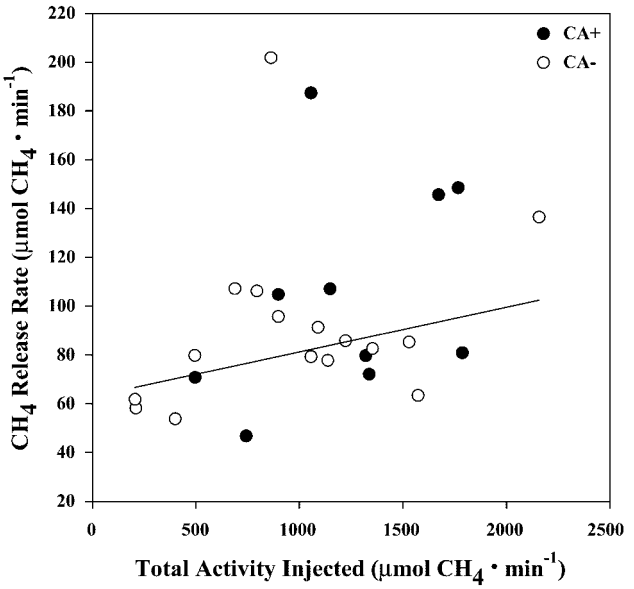


Fig. 4. Mean methane release rate (μmol $CH_4$/min) from pigs injected with *M. smithii*, with (CA+INJ+; solid circles) and without (CA−INJ+; open circles) administration of caffeine, as a function of injected activity of *M. smithii*. Data were collected during an exposure of these animals to 24 atm $H_2$-$O_2$ mixture for 3 h. These 2 data sets are not statistically different from each other ($P > 0.30$, ANCOVA). Line represents robust regression for the pooled data: $y = 63 + 0.018x$, $P < 0.05$. Data from pigs without caffeine are from Kayar et al. (11).

augmented $H_2$ uptake but kept $H_2$ 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 $\dot{V}_{O_2}$. Caffeine administration also increased $\dot{V}_{CH_4}$ during hyperbaric $H_2$ 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 $H_2$ uptake without a matching $H_2$ 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.

We are grateful as ever to the Phase III support team, Richard Ayres, Jerry Morris, Roland Ramsey, and Chief Anthony Ruopoli for devotion to doing things well. We also thank Diana Temple for editorial assistance and critical reading of this manuscript.

This work was funded by the Naval Medical Research and Development Command Work Unit no. 61153N MR04101.00D-1103. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department and the naval service at large.

Present address of A. Fahlman: School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK.

## REFERENCES


1. **Abraini JH, Gardette-Chauffour MC, Martinez E, Rostain JC, and Lemaire C.** Psychophysiological reactions in humans during an open sea dive to 500 m with a hydrogen-helium-oxygen mixture. *J Appl Physiol* 76: 1113–1118, 1994.
2. **Armstrong RB, Delp MD, Goljan EF, and Laughlin MH.** Distribution of blood flow in muscles of miniature swine during exercise. *J Appl Physiol* 62: 1285–1298, 1987.
3. **Balch WE, Fox GE, Magrum LJ, Woese CR, and Wolfe RS.** Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 43: 260–296, 1979.
4. **Bartholomew GA, Vleck D, and Vleck CM.** Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J Exp Biol* 90: 17–32, 1981.
5. **Bauer J, Maier K, Linderkamp O, and Hentschel R.** Effect of caffeine on oxygen consumption and metabolic rate in very low birth weight infants with idiopathic apnea. *Pediatrics* 107: 660–663, 2001.
6. **Fahlman A, Tikuisis P, Himm JF, Weathersby PK, and Kayar SR.** On the likelihood of decompression sickness during $H_2$ biochemical decompression in pigs. *J Appl Physiol* 91: 2720–2729, 2001.
7. **Graham TE, Sathasivam P, and MacNaughton KW.** Influence of cold, exercise, and caffeine on catecholamines and metabolism in men. *J Appl Physiol* 70: 2052–2058, 1991.
8. **Greer F, McLean C, and Graham TE.** Caffeine, performance, and metabolism during repeated Wingate exercise tests. *J Appl Physiol* 85: 1502–1508, 1998.
9. **Katzung BG.** *Basic & Clinical Pharmacology*. Norwalk, CT: Appleton & Lange, 1992, p. 284.
10. **Kayar SR and Fahlman A.** Decompression sickness risk reduced by native intestinal flora in pigs after $H_2$ dives. *Undersea Hyperb Med* 28: 89–97, 2001.
11. **Kayar SR, Fahlman A, Lin WC, and Whitman WB.** Increasing activity of $H_2$-metabolizing microbes lowers decompression sickness risk in pigs during $H_2$ dives. *J Appl Physiol* 91: 2713–2719, 2001.
12. **Kayar SR, Miller TL, Wolin MJ, Aukhert EO, Axley MJ, and Kiesow LA.** Decompression sickness risk in rats by microbial removal of dissolved gas. *Am J Physiol Regul Integr Comp Physiol* 275: R677–R682, 1998.
13. **LeBlanc J, Jobin M, Cote J, Samson P, and Labrie A.** Enhanced metabolic response to caffeine in exercise-trained human subjects. *J Appl Physiol* 59: 832–837, 1985.
14. **Miller TL.** Biogenic sources of methane. In: *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*, edited by Rogers JE and Whitman WB. Washington DC: Am. Soc. for Microbiology, 1991, p. 175–187.
15. **Prosser CL.** *Comparative Animal Physiology*. Philadelphia, PA: Saunders, 1973.
16. **Roy BD, Bosman MJ, and Tarnopolsky MA.** An acute oral dose of caffeine does not alter glucose kinetics during prolonged dynamic exercise in trained endurance athletes. *Eur J Appl Physiol* 85: 280–286, 2001.
17. **Sewester CS.** *Drug Facts and Comparisons*. St. Louis, MO: Wolters Kluwer, 1995, p. 1166–1168.
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.