Effects of prolonged CO\textsubscript{2} inhalation on shivering thermogenesis during cold-water immersion

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Lun V, Sun J, Passias T, Mekjavić IB. Effects of prolonged CO\textsubscript{2} inhalation on shivering thermogenesis during cold-water immersion. Undersea & Hyperbaric Med 1993; 20(3):215–224.—We investigated the effect of prolonged hypercapnia on human thermoregulation during immersion of seven male subjects in a 15°C water bath until their esophageal temperature dropped to 35°C or until 1 h had elapsed. In the control trial, subjects inspired room air, whereas in the other trial the inhaled gas mixture was a 4% CO\textsubscript{2}:20% O\textsubscript{2}:76% N\textsubscript{2} gas mixture. Oxygen uptake (VO\textsubscript{2}, liter · min\textsuperscript{-1}), inspired minute ventilation (V\textsubscript{I}, liter · min\textsuperscript{-1}), esophageal temperature (T\textsubscript{es}, °C), mean unweighted skin temperature (T\textsubscript{sw}, °C), mean heat flux (Q, W · m\textsuperscript{-2}), and electromyographic (EMG, mV) activity of the trapezius muscle were recorded. VO\textsubscript{2} and integrated EMG (IEMG) activity were used as the primary indicators of shivering thermogenesis. There was a tendency for elevated VO\textsubscript{2}, albeit not significant, in the CO\textsubscript{2} trial compared to the air trial. We observed no significant differences in the IEMG between the air and CO\textsubscript{2} trials. These results suggest that prolonged inhalation of a gas mixture containing 4% CO\textsubscript{2} does not have a significant inhibitory effect on shivering thermogenesis and does not enhance the cooling rate of the body core. The absence of any shivering attenuation is most likely due to the small blood P\textsubscript{CO\textsubscript{2}} increase incurred by inhalation of 4% CO\textsubscript{2}, compensation of hypercapnic-induced respiratory acidosis, and a strong thermal drive from core and peripheral regions. It is unlikely that elevated P\textsubscript{t\textsubscript{CO\textsubscript{2}}} levels contribute significantly to the etiology of hypothermia in divers.

temperature regulation, hypercapnia, hypothermia, diving, cooling

Numerous animal studies (1, 2) have demonstrated that inhalation of gas mixtures containing elevated concentrations of CO\textsubscript{2} during cold exposure causes inhibition of shivering tremor and reduction of oxygen consumption. Direct exposure of isolated frog skeletal muscle to elevated CO\textsubscript{2} has also been reported to decrease heat production (3). The effect of CO\textsubscript{2} inhalation on thermal balance in humans, however, is less clear. Bullard and Crise (4) demonstrated a transient depression of heat production and shivering in male subjects exposed to an ambient temperature of 5°C while inhaling for 30 min a gas mixture containing 6% CO\textsubscript{2}; with continued cold exposure, the inhibitory effects of CO\textsubscript{2} inhalation were overcome and heat production and shivering were resumed.
In contrast, Wagner et al. (5) did not observe such suppression of shivering thermogenesis in cold-exposed subjects inhaling 4% CO₂ gas mixtures, as indicated by the absence of significant differences in heat production between the air and CO₂ trials, as well as from accelerometer recordings of shivering activity. The greater rate of core temperature drop observed during the CO₂ trial was attributed to increased respiratory heat loss associated with increased rate of ventilation. However, under conditions of stable skin temperature, this greater rate of core temperature cooling, regardless of the mechanism of heat loss, should have precipitated an elevation in O₂ consumption above that measured in the air inhalation trial. The observation that O₂ consumption was not significantly elevated in the CO₂ trial compared to the air trial suggests that shivering thermogenesis was suppressed.

The present study examined whether cold-water divers would be more susceptible to hypothermia during exposure to hypercapnia. In diving operations, hypercapnia may arise for numerous reasons: the breathing apparatus may increase the dead space of respiration and thus reduce normal CO₂ elimination, consequently increasing FICO₂; in underwater submersibles and atmospheric diving suits, hypercapnia may arise as a result of inefficient CO₂ removal by CO₂ scrubbing systems; body P CO₂ may also increase as a result of increased physical activity or, as documented in some population groups, increased retention of CO₂, or both (6).

We postulated that should hypercapnia attenuate shivering, the rate of core temperature cooling and consequently the progression to hypothermia would be accelerated, as reflected by the O₂ uptake and shivering tremor. Also, the hypercapnia-induced hyperventilation could cause an increase in respiratory heat loss and thus enhance the rate of core cooling during cold-water immersion.

METHODS

The level of hypercapnia (4% CO₂) imposed on the subjects was above that normally anticipated in divers and greatly exceeded permissible levels of exposure (7). Nevertheless, such levels may develop as a result of failure of CO₂ scrubbing systems in manned submersibles, atmospheric diving suits, and diving bells.

Protocol

Seven male subjects (Table 1) were immersed to the neck in a 15°C water bath on two separate occasions. In the control trial, subjects inspired room air; in the CO₂ trial they inhaled 4% CO₂:20% O₂:76% N₂ gas mixture. They remained immersed until their esophageal temperature fell to 35.5°C or until 60 min of immersion had elapsed. The subjects were then removed from the water bath and rewarmed. The order of the two experimental trials was randomly assigned for each subject. The two trials were conducted 1 wk apart and at similar times of the day on each occasion. The protocol was approved by the Ethics Review Committee of Simon Fraser University.

Instrumentation

Esophageal temperature (Tₑₑₑ, °C) was measured with a YSI 702 thermistor probe (Yellow Springs Instruments), which was inserted through a nostril to a depth deter-
Table 1: Subjects' Physical Characteristics

<table>
<thead>
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<th>Subject</th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
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<tbody>
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<tr>
<td>2</td>
<td>24</td>
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</tr>
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<td>6</td>
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</tr>
<tr>
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</tr>
<tr>
<td>± SD</td>
<td>4.2</td>
<td>0.05</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Determined from a regression equation based on sitting height (8). Heat flux (Q, W m⁻²) and skin temperature (Tₘₚₜ, °C) measurements were obtained with heat flux transducers (Concept Engineering) attached with waterproof tape to 6 sites (arm, chest, abdomen, thigh, calf, and back). Electromyograms (EMG) were recorded from the skin overlying the trapezius muscle with 2 2-mm diameter AgCl disc electrodes (Beckmann) spaced 2 cm apart and covered with waterproof tape. Raw surface EMG signals were band-pass filtered (3-5 kHz) and amplified (100 times) with a common mode rejection preamplifier (Grass Instruments, P15D), and recorded with an FM tape recorder (Hewlett Packard, 3968A). The raw analog EMG (mV) was subsequently digitized at a sampling rate of 1,000 Hz, and the DC bias was removed by subtracting the mean of the EMG signal. The digitized EMG was then rectified and integrated over consecutive 10-s periods, and minute values obtained by averaging the integrated EMG (IEMG, mV s⁻¹) of six consecutive 10-s periods. Minute IEMG values were summed for the immersion period, and the IEMG at any given minute was normalized by expressing it as a percentage of the total immersion IEMG (NIEMG, %).

In each trial the inspired gas mixtures were humidified. Inspiratory gas flow was measured using a flow transducer (Hewlett Packard, 47304A) in combination with a pneumotachometer (Hewlett Packard, 21073B). Inspiratory volume (Vᵢ, liter s⁻¹; ATPS) was determined by integrating the inspired flow with a low-drift integrator. Expired gases were directed to a fluted 5-liter Plexiglas mixing box from which a continuous sample was drawn for analysis of mixed expired O₂ (Applied Electrochemistry S-3A oxygen analyzer) and CO₂ (Statham Godart capnograph) concentrations. Oxygen consumption (VO₂, liter s⁻¹; STPD) was calculated from measured values of Vᵢ, F̅O₂, F̅CO₂, and known values of F̅O₂ and F̅CO₂.

Statistical analyses

The VO₂, Vᵢ, TEₘₚₜ, TAₘₚₜ, and Q were compared between the control and CO₂ trials with repeated measures analysis of variance. A linear regression analysis was conducted on the linear portion of the VO₂ response to decreased TEₘₚₜ, and the VO₂-TEₘₚₜ relation compared between the control and CO₂ trials. The IEMGs at TEₘₚₜ of 36.0°.
35.75°, 35.5°, and 35.25°C were compared between the control and CO₂ trials with a two-tailed paired t test.

RESULTS

Results are reported as the mean (±SD) response of the subject group. During the course of the study, ambient barometric pressure was 734.7 (± 2.1) mmHg and temperature 24.2° (± 1.0°) C.

During the rest phase of the experiments, the measured variables were very similar between the control and CO₂ conditions, except that both VO₂ and V̇ indicated significantly elevated by 36 and 31%, respectively, in the CO₂ trial.

Esophageal temperature (Tₑₑ, °C) decayed in a similar manner in both the control and CO₂ trial (Fig. 1 top), reaching end-immersion values of 35.1° (± 0.2°) C in the control and 35.2° (± 0.1°) C in the CO₂ trial. The differences in Tₑₑ and the rate of cooling of Tₑₑ between the two trials were not statistically significant.

Mean unweighted skin temperature (Tₘₚ, °C) values were used rather than weighted values, because weighting factors do not reflect heat production in individual regions (9). Skin temperature displayed a similar time course of response in both trials, plateauing at end-immersion values of 17.4° (± 0.8°) C in the control and 16.3° (± 1.3°) C in the CO₂ trial (Fig. 1 middle). The differences were not statistically significant.

Heat flux (Q, W m⁻²) in both conditions showed a sharp transient increase within the first several minutes of immersion, thereafter decaying in a curvilinear manner to asymptotic values of 222 (± 13) W m⁻² in the control and 235 (± 14) W m⁻² in the CO₂ trial (Fig. 1 bottom). The differences were not statistically significant.

In both trials, oxygen uptake (VO₂, liter · min⁻¹) increased with progressive cooling of Tₑₑ, reaching end-immersion values of 0.86 (± 0.16) liter · min⁻¹ for the control and 1.03 (± 0.18) liter · min⁻¹ for the CO₂ trial (Fig. 2 top). The difference in VO₂ between the trials was not statistically significant. A comparison of the ΔVO₂—ΔTₑₑ relation between both trials, using a test of parallelism, also revealed no significant differences.

Ventilation (V̇, liter · min⁻¹) increased to end-immersion values of 22.61 (± 4.28) liter · min⁻¹ in the control and 34.61 (± 5.63) liter · min⁻¹ in the CO₂ trial. The differences between the control and CO₂ trials were not statistically significant.

No EMG (mV) activity was apparent during the resting phase of the experiment except during voluntary movements. After entry into the water bath, tonic shivering was observed to increase gradually. With continued cooling, the level of tonic shivering further increased and bursts of large amplitude clonic activity began to appear. With decreasing Tₑₑ, this clonic activity grew in frequency, intensity, and amplitude, often to the point where tonic activity was not distinguishable. This pattern of shivering was generally observed in most of the subjects, especially those who were leaner and less muscular. In some of the more muscular subjects, clonic shivering did not appear, although the intensity of tonic shivering seemed to increase.

The IEMG between the two conditions was compared by examining the differences in the rate of increase of IEMG with decreasing Tₑₑ (Fig. 3). A test for parallelism indicated no significant difference in the IEMG—Tₑₑ relation between the control and CO₂ trials.
DISCUSSION

The present study demonstrates that prolonged inhalation of a gas mixture containing 4% CO₂ during cold-water immersion does not attenuate shivering thermogenesis. Both shivering of the trapezius muscle and O₂ uptake were similar for the air and CO₂ trials. Taking into account the added metabolic cost of breathing in the CO₂ trial, any difference in shivering VO₂ between the two trials is further reduced. The absence of an inhibitory effect of CO₂ on heat production confirms the observations of Wagner et al. (5) for exposures to 5°C air, but the significantly greater rate of core temperature cooling that they observed during CO₂ inhalation was attributed to increased respiratory heat loss. In the present study, humidification of the inhaled gas minimized differences in respiratory heat loss. Thus, in the absence of an imbalance between heat loss and heat production, it is not surprising that core cooling rates were similar in the two conditions.

Aside from the effects of CO₂ on heat production, the peripherally induced vasodilative effects of CO₂ would have been expected to also enhance heat loss. However, during the resting phase, measures of cutaneous heat loss, such as skin heat flux and temperature, were found to be maintained at similar levels in the two trials, indicating that even at thermoneutral ambient temperatures, cutaneous vasodilatation was not increased by inhalation of 4% CO₂. Increased heat loss during immersion would thus not be expected and was not observed.

Bullard and Crise (4) reported a noticeable decrease in the heat production of cold-exposed humans after the switch to 30-min inhalation of 6% CO₂ from air. This inhibition was subsequently reversed after 6 min of CO₂ inhalation. In view of the results of earlier studies (4, 5) and the present work, it seems that gas mixtures containing more than 4% CO₂ are required to elicit any noticeable inhibitory effect on shivering thermogenesis in humans. However, the inhibitory effects of higher concentrations (i.e., 6% CO₂) are quickly overcome. Assuming that the inhibitory effects of CO₂ arise secondarily to a decrease in pH of blood and cerebrospinal fluid, with subsequent effects on the proper function of the neural elements involved in thermoregulatory processes, then adequate compensation for acid-base imbalances may explain the absence of inhibitory effects by CO₂. Brackett et al. (10) demonstrated that the respiratory acidosis stemming from inhalation of 7 and 10% CO₂ is quickly compensated for by a rapid rise in plasma bicarbonate levels within 5–10 min of the onset of hypercapnia. Although the steady-state plasma pH and PaCO₂ resulting from inhalation of such mixtures is beyond that considered normal (11), the disinhibition of heat production observed by Bullard and Crise (4) during inhalation of 6% CO₂ suggests that the compensation is sufficient. The inhalation of 4% CO₂, as in the present study, would result in a PaCO₂ of approximately 47 mmHg when estimated from an exponential curve fit of the PaCO₂ values resulting from inhalation of gas mixtures containing 7 and 10% CO₂ (10). As this value lies within the normal physiologic range of blood Pco₂ of 41–49 mmHg (11), inhalation of 4% CO₂ would not be expected to incur any significant alterations in acid-base balance. Consequently, the absence of significant inhibitory effects of CO₂ is not surprising. In our experiment, the evaluation of acid-base homeostasis is further complicated because during hypothermia there are tendencies toward both metabolic acidosis and alkalosis. Metabolic acidosis arises from the hypoxic metabolism of shivering muscles, with resulting lactic acidosis, due to vasoconstriction of surrounding vasculature with cold exposure.
FIG. 1—Response (mean ± SD) to middle, unweighted mean skin temperature, bottom, heat flux during immersion in 15°C water. Subjects inspired either room air (open circle) or a breathing mixture containing 5% CO₂ (closed circle).
FIG. 2—Oxygen uptake (top) and ventilation (bottom) during immersion in 15°C water (values are mean ± SD). Subjects inspired either room air (open circles) or a breathing mixture containing 4% CO₂ (closed circles).
PROLONDED CO₂ INHALATION IN COLD-WATER IMMERSION

![Graph showing normalized IEMG (%)](image)

FIG. 3—Minute normalized IEMG (mean ± sd, %) recorded from the trapezius muscle at Teₐ of 36°C, 35.75°C, 35.5°C, and 35.25°C during immersion in 15°C. Subjects inspired either room air (open circles) or a breathing mixture containing 4% CO₂ (closed circles).

Moreover, the lactic acid elimination capacity of the liver is compromised with core temperature cooling, permitting further elevation of plasma lactic acid levels. Decrease in blood temperature during hypothermia results in an increase in the solubility of CO₂, leading to a decrease in PCO₂, as well as an increase in the buffering efficiency of protein buffers, which constitute the main non-bicarbonate, extra-renal buffer in whole blood and cytoplasm. Both of these combine to bring about an increase in the plasma pH (14). Without proper assessment of acid-base balance with measures of blood pH, PaCO₂, and bicarbonate levels it is difficult to interpret the results of the present study.

The inhibitory effects of CO₂ may also have been overcome by the increasing thermal drive to central thermoregulatory centers that would be expected with decreasing core temperature. In our study it is assumed that with cold-water immersion, Tₘₙ is clamped and thus peripheral thermal drive is relatively constant. Consequently, the gradual decrease in core temperature induced by immersion would serve as an added thermal drive for shivering thermogenesis, in addition to that emanating from skin thermoreceptors, possibly helping to overcome the central inhibitory effects of CO₂ inhalation. From studies of heat production of goats during core temperature cooling with skin temperature clamped above thermoneutral, Jessen (15) estimated that the relative contribution of core and skin temperatures in control of heat production is about 3:1. This lends further support to our conclusion.

In our study, inhalation of a hypercapnic gas mixture did not affect thermal balance. In open-water diving, however, hypercapnia-induced hyperventilation may render divers susceptible to hypothermia by increasing respiratory heat loss. The magnitude of the effect will depend on the ambient pressure and the temperature and humidity of the inspired gas mixture.

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