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THE EFFECT OF CARBON DIOXIDE ON SHIVERING THERMOGENESIS

BY

VICTOR LUN

B.Sc. (Kinesiology)

Simon Fraser University

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the School of Kinesiology

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APPROVAL

NAME: Victor Lun

DEGREE: Master of Science

TITLE OF THESIS: The effect of carbon dioxide on shivering thermogenesis

EXAMINING COMMITTEE:

Chairman: Dr. Glen Tibbits

Dr. Igor Mekjavic
Senior Supervisor

Dr. Tom Richardson

Dr. Ola Eiken
External Examiner
Department of Clinical Physiology
Huddinge Hospital, Huddinge, Sweden

Date Approved: Sept. 4, 1992

ABSTRACT

The effects of both prolonged and acute hypercapnia on human thermoregulation during cold exposure were investigated in two sets of experiments in which hypothermia was induced by immersion of subjects to the neck in a 15°C water bath until their core temperatures dropped to 35°C or until one hour of immersion had elapsed. In the first series of experiments, designed to assess the effects of prolonged exposure to hypercapnia, seven male subjects were immersed on two separate trials. During the AIR trial (control), subjects inhaled compressed air (0.03% CO_2, 20.93% O_2, and balance N_2), while in the CO_2 trial, the inhalate was a 4% CO_2, 20% O_2, and 76% N_2 gas mixture. In the second series of experiments, eight male subjects were immersed and the effects of acute hypercapnia were tested by switching the inhaled gas mixture from compressed air to one containing 4% CO_2, 20% O_2, and 76% N_2 for a 15 minute period beginning when subject core temperatures had attained 36.5°C. During both sets of experiments, oxygen uptake ($\dot{V}O_2$, L-min^{-1}), inspired minute ventilation ($\dot{V}_I$, L-min^{-1}), esophageal and rectal temperatures (Tes and Tre, respectively, °C), mean unweighted skin temperature (Tsk, °C), mean heat flux ($\dot{Q}$, W-m^{-2}), and electromyographic activity (EMG, millivolts) of the trapezius muscle were recorded, from which $\dot{V}O_2$ and integration of EMG activity (IEMG) were used as the primary indicators of shivering thermogenesis.

In the first set of experiments, the difference in the level of IEMG of the trapezius muscle at esophageal temperatures of 36.0, 35.75, 35.5, and 35.0°C were not significantly different between the AIR and CO_2 trials. $\dot{V}O_2$ was slightly higher in the CO_2 trial compared to the AIR trial, but differences were not significant.

In the second series of experiments, although the raw EMG appeared to be noticeably depressed in chart recordings, the IEMG of the trapezius muscle immediately following switch of the inhaled gas mixture from air to 4% CO_2 was not found to be significantly lower compared to the IEMG just before the switch. Moreover, the iEMG immediately after switching the inhalate back to air from CO_2 was not significantly different compared to the IEMG just before the switch. $\dot{V}O_2$ was also not significantly suppressed during inhalation of the 4% CO_2 gas mixture.

The combined results of the study suggest that both acute and prolonged exposures to moderately elevated levels of CO_2 do not have a significant inhibitory effect on shivering thermogenesis. The absence of any shivering attenuation is probably due to the small blood PCO_2 increase incurred by inhalation of 4% CO_2, sufficient compensation of hypercapnic-induced respiratory acidosis, and strong thermal drive from core temperature cooling and cold skin temperature.

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I. INTRODUCTION

The exposure to cold environmental temperatures, such as those often experienced in underwater diving, may lead to an excessive loss of body heat, resulting in a gradual decrease in body core temperature, and ultimately, a state of hypothermia. Many non-thermal factors such as anesthetics, alcohol, and drugs may potentiate hypothermia by preventing or attenuating thermogenic responses to cold exposure (Maclean and Emslie-Smith, 1977). In animal and some human studies (reviewed in Stupfel, 1974; Bullard and Crise, 1961), CO_2 has been reported to suppress shivering thermogenesis. Consequently, it would be expected that the simultaneous exposure to both cold ambient temperatures and elevated CO_2 would lead to a much faster rate of core temperature cooling and thus, a greater susceptibility to hypothermia. Contrary to previous studies, however, Wagner *et al.* (1986) did not observe the inhibition of shivering in hypercapnic human subjects exposed to cold. Thus, the purpose of the present study was to further clarify the effects of hypercapnia on thermoregulatory responses to cold exposure in two separate series of experiments. Unlike previous human studies and most animal studies, cold water immersion was used as the cold stimulus, which permitted rapid cooling of body core temperature, and thus examination of hypercapnic effects at hypothermic levels of core temperature. Cold water immersion also allowed for clamping of the skin temperature and thus control of peripheral contributions to thermoregulatory drive.

The study is presented in four sections:

Section II: Briefly reviews thermoregulation in mammals and offers short discussion about hypothermia, particularily as it relates to underwater diving.

Section III: Reviews the effects of elevated body CO_2 on the function of numerous body systems, with emphasis on how these effects might influence

maintenance of body heat balance and mechanisms of heat production and retention.

Section IV: In this section the effect of prolonged inhalation of 4% CO_2 on shivering thermogenesis was investigated by immersion of 7 subjects on two separate occasions: once while breathing air (control trial) and the other while breathing a 4% CO_2 gas mixture. Shivering thermogenesis of the two trials was compared with measurements of oxygen uptake, IEMG, and rate of core temperature cooling. It is hypothesized that inhalation of 4% CO_2 will inhibit shivering and thus, IEMG and oxygen uptake will be reduced in the CO_2 inhalation trial compared to the air trial, with a consequent faster rate of core temperature cooling.

Section V: The effect of a ten minute period of inhalation of a 4% CO_2 gas mixture on shivering thermogenesis in 8 cold water immersed subjects was assessed with measurements of oxygen uptake and integrated EMG activity. It is again expected that shivering will be suppressed, resulting in an attenuated oxygen uptake and IEMG.

Although sections IV and V are presented as separate studies for the purpose of clarity, the discussions and conclusions drawn from each are complimentary and, as such, the two studies should be considered together in order to obtain a more integrated view of the effect of CO_2 on thermogenesis.

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II. THERMOREGULATION IN MAMMALS

A. Body Heat Balance

The body temperature of humans is maintained within a constant range of temperatures by balancing heat loss to the environment with mechanisms of heat production and heat retention. Heat is lost from the body largely from the skin via mechanisms of conduction, convection, radiation, and evaporation of sweat from the skin. The contribution of each of the above pathways is dependant upon the velocity and specific heat capacity of the medium surrounding the body; the temperature gradient between the body and the surrounding medium; and the rate of sweating. Moreover, the amount of heat lost from the skin may be regulated by altering the vasomotor tone of cutaneous blood vessels. Increasing peripheral blood flow by vasodilation increases heat flow from the core and, when coupled with sweating, further enhances heat loss. Contrarily, a decrease in peripheral blood flow by vasoconstriction reduces heat flow from the core and increases the thickness of the body's insulative shell. Although of lesser significance, heat may also be lost through evaporation of moisture from upper respiratory airways, amounting to an equivalent of 10% of total heat production at rest (Webb, 1982). Heat loss by this mechanism is dependant upon the rate of respiration, the specific heat capacity of the respired gas, and the difference in temperature between the inspired and expired gases (Pasche, 1986).

In mammals, body heat is produced by virtue of the metabolic activity of cells. Body heat may be produced above and beyond that generated by basal metabolism by voluntary skeletal muscle activity and shivering thermogenesis. Shivering thermogenesis is initiated by the appropriate stimulation of neurons localized in the posterior hypothalamus. The arrhythmic descending influences from this shivering center causes oscillations of the stretch reflex as a result of modification of muscle spindle afferent activity. This oscillation in turn allows for

synchronous discharge of spinal motorneurons and consequently, the involuntary shivering tremor that is normally manifested with cold exposure (Kleinebeckel and Klussman, 1990). It is generally reported that the predominant frequency of shivering, as determined from power spectral analysis of the shivering EMG activity, ranges from 7 to 12 Hz (Bawa *et al.*, 1987 and Sessler *et al.*, 1988). However, Muza *et al.* (1986) have measured a predominant shivering frequency of approximately 200 Hz in the masseter muscle, suggesting that shivering frequency is muscle dependant. It is hypothesized that this rhythm of shivering is generated by Renshaw cell-mediated recurrent inhibition of motor neurons (reviewed in Kleinebeckel and Klussman, 1990).

While non-shivering thermogenesis (NST), that is, oxidation of brown adipose tissue, has been identified to be an important mechanism of thermogenesis in smaller mammals such as mice, rats, and rabbits, and possibly in infants, NST is generally not recognized to make a significant contribution to heat production in adult humans (Johnson *et al.*, 1963 and Astrup *et al.*, 1985).

Besides increasing net heat production, body heat may also be increased by retention of the heat produced largely through vasoconstriction of cutaneous blood vessels, effectively increasing the thickness of the insulative shell around the body. Finally, behavioural responses may further augment heat production/retention mechanisms to increase body heat.

B. Mammalian Thermoregulatory System

The mammalian thermoregulatory system has been modelled as a single integrator with numerous inputs from thermoreceptors and outputs to thermoeffectors (Bligh, 1984). Thermoreceptors and thermosensitive neurons

have been located in the body periphery, body core, and in the central nervous system (CNS). Peripherally, thermoreceptors are primarily found in the skin, but have also been reported in skeletal muscle (Jessen *et al.*, 1983). Cutaneous thermoreceptors occur as free nerve endings in the subcutaneous tissue and their variation in depth (between 0.1 and 2.5 mm) beneath the skin surface suggests the ability not only to detect absolute temperature, but also the direction and rate of heat flow (Ivanov, 1990). Thermoreceptors in the body core have been reported in the tissue enclosing the adrenal gland and the root of the superior mesenteric artery and in the abdomen of the ewe (Rawson and Quick, 1972). In the CNS, thermosensitive neurons are located primarily in the spinal cord, medulla, and the hypothalamus (Chai and Lin, 1973; Jessen and Ludwig, 1974). Thermoreceptors and thermosensitive neurons are generally classified as being cold or warm sensitive based on increased or decreased firing rate in response to localized cooling and warming or warming and cooling, respectively.

Although there is evidence to indicate that thermo-integrative centers exist in several areas of the CNS (Satinoff, 1983), thermal balance in humans is regulated primarily by the thermosensitive neurons in the preoptic and anterior (PO/A) areas of the hypothalamus. Neurons in this region are warm or cold sensitive and localized warming or cooling of the these neurons elicits appropriate thermo-regulatory responses. Reports of the proportion of warm to cold receptors in the PO/A range between 26 and 31% to 7 and 10%, respectively (reviewed in Boulant and Dean, 1986). As cold and warm effector mechanism are reciprocally cross inhibited, net increases in cold or warm receptor activity leads to an inhibition (or activation) of heat loss mechanisms and activation (or inhibition) of heat production mechanisms, respectively. As the hypothalamus is also involved in other homeostatic functions, it is not

surprising that thermosensitive neurons have also been found to be sensitive to non-thermal stimuli such as blood pressure, osmolarity, and blood glucose levels, and input from extra-hypothalamic areas (reviewed in Hori, 1991).

Within a range of core temperatures, the core temperature null zone, alterations in cutaneous vasomotor tone may be sufficient to maintain thermal balance (Mekjavic *et al.*, 1991). Deviations of core temperature above or below this core temperature range causes the activation of heat loss and heat production/retention responses. Thermoregulatory effector responses are both autonomic and behavioral in nature (Mekjavic and Bligh, 1987). Autonomic responses include alterations in vasomotor tone, sympathetic drive, respiration, metabolic rate, and shivering or sweating. Behavioral responses include postural changes, donning or removal of clothing, shelter building etc..

C. Underwater Diving: Heat Balance and Hypothermia

Underwater divers and workers in other related activities which take place in ocean waters have the potential risk of increased susceptibility to body heat loss. This problem arises because of several characteristics of the water environment. Firstly, ocean waters are generally cold. Water temperatures are highest near the equator (approaching 30°C at the water surface), gradually decreasing with increasing depth and proximity to the North and South poles (close to 0°C at the surface) (Webb, 1982). Consequently, during immersion, a large thermal gradient is established between the body and water, and combined with the high thermal-conductivity of water, heat loss from the body occurs primarily by circulation of water around the body (convective heat loss), and to a lesser extent by conductive and radiative heat loss. In contrast to sea-level, respiratory evaporative heat loss is an important pathway of heat loss in diving. Heat loss by this pathway is dependant upon the rate of respiration, the

difference in temperature between the inhaled and exhaled gas, and density and specific heat capacity of the inhaled gas (Pasche, 1986). As compressed gas mixtures are usually cool and dry, the necessity of endogenous warming and humidification of inhaled gas mixtures, combined with higher rates of respiration, may lead to significant levels of respiratory evaporative heat loss. In fact, Piantadosi *et al.* (1981) observed that inhalation of cooled He-O_2 caused a noticeable drop in the rectal temperature of subjects despite increased $\dot{V}O_2$ and a maintenance of a warm ambient temperature. Moreover, the thermo-conductivity of inhaled gases is enhanced as the density of inhaled gases increases with elevation of ambient pressure experienced during diving, which may further enhance ventilatory heat loss.

Ultimately, uncompensated heat loss, regardless of environment, leads to onset of hypothermia. Generally, mild hypothermia is considered to have occured when body core temperature drops below 35°C. Clinical signs associated with hypothermia are numerous and may include mental confusion, lethargy, poor speech articulation, muscle rigidity, and loss of tendon reflexes (reviewed in Webb, 1982 and MacLean and Emslie-Smith, 1977). Aspects of motor and mental performance such as manual dexterity, tactile sensation, tracking efficiency, reaction time, and arithmetic and logical reasoning are also greatly impaired. These consequences of hypothermia are potentially life threatening and as such, all factors which may contribute or potentiate hypothermia must be recognized in order that proper precautions may be taken to prevent its onset.

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III. THE EFFECTS OF CARBON DIOXIDE ON THE FUNCTION OF VARIOUS BODY SYSTEMS

A. Introduction

To date, most hypothermia research has focussed on the contribution of thermal factors, namely the factors directly responsible for heat loss in cold environments, the innovation of insulative garments to reduce heat loss, and methods of exogenous heat production. There is much less work on how various non-thermal factors may affect thermoregulatory centers and mechanisms of heat production and heat retention, which in turn influence body heat balance and the onset of hypothermia. For example, it is well established that anesthetics induce body cooling both by increasing heat loss through cutaneous vasodilation and suppression of heat production by attenuation of shivering thermogenesis (reviewed in Passias, 1990). Carbon dioxide (CO_2) is another such non-thermal factor which has been implicated in preventing proper thermoregulatory responses during cold exposure (reviewed in Stupfel, 1974). However, unlike most other non-thermal factors, CO_2 is a normal constituent of the body as it is generated by oxidative metabolism,

$$\text{glucose} + O_2 \dashrightarrow ATP + H_2O + CO_2 \qquad [1]$$

Although primarily a waste product, CO_2 plays an important role in the function of several body systems. For example, in its bicarbonate ion form, which is produced as seen in the following reactions, with involvement of the enzyme, carbonic anhydrase (CA),

$$CO_2 + H_2O \overset{CA}{\Longleftrightarrow} H_2CO_3 \Longleftrightarrow H^+ + HCO_3^- \qquad [2]$$

CO_2 serves as a principal buffer of the body. CO_2 is also a substrate for the production of oxaloacetate from pyruvate. Finally, through H^+, produced as

shown in equation 2, CO_2 indirectly acts to drive peripheral and central chemoreceptors to stimulate breathing (Lambertsen, 1980a).

Thus, as CO_2 is not physiologically inert, its level in the body must be maintained constant. The partial pressure of CO_2 (PCO_2) in the body normally ranges between 25 to 60 mmHg (Lambertsen, 1980c). The maintenance of the body's PCO_2 within this range is dependant upon the rate of endogenous CO_2 production, the amount of CO_2 that is inspired, and the rate of elimination of CO_2.

The main contributor to the body's CO_2 stores is that which is produced metabolically in the body's tissues, normally amounting to a partial pressure of 46 mmHg in the venous blood ($PvCO_2$). It is transported to the lungs and subsequently eliminated. A large amount of CO_2 remains in the blood after it has passed through the lungs, however, as indicated by a partial pressure of CO_2 in the arterial blood ($PaCO_2$) of 40 mmHg. Inspired ambient air has a negligible contribution to the body's CO_2 stores as the partial fraction of CO_2 in ambient air (F_ICO_2) is 0.03%, which only gives an inspired CO_2 partial pressure (P_ICO_2) of 0.23 mmHg. The expired partial pressure of CO_2 (P_ECO_2) is close to 40 mmHg because of the equilibration of the partial pressure of alveolar CO_2 (P_ACO_2) with the $PaCO_2$. These various measures of body PCO_2 represent resting values, and as the rate of CO_2 production ($\dot{V}CO_2$) may actually range between 0.36 $L \cdot min^{1}$ at rest to 5.0 $L \cdot min^{-1}$ during maximal exercise, body PCO_2 may also fluctuate accordingly.

Extreme endogenously or exogenously induced variation of body PCO_2 above (hypercapnia) or below (hypocapnia) its normal range of values has effects on many of the body's systems. Of particular importance to the present investigation is the condition of elevated body PCO_2, which may occur, if the rate of CO_2 production increases, for example as seen in exercise; if P_ICO_2 is

increased and; if CO_2 elimination is reduced or prevented by respiratory disease such as emphysema or use of depressant drugs (Lambertsen, 1980b).

This investigation focusses on the effects of increasing P_ICO_2, brought about by elevating the F_ICO_2. Increasing P_ICO_2 not only increases CO_2 intake, but also prevents normal elimination of metabolically produced CO_2. Consequently, the effect of elevating P_ICO_2 is to elevate P_ACO_2, and subsequently $PaCO_2$, resulting in a condition of hypercapnia. The effect of elevating P_ICO_2 on P_ACO_2 is dependant upon rate of ventilation and $\dot{V}CO_2$, as seen in the following formula (Lanphier and Camporesi, 1978):

$$P_ACO_2 = k \cdot \frac{\dot{V}CO_2}{V_A} + P_ICO_2 \qquad [3]$$

As inequalities occur between alveolar ventilation and perfusion, $PaCO_2$ is not the same as P_ACO_2, although they are generally assumed to be equal. $PaCO_2$ can be accurately predicted using regression formulas determined from simultaneous measurement of end-tidal P_{CO_2} ($P_{ET}CO_2$), an estimate of P_ACO_2, and from measurements of V_T and $PvCO_2$ (Jones *et al.*, 1979):

$$PaCO_2 = 2.3 + 0.75P_{ET}CO_2 - 0.00205V_T + 0.14PvCO_2 \qquad [4]$$

B. Physiological Effects of Hypercapnia

1. Biochemical

A major secondary effect of elevated body PCO_2 is an increase in the concentration of H^+ ions (due to the action of carbonic anhydrase, as indicated in equation 2), resulting in a condition of respiratory acidosis. Thus, it should be

noted that the physiological effects of CO_2 may not only be due to a direct effect of CO_2 itself, but may also be due to the secondary effects of decreased blood pH. Aside from elevated levels of P_ICO_2, respiratory acidosis may also be brought about by breath holding, respiratory obstruction, respiratory disease (for example, emphysema), or depression of the respiratory center by drugs (Lambertsen, 1980c and Rose, 1989).

2. Cardiovascular System

Hypercapnia has both peripheral and central effects on the cardiovascular system. CO_2 locally and directly causes vasodilation of peripheral blood vessels. Diji (1959) initially demonstrated this action by calorimetrically determining heat elimination from the hands of subjects which were immersed in separate 29 °C water baths, one of which was saturated with CO_2. A 40% greater heat elimination was measured from the hand immersed in water saturated with CO_2. This was attributed to the local vasodilatatory effect of CO_2 on the hand's cutaneous blood vessels. Centrally, hypercapnia indirectly enhances the stimulation of aortic and carotid chemoreceptors through an increase in the concentration of H^+ ions, resulting in increased sympathetic nervous outflow. This subsequently leads to increases in circulating catecholamines, peripheral blood vessel vasoconstriction, systolic and diastolic blood pressure, and heart rate (Price, 1960). Evidence from current literature suggests that the local vasodilatatory effects of CO_2 predominate over the aforementioned neurogenically induced vasoconstriction, giving rise to decreased peripheral resistance despite increased sympathetic outflow. Sokoloff (1960) observed a 50% increase in cerebral blood flow during inhalation of a 5-7% CO_2. Sokoloff further suggests that the increase in brain volume arising from increased blood flow may be responsible for the

headaches that human subjects often experience following CO_2 inhalation. In dogs ventilated with 0 to 5% CO_2, Brickner *et al.* (1956) observed an initial decrease in mesenteric blood flow (MBF) and increase in blood pressure (BP) upon administration of CO_2, which was shortly followed by an increase in MBF and a decrease in BP. This observation was attributed to a centrally elicited increase in vasomotor activity immediately following CO_2 administration, resulting in decreased MBF and increased BP, which was subsequently over-ridden by local vasodilatatory effects of CO_2 directly on the mesenteric vasculature, resulting in a reversal of MBF and BP. In other vascular circulations - skeletal muscle, splanchnic, and renal - local vasodilation is balanced by reflexive and central effects and thus, there is little change in peripheral resistance in these vascular beds (Price, 1960).

3. Nervous System

Hypercapnia causes both stimulation and depression of the nervous system depending on the acuity, duration, and concentration of the CO_2 that is administered. Woodbury *et al.* (1958) used the threshold for electrically induced seizures (electro-shock seizure threshold, EST) to measure the effect of various concentrations of CO_2 on the excitability of the brain of rats and mice. With increasing F_ICO_2, the EST was observed to gradually increase, peak at 12.5% for mice and 15% for rats, and then gradually decrease to below pretreatment levels at F_ICO_2's of 30-40%. Functionally, this was a demonstration of a triphasic effect of CO_2: at low F_ICO_2 there was direct depression of cortical excitation; at intermediate levels (12-20%) there was enhanced cortical excitability, due to the activation of subcortical centers (hypothalamus and reticular system) and increased release of sympatho-adrenal catecholamines; at high P_ICO_2 (25%+) anesthesia was induced,

attributed to depression of cortical and subcortical areas. In dogs, P_aCO_2 greater than 95 mmHg (12.5% CO_2) progressively gives rise to inert gas-like narcosis and complete anesthesia is achieved at P_aCO_2 of 245 mmHg (Eisele *et al.*, 1967). The narcotic effect of hypercapnia has been attributed entirely to the decrease in cerebral pH, with narcosis being induced when cerebrospinal fluid pH drops below 7.1 and reaching a maximum at a pH of 6.8. (Atkinson *et al.*, 1987; Eisele *et al.*, 1967). In humans, exposure to F_ICO_2 less than 4% does not produce significant effects and may even go unnoticed (Lambertsen, 1980c). Elevation to 4 to 7% produces mild dyspnea, but is generally tolerable. With further increases up to 10% , respiration is greatly stimulated and subjects may experience headaches, depression, dizziness, and confusion. Further increases in F_ICO_2 lead to loss of voluntary activity, loss of consciousness, and convulsions. McAleavy *et al.* (1961) demonstrated a reduction in the concentration in the nitrous oxide (N_2O) required to reach a threshold of unconsciousness as the concentration of inhaled CO_2 was increased. The 40 mmHg decrease in PN_2O concentration for every 10 mmHg increase in PCO_2 led the authors to estimate that CO_2 has anesthetic potency four times that of N_2O.

At the neuronal level, the effects of hypercapnia are generally depressive. Lorente de No (referenced from Carpenter *et al.*, 1974) observed that exposure of the frog sciatic nerve to 5% CO_2 caused an increase in the amplitude of the nerve's threshold stimulus, a decrease in the nerve's conduction velocity, an increase in the duration of action potentials, and an increase in the resting membrane potential. The effects of hypercapnia on cortical and spinal neurons has also been studied. Neurons of the sensori-motor cortex (Carpenter *et al.*, 1974) and the membrane potentials of neo-cortical neurons (Lehmenkuhler *et al.*, 1989) of cats demonstrated

hyperpolarization during exposure to 10% CO_2 and during apnea induced by breathing pure oxygen, respectively. The firing rate of neurons in the pre-optic (PO) area of rats, measured *in vivo*, was seen to increase with exposure to 5% CO_2 (Matsumara *et al.*, 1987). In contrast, Tamaki *et al.* (1989) observed a decrease in the firing rate of neurons in the PO area when measured *in vitro* from rat brain slices exposed to 5% CO_2. The authors concluded that CO_2 has a direct inhibitory effect on neurons but synaptic inputs to the PO area during *in vivo* CO_2 exposure produce excitation of PO neurons, as observed by Matsumara *et al.* (1987). Membrane potentials of cultured and isolated spinal ganglion cells measured *in vitro* demonstrated depolarization during hypercapnia (Lehmenkuhler *et al.*, 1989). However, when pH changes of fluid bathing the cells were minimized by including a buffer, membrane potential hyperpolarization was observed, giving rise to the conclusion that while an extracellular decrease in pH causes depolarization, CO_2 has a direct effect of hyperpolarization. Carpenter *et al.* (1974) showed that depression of the monosynaptic reflex in cats artificially ventilated with 10% CO_2 was brought about by a transient hyperpolarization of Ia terminals.

4. Neurohormonal

Hypercapnia, as already alluded to, causes a significant increase in the release of catecholamines, specifically, from the adrenal medulla and heart (Tenney, 1960 and Sechzer, 1960). One significant consequence of increased catecholamine release is stimulation of the cardiovascular system, namely increased cardiac output, myocardio-contractility, heart rate, and systolic and diastolic blood pressure (reviewed in Price, 1960). In addition, it has also been reported that CO_2 causes the increased release of ACTH from the anterior pituitary which leads to an increase in the release of cortisol from the adrenal

cortex. Tenney (1960) suggests that the increased secretion of cortisol circumvents any attenuating effect that decreased pH has on the sympathetic actions of epinephrine (Tenney, 1960).

Hypercapnia also affects the metabolism of acetylcholine (Ach), which appears in both the sympathetic and parasympathetic divisons of the autonomic nervous system, as well as in the somatic nervous system. The synthesis of Ach and activity of acetylcholinesterase, which is the enzyme responsible for breaking down Ach, are both reduced by decreases in pH, which arises secondarily to increased PCO_2 (reviewed in Tenney, 1960).

5. Mental Performance

Sayers *et al.* (1987) examined the effects of inhaling different concentrations of CO_2 on mental performance and found that a threshold level of CO_2 was required to elicit decrements in mental performance. The authors measured significant increases in the times required to complete subtraction and logic problems only when $P_{ET}CO_2$ was greater than 51 mmHg, which was elicited by breathing at least 6.5% CO_2. Short term memory was not noticeably affected. The decrement in speed of performance was attributed to a narcotic effect of CO_2, as well as to the stress caused by tachypnea. The threshold effect reported by Sayers *et al.* (1987) is indirectly supported by Storm and Gianetta (1974), who reported that chronic exposure to 4% CO_2 was not sufficient to cause any significant decrement to problem solving ability, as well as to complex tracking and eye-hand tasks. Contrarily, Vercruyssen (1984), using various measures of psycho-motor performance, showed that inhalation of 4% CO_2 caused a significant increase in information processing time by impairing the response selection stage of processing.

6. Respiratory System

As CO_2 is normally the primary stimulus for breathing, hypercapnia further augments central and peripheral chemoreceptor drive, stimulating breathing, as evidenced by the increase in respiratory minute volume ($\dot{V}_E$) brought about by elevations in both respiratory rate (f) and tidal volume (V_T). The relative contributions of increasing f and V_T to the elevation of $\dot{V}_E$ are dependant upon the concentration of CO_2 and duration of exposure. Reynolds *et al.* (1972) demonstrated that during inhalation of 3% CO_2, increases in $\dot{V}_E$ are almost entirely due to increase in V_T. However, it was observed that during inhalation of gas mixtures containing 5-7% CO_2, although V_T and f both initially increase, increases in V_T quickly reach a plateau, after which further increases in ventilation are brought about by elevating f. Furthermore, increases in $\dot{V}_E$, f, and V_T brought about by inhalation of 2, 4, and 6% CO_2, are linearily related to $PaCO_2$, thus indicating that CO_2 is continually active in stimulating respiration and that no threshold exists for a respiratory response to CO_2 (Lambertsen, 1980b). Acute inhalation of excessive concentrations of CO_2 (30%) bring about depression of respiration, not due to respiratory center inhibition, but because of interference of respiration by the onset of convulsions (Lambertsen, 1980b).

Schaeffer (1958) has distinguished two patterns of respiratory response to CO_2 inhalation - low and high ventilation groups - based on the response to inhalation of 5.4 and 7.4% CO_2. The low ventilation group had a relatively lower increase in $\dot{V}_E$, higher increase in V_T, and higher $PaCO_2$ compared to the high ventilation group. This pattern of breathing was consistent with that which was observed during air breathing. This variation in the respiratory response to hypercapnia suggests individual differences in tolerance and retention of CO_2. That is, "CO_2 retainers" are individuals who are able tolerate elevated levels of body PCO_2 and thus have lower ventilatilatory responses to

hypercapnia. This phenomenon has been described particularly in divers (Florio *et al.*, 1979), and is suggested to be part of the adaptive response to prolonged exposures to CO_2.

B. Effects of Hypercapnia on Thermoregulation in Mammals During Cold Exposure

Studies in small animals (Lyszczarz *et al.*, 1980; Plewes and Jennings, 1972; Schaefer and Wunnenberg, 1976; Schaefer *et al.*, 1975; and reviewed in Stupfel, 1974) and humans (Bullard and Crise, 1961; reviewed in Stupfel, 1974; and Wagner *et al.*, 1983) have demonstrated that hypercapnia during cold exposure leads to a faster rate of core temperature (Tc) cooling compared to inhalation of air, and if persisted at sufficiently high CO_2 concentrations and for long enough duration, leads to mortality. Decreases in Tc are generally paralleled by increases in respiration rate ($\dot{V}_E$) and circulating catecholamines, decreases in blood pH, and most significantly, decreases in oxygen consumption ($\dot{V}O_2$). As shivering is the predominant mechanism for thermogenesis during cold exposure, and $\dot{V}O_2$ is an indirect indicator of metabolic and muscular activity, a decrease in $\dot{V}O_2$ during CO_2 exposure would be an indication of suppression of both shivering and non-shivering thermogenic activity. Indeed, Schaefer *et al.* (1975) reported a drop in $\dot{V}O_2$ below basal levels in guinea pigs exposed to 15% CO_2. Bullard and Crise (1961) observed a transient reduction in body metabolism of human subjects at the onset of an acute 30 minute period of inhaling 6% CO_2 while exposed to 5°C. The authors additionally reported that shivering was not visually detectable. However, unlike any of the animal studies, 6 minutes after beginning the CO_2 inhalation period, disinhibition of shivering and oxygen consumption occurred. The authors suggested that this disinhibition resulted

from increased respiratory evaporative heat loss and depressed heat production creating a sufficiently large thermal drive to overcome the inhibitory effects of hypercapnia. The inhibitory effects of CO_2 have been further substantiated by studies using more direct measures of shivering muscular activity. Lyszczarz *et al.* (1980), using EMG, observed a considerable depression of electrical activity in the quadriceps muscle of rabbits inhaling 6% CO_2 and resumption of activity after return to air inhalation. Kitano (1989) reported that exposure of frog skeletal muscle to 5% CO_2 increased muscle relaxation and reduced maximum force of contraction, and consequently, reduced the amount of stable heat produced during steady contraction. This observation was attributed to a decrease in the intracellular pH and the resulting reduction in the rate of activity of the enzymes myosin and Ca^{2+} ATPase.

Pepelko and Dixon (1974) used $\dot{V}O_2$ as an estimate of nonshivering thermogenesis (NST) in cold acclimatized rats. Inhalation of 5, 10, and 20% CO_2 lead to a progressive decrease in $\dot{V}O_2$, and NST was completely eliminated during inhalation of CO_2 concentrations greater than 10%. NST generally does not make a significant contribution to heat production in adult humans (Johnson, 1963 and Astrup *et al.*, 1985).

Besides the suppression of shivering, Plewes and Jennings (1972), Schaefer and Wunnenberg (1976), and Wunnenberg and Baltruschat (1982) additionally noted that CO_2 inhalation causes decreases in the core threshold temperature of shivering onset in dogs and guinea pigs, and of non-shivering thermogenesis in hamsters, respectively. The authors of the first and last studies suggest that this observation is due to a shift in the hypothalamic set point temperature for thermoregulatory responses to cold. Schaefer and Wunnenberg (1976), however, attribute the decreased threshold shivering

temperature to a transient decrease in hypothalamic norepinephrine observed immediately following the start of chronic exposure of guinea pigs to 15% CO_2. The return of hypothalamic norepinephrine levels to control values was paralleled by a return of threshold shivering temperatures to control values. The importance of hypothalamic norepinephrine levels in thermoregulation is further supported by Zeisberger and Bruck (1971) who were able to elicit shivering in guinea pigs above normal threshold temperatures after intra-hypothalamic injections of norepinephrine.

In contrast to previous animal and human studies, Wagner *et al.* (1983) did not observe a suppression of shivering or oxygen consumption in human subjects inhaling 4% CO_2 while exposed to 5°C air. Enhanced shivering was observed after cessation of CO_2. The authors attributed the greater rate of core temperature drop observed during periods of CO_2 inhalation to increased respiratory evaporative, convective, and conductive heat loss associated with hyperventilation induced by CO_2 inhalation.

C. Effects of CO_2 on Mechanisms of Body Heat Maintenance

As discussed earlier, hypercapnia leads to increases in the rate of respiration, tidal volume, and vasodilation of cerebral and skin blood vessels, and consequently, would be expected to cause increases in respiratory evaporative, radiative, and convective heat loss. Evidence from both human and animal studies suggest that during hypercapnia, respiratory evaporative heat loss is more significant than cutaneous and scalp heat loss. Stupfel (1974) found diminished skin blood flow but enhanced respiratory heat loss in rats exposed to 10 and 33% CO_2. Bullard and Crise (1961) estimated that the average rate of respiratory evaporative heat loss of 40 $cal \cdot m^{-2} \cdot hr^{-1}$ in humans at the end of a 30 minute period of inhalation of 6% CO_2 was about four times

that of the average control value. Based on skin temperature measurements, the authors also concluded that CO_2 inhalation had not prevented the cutaneous vasoconstriction that would have been expected during the exposure to an ambient temperature of 5°C, and thus, convective heat loss from the skin would not have been significant.

Several investigators have demonstrated that CO_2 and its concomitant acidosis also has effects on general metabolism (reviewed in Nahas, 1974). *In vivo* and *in vitro* experiments demonstrated that despite increases in circulating catecholamines during hypercapnia, there is a decrease in the level of cellular lipolysis and serum free fatty acids, decreased cellular glucose uptake, and inhibition of phosphofructokinase. The consequent reduction in oxidative activity would lead to a decrease in cellular respiration and thus possibly contribute to the observed decrease in oxygen utilization during hypercapnia.

Some investigators (reviewed in Stupfel, 1974; Schaefer *et al.*, 1975) have observed that the exposure of small mammals to low concentrations of CO_2 actually leads to an increase in $\dot{V}O_2$ and a transient increase in body temperature. This has been attributed mainly to the increase in the amount of respiratory work due to the increased rate of ventilation induced by hypercapnia. Another aspect of CO_2 inhalation which may also enhance heat retention is the lower thermal conductivity of gas mixtures with higher FCO_2 , which would reduce the heat carrying capacity of inhaled gas mixture, and consequently the amount of heat lost from respired gases. Also, the higher density of CO_2 rich gas mixtures (1.248 $g \cdot L^{-1}$ for 7% CO_2 , balance air gas mixture compared to 1.205 $g \cdot L^{-1}$ for only air; Macdonald and Wann, 1978) would also increase the work of breathing and consequently increase oxygen consumption. In fact, Glauser *et al.* (1967) have reported that the oxygen cost of

ventilation of a 7% CO_2 gas mixture is 80 mL-min^{-1} (STPD) greater than ventilation of air (0.03% FCO_2).

D. Acclimatization to Chronic Hypercapnia

1. Body Temperature

Exposure of guinea pigs and rats to 15% CO_2 for 7 days (Schaefer *et al.*, 1975) and dogs to 5% CO_2 for 2 and 14 days (Jennings, 1979) led to an initial drop in body temperature, followed by a return to control values within 3 and 2 days, respectively. The maximal drop in body temperature of the guinea pigs and rats occured 6 hours after beginning CO_2 administration.

2. Respiration

Clark *et al.* (1971) and Jennings (1979) demonstrated an elevation of the threshold $PaCO_2$ required to elicit a ventilatory response in human subjects after being exposed to 4% CO_2 for 24 hours and in dogs after being exposed to 5% CO_2 for 2 days, respectively. The threshold $PaCO_2$ returned to control levels within 14 days of beginning exposure in the dog, while in the human subjects, the threshold $PaCO_2$ remained elevated until after CO_2 inhalation was terminated.

The pattern of respiratory response has been observed to change with acclimatization to chronic hypercapnia. Clarke *et al.* (1971) saw a two to three fold increase in $\dot{V}_E$ initially following hypercapnia which returned to control values within 24 hours. Schaefer *et al.* (1963a) reported similar findings in humans, although the return of $\dot{V}_E$ to control values occurred over the period of a 42 day exposure to 1.5% CO_2, and even fell below control values. During the exposure period, the authors additionally observed a gradual decrease in the respiratory rate which was paralleled by an increase in tidal volume and

respiratory dead space. The increase in dead space was attributed to the dilation of pulmonary airways resulting from relaxation of smooth muscle by a direct and local effect of CO_2. The results of the study also indicate that respiratory acidosis was uncompensated for the first 23 days of the 42 day exposure, indicating a high tolerance for CO_2 retention.

3. Acid-Base Balance

Schaefer *et al.* (1975) observed that after beginning exposure to 15% CO_2, the maximal drop of pH and body temperature in guinea pigs (0.4 pH units) and rats (0.330 pH units) occured between 1 and 6 hours . By the end of the 7 day exposure, pH had almost returned to control levels. In a 5 day exposure of human subjects to 4% CO_2, Clarke *et al.* (1971) observed an immediate decrease in arterial pH upon CO_2 exposure, reaching a maximal drop after 20 hours and then return back to control values within 5 days. Arterial bicarbonate ion (HCO_3^-) concentrations paralleled the observed changes in arterial pH. In contrast, Schaefer *et al.* (1963b) observed a decrease in plasma pH of 0.06 units in human subjects, which remained uncompensated for 23 days after beginning exposure to 1.5% CO_2. The authors observed an additional release of CO_2 from body stores 8-9 days after beginning recovery from CO_2 exposure. The authors suggest that the high correlation of plasma calcium concentrations with CO_2 excretion, that is, decrease during the first 23 days CO_2 exposure, a return to normal levels, and then a subsequent increase to above control levels 8-9 days after exposure, indicate that storage of CO_2 in bone plays a significant role in acclimatization and deacclimatization to chronic hypercapnia. Further studies (reviewed in Schaefer, 1979) suggest that during the initial stages of chronic exposure to low CO_2 concentrations (0.7-1.5%), bone plays a comparatively more dominant role in acid-base balance than the

kidney. The release of CO_2 from bone, however, is of sufficient magnitude to elicit the participation of the kidney. Bone CO_2 retention is cyclical, with the period of uptake and release being between 15-25 days and 10-15 days, respectively (Schaefer, 1979).

In summary, hypothermia in humans may be brought about by excessive heat loss to the environment or insufficient heat production. Evidence has been presented which demonstrate that elevated levels of body PCO_2 may lead to both enhanced heat loss, through increased rate of respiration and cutaneous vasodilation, and suppression of heat production, primarily by the attenuation or inhibition of shivering thermogenesis. Consequently, CO_2 cannot be disregarded as a contributing factor to hypothermia in humans.

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IV. THE EFFECTS OF PROLONGED CO_2 INHALATION ON SHIVERING THERMOGENESIS

A. Introduction

Numerous animal studies (Lyszcarz *et al.*, 1980 and Stupfel, 1974) have demonstrated that inhalation of gas mixtures containing elevated concentrations of CO_2 during cold exposure causes inhibition of shivering tremor and reduction of oxygen consumption. Furthermore, direct exposure of isolated frog skeletal muscle to elevated CO_2 has been reported to decrease heat production (Kitano, 1988). The effect of CO_2 inhalation on thermal balance in humans, however, is less clear. Bullard and Crise (1961) demonstrated a transient depression of heat production and shivering in male subjects exposed to an ambient temperature of 5°C during a 30 minute period of inhaling a gas mixture containing 6% CO_2. However, with continued cold exposure, the inhibitory effects of CO_2 inhalation were overcome and heat production and shivering were resumed. In a more recent study, however, Wagner *et al.* (1983) did not observe suppression of shivering thermogenesis in cold exposed subjects inhaling 4% CO_2 gas mixtures for one hour periods. This was concluded from the absence of significant differences in heat production between the air and CO_2 trials, as well as from accelerometer recordings of shivering activity. The greater rate of core temperature drop that they observed during the CO_2 breathing periods, compared to the air breathing periods, was attributed to increased respiratory convective, conductive, and evaporative heat loss associated with increased rate of ventilation induced by CO_2 inhalation.

It still remains unclear, however, whether CO_2-induced depression of shivering can be eliminated as a contributing factor to enhanced core temperature cooling during cold exposure. Under conditions of stable skin temperature, the greater rate of core temperature cooling that was observed by Wagner *et al.* (1983) during CO_2 breathing, regardless of the mechanism of heat loss, should have precipitated an elevation in oxygen consumption above

that measured in the air inhalation trial. The observation that oxygen consumption was not significantly elevated in the CO_2 trial compared to the air trial, would in fact suggest that shivering thermogenesis was suppressed. Therefore, the present study was designed to examine the effects of CO_2 on heat production during cold exposure while minimizing the contribution of respiratory heat loss to overall heat loss.

The present study was also designed to assess whether cold water divers would be more susceptible to hypothermia during hypercapnic exposure. In diving operations, hypercapnia may arise for numerous reasons: the breathing apparatus may increase the dead space of respiration and thus reduce normal CO_2 elimination, consequently increasing F_ICO_2; in underwater submersibles hypercapnia may arise as a result of inefficient CO_2 removal by CO_2 scrubbing systems; body PCO_2 may also increase as a result of increased physical activity, and /or, as documented in some population groups, increased retention of CO_2 (Florio *et al.*, 1979).

In the present study, thermal balance of subjects immersed in 15°C water and breathing normal air was compared to that observed when the inhalate was comprised of 4% CO_2, 20% O_2, and 76% N_2. These conditions were not chosen to simulate normal diving operations, but to examine the responses during extremes of exposure to cold and hypercapnia. Namely, divers using dry suits or wet suits perfused with warm water are unlikely to encounter a cold water stimulus of 15°C, and are thus less vulnerable to hypothermia than divers using regular wet suits. Divers in manned submersibles, atmospheric diving suits and diving bells are also insulated from the surrounding cold water, and though the cabin air temperature may drop to levels slightly above that of the surrounding water, skin temperature is normally maintained well above the air

temperature. In terms of thermal stress, the present study simulates the worst case situation a cold water diver wearing a wet-suit may experience.

Similarly, the level of hypercapnia imposed on the subjects is above levels normally anticipated in divers. Indeed, inhalation of 4% CO_2 greatly exceeds permissable levels of exposure (Miller, 1991). Although this hypercapnic exposure would not reflect a normal diving scenario, it is possible that such levels could develop as a result of failure of CO_2 scrubbing systems in manned submersibles, atmospheric diving suits and diving bells.

It was postulated that should hypercapnia attentuate shivering, the rate of core temperature cooling and consequently the progression to hypothermia would be accelerated, as reflected by the oxygen uptake and shivering tremor. As well, the hypercapnia-induced hyperventilation may cause an increase in respiratory heat loss and thus enhance the rate of core cooling during cold water immersion.

B. Methods

1. Subjects

Seven male subjects were recruited to participate in this investigation. Their participation was subject to a physician's approval. Before signing an informed consent form, each subject was familiarized with the experimental protocol and reminded that they could withdraw from the study at any time.

2. Protocol

Clad only in swim trunks, subjects were instrumented while lying supine. Following recording of resting values for 10 minutes, subjects were seated in a webbed chair and immersed to the neck in a 15°C waterbath in two separate experimental trials. In the AIR trial (control), subjects inhaled air (0.03% CO_2, 20.93% O_2, and 79.04% N_2), while in the CO_2 trial they inhaled a 4% CO_2, 20% O_2, and 76% N_2 gas mixture. Subjects were immersed to the neck until their core temperature, as measured in the esophagus, fell to 35.5°C, or until 60 minutes of immersion had elapsed. The subjects were then removed from the water bath and rewarmed passively for 30 minutes while lying supine and covered with an insulated blanket. Upon completion of the rewarming period, the experiment was terminated, instrumentation removed, and the subjects' heat content was reinstated with immersion in a hot bath.

The order of the two experimental trials was randomly assigned for each subject. The two trials were conducted one week apart, and at similar times of the day on each occasion, to prevent acclimitization and circadian rhythm effects, respectively. Subjects were instructed not to undergo heavy exercise on the days of their trials and to consume a light meal 2-3 hours before each trial.

The protocol used in the present study was approved by the Ethics Review Committee of Simon Fraser University.

3. Instrumentation

Immersion Tank. The immersion tank was a 2.1 m x 1.05 m x 2.1 m fiberglass shell and was normally filled with approximately 4000 L water. The water was constantly circulated with a pump (Swimquip Spa-Support Systems) and filtered (Triton). Circulated water was passed through a refrigeration unit controlled by a thermoregulation system (Honeywell) to maintain the water temperature constant at 15°C.

Core Temperature. Rectal temperature (Tre, °C) was measured with a rectal thermistor probe (YSI 701, Yellow Springs Instruments) inserted 15cm beyond the anus by the subject. Another thermistor probe (YSI 702, Yellow Springs Instruments) was inserted through the nostril and subsequently "swallowed" into the esophagus for recording esophageal temperature (Tes, °C). The insertion length of the esophageal probe was determined from a regression equation based on sitting height, as suggested by Mekjavic and Rempel (1990), and situated the thermistor in the region of the esophagus nearest the left ventricle and atria. The esophageal probe was inserted with the assistance of the experimenter with the subjects sitting upright and head slightly tilted back. After esophageal probe insertion, the subjects rested in a supine position and the remaining instrumentation was completed.

Heat Flux and Skin Temperature. Heat flux transducers, with imbedded thermistors (Concept Engineering), were used to simultaneously measure heat flux ($\dot{Q}$, $W \cdot m^{-2}$) and skin temperature (Tsk, °C), respectively, at six sites: arm

(upper lateral aspect, over medial head of deltoid muscle), chest (right lateral mid-clavicular line at the second intercostal space), abdomen (several centimeters on right lateral side of the umbilicus), thigh (right anterior surface of mid-thigh), calf (upper lateral aspect), and back (several centimeters above the iliac crest and several centimeters lateral to the vertebral column). The electrodes were covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion.

Heart Rate. Electrocardiograms (ECG) were measured with an electrocardiograph (Physio-Control Systems, Seattle, WA) linked to disposable, self-adhesive ECG electrodes (Medi-Trace) by a shielded patient cable. The ECG electrodes were attached to the chest in a standard lead II configuration. ECG signals were recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis. The electrodes were covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion. ECGs were examined for arrythmias and the heart rate (beats per minute) was determined from average R-R interval for each minute of the experiment.

Electromyography. Skin surface electromyograms (EMG) were recorded from the skin overlying the trapezius muscle (shoulder) with two 2 mm diameter AgCl disc electrodes (Beckmann) spaced 2 cm apart. A before, the electrodes were covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion. Raw surface EMG signals from the electrodes were band pass filtered (3-3kHz) and amplified (100x) with a common mode rejection preamplifier (Grass Instruments, P15D), and recorded with an FM tape recorder (Hewlett Packard, 3968A) for later

analysis. Throughout each experiment EMG was also continuously monitored by display on a digital oscilliscope (Philips, PM 3206) as well as plotted on a chart recorder (Hewlett Packard, 7404A).

Ventilation and Mixed Expired Gas Concentrations. During each trial subjects wore a noseclip and breathed from a mouthpiece which was connected to a low resistance two-way respiratory valve. Inhaled gas mixtures in each trial were humidified before delivery to the subject by bubbling the gas mixtures supplied from compressed gas cylinders (Union Carbide Limited) through a water bath. The water bath was maintained at room temperature and its top was encapsulated with a Douglas meteorological bag. When the bag was sufficiently expanded, gas mixtures were directed from the top of the bag to the inspiratory side of the respiratory valve with ventilation hosing. The flow rate of the inspired gas mixtures ($\dot{V}_I$) was measured using a flow transducer (Hewlett-Packard, 47304A) in combination with a pneumotachometer (Hewlett-Packard, 21073B). Inspiratory volume ($\dot{V}_I$, L-min^{-1}) was determined by integrating the inspired flow with a low drift integrator. Expired gases were directed to a fluted 5L plexiglass mixing box from which a continuous sample was drawn for analysis of mixed expired O_2 (Applied Electrochemistry S-3A Oxygen Analyzer) and CO_2 (Statham Godard Capnograph) concentrations, FEO_2 and $FECO_2$, respectively. Expired gas was also sampled from an outlet tube on the subjects' mouthpeice in order to measure the end-tidal concentration of CO_2 ($F_{ET}CO_2$).

4. Data Acquisition

$\dot{Q}$, Tes, Tre, Tsk, $\dot{V}_I$, FEO_2, and $FECO_2$ were measured on-line with a data acquisition system (Hewlett-Packard 3497A) controlled by a Macintosh II

microcomputer (Apple) with Labview software (Version 2.2, National Instruments). The above parameters were sampled every 10s and stored in a spreadsheet program (MS Excel) for later analysis.

EMG, $F_{ET}CO_2$, and heart rate were continuously sampled and recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis.

5. Data Processing and Analysis

Oxygen consumption ($\dot{V}O_2$, L-min^{-1}) was calculated from measured values of $\dot{V}_I$, FEO_2, $FECO_2$, and known values of FIO_2 and $FICO_2$.

The raw analog EMG was digitized at a sampling rate of 1000 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 ten second periods, after which minute values were obtained by averaging the IEMG of six consective ten second periods. For both trials, the minute levels of IEMG corresponding to Tes of 36.0, 35.75, 35.5, and 35.25°C were then plotted with respect to that Tes, and the resulting slopes of each trial were compared.

6. Statistical Analyses

The responses of $\dot{V}O_2$, $\dot{V}_I$, Tes, Tre, Tsk, and $\dot{Q}$ were compared between the control and CO_2 trials with repeated measures analysis of variance (ANOVA). As well, linear regression analysis was conducted to compare the linear portions of the rates of Tes cooling with respect to time and $\dot{V}O_2$ between the control and CO_2 trials. The heart rate at Tes of 36.0, 35.75, 35.5, and 35.25°C were compared between the control and CO_2 trials with two-tailed, paired T-tests, as was the difference in the slope of the plot of minute IEMG with respect to Tes.

C. Results

Although the subjects were naive as to inhalate during each trial, some of the subjects were able to distinguish the CO_2 gas mixture because of the elevated rate of respiration caused by CO_2 inhalation. Data measurements were not collected during the transition from resting to immersion and from immersion to rewarming due the necessity of disconnecting some pieces of equipment to facilitate easier transfer of the subject to and from the water bath.

During the rest phase of the experiments, the measured variables were very similar between the control and CO_2 conditions except for $\dot{V}O_2$ and $\dot{V}_I$, both of which were significantly elevated by 36 and 31%, respectively, in the CO_2 trial compared to the control trial.

All results are reported as the mean $\pm$ S.D. response of the the subject group.

Immersion Phase

Esophageal temperature (Tes, °C). Tes in the control and CO_2 trial was maintained near the pre-immersion value for the first 6-7 minutes of immersion (Figure 1a). Thereafter, Tes steadily dropped, reaching end-immersion values of 35.1±0.2 and 35.2±0.1°C in the AIR and CO_2 trial, respectively. Although not statistically significant, Tes was consistently higher in the CO_2 trial compared to the AIR trial. Further analysis of the difference between the average rate of drop of the linear portion of Tes (that is, approximately, minutes 6 to 22 of immersion) with respect to time between the control and CO_2 trial was not statistically significant.

Mean unweighted skin temperature (Tsk, °C). Mean unweighted Tsk values were used rather than weighted values because the the thermal sensitivity of

different body areas does not always correspond to the heat that is generated by that area (Timbal, 1976). Tsk displayed a similar time course of response in both trials, beginning from pre-immersion values of 33.2±0.9 and 31.3±0.9°C and plateauing at end-immersion values of 17.4±0.8 and 16.3±1.3°C in the control and CO_2 trial, respectively (Figure 1b). The higher plateau temperature observed in the control trial was not statistically significant.

Heat Flux ($\dot{Q}$, $W.m^{-2}$). Heat flux in both conditions showed a sharp transient increase within the first several minutes of immersion, beginning from pre-immersion values of 40±5 and 34±3 $W \cdot m^{-2}$ and peaking at 669±112 $W \cdot m^{-2}$ after 3 minutes of immersion and 619±125 $W \cdot m^{-2}$ after 4 minutes of immersion in the control and CO_2 trial, respectively (Figure 1c). The heat flux then decayed in a curvi-linear manner with continued immersion, reaching asymptotic values of 222±13 and 235±14 $W \cdot m^{-2}$ in the control and CO_2 trial, respectively. The difference in heat flux between the two trials was not statistically significant.

Oxygen consumption ($\dot{V}O_2$, $L \cdot min^{-1}$). Upon immersion $\dot{V}O_2$ exhibited a transient elevation in both control and CO_2 trials to 0.77±0.16 and 0.58±0.10 $L \cdot min^{-1}$, respectively (Figure 2a). $\dot{V}O_2$ then showed a slight decrease over the following 10 minutes and then steadily increased for the duration of the immersion phase, reaching end-immersion values of 0.86±0.16 and 1.03±0.18 $L \cdot min^{-1}$ for the control and CO_2 trials, respectively. The differences in $\dot{V}O_2$ between the control and CO_2 trials were not statistically significant except in the first few minutes of immersion ($p=0.05$).

Ventilation ($\dot{V}_I$, L·min^{-1}). $\dot{V}_I$ displayed a more gradual increase, compared to $\dot{V}O_2$, plateauing after five minutes of immersion for about seven minutes (Figure 2b). $\dot{V}_I$ then continued to increase, reaching end-immersion values of 22.61±4.28 and 34.61±5.63 L·min^{-1} for the control and CO_2 trials, respectively. The differences in $\dot{V}_I$ between the control and CO_2 trials were not statistically significant except in the first few minutes of immersion ($p=0.05$).

Heart Rate (min^{-1}). Heart rate increased from average resting values of 65±5 and 75±7 min^{-1} to 85±8 and 83±8 min^{-1} at Tes of 35.25°C, in the control and CO_2 trial, respectively. The differences in heart rate between the two conditions were not statistically significant.

Electromyography (EMG, mV). No EMG activity was apparent during the resting phase of the experiment except during voluntary movements. After entry into the water bath, tonic shivering activity was observed to gradually increased. With progression of the immersion phase the level of tonic shivering activity further increased and bursts of large amplitude clonic activity began to appear. With decrease in Tc, 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 of lower muscularity. In some of the subjects with greater muscle mass, clonic shivering activity did not appear, although the intensity of tonic shivering seemed to increase.

Tonic shivering activity with superimposing clonic tremor continued in the early stages of the rewarming phase of the experiment. As rewarming progressed, the intensity of clonic activity gradually diminished in frequency, duration and amplitude. Tonic shivering tremor also gradually decreased in

intensity. Near the latter stages of rewarming, both clonic and tonic activity were almost completely absent.

The IEMG between the two conditions was compared by examining the differences is the rate of increase of IEMG with decreasing Tes (Figure 3). A test for parallelism indicated no significant difference in the IEMG-Tes relation between the control and CO_2 trials.

VO_2 -Tes relation. The increase in oxygen uptake with decreasing Tes during the control trial was compared with that observed during the CO_2 trial (Figure 4). A test of parallelism revealed no significant differences between the VO_2-Tes relations observed in the control and CO_2 trials.

Rewarming Phase

$\dot{V}O_2$. $\dot{V}O_2$ continued to increase in both AIR and CO_2 trials, reaching peak values of 1.14±0.26 and 1.10±0.18 L-min^{-1} after 2 and 3 minutes of rewarming, respectively (Figure 5). Thereafter, $\dot{V}O_2$ in both trials exhibited a non-linear decrease to end-rewarming values of 0.23±0.03 and 0.37±0.03 L-min^{-1} for the AIR and CO_2 trial, respectively. The differences in $\dot{V}O_2$ between the two trials was not statistically significant.

$\dot{V}_I$. $\dot{V}_I$ remained stable during the first three and five minutes of rewarming in the AIR and CO_2 trials, respectively, after which it began to gradually drop in an almost linear fashion, eventually plateauing at 11.29±1.25 and 16.17±2.90 L-min^{-1} for the AIR and CO_2 trial, respectively (Figure 6).

Tes. Tes continued to drop in both AIR and CO_2 trials, reaching values of 34.5±0.1 and 34.6±0.2°C after 6 and 7 minutes of rewarming, respectively

(Figure 7). Thereafter, Tes gradually increased, attaining end-rewarming values of 35.2±0.2 and 34.9±0.1°C in the AIR and CO_2 trials, respectively.

Tre. Tre dropped to 35.1±0.3 and 35.3±0.4°C at minute 3 of rewarming and then rebounded, reaching peak values of 36.0±0.2 and 36.5±0.2°C in the AIR and CO_2 trials, respectively. Tre then steadily dropped, finally stabilizing about 34.8±0.2°C in both AIR and CO_2 trials (Figure 8).

Tsk. Tsk in both conditions demonstrated a non-linear increase during rewarming, although a plateau was not reached due to the brevity of the rewarming phase (Figure 9). Tsk was consistently higher in the CO_2 trial compared to the AIR trial, starting at initial values of 17.9±0.7 and 16.2±1.3 °C, and reaching end-rewarming values of 28.1±1.0 and 27.0±1.9 °C, respectively. However, the differences were not statistically significant.

$\dot{Q}$. The course of heat flux during rewarming was almost identical in both AIR and CO_2 trials, beginning at values of 143±33 and 189±15 $W \cdot m^{-2}$ and reaching end-rewarming values of 24±3 and 27±3 $W \cdot m^{-2}$, respectively (Figure 10). Again differences in HF values between the AIR and CO_2 trials were not statistically significant.

EMG. Tonic shivering activity with superimposing clonic tremor continued in the early stages of the rewarming. As rewarming progressed, the intensity of clonic activity gradually diminished in frequency, duration and amplitude. Tonic shivering tremor also gradually decreased in intensity. Near the latter stages of rewarming, both clonic and tonic activity were almost completely absent.

D. Discussion

The present study demonstrates that prolonged inhalation of a gas mixture containing 4% CO_2 during cold water immersion does not attenuate shivering thermogenesis. Both shivering activity of the trapezius muscle and oxygen uptake were similar for the AIR and CO_2 trials. Furthermore, although the hyperventilation observed in the CO_2 trial, indicated by a higher $\dot{V}_I/\dot{V}O_2$, most likely enhanced respiratory heat loss, the rate of core cooling in the two trials was not significantly different (Figure 1). Wagner et al. (1983) concluded that the increase in respiratory heat loss during inhalation of 4% CO_2 in a 5°C air environment resulted in a greater core cooling rate compared to that observed during air inhalation. In the present study the differences in $\dot{V}_I$ and $\dot{V}O_2$ between the AIR and CO_2 trials were not significant. Therefore, in the absence of an imbalance between heat loss and heat production, which could be mediated by the hypercapnia-induced hyperventilation, it is not surprising that core cooling rates were similar in the two conditions. The findings of the present study confirm the observations made by Wagner et al. (1983) for exposures to 5°C air. Therefore, it can be concluded that the greater rate of core temperature cooling observed by Wagner et al. (1983) during CO_2 inhalation, may indeed be attributed to increased respiratory heat loss.

To examine the contribution of the oxygen cost of breathing to the overall oxygen uptake during the CO_2 trial, the oxygen uptake was monitored in one subject while resting in air at room temperature and breathing at rates of 20, 40 and 60 $L \cdot min^{-1}$ .Accounting for the metabolic cost of breathing in this manner (see Appendix B), the difference in shivering $\dot{V}O_2$ between the two trials was further reduced. The insignificant difference in the slopes of the IEMG - Tes relation between the AIR and CO_2 trials (Figure 3) also indicates that shivering inhibition is not significant.

Aside from the effects of CO_2 on heat production, the peripherally induced vasodilative effects of CO_2 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 vasodilation was not increased by inhalation of 4% CO_2. Increased heat loss during the immersion would thus not be expected, and indeed, was not observed.

The results of the present series of experiments are in agreement with that reported by Wagner et al. (1983), who also failed to observe any inhibition of shivering in humans inhaling 4% CO_2 while exposed to 5°C air for 60 minutes. Bullard and Crise (1961), however, observed a noticeable decrease in the heat production of cold exposed humans following the switch to a 30 minute period of inhalation of 6% CO_2 from air. This inhibition was subsequently reversed after six minutes of CO_2 inhalation. The results of these studies, along with those of the present experiments, would suggest that gas mixtures containing between 4 and 6% CO_2 are required to elicit any noticeable inhibitory effect on shivering thermogenesis in human subjects. However, even then, the inhibitory effects of 6% are quickly overcome. Assuming that the inhibitory effects of CO_2 probably arise secondarily to a decrease in blood and pH of the cerebrospinal fluid (CSF), 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 CO_2. Brackett et al. (1965) have in fact demonstrated that the respiratory acidosis stemming from the inhalation of 7 and 10% CO_2 is quickly compensated for by a rapid rise in plasma bicarbonate levels within 5 to 10 minutes of the onset of hypercapnia.

Although the steady-state plasma pH and $PaCO_2$ resulting from inhalation of such mixtures is beyond that considered normal (Gennari *et al.*, 1982), the disinhibition of heat production observed by Bullard and Crise (1961) during inhalation of 6% CO_2 would suggest that the compensation is sufficient. The inhalation of 4% CO_2, as in the present study, would result in a $PaCO_2$ of approximately 47 mmHg, when estimated from an exponential curve fit (see Appendix C) of the $PaCO_2$ values resulting from inhalation of gas mixtures containing 7 and 10% CO_2 which were reported by Brackett et al. (1965). As this value lies within the normal physiological range of blood PCO_2 of 41 to 49 mmHg (Gennari *et al.*, 1982), inhalation of 4% CO_2 would not be expected to incur any significant alterations in blood acid-base balance. Consequently, the absence of significant inhibitory effects of CO_2 is not surprising. In the present experiment, however, the evaluation of acid-base homeostasis is further complicated as during hypothermia there are tendencies toward both metabolic acidosis and alkalosis. Metabolic acidosis arises during hypothermia from the hypoxic metabolism of shivering muscles, with resulting lactic acidosis, due to vasoconstriction of surrounding vasculature with cold exposure (Thompson and Henrich, 1989). Moreover, the lactic acid elimination capacity of the liver is compromised with core temperature cooling, permitting further elevation of plasma lactic acid levels (Ballinger *et al.*, 1961). Decrease in blood temperature during hypothermia results in an increase in the solubility of CO_2, leading to a decrease in PCO_2, 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 (Reeves, 1985). Without proper assessment of acid-base balance with measures of blood pH, $PaCO_2$, and bicarbonate levels, it is difficult to conclusively interpret the results of the present study.

Aside from adequate compensation for perturbations in acid-base balance, the inhibitory effects of CO_2 may also have been overcome by the increasing thermal drive to central thermoregulatory centers that would be expected with decreasing core temperature. In the present study, it is assumed that with cold water immersion, Tsk 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, in addition to that emanating from skin thermoreceptors, for shivering thermogenesis, possibly helping to overcome the central inhibitory effects of CO_2 inhalation. From studies of heat production of goats during core temperature cooling with skin temperature clamped above thermoneutral, Jessen (1981) estimates that the relative contributions of core and skin temperatures in control of heat production is about 3:1. This would lend further support to this conclusion.

During the rewarming phase, the elevated level of $\dot{V}O_2$ in the CO_2 trial (Figure 5) may be attributed to the increased metabolic cost of breathing, as evidenced by the higher inspired minute ventilation (Figure 6). Moreover, since heat production is also governed by thermal drives emanating from both peripheral and central thermoreceptors, it is possible that the tendency of a lower $\dot{V}O_2$ in the AIR trial arose because of the correspondingly higher levels of Tes and Tsk (Figures 7 and 9, respectively). The response of rectal temperature (Figure 8), however, does not support this suggestion.

In summary, prolonged inhalation of 4% CO_2 did not attenuate shivering thermogenesis or enhance heat loss in cold water-immersed human subjects, as indicated by similar levels of $\dot{V}O_2$ and the rate of core temperature cooling. This finding further suggests that, within the context of activities in which there is

cold exposure, such as underwater diving, prolonged introduction of gas mixtures containing up to 4% CO_2 does not significantly affect body thermal balance. Consequently, CO_2 per se at this concentration would not be considered a significant contributor to hypothermia during cold exposure. It should be emphasized, however, that CO_2 inhalation may affect thermal balance by inducing hyperventilation. The contribution of this mechanism is dependant upon the pressure, temperature, and humidity of the inspired gas mixture.

The absence of any inhibitory effect is most likely due to the combined effects of efficient buffering of CO_2 inhalation-induced acidosis, the relatively small increase in blood PCO_2 associated with inhaling 4% CO_2, and an increasing thermal drive for heat production during rapid core temperature cooling.

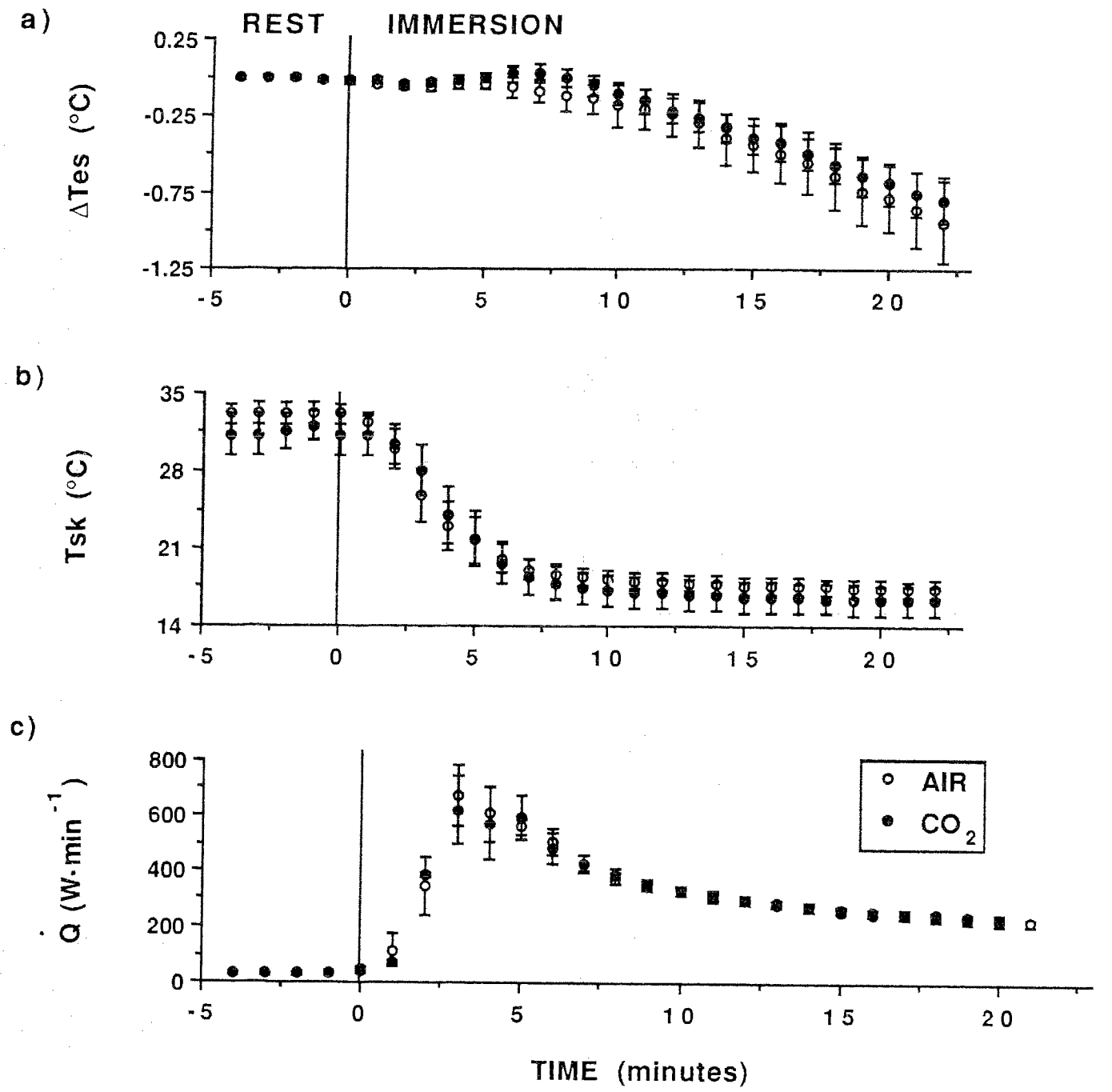

Figure 1. Responses (mean±S.E.) of a) esophageal temperature (ΔTes, °C), unweighted mean skin temperature (Tsk, °C), and c) heat flux ($\dot{Q}$, $W \cdot m^{-2}$) during immersion in 15°C water. Subjects (n=7) inspired either room air (AIR, open circles) or a breathing mixture containing 4%CO_2-20%O_2-76%N_2 (CO_2,closed circles).

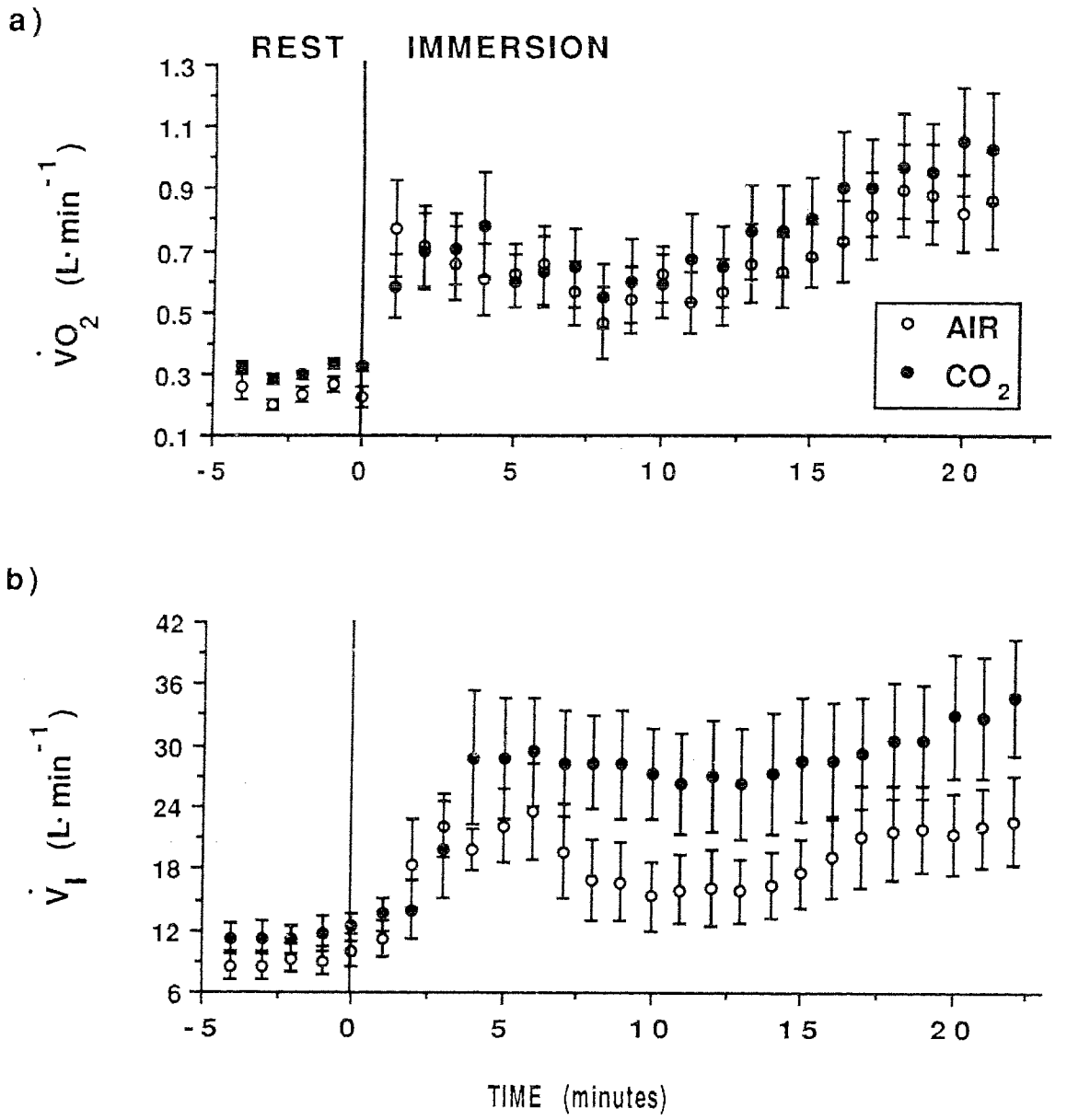

Figure 2. Responses (mean±S.E.) of a) oxygen uptake ($\dot{V}O_2$, L·min^{-1}), and b) ventilation ($\dot{V}_I$, L·min^{-1}), during immersion in 15°C water. Subjects (n=7) inspired either room air (AIR, open circles) or a breathing mixture containing 4%CO_2-20%O_2-76%N_2 (CO_2, closed circles).

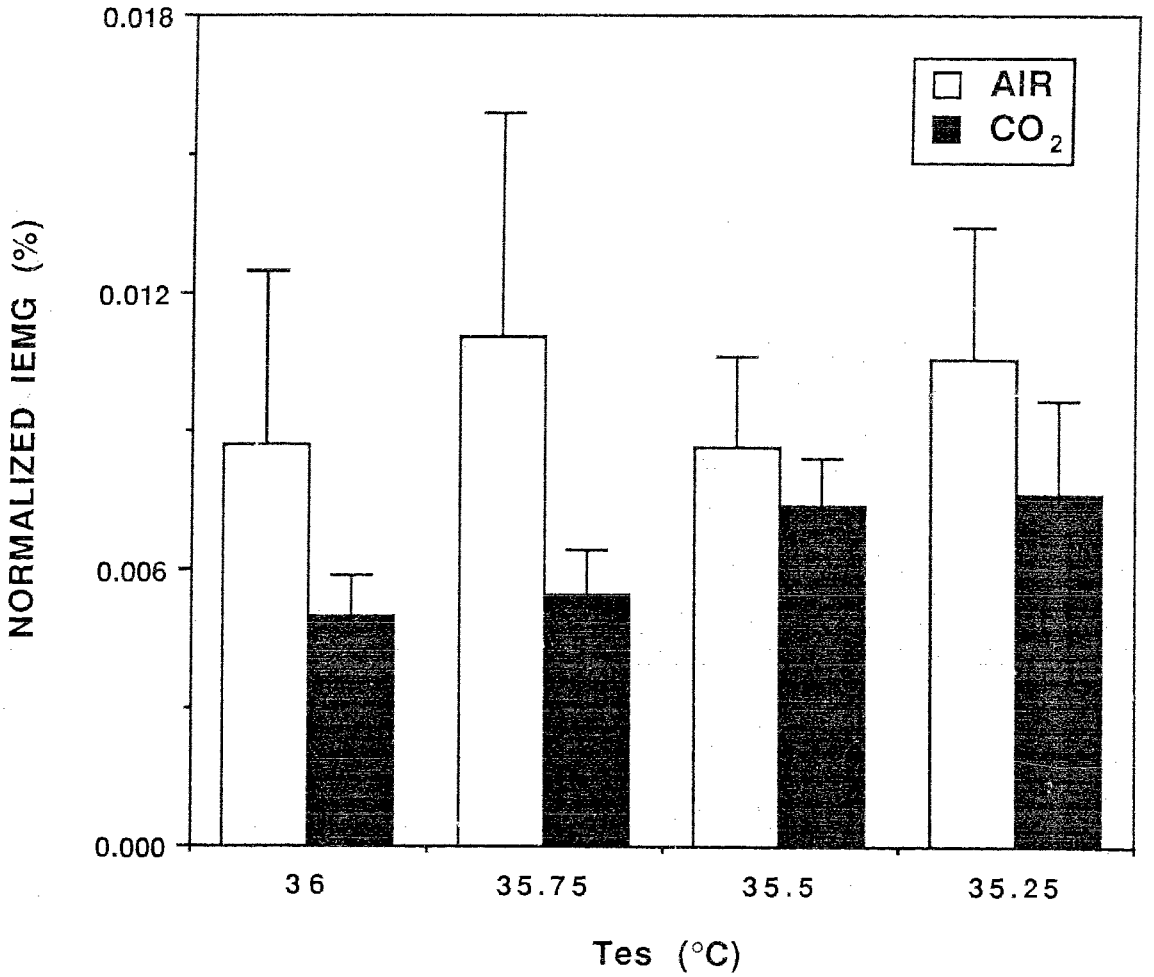

Figure 3. Minute normalized IEMG (mean±S.E., %) recorded from the trapezius muscle at Tes of 36, 35.75, 35.5, and 35.25°C during immersion in 15°C water. Subjects (n=7) inspired either room air (AIR, open bars) or a breathing mixture containing 4%CO_2-20%O_2-76%N_2 (CO_2, closed bars).

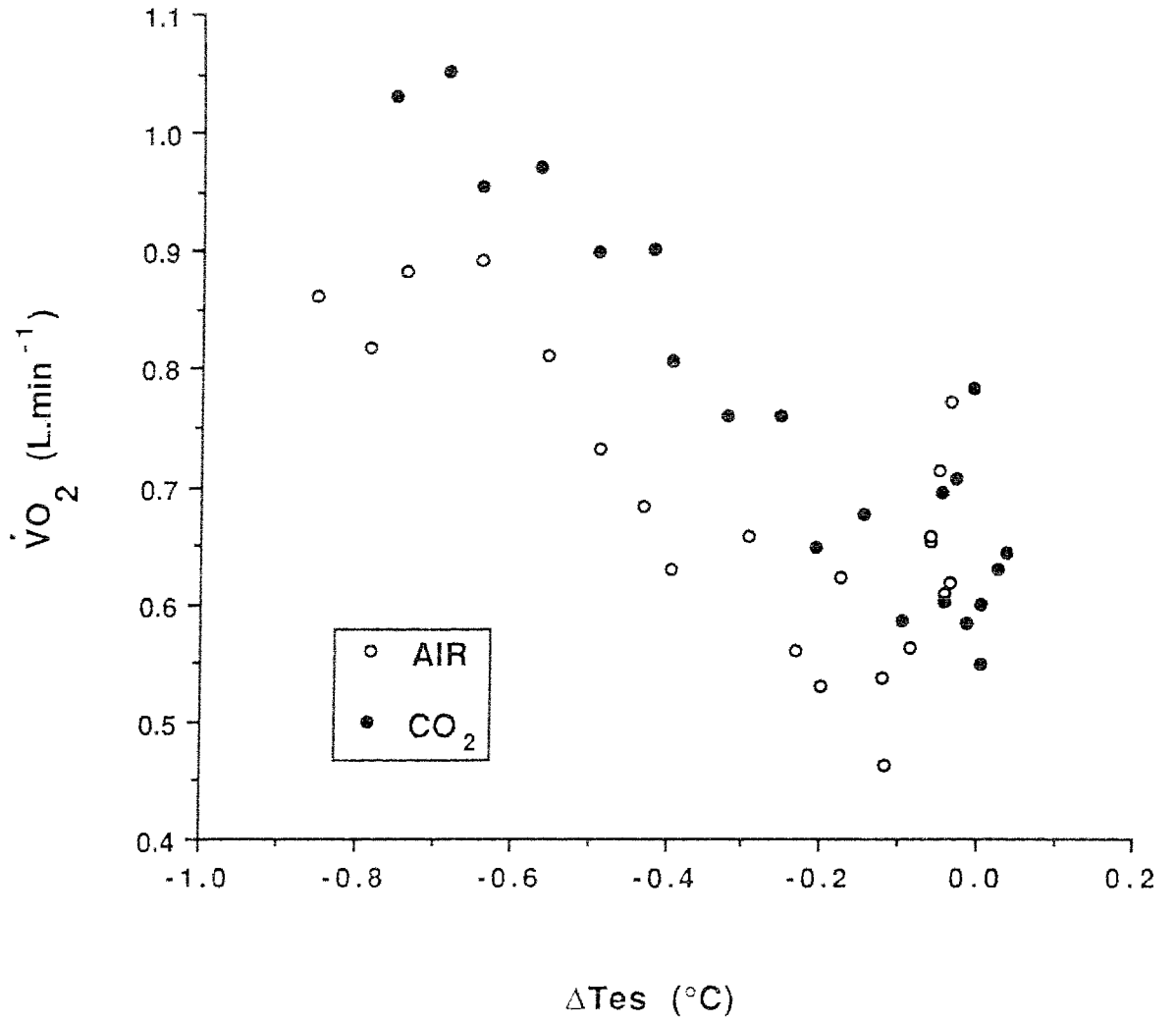

Figure 4. The oxygen uptake ($\dot{V}O_2$, L·min^{-1}).) response to a change in esophageal temperature (ΔTes, °C) during immersion in 15°C water. Subjects (n=7) inspired either room air (AIR, open circles) or a breathing mixture containing 4%CO_2-20%O_2-76%N_2 (CO_2, closed circles).

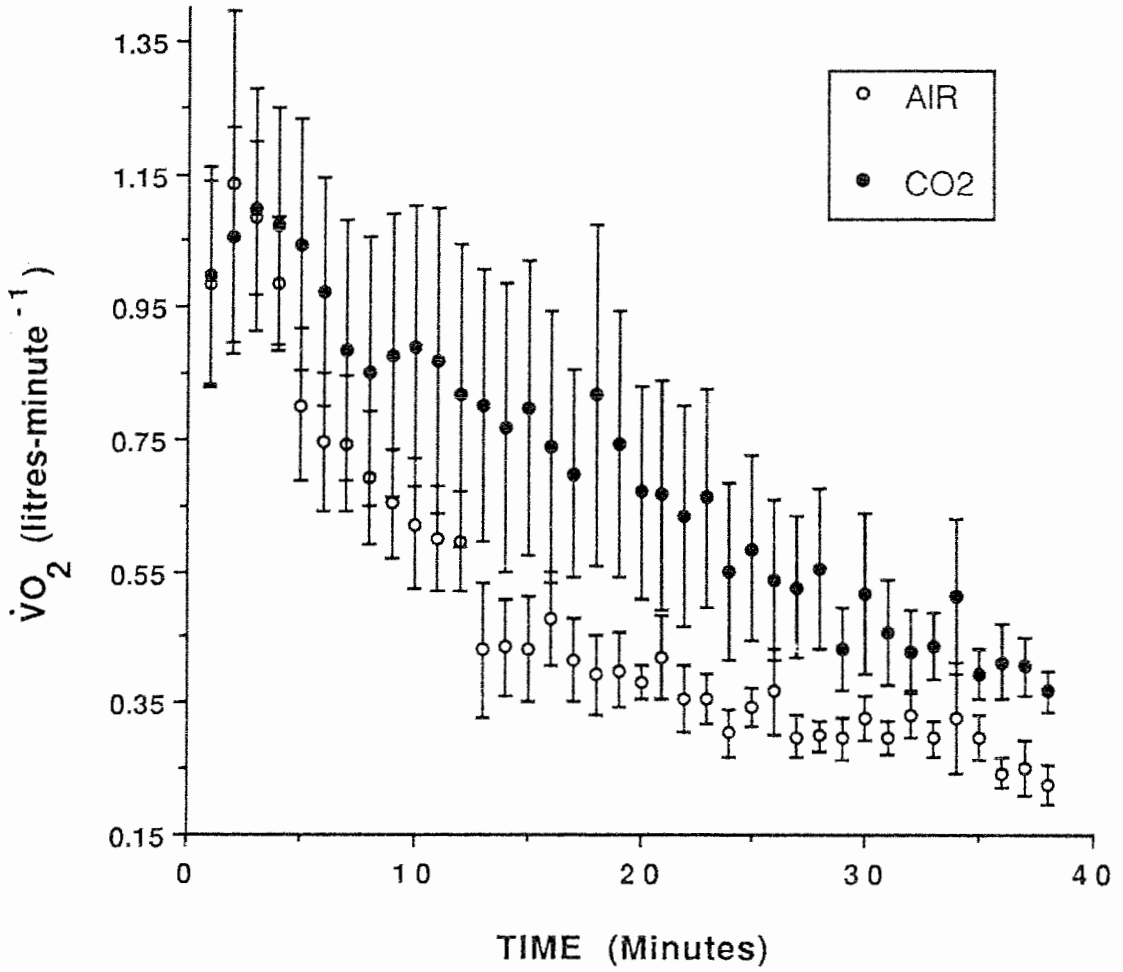

Figure 5. Response of oxygen uptake (mean±SE, litres-min^{-1}) during rewarming while breathing air and 4% CO_2 (n=7).

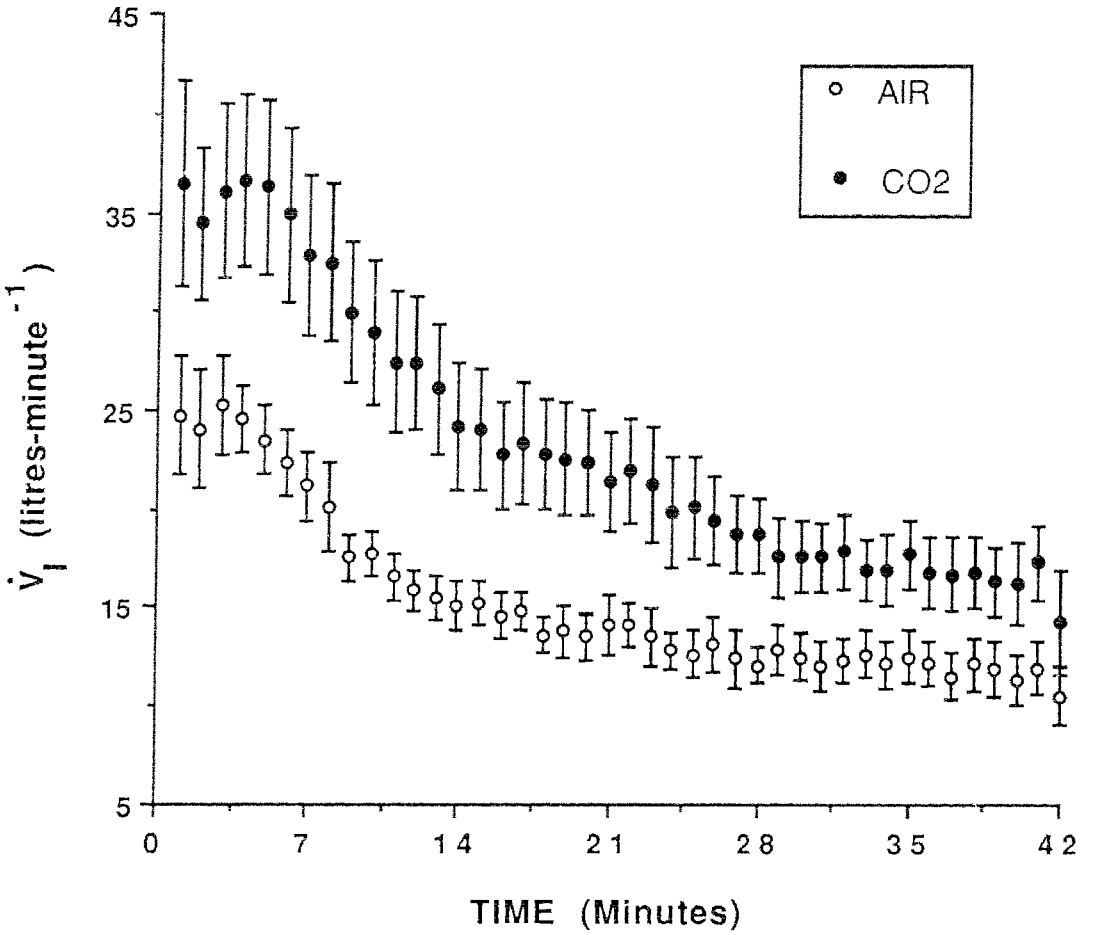

Figure 6. Response of minute inspired ventilation (mean±SE, litres-min^{-1}) during rewarming while breathing air and 4% CO_2 (n=7).

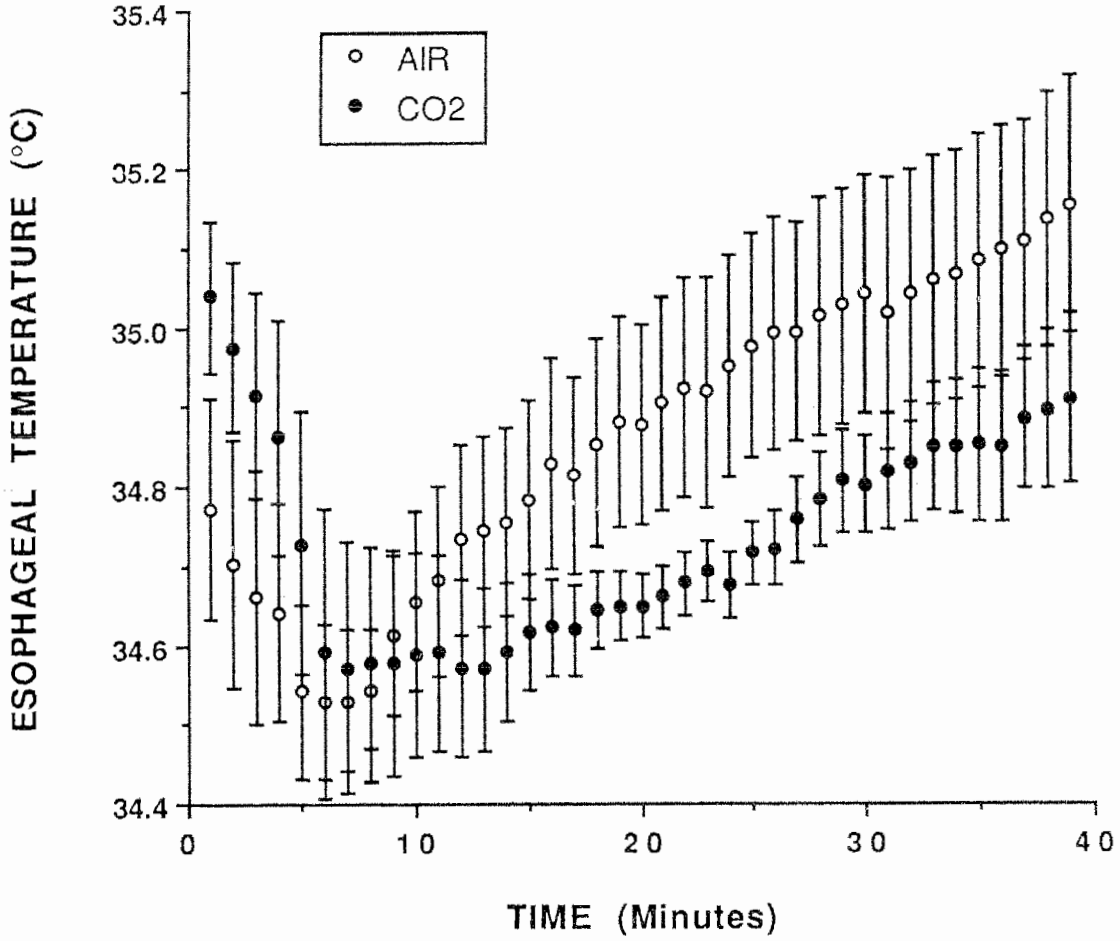

Figure 7. Response of esophageal temperature (mean±SE, °C) during rewarming while breathing air and 4% CO_2 (n=5).

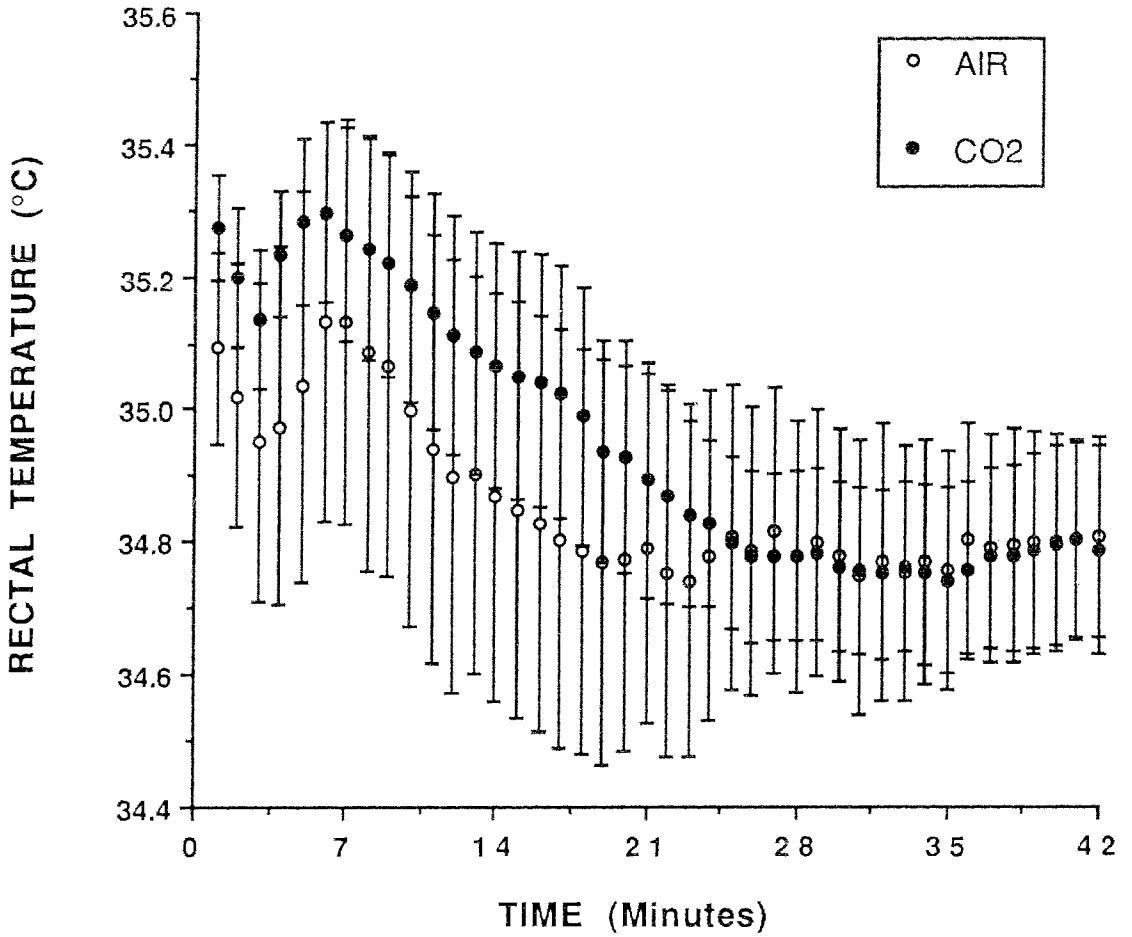

Figure 8. Response of rectal temperature (mean±SE, °C) during rewarming while breathing air and 4% CO_2 (n=7).

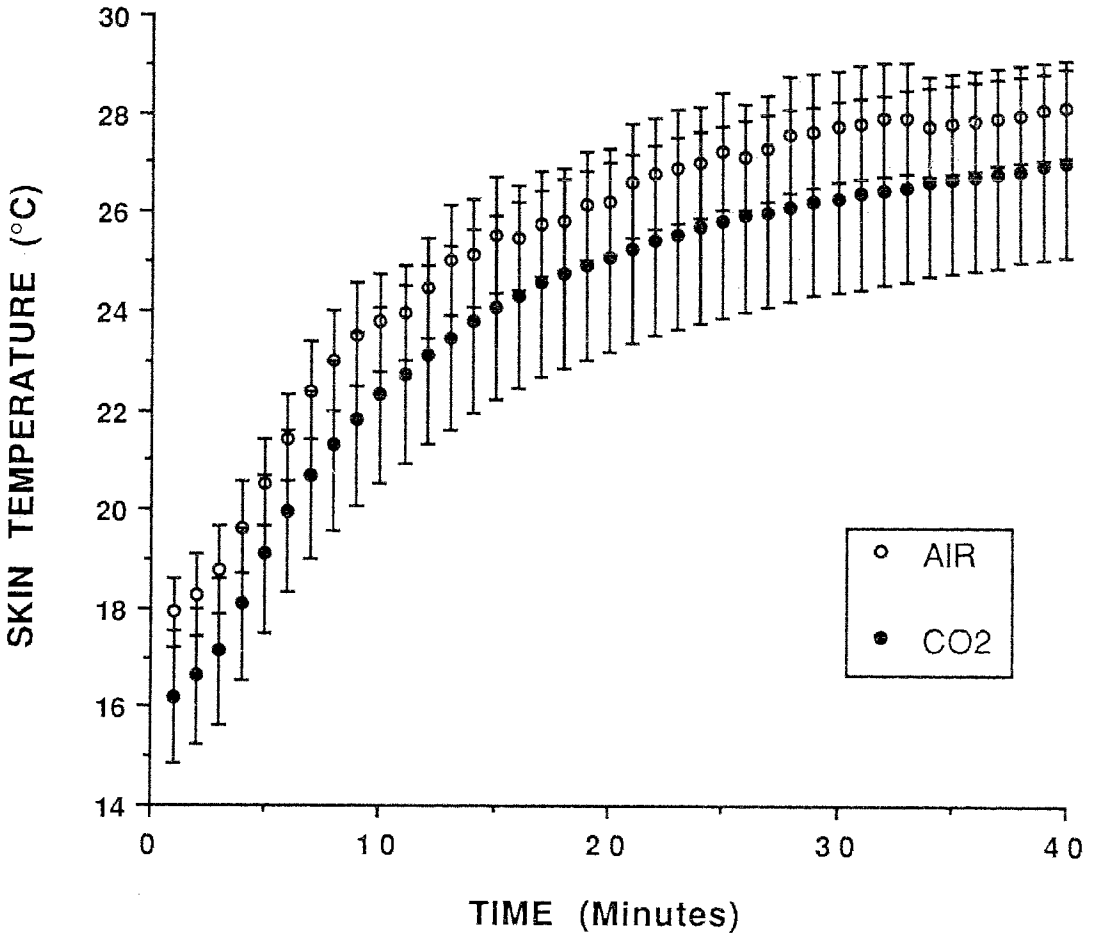

Figure 9. Response of skin temperature (mean±SE, °C) during rewarming while breathing air and 4% CO_2 (n=7).

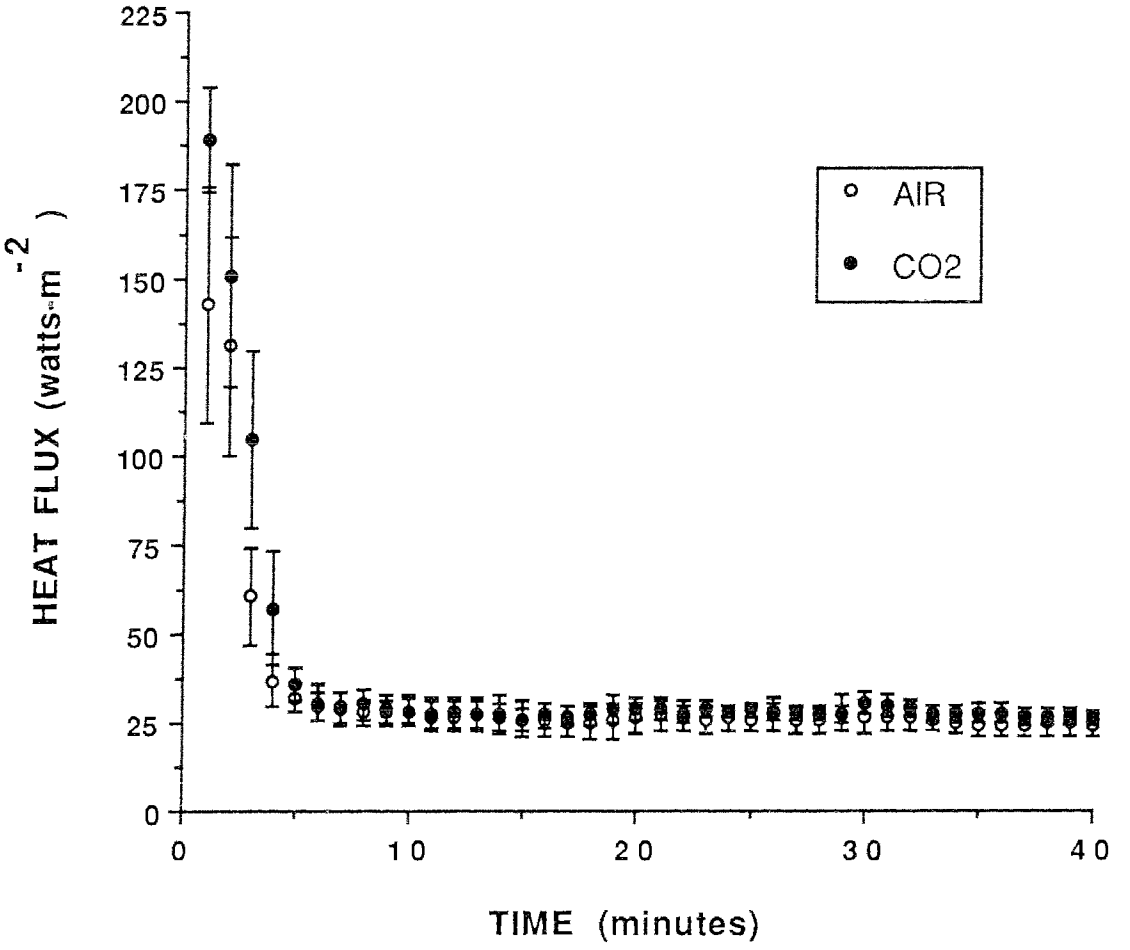

Figure 10. Response of heat flux (mean±SE,watts-m^{-2}) during rewarming while breathing air and 4% CO_2 (n=7).

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V. THE EFFECTS OF INTERMITTENT INHALATION OF CO_2 ON SHIVERING THERMOGENESIS

A. Introduction

Animal studies (Kitano, 1988; Plewes and Jennings, 1972; Schaefer and Wunnenberg, 1976; reviewed in Stupfel, 1974) have reported that hypercapnia impairs thermal homeostasis during cold exposure by acting on central regions involved with temperature regulation, as well as acting directly on the thermoregulatory effector organs, namely attenuating heat production in skeletal muscle and instigating cutaneous vasodilation, thus enhancing heat loss, and precipitating hypothermia. The effect of CO_2 inhalation on thermal balance in humans, however, is less clear. Bullard and Crise (1961) demonstrated a transient depression of heat production and shivering in male subjects exposed to an ambient temperature of 5°C during a 30 minute period of inhaling a gas mixture containing 6% CO_2. However, with continued cold exposure, the inhibitory effects of CO_2 inhalation were overcome and shivering resumed. This disinhibition was attributed to increased respiratory heat loss resulting from hypercapnia-induced hyperventilation. In more recent studies, however, Section IV and Wagner *et al.* (1983) have reported that the shivering tremor of subjects exposed to 5°C air or immersed in a 15°C water bath, respectively, was not suppressed during prolonged inhalation of 4% CO_2 gas mixtures. However, in light of evidence (Bullard and Crise, 1961) of significant effects of CO_2 on thermoregulatory responses during switching of the inhalate from a normocapnic mixture to a hypercapnic one, concomitant with cold air exposure, it is conceivable that prolonged inhalation of CO_2 (Section IV and Wagner *et al.*, 1983) may result in adaptation thus masking any transient effects.

The present study therefore examined the effect of transient exposure to CO_2 on thermal balance during cold exposure, especially within the context of cold and hypercapnic exposures encountered in activities such as underwater

diving. Consequently, in the present study, subjects were exposed to extremes of cold (immersion in 15°C water) and hypercapnia (inhalation of 4% CO_2).

B. Methods

1. Subjects

Eight male subjects were recruited to participate in this investigation. Their participation was subject to a physician's approval. Before signing an informed consent form, each subject was familiarized with the experimental protocol and reminded that they could withdraw from the study at any time.

2. Protocol

Clad only in swim trunks, subjects were instrumented while lying supine and, following recording of resting values for 10 minutes, the subjects were seated in a chair suspended from a compressed air winch. They were then transferred into a water bath maintained at 15°C. The subjects remained immersed to the neck until their core temperature, as measured in the esophagus, fell to 35.0°C, or until 60 minutes of immersion had elapsed. The subjects were then removed from the water bath, dried with a towel, and rewarmed while lying supine and covered with an insulated blanket.

Air (0.03% CO_2 , 20.93% O_2, and 79.04% N_2) was inspired by the subjects throughout each experiment except for a 15 minute period during which a 4% CO_2, 20% O_2, and 76% N_2 gas mixture was introduced when the subject's core temperature had dropped to 36.5°C. During each experiment, appropriate measures were taken to keep subjects naive as to time elapsed and of when the gas mixtures were switched.

Subjects were instructed not to undergo heavy exercise on the days of their trials and to consume a light meal 2-3 hours before each trial.

The protocol used in the present study was approved by the Ethics Reveiw Committee of Simon Fraser University.

3. Instrumentation

Immersion Tank. The immersion tank was a 2.1 m x 1.05 m x 2.1 m fiberglass shell and was normally filled with approximately 4000 L water. The water was constantly filtered (Triton) and circulated with a pump (Swimquip Spa-Support Systems). Circulated water was passed through a refrigration unit controlled by a thermoregulation system (Honeywell) to maintain the water temperature constant at 15°C.

Core Temperature. Rectal temperature (Tre, °C) was measured with a rectal thermistor probe (YSI 701, Yellow Springs Instruments), inserted 15cm beyond the anus. An esophageal thermistor probe (YSI 702, Yellow Springs Instruments, Yellow Springs, Ohio) was inserted via a nostril into the esophagus for recording esophageal temperature (Tes, °C). The insertion length of the esophageal probe was determined from a regression equation based on sitting height, as suggested by Mekjavic and Rempel (1990), which situated the thermistor in the region of the esophagus nearest the myocardium. After insertion of the esophageal probe, the subjects rested in supine position and the remaining instrumentation was completed.

Heat Flux and Skin Temperature. Heat flux transducers, with embedded thermistors (Concept Engineering), were used to simultaneously measure heat flux ($\dot{Q}$, $W \cdot m^{-2}$) and skin temperature (Tsk, °C), respectively, at six sites: arm (upper lateral aspect, over medial head of deltoid muscle), chest (right lateral mid-clavicular line at the third intercostal space), abdomen (several centimeters on right lateral side of the umbilicus), thigh (right anterior surface of mid-thigh), calf (upper lateral aspect), and back (several centimeters above the iliac crest and several centimeters lateral to the vertebral column). The electrodes were

covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion.

Electromyography. Skin surface electromyograms (EMG) were recorded from the skin overlying the right trapezius muscle and left masseter muscle with two 2 mm diameter AgCl disc electrodes (Beckmann) spaced 2 cm apart at each site. The electrodes were covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion. Raw surface EMG signals from the electrodes were band pass filtered (3-3kHz) and amplified (100x) with a common mode rejection preamplifier (Grass Instruments), and recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis. Throughout each experiment EMG was continuously monitored by display on a digital oscilliscope (Philips, PM 3206) as well as plotting on a chart recorder (Hewlett Packard 7404A).

Ventilation and Mixed Expired Gas Concentrations. During each trial subjects respired through a two way valve connected to an oro-nasal mask. Both inhaled gas mixtures were separately humidified before delivery to the subject by passing the gas mixtures, supplied from compressed gas cylinders (Union Carbide Limited), through separate water baths. The water baths were maintained at room temperature and the top of each water bath encapsulated with a meteorological balloon. A valve permitted switching of the gas mixtures, which were then delivered to the inspiratory side of the respiratory valve with corrugated respiratory hosing (Collins). The inspired minute ventilation ($\dot{V}_I$, $L \cdot min^{-1}$) was measured using a turbine flow meter (Alpha Technologies, Turbine Ventilation Module, model VMM110). Expired gas was directed from the subject's mouthpiece to a 5L fluted plexiglass mixing box. A continuous sample

(0.5 $L \cdot min^{-1}$) was drawn from the mixing box for analysis of mixed expired O_2 (Applied Electrochemistry S-3A Oxygen Analyzer) and CO_2 (Statham Godard Capnograph) concentrations (F_EO_2 and F_ECO_2, respectively). Expired gas was also sampled from an outlet tube on the expiratory valve of the subject's oro-mask in order to measure the end-tidal concentration of CO_2 (F_ETCO_2).

4. Data Acquisition

With the exception of EMG and F_ETCO_2, all variables were measured on-line with a data acquisition system (Hewlett-Packard 3497A) controlled by a Macintosh II microcomputer (Apple) with Labview software (Version 2.2.1, National Instruments). The above parameters were sampled every 10s and stored in a spreadsheet program for later analysis.

EMG and F_ETCO_2 were continuously sampled and recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis.

5. Data Processing and Analysis

The analog raw EMG signal during the immersion phase of the experiment was digitized at a sampling rate of 1000 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 ten second periods, after which minute values were obtained by averaging the IEMG of six consective ten second periods. Minute IEMG values were then summed to give the total IEMG of immersion. Normalized minute IEMG values (NIEMG) were then calculated by dividing the minute IEMG values by the total IEMG of immersion. The minute values of NIEMG in the two minutes preceding the switch to the CO_2 gas mixture were compared to the minute NIEMG values of the two minutes following the switch to CO_2 breathing (AIR-CO_2 difference). Similarily, the

minute values of NIEMG in the two minutes preceding the switch back to air breathing were compared to the minute IEMG values of the two minutes following the switch back to air breathing (CO_2 -AIR difference). As well, the minute value of NIEMG preceding the switch to CO_2 (pre-CO_2) was compared to the lowest NIEMG value during the CO_2 inhalation period (minimum-CO_2) and to the NIEMG immediately following the end of the CO_2 inhalation period (post-CO_2).

6. Statistical Analyses

The AIR-CO_2 and the CO_2-AIR differences in the trapezius muscle NIEMG was compared with repeated measures one-way analysis of variance (ANOVA). The corresponding values for Tes, $\dot{V}_I$, and $\dot{V}O_2$ were analyzed in the same manner. The differences between the pre-, minimum, and post-CO_2 inhalation NIEMG values were assessed with a two-tailed, paired t-test.

C. Results

Data measurements were not collected during the transition from resting to immersion due to the necessity of disconnecting some of the transducers to facilitate easier transfer of the subject to and from the water bath.

All results are reported as the mean response (±S.E.) of the subject group. As such the total number of minutes of results that could be reported was limited by the subject with the shortest duration of the immersion, in this case 32 minutes.

Oxygen Consumption ($\dot{V}O_2$, $L \cdot min^{-1}$). $\dot{V}O_2$ exhibited an initial transient elevation which peaked at 1.05±0.43 $L \cdot min^{-1}$, and then rapidly dropped to slightly above the pre-immersion value of 0.59±0.19$L \cdot min^{-1}$. This was followed by a gradual increase to an end-immersion value of 1.08±0.37 $L \cdot min^{-1}$. During the CO_2 inhalation period, $\dot{V}O_2$ remained near pre-AIR-CO_2 switch levels, with a slight increase in the latter 5 minutes of CO_2 exposure (Figure 1a). Both the AIR-CO_2 and CO_2-AIR differences were not statistically significant.

Inspired minute ventilation ($\dot{V}_I$, $L \cdot min^{-1}$). Following an initial rapid rise in ventilation, peaking at 33.27±5.32 $L \cdot min^{-1}$, $\dot{V}_I$ gradually decreased to a minimum value of 14.35±1.45 $L \cdot min^{-1}$, and then continued to steadily rise to an end-immersion value of 31.84±5.70 $L \cdot min^{-1}$. From the onset of the CO_2 inhalation period, $\dot{V}_I$ displayed a gradual increase from a mean pre-AIR-CO_2 switch value of 21.35±3.95 $L \cdot min^{-1}$ and then palateaued at about 51.69±5.03 $L \cdot min^{-1}$, beginning at minute twelve of the CO_2 inhalation period (Figure 1b). Within the first minute of returning to air inhalation, $\dot{V}_I$ dropped to a mean value of 39.38±6.26 $L \cdot min^{-1}$. Whereas the AIR-CO_2 switch difference in $\dot{V}_I$ was not significant, the CO_2-AIR switch difference was statistically significant ($p \leq 0.05$).

Core Temperature: esophageal (Tes, °C) and rectal (Tre,°C) temperatures. Tes fluctuated about pre-immersion values for the first 10 minutes of immersion, after which it steadily decayed. During immersion, Tre displayed a gradual decline from a pre-immersion value of 36.9±0.1°C to an end-immersion value of 36.2±0.2°C.

Mean Unweighted Skin Temperature (Tsk, °C). Mean unweighted Tsk values were used rather than weighted values because the thermosensitivity of different areas of the body does not exactly match heat generating capability of the correponding body area in response to cold stimulus, (Timbal *et al.*, 1976). Tsk stabilized at about 16.0±0.4°C following a rapid and immediate drop from a pre-immersion value of 33.0±0.8°C. The AIR-CO_2 and CO_2-AIR differences in Tsk were not statistically significant.

Heat Flux ($\dot{Q}$, $W \cdot m^2$). $\dot{Q}$ showed a sharp increase, peaking in the second minute of immersion at 829±121 $W \cdot m^{-2}$. Thereafter it declined in a non-linear manner with continued immersion, reaching an asymptotic value of 243±19 $W \cdot m^{-2}$ at the end of immersion. The AIR-CO_2 and CO_2-AIR differences in heat flux were not statistically significant.

Electromyography (EMG, mV). No EMG activity was apparent during the resting phase of the experiment except during voluntary movements. After several minutes of immersion, intense involuntary shivering tremor appeared. Following stabilization of Tsk, shivering activity subsided to a diminished tonic level. With progression of the immersion phase the level of this tonic shivering activity gradually increased and bursts of intense shivering tremor began to appear. This clonic activity grew in frequency, intensity and amplitude with

decrease in Tc. Once the subjects were switched to breathing the hypercapnic gas mixture, both tonic and clonic shivering activity became markedly reduced (Figure 3) after a lag time ranging between 8.5 and 43.8 seconds. The shivering activity generally returned after several minutes of CO_2 inhalation. The shivering activity was not noticeably different after the subject's inhalate was switched from the hypercapnic to the normocapnic one.

Minute values of NIEMG activity of the masseter and trapezius muscles from several minutes before switching the inspired gas mixture to 4% CO_2 to several minutes after switch of the inhalate back to air are presented with corresponding values of Tes (Figure 2). The NIEMG in both muscles appeared to remain at near pre-AIR-CO_2 switch levels for the first seven minutes of CO_2 inhalation. The NIEMG then rose to a new level, at which it was maintained for the remainder of the CO_2 inhalation period and for the first several minutes after the switch back to air inhalation. This rise in the level of NIEMG corresponded to an increase the raw EMG activity. The AIR-CO_2 and CO_2-AIR differences in the NIEMG of both muscles were both not statistically significant. However, there was a statistically significant elevation in IEMG in both muscles at the eigth minute of CO_2 inhalation.

The NIEMG value during CO_2 inhalation was found to be 15% less than the NIEMG value preceding CO_2 inhalation, while the post-CO_2 inhalation NIEMG value was found to 55% greater than the IEMG value preceding CO_2 inhalation (Figure 4). These changes in the NIEMG level were statistically significant in both cases ($p \leq 0.05$).

D. Conclusion

The present study demonstrated a noticeable decrease in the EMG activity of the masseter and trapezius muscles following CO_2 inhalation, when observed in the raw EMG record (Figure 3), which suggests an inhibition of shivering. The magnitude of attenuation and the time of onset of attenuation from time of introduction of CO_2 was quite varied between subjects. This variation probably arises from individual differences in sensitivity to sudden CO_2 loading. The observed decrease in EMG is not unlike that reported by Lyszczaraz *et al.* (1980) who observed a considerable depression of the amplitude of EMG activity recorded from the quadriceps muscle of rabbits inhaling 6% CO_2, and resumption of activity after return to air inhalation. In the present study, however, EMG activity returned before the switch back to air inhalation. In this study, with further quantification of the shivering activity by integration of EMG, neither a significant reduction of shivering activity following acute breathing of CO_2, nor an elevation in activity after return to air inhalation (Figure 2a and 2b) was revealed. The noticeable drop in EMG activity observed in the raw EMG record is not as evident in the average minute IEMG probably because the time of onset of shivering inhibition from the time of introduction of CO_2 was highly variable between subjects (9 to 43 seconds), and thus averaging of overlapping non-inhibited and inhibited EMG made the inhibitory effect of CO_2 much less dramatic. This is supported by the significantly lower average minimum IEMG value during the fifteen minute period of CO_2 inhalation compared to the IEMG preceding the CO_2 inhalation period (Figure 4). Moreover, the maintenance of IEMG at pre-AIR-CO_2 switch levels in the first 7 minutes of CO_2 inhalation, despite a presumably strong thermal drive from decreasing core temperature and cold skin temperature, is suggestive of a mild inhibitory effect of CO_2 inhalation. Bullard and Crise (1961) observed a

disinhibition of heat production in cold exposed humans after six minutes of inhalation of 6% CO_2. This may also be indicated in the present experiment by the significant rise in IEMG in the eighth minute of the CO_2 inhalation period in both the masseter and trapezius muscles ($p \leq 0.05$). The short duration of shivering inhibition observed by Bullard and Crise (1961) and that suggested by the present experiments may reflect, as discussed in Section IV, the rapid compensation of hypercapnia-induced acidosis and the large stimulus for thermogenesis emanating from central and peripheral thermoreceptors.

The results of the present investigation suggest that acute inhalation of 4% CO_2 during cold exposure may produce a short-lived and mild attenuation of shivering thermogenesis. This mild and short duration inhibition would not be expected to greatly perturb body thermal balance. As such, an acute inhalation of up to 4% CO_2 during cold exposure, such as that encountered during underwater diving, would not be considered a significant potentiator of hypothermia.

The absence of prolonged shivering inhibition may be attributed to adequate compensation of the respiratory acidosis induced by CO_2 inhalation as well as to increasing central thermoregulatory drive with core temperature cooling and cold skin temperature.

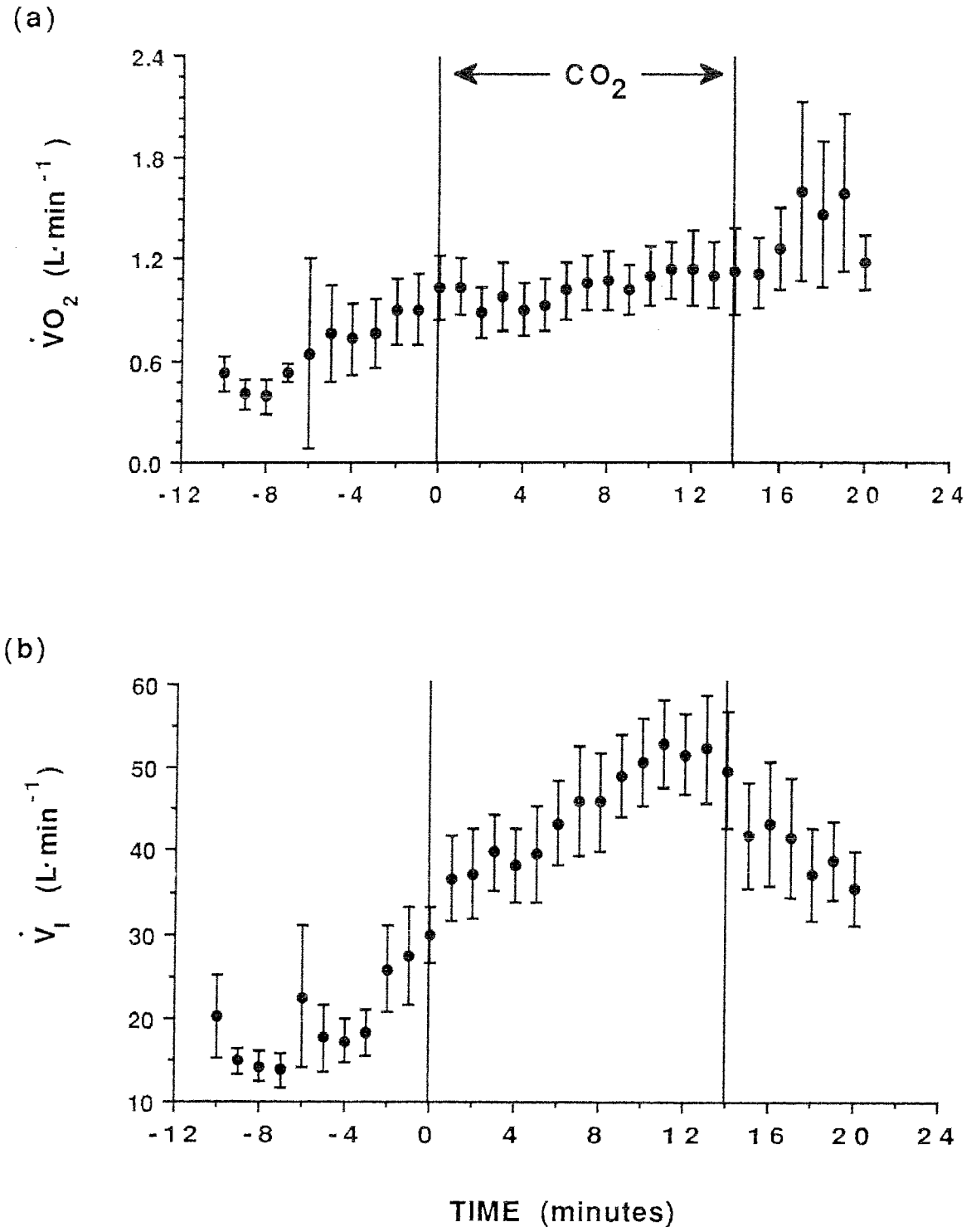

Figure 1. Responses (mean±S.E.) of a) oxygen uptake ($\dot{V}O_2$, L·min^{-1}) and b) ventilation ($\dot{V}_I$, L·min^{-1}) during immersion in a 15°C water bath while breathing room air and 4% CO_2 (n=8).

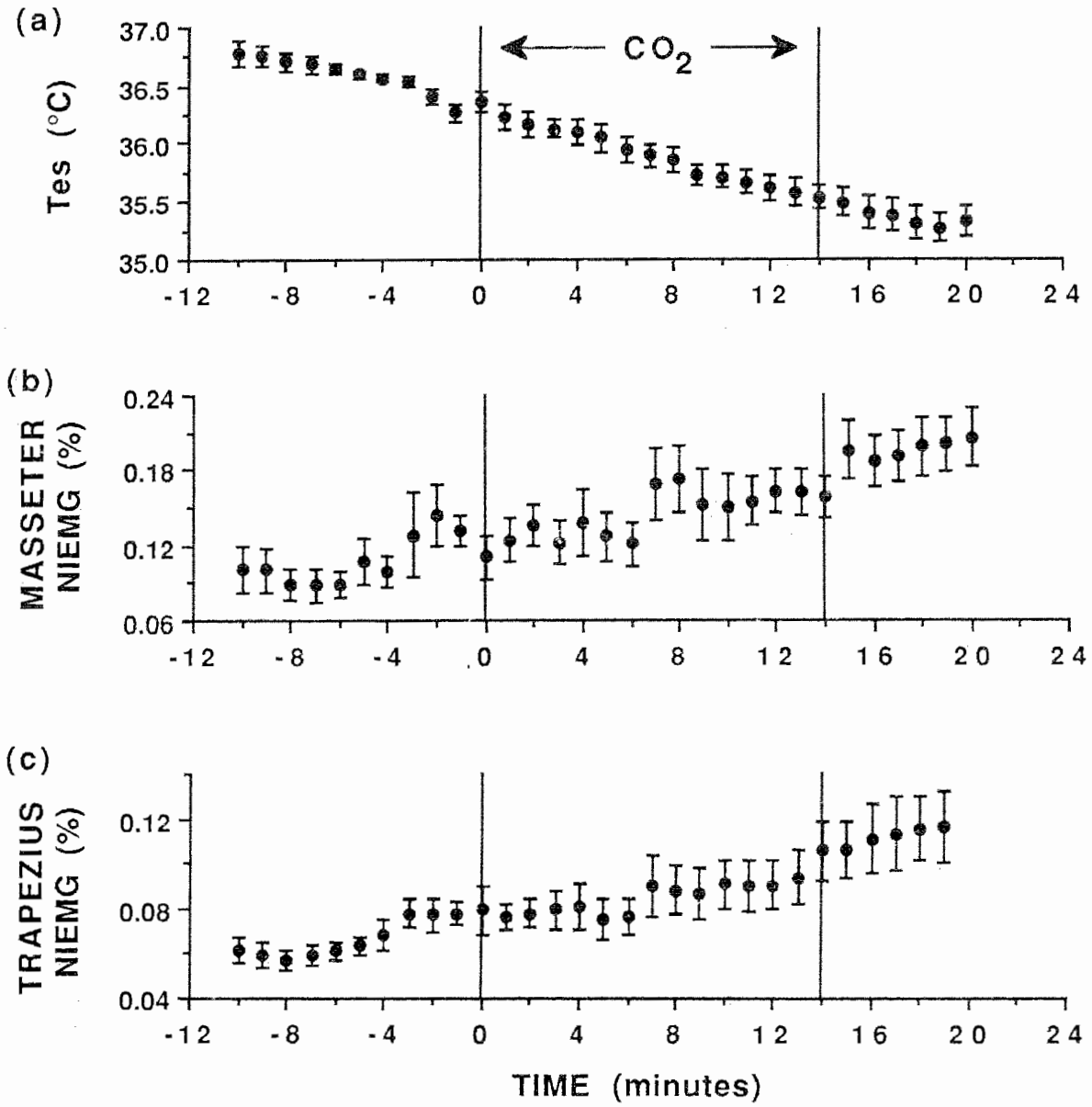

Figure 2. Response (mean±S.E.) of a) esophageal temperature (Tes, °C) and minute normalized IEMG (NIEMG, %) recorded from b) masseter and c) trapezius muscle during immersion in a cold water bath while breathing room air and 4% CO_2 (n=8).

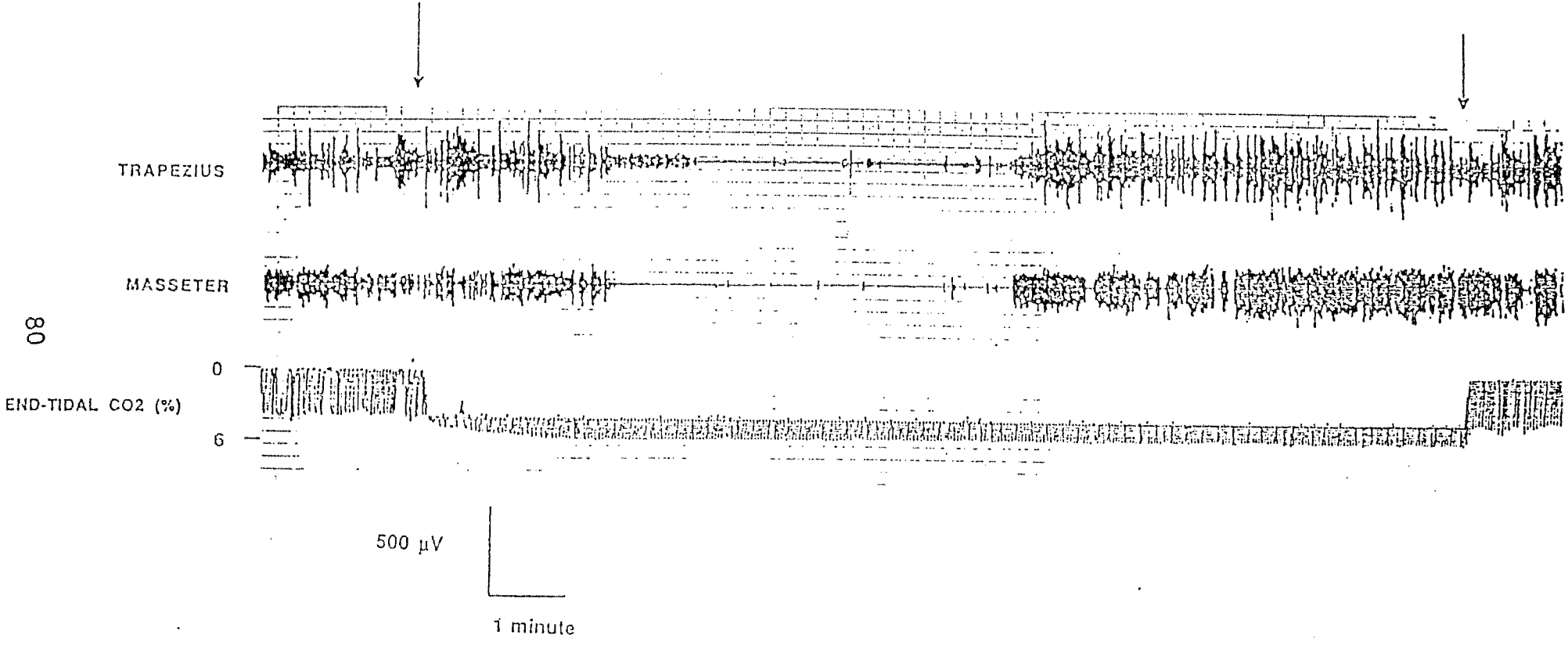

Figure 3. Raw EMG of the trapezius and masseter muscle and corresponding end-tidal CO2 concentrations prior to and during a fifteen minute period of inhalation of 4% CO_2 (between arrows).

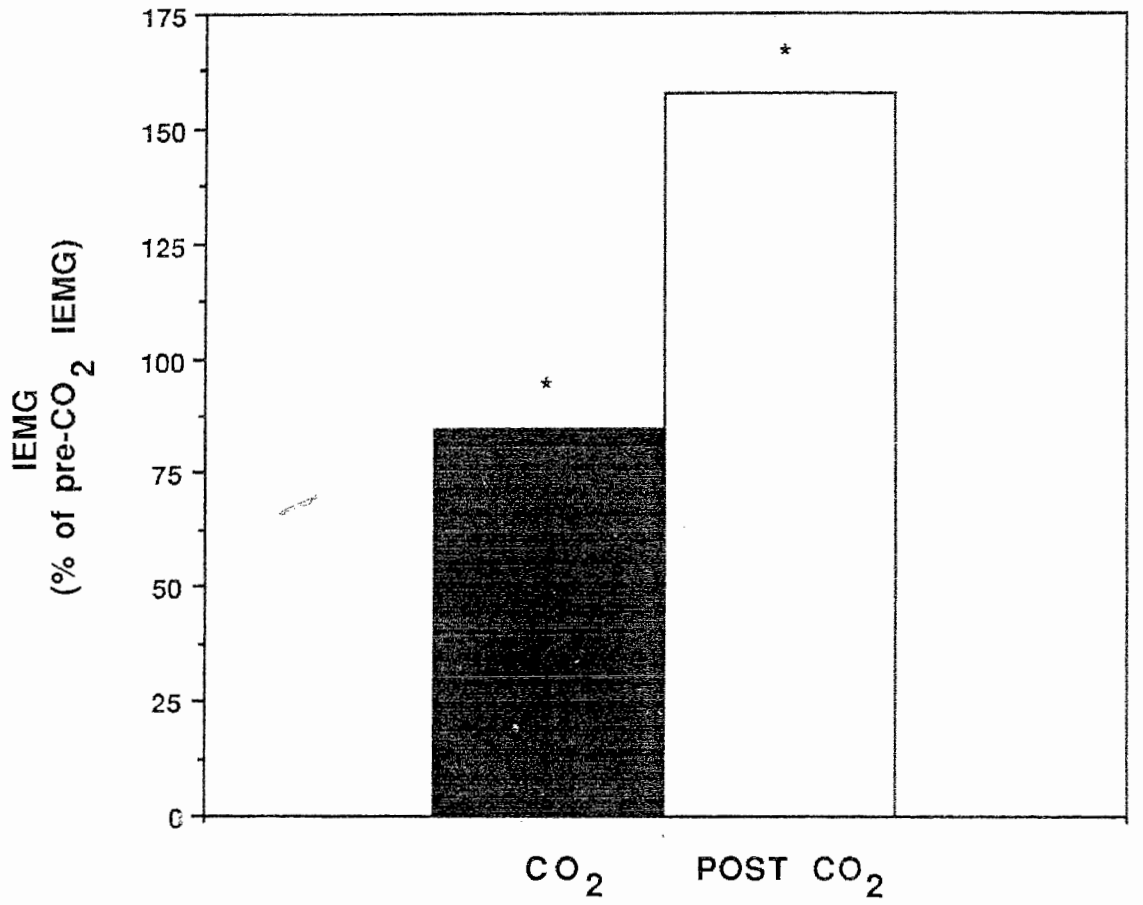

Figure 4. Reduction of IEMG value (mean±S.E.) during CO_2 inhalation by 15% below pre-CO_2 inhalation IEMG value and 55% elevation of IEMG value above pre-CO_2 inhalation IEMG value after return to air inhalation (post-CO_2) (n=8; *, statistically significant at $p \leq 0.05$).

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VI. OVERALL SUMMARY

The combined results of the two experiments in the present study indicate that both prolonged and acute inhalation of 4% CO_2 does not cause a significant inhibition of shivering thermogenesis in hypothermic human subjects. The absence of a significant inhibition may be attributed to the combination of:

1. *Minimal alterations in blood PCO_2 induced by inhalation of 4% CO_2.* As estimated in Appendix B, the inhalation of 4% CO_2 causes elevation of $PaCO_2$ to 46.5mmHg, an increase of 6.5mmHg. Possibly, for a given combination of core and skin temperature, a threshold $PaCO_2$ exists, beyond which shivering may be significantly suppressed. As Wagner et al. (1983) and the present study did not show shivering inhibition with inhalation of 4% CO_2 and Bullard and Crise (1961) observed a transient decrease in heat production with inhalation of 6% CO_2, which would cause $PaCO_2$ to increase by 9 mmHg, then inhalation of at least 6% CO_2 would be required to have any effect. However, as later discussed in (3) the amount of CO_2 needed to have an effect would ultimately depend on the magnitude of the central and peripheral thermal drives.

2. *Compensation of respiratory acidosis resulting from CO_2 inhalation.* Although the acute respiratory acidosis brought about by CO_2 inhalation is not a common physiological condition, the CO_2 titration curves reported by Brackett et al. (1965) demonstrate that an acidosis brought about in this manner is quickly compensated and a new steady state level of acid-base homeostasis is attained within 5-10 minutes. Thus, it may be suggested that the inhalation of 4% CO_2 induces only very small perturbations of acid-base balance which are insufficient to cause an inhibition of shivering.

3. *Increased thermal drive.* Studies to date investigating the effect of CO_2 on temperature regulation have been limited to cold air exposures,

inducing minimal cooling of core and skin regions. The substantially lower absolute core and skin temperatures and faster rates of core temperature cooling attained in the present study with cold water immersion would provide a much greater thermal stimulus for shivering thermogenesis. This increased thermal stimulus may have been a significant factor in overriding any attenuating effects of CO_2.

The results of the study further support that the introduction of elevated inspired PCO_2 of up to 4% in conditions of low ambient temperature probably does not have a significant effect on maintenance of body thermal balance. Consequently, within the context of activities such as underwater diving, during which, cold and hypercapnic conditions are often encountered, inadverdent prolonged or acute inhalation of up to 4% CO_2 would not be considered an important contributor to perturbations of body thermal balance and onset of hypothermia.

Further studies which may stem from the present investigation may include:

1. The effects of combined hypothermia and hypercapnia on body acid-base balance. Acid-base homeostasis during simultaneous thermal and hypercapnic stresses should be assessed with appropriate measures of blood pH, blood bicarbonate, and $PaCO_2$.

2. The influence of individual sensitivity to elevated PCO_2 on the effects of hypercapnia on thermoregulation. Presumably, the effect of a given PCO_2 on thermoregulation for a given individual will be correlated to their CO_2 sensitivity, as determined by the ventilatory response to various levels of inspired PCO_2.

3. The effect of inspiration of gas mixtures with higher concentrations of CO_2. A greater perturbation of acid-base balance with greater concentrations of CO_2 would be expected to disrupt the milleau of neural structures involved in thermoregulation and thus produce greater disturbances of thermal balance.

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APPENDIX A

Influence of CO_2 on Frequency Components of Shivering EMG Activity

A. Introduction

Power spectral analysis (PSA) of the frequency components of electromyographic (EMG) activity recorded from skeletal and smooth muscle has been extensively used to characterize patterns of muscle activity under numerous conditions including various forms of fatiguing and non-fatiguing muscle contractions (Kadefors *et al.*, 1968; Merletti *et al.*, 1990; Petrofsky and Lin, 1980a) and localized muscle ischemia and cooling (Merletti et al., 1984 and Petrofsky and Lind, 1980b). In addition, PSA has been used to assess motor unit firing rates (Fuglslang and Ronager, 1988) and recruitment strategies (Solomonow *et al.*, 1990) and muscle fiber conduction velocities (Holger *et al.*, 1985 and Stulen and de Luca, 1981). More recently, PSA has also been used in the frequency analysis of shivering muscle tremor induced by cold exposure. Bawa *et al.* (1987), used PSA to analyze the surface EMG recorded from the biceps and triceps of human subjects exposed to 5°C. The predominant frequency of shivering tremor of these muscles groups was between 5 and 12 Hertz, and further cross-correlational analysis indicated that the activity of these two antagonistic muscles was approximately 180° out of phase. Muza *et al.* (1986) analyzed the EMG shivering tremor recorded from the masseter muscle of cold exposed human subjects with PSA. The median frequency of shivering tremor was found to fluctuate in range between 170 and 190 Hertz during 80 minutes of cold exposure, with the frequency band of 60-120 Hz containing the most power.

Lyszczaraz *et al.* (1980) reported a considerable depression of the amplitude of EMG activity recorded from the quadriceps muscle of rabbits inhaling 6%CO_2 and a return of the EMG amplitude to pre-CO_2 levels after return to air inhalation. Elevated body PCO_2 has been demonstrated to have effects on both the central and peripheral structures involved in maintenance of body thermal balance, including thermosensitive-hypothalamic neurons, spinal neurons, first-order thermal receptor afferent synpases, and even on skeletal muscle itself. The alteration of the function of these structures then ultimately leads to a reduction in the level of EMG shivering tremor. It is hypothesized that the frequency components of shivering tremor may also be affected by increased body CO_2. Thus, the aim of the present experiment was to determine, using PSA, the effects of CO_2 inhalation on the freqeuncy components of shivering tremor induced by cold water exposure.

B. Methods

1. Subjects

Seven male subjects were recruited to participate in this investigation. Their participation was subject to a physician's approval. Before signing an informed consent form, each subject was familiarized with the experimental protocol and reminded that they could withdraw from the study at any time.

2. Protocol

Clad only in swim trunks, subjects were instrumented while lying supine and following recording of resting values for 10 minutes, subjects were immersed in a 15°C waterbath in two separate experimental trials. In the AIR trial (control), subjects inhaled compressed air (0.03% CO_2, 20% O_2, and 79.97% N_2), while in the CO_2 trial they inhaled a 4% CO_2, 20% O_2, and 76% N_2 gas mixture. Subjects were immersed to the neck until their core temperature, as measured in the esophagus, fell to 35.5°C, or until 60 minutes of immersion had elapsed. The subjects were then removed from the water bath and permitted to rewarm passively for 30 minutes while lying supine and covered with an insulated blanket. Upon completion of the rewarming period, the experiment was terminated, instrumentation removed, and the subjects' heat content was reinstated with immersion in a hot bath.

The order of the two experimental trials was randomly assigned for each subject. The two trials were conducted one week apart, and at similar times of the day on each occasion, to prevent acclimitization and circadian rhythm effects, respectively. Subjects were instructed not to undergo heavy exercise on the days of their trials and to consume a light meal 2-3 hours before each trial.

The protocol used in the present study was approved by the Ethics Review Committee of Simon Fraser University.

4. Instrumentation

Immersion Tank. The immersion tank was a 2.1 m x 1.05 m x 2.1 m fiberglass shell and was normally filled with approximately 4000 L water. The water was constantly circulated with a pump (Swimquip Spa-Support Systems) and filtered (Triton). Circulated water was passed through a refrigration unit controlled by a thermoregulation system (Honeywell) to maintain the water temperature constant at 15°C.

Core Temperature. A thermistor probe (YSI 7012A, Yellow Springs Instruments) was inserted through the nostril and subsequently "swallowed" into the esophagus for recording esophageal temperature (Tes, °C) as an indicator of core temperature (Tc, °C). The insertion length of the esophageal probe was determined from a regression equation based on sitting height, as suggested by Mekjavic and Rempel (1989), and situated the thermistor in the region of the esophagus nearest the left ventricle and atria. The esophageal probe was inserted with the assistance of the experimenter with the subjects sitting upright and head slightly tilted back. After esophageal probe insertion, the subjects rested supine position and the remaining instrumentation was completed.

Electromyography. Skin surface electromyograms (EMG) were recorded from the skin overlying the trapezius muscle (shoulder) with two 2 mm diameter AgCl disc electrodes (Beckmann) spaced 2 cm apart . The electrodes were covered with waterproof tape to minimize movement of the electrodes and prevent water penetration during cold water immersion. Raw surface EMG

signals from the electrodes were band pass filtered (3-3kHz) and amplified (100x) with a common mode rejection preamplifier (Grass Instruments), and recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis. Throughout each experiment EMG was continuously monitored by display on a digital oscilliscope (Philips PM 3206) as well as plotting on a chart recorder (Hewlett Packard 7404A).

4. Data Acquistion

Tes was measured on-line with a data acquisition system (Hewlett-Packard 3497A) controlled by a Macintosh II microcomputer (Apple) with Labview software (Version 2.2, National Instruments).

EMG was continuously sampled and recorded with an FM tape recorder (Hewlett Packard 3968A) for later analysis.

5. Data Processing and Analysis

Power spectral analysis was performed on the shivering EMG activity corresponding to core termperatures of 36.0, 35.75, 35.5 and 35.25°C. 15 second segments of raw analog EMG occuring within 30 seconds before or after the aforementioned core temperatures were digitized at a sampling rate of 1024 Hz and the DC bias removed by subtracting the mean of the EMG segment from the raw signal. From the 15 second digitized EMG samples, one 1 second segment containing only tonic activity was selected and the power spectral density of the segment was determined. One 5 second segment containing predominantly clonic activity was also selected from the 15 second digitized EMG sample. The 5 second segment was then subdivided into 5 one second segments and the power spectral density of each was determined and averaged. The power spectra were then described in terms of their median

frequency (frequency at which the power of the spectrum was equally divided into two, F_{med}) and mean power frequency:

$$Fmean = \frac{sum[\ f(i) \times S(i)\ ]}{sum\ [S(i)]} \qquad [1]$$

Where S(i) is the power spectral density function.

C. Results

No EMG activity was apparent during the resting phase of the experiment except during voluntary movements. Upon entry into the water bath, intense involuntary shivering tremor immediately appeared. Following stabilization of Tsk, shivering activity subsided to a diminished tonic level. With progression of the immersion phase the level of this tonic shivering activity gradually increased and bursts of intense shivering tremor began to appear. With decrease in Tc, this clonic activity grew in frequency, intensity and amplitude, often to the point where tonic activity was not distinguishable.

Initial power spectal analysis of shivering tremor revealed a sharp, high power peak which consistently occured below 10 Hz at the various core termperatures well as a much more dispersed (at least over 100 Hz), low power peak which fluctuated about 50 to 70 Hz during the experiment. In light of what was observed in the segment of raw EMG during PSA, it became apparent that the sharp, lower frequency peak was due to the clonic-type shivering tremor (Figure 1a) while dispersed higher frequency peaks arose from the tonic-type shivering activity (Figure 1b). Consequently, it was decided that it was more appropriate to analyze the clonic and tonic shivering tremor independently. Results, however, are only reported for two subjects who had analyzable tonic and clonic activity at most of the aforementioned temperatures in both the AIR and CO_2 conditions. Most subjects did not display sufficient amounts of both types of shivering at all the core temperatures of analysis, in both trials, and thus had incomplete data sets. Consequently, with such a low sample number, statistical analysis of pooled mean data was not conducted. Interestingly, however, it seemed that the leaner, lower muscle mass subjects generally displayed an early onset of clonic shivering that was maintained throughout most of each trial. The shivering activity of two heavier and higher muscle

massed subjects, on the other hand, seemed to consist of only tonic activity and very little, if any clonic activity. The power spectra for tonic shivering during air and CO_2 inhalation at Tes of 35.75 and 35.25°C of Subject DB are presented in Figures 2 and 3, respectively. The analyzed frequency parameters of the spectra are presented in Table 1. With core temperature cooling, the median frequency increased in both AIR and CO_2 trials. The mean frequency increased in the AIR trial but decreased in the CO_2 trial with decreasing core temperature. Between AIR and CO2 differences were noticeably greater only in the mean frequency at Tes of 35.75. The power spectra for clonic shivering during air and CO_2 inhalation at Tes of 36.0 and 35.25°C for Subject SC are presented in Figure 4 and 5, respectively. The analyzed frequency parameters of the spectra are presented in Table 2. Both mean and median frequencies were higher in the CO_2 trial compared to the AIR trial at Tes of 35.5°C but at Tes of 35.25°C the opposite was true. The between AIR and CO_2 trial and between temperature differences seem to be significant in mean frequency, but not in median frequency.

D. Discussion

The results of the present study suggest that inhalation of gas mixtures containing elevated concentrations of CO_2 does not significantly affect the frequency components of surface tonic and clonic EMG activity recorded from shivering muscle. This is indicated by the relatively similar median frequencies in both AIR and CO_2 trials at different core temperatures. Numerous investigators have reported a decrease in the median frequency of the power spectra of muscle performing various fatiguing exercises (Kadefors *et al.*, 1968 and Merletti *et al.*, 1990) and of those locally cooled during contraction (Merletti *et al.*, 1984 and Petrofsky, 1980), indicating a reduction of muscle fiber conduction velocity due to fatigue and cooling, respectively. In the present study, the trapezius muscle would be expected to be somewhat cooled as a result of cold-induced vasoconstriction and the close proximity to cold water, but the relatively low work of shivering would probably not have fatigued the muscle. The failure to show any changes in the frequency parameters of the power spectra may suggest that within the conditions of the present study, the shivering muscle was neither significantly cooled nor fatigued. Moreover, the involuntary nature of the shivering tremor may also have some influence on the motor control of the muscle, perhaps making it less susceptible to the effects of fatigue and temperature changes. Lyszczarz *et al.* (1980) showed a drop in intensity and amplitude of shivering EMG during CO_2 inhalation, and combined with the present results may be an indication that CO_2 only affects the intensity of muscle activity but not its recruitment and activation. Further evidence of this is indicated by the noticeable decrease in the maximum contraction force, and consequently heat production of frog skeletal muscle when directly exposed to elevated concentrations of CO_2 *in vitro* (Kitano, 1988). However, with breathing 4% CO_2, there are relatively small alterations in acid-base balance (see

Discussion, Section IV) and as such, any inhibitory influences are probably greatly reduced.

With core temperature cooling the median frequency tended to increase in both AIR and CO_2 trials, indicating, perhaps an increase in motor unit recruitment with increasing intensity of shivering as core temperature cooled. This is supported by Solomonow *et al.* (1990), who found an increase in the median frequency of the power spectra of the intramuscular EMG recorded from the gastrocnemius muscle of cats artificially stimulated at different levels of motor unit recruitment. This result, however, is contrary to that reported by Muza *et al.* (1986) who also analyzed the surface shivering EMG of the masseter muscle with PSA and did not find any alterations in the power spectrum with prolonged shivering. However, as the mean core temperature of the subjects in Muza *et al.*'s study (1986) was actually higher at the end of cold exposure than at the beginning of cold exposure, it is possible that the thermal drive of only low skin temperature was not sufficient to cause a dramatic increase in shivering intensity. Thus, an increase in motor unit recruitment was not observed in their study.

In the present study, we have also separated the shivering activity of the trapezius muscle into a sharp, low frequency, high power clonic component and a dispersed, higher frequency, low power tonic component. Although previous investigators have qualitatively reported that shivering contains at least three patterns of activity, namely, continuous, high frequency, asynchronous tonic activity; rhythmic, synchronized group discharges; and low frequency, burst-like activity (reviewed in Kleinebeckel and Klussmann, 1990), the quantitative separation of the shivering into these components with PSA has not been described in humans to date. As mentioned above, the appearance of clonic activity seemed to depend on the mass and muscularity of the subject, that is,

with greater mass and muscularity, there was a lower degree of clonic shivering, presumably due to a greater peripheral insulative shell. In general, though, the tonic component was present throughout the cold exposure period while the clonic component appeared at the onset of cold exposure, subsided and then gradually reappeared with increasing frequency, duration, and amplitude with continued cold exposure.

In summary, chronic inhalation of 4% CO_2 and prolonged shivering does not affect the frequency components of shivering in human subjects immersed in cold water. However, the frequency of shivering was found to increase with core temperature cooling. The absence of any affects during CO_2 inhalation may be due to the involuntary nature of shivering, or perhaps because CO_2 causes inhibition of the intensity of muscle activity and not its recruitment. However, inhibitory effects are probably limited because the acid-base perturbation induced by inhalation of 4% CO_2 is small and probably quickly compensated.

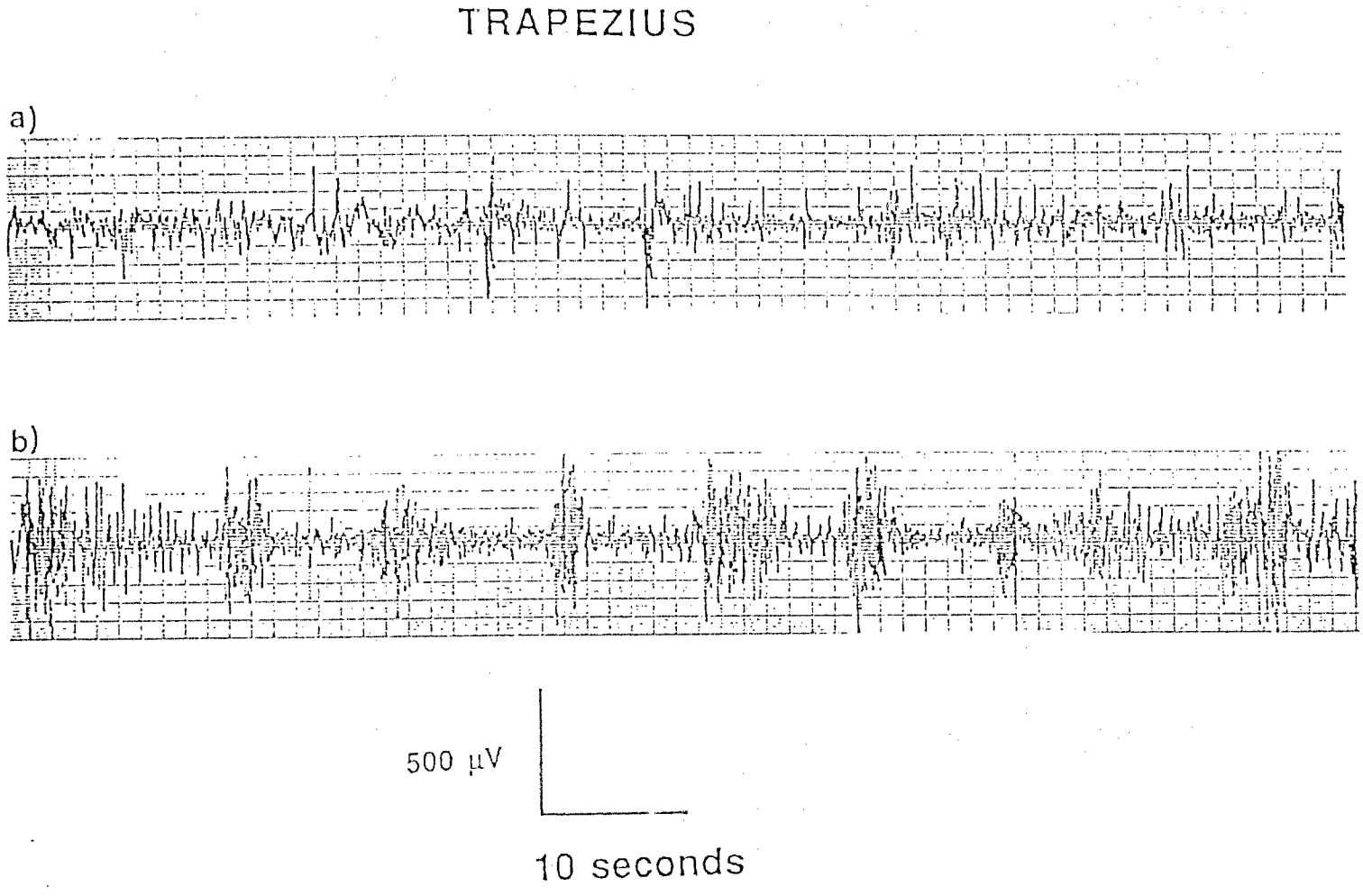

Figure 1. Raw EMG record of tonic and clonic shivering activity (a and b, respectively) recorded from the trapezius muscle of Subject IM.

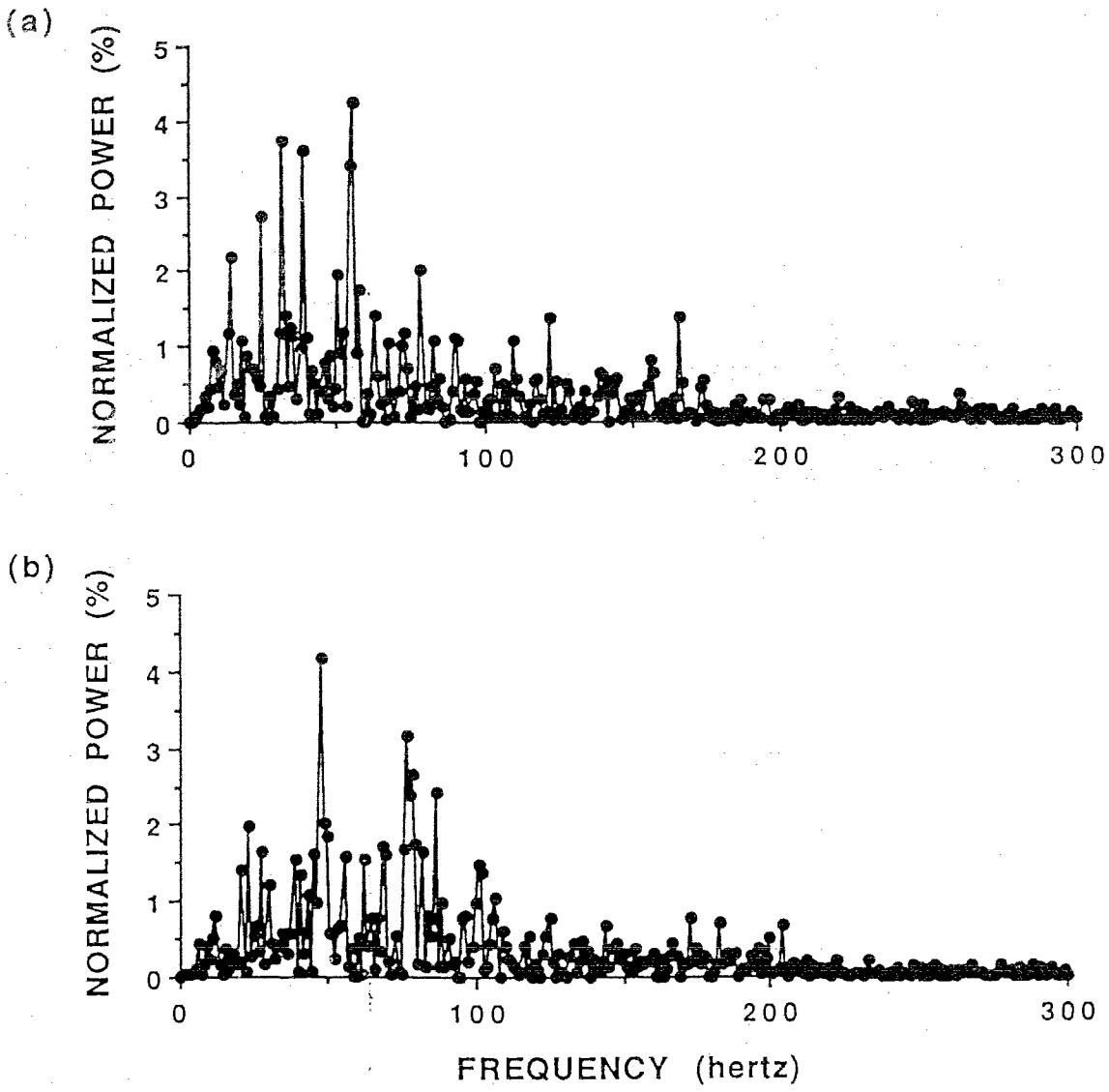

Figure 2. Power spectra of tonic shivering activity at Tes of 35.75 and 35.25°C (2a and 2b, respectively) recorded from trapezius muscle of while breathing air (Subject DB).

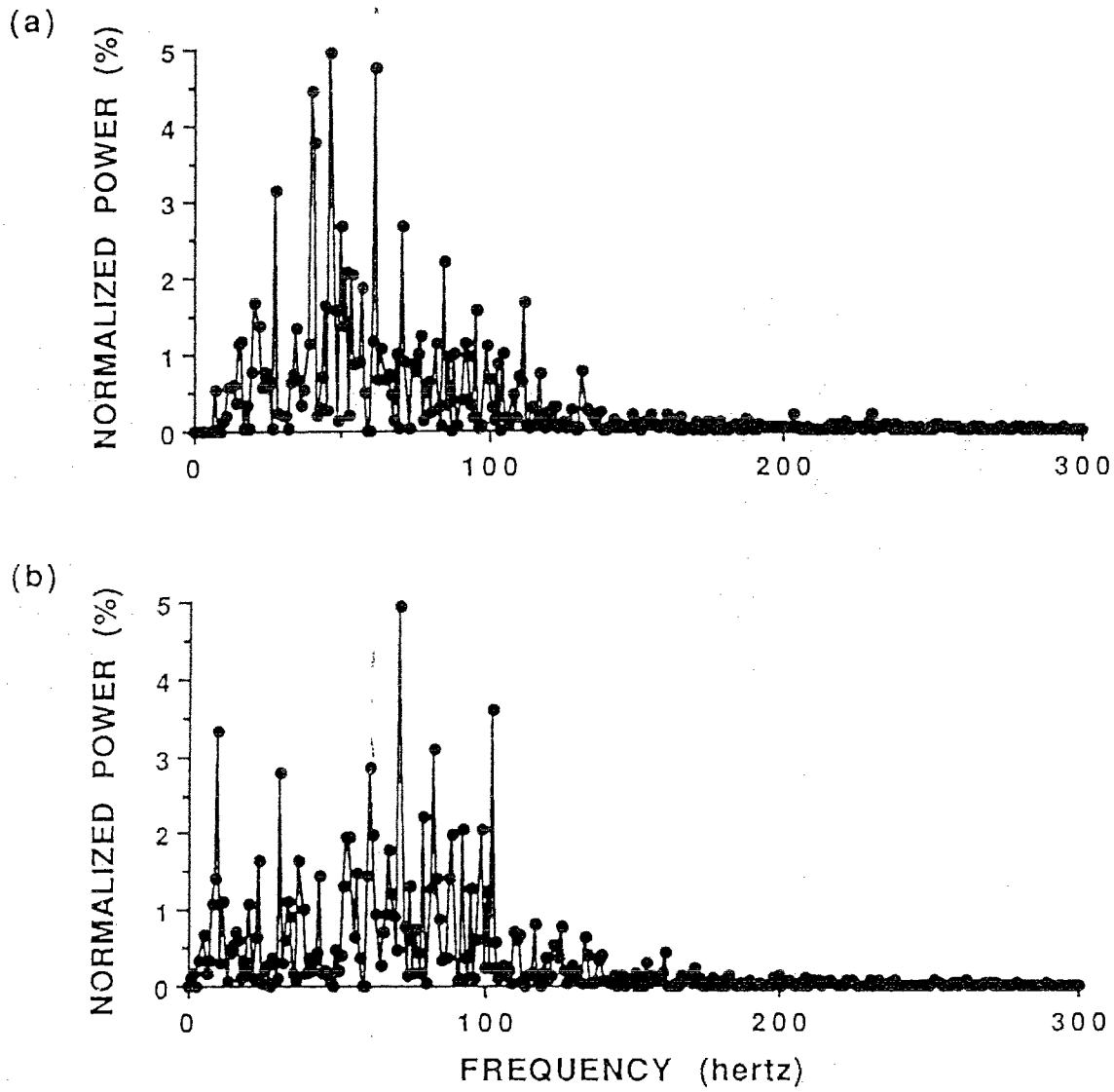

Figure 3. Power spectra of tonic shivering activity at Tes of 35.75 and 35.25°C (3a and 3b, respectively) recorded from trapezius muscle while breathing 4% CO_2 (subject DB).

Table 1. Analyzed frequency parameters of the power spectra of tonic shivering activity at Tes of 35.75 and 35.25°C recorded from trapezius muscle while breathing air and CO_2 (Subject DB).

	Core temperature	
Parameter	35.75	35.25
Med Freq. (Hz)		
AIR	58.0	63.3
CO_2	57.1	70.3
Mean Freq. (Hz)		
AIR	82.3	79.8
CO_2	68.3	72.4

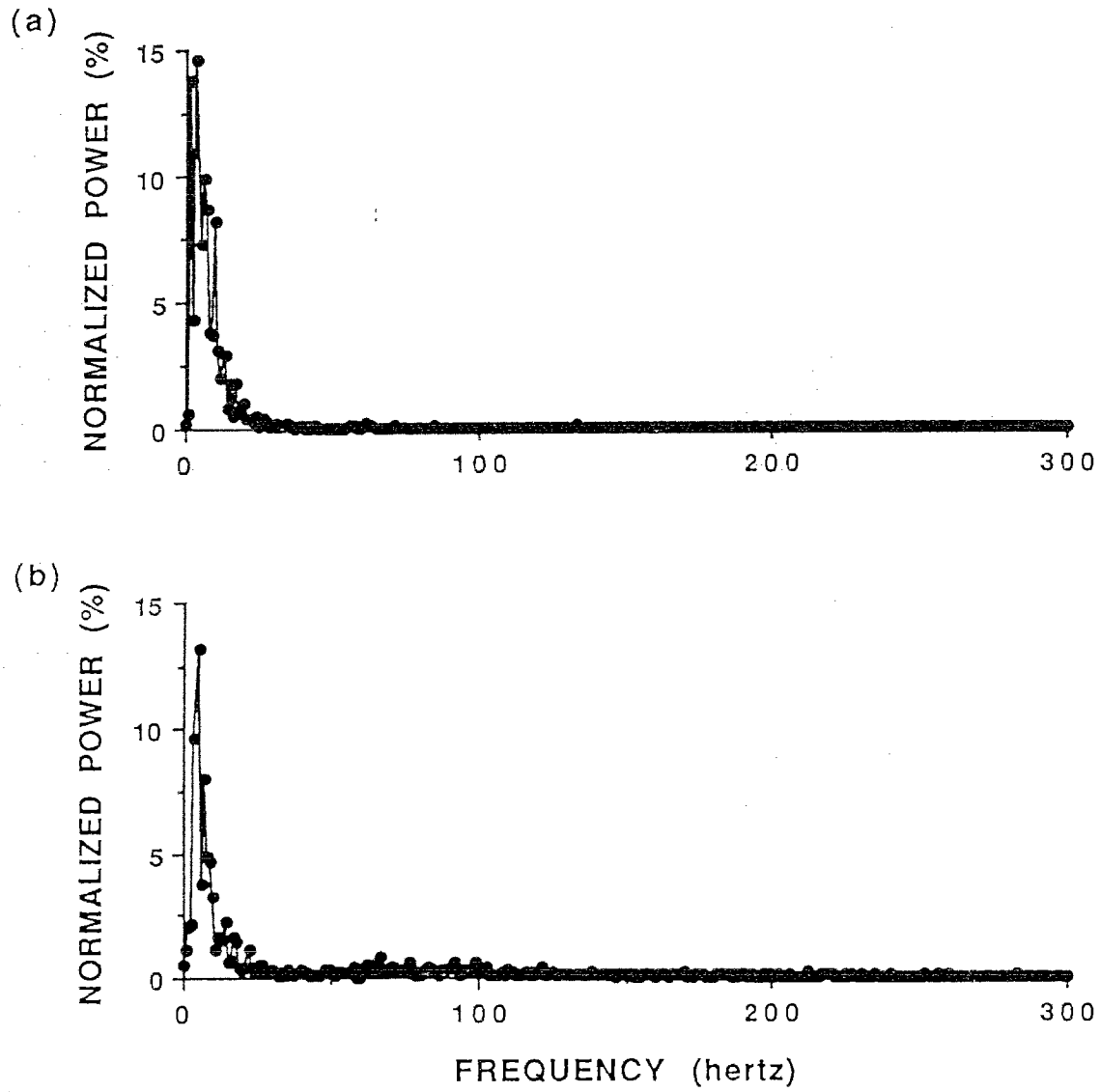

Figure 4. Power spectra of clonic shivering activity at Tes of 36.0 and 35.75°C (4a and 4b, respectively) recorded from trapezius muscle while breathing air (subject SC).

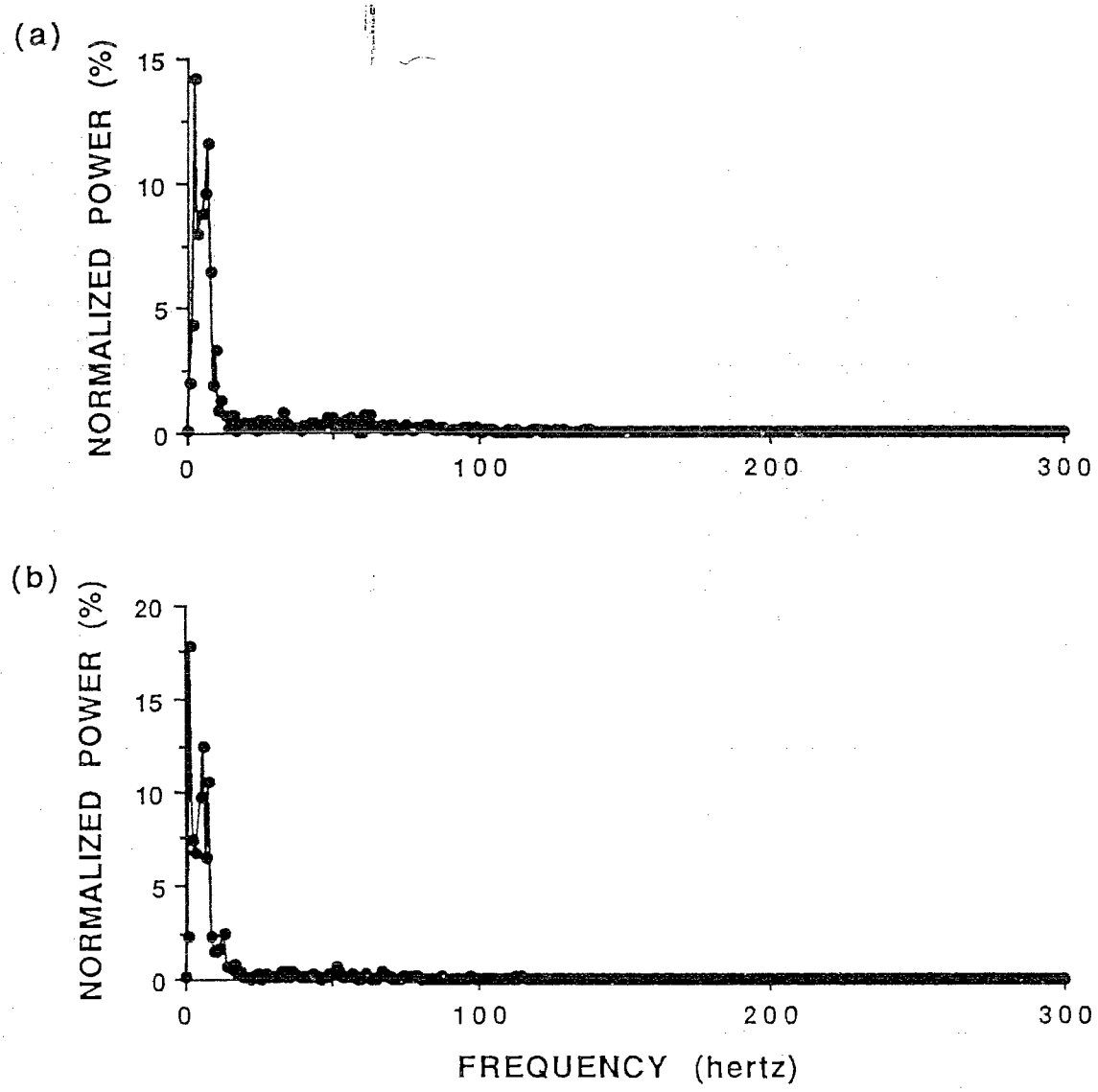

Figure 5. Power spectra of clonic shivering activity at Tes of 36.0 and 35.75°C (5a and 5b, respectively) recorded from trapezius muscle while breathing 4% CO_2 (subject SC).

Table 2. Analyzed frequency parameters of the power spectra of clonic shivering activity at Tes of 36.0 and 35.25°C recorded from trapezius muscle while breathing air and 4% CO_2 (Subject SC).

	Core temperature	
Parameter	36.0	35.25
Med Freq. (Hz)		
AIR	6.4	9.7
CO_2	6.8	6.0
Mean Freq. (Hz)		
AIR	15.3	40.5
CO_2	20.7	13.9

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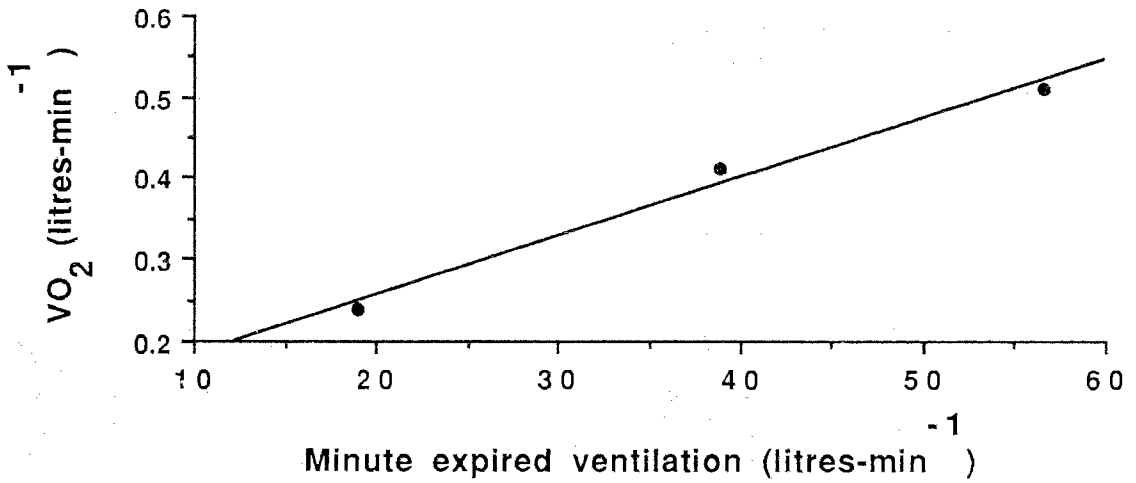
APPENDIX B

METABOLIC COST OF SHIVERING

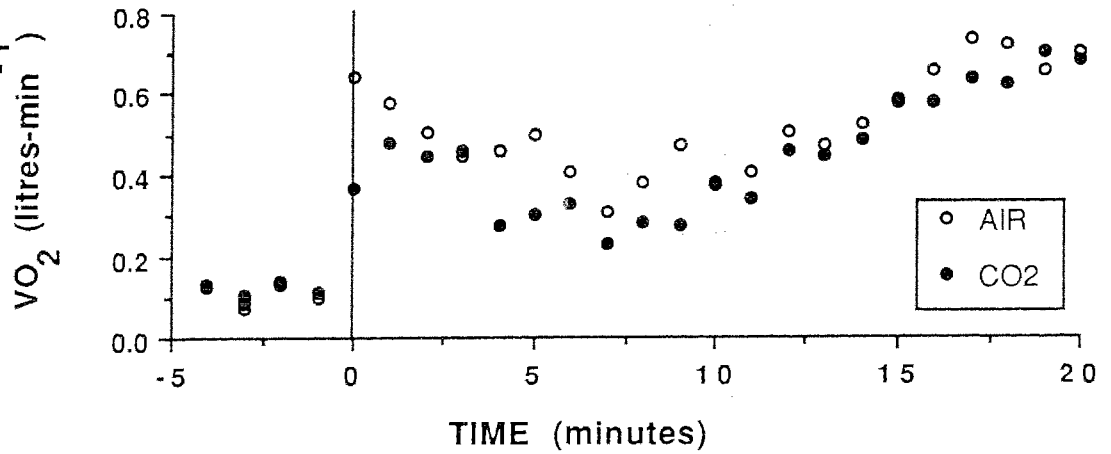
It is well documented that the oxygen cost of breathing increases exponentially with increasing minute ventilation (reviewed in Otis, 1964). Thus a more accurate assessment of the differences in the metabolic cost of shivering between the AIR and CO_2 trials could be made by subtracting the metabolic cost of respiration from the measured total $\dot{V}O_2$. The $\dot{V}O_2$ of breathing at 20, 40, and 60 litres-min^{-1} was measured in one subject during rest is presented in the following figure:



From a curve fit of the data,

$$\text{Work of breathing} = 7.1818 \times e^{-3x} + 0.11275$$

The resulting differences VO_2 between the AIR and CO_2 trial were again not significant and are presented in the following figure:



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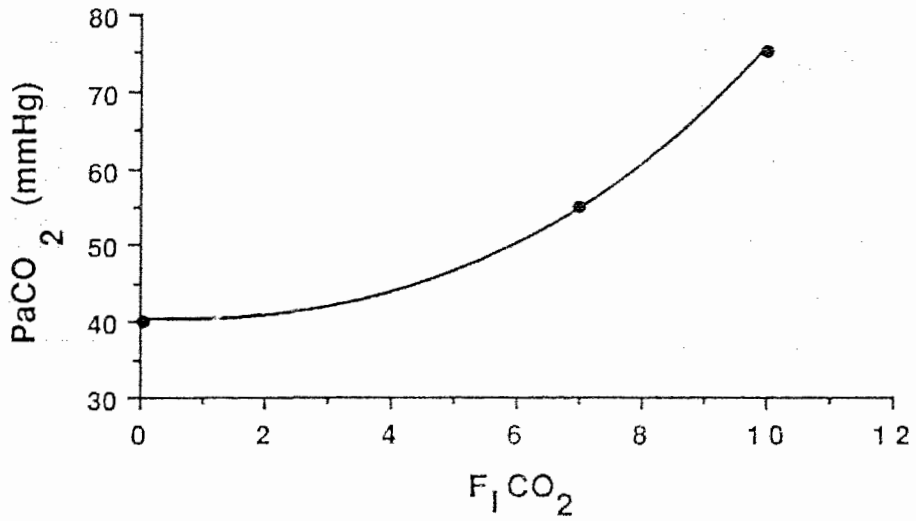
APPENDIX C

PREDICTION OF $PaCO_2$ FROM $FICO_2$

In order to estimate the $PaCO_2$ that would result from inhalation of 4% CO_2, an exponential curve was fit to the $PaCO_2$ values reported by Brackett *et al.* (1965) resulting from inhalation of gas mixtures containing 7 and 10% CO_2:



Thus:

$$PaCO_2 = F_ICO_2 \times e^{(FICO2/8.2)} + 40$$

For $F_ICO_2 = 4\%$:

$$PaCO_2 = 4 \times e^{(4/8.2)} + 40$$

$$PaCO_2 = 46.52 \text{ mmHg}$$

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