

ESTIMATED CARBON DIOXIDE PRODUCTION AND PHYSIOLOGICAL
ADAPTATION OF SURVIVORS IN A SIMULATED DISABLED
SUBMARINE

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EXECUTIVE SUMMARY

Seven volunteer US Navy personnel were exposed for one week to conditions simulating those expected to develop within a disabled submarine (DISSUB) in the hypobaric facility at the United States Army Research Institute of Environmental Medicine (USARIEM). The primary purpose of the study was to obtain a more reliable estimate of the rate at which DISSUB survivors consume oxygen (VO_2) and generate carbon dioxide (VCO_2). This information will be used to provide improved estimates of the amount of lithium hydroxide (LiOH) required aboard submarines for emergency CO_2 scrubbing, and to calculate likely survival times based on the quantity of LiOH available and the number of surviving crew.

The environmental conditions used in this study were 4°C (39°F), 16.75% O_2 , 2.5% CO_2 , 1 atmosphere absolute pressure (ata), and 80-85% relative humidity. In accordance with recommended DISSUB procedures, the subjects were instructed to rest in their bunks whenever they were not involved in an experimental procedure, approximately 16-20 hours per day. They were provided with a diet that would be available in the forward compartment of a 688-class DISSUB. However, quantities of food and macronutrient composition ratios of the diet were controlled at constant levels designed to avoid dietary-related changes in VCO_2 . None of the food was cooked or heated and the volunteers had free access to fluids. Besides measuring respiratory exchange and metabolism parameters, a variety of other studies were conducted to document further the expected effects of DISSUB conditions on survivors: anthropometric measures; thermoregulation; fluid balance; hand grip strength and endurance; subjective and cognitive function. It was anticipated that the results of these experiments might provide some insight into the likely capability of surviving DISSUB crewmembers to assist in the effort to rescue them.

The principal findings were:

- The rate of carbon dioxide production by DISSUB survivors is critically dependent on their level of activity and is likely to fall in the range $0.08\text{-}0.12 \text{ lb}\cdot\text{hr}^{-1}$ ($0.29\text{-}0.46 \text{ l}\cdot\text{min}^{-1}$).
- The rate of oxygen consumption is likely to fall in the range $0.64\text{-}1.18 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F , 1 ata, dry ($0.69\text{-}1.27 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F ; $0.30\text{-}0.56 \text{ l}\cdot\text{min}^{-1}$).
- A diet of 2000 kcal per day results in a negative energy balance, but is adequate for the limited period that the crew will have to survive in a DISSUB.
- The volunteers required a considerable amount of insulation to keep warm. They had an average of 5 clo of insulation split almost evenly between clothing and bedding, and this was adequate to maintain their core temperature throughout the trial.
- There were no operationally significant effects of this trial on the other variables measured.

ACKNOWLEDGEMENTS

This is a report of a study that was undertaken collaboratively with the United States Army Research Institute of Environmental Medicine (USARIEM). The authors would like to acknowledge at the outset the tremendous assistance provided by the staff of USARIEM in the execution of the project. In particular, we would like to recognize the substantial contribution of MAJ M.E. Bovill, Sharon Kavanaugh, and Mr. P. Niro from USARIEM. We would also like to recognize the hard work of ENS S. Foster who analyzed much of the ventilation data, as well as the efforts of COL K. Keenan and LCDR J. Kane who served as the medical monitors, around the clock, throughout the study. No acknowledgment would be complete without a mention of the subjects who volunteered to participate in this study. Despite extensive briefing on the unpleasant nature of the conditions to which they would be exposed, and their subsequent realization that there had been no exaggeration, these remarkable men voluntarily remained isolated in a study chamber in which the likely conditions in a disabled submarine were replicated. We would like to express our admiration for their selfless effort and acknowledge that without them there would be no findings to report.

INTRODUCTION

A disabled submarine (DISSUB) unable to surface under its own power will quickly become a hostile environment. Even if watertight integrity is maintained and the products of combustion, radioactive species or other toxic contaminants do not pollute the atmosphere, the survivors will face other hazards. For one, the DISSUB will progressively cool to the temperature of the water surrounding the boat and this is likely to be on the order of 4°C on the continental shelf in most parts of the world. These conditions exacerbate body heat loss, challenging the thermoregulatory system's defense of normal body temperature. The humidity will increase quickly to about 100% as a result of the cooling atmosphere, the large amount of water released into the air from the survivors, and the reaction between carbon dioxide (CO₂) and lithium hydroxide (LiOH), the emergency CO₂ scrubbing agent carried on U.S. Navy (USN) submarines. The high humidity will further exacerbate body heat loss in the survivors by reducing the thermal insulation of clothing.

Current advice is to maintain the O₂ levels in submarines during normal operations somewhat below normal sea level values (approximately 18%) to help suppress outbreaks of fires, and the O₂ level may fall to as low as 16% in a DISSUB before escape is considered¹. This potentially will lead to hypoxemia. The most important factor, however, in determining the length of time that the crew will be able to survive in a DISSUB will probably be the level of carbon dioxide in the atmosphere. Atmospheric CO₂ levels typically average 0.03-0.09% outdoors and in unconfined spaces, but when levels exceed about 3% for several hours or more, a variety of pathophysiological effects begin to develop. These include progressively severe headaches, loss of ability to concentrate, air hunger, sweating, anxiousness, nausea, dizziness, tremors and burning eyes. Prolonged exposure to 10% CO₂ results in loss of consciousness and death within a few hours¹.

Exactly how long the CO₂ can be maintained at safe levels within a DISSUB will depend on a number of factors:

- a. The capacity (throughput and efficiency) of any scrubbing device for removing CO₂ from the atmosphere;
- b. The volume of the compartment;
- c. The number of survivors;
- d. The survivors' carbon dioxide production rate.

USN submarines have very limited storage space for CO₂ scrubbing material (LiOH). Currently, the amount available in a 688-class boat is about 950 lb, sufficient to absorb about 700 lb of CO₂ in the cold, humid conditions in a DISSUB. The current assumption is that each survivor generates about 0.1 lb·hr⁻¹ of CO₂ (which equates to approximately 0.38 l·min⁻¹ of CO₂ at 0°C and 1 atmosphere absolute pressure [ata], dry). This translates to a total scrubbing capacity of about 290 man-days (assuming 100% scrubbing efficiency). With the forward compartment likely to contain as many as 120 survivors, the scrubbing capacity amounts to no more than 58 hours of CO₂ production in a worst-case scenario. Since the efficiency of the current passive scrubbing method is half that of the electrically powered system¹, even this figure is optimistic.

The survivors' rate of CO₂ production depends on their metabolic rate and respiratory quotient (RQ) which, in turn, are dependent on their diet, activity level, thermal balance, and levels of O₂ and CO₂ in the inspired air. Clearly, these interdependent variables that determine the rate of CO₂ production (VCO₂) of the crew will be influenced by conditions prevailing in the DISSUB. However, the assumed value for CO₂ production currently used by the USN to estimate scrubbing capacity was based on CO₂ production measurements made on subjects exposed to conditions that do not adequately reflect those likely to prevail on the DISSUB. Unfortunately, there is no physiological model known to the authors which is sufficiently robust to quantify the interactions of all the factors likely to influence CO₂ production under DISSUB conditions. Thus, in this investigation, the VCO₂ of humans was measured directly during several days of exposure to conditions closely simulating a DISSUB to provide a better estimate of CO₂ production in DISSUB survivors for use by operational planners devising rescue protocols.

BACKGROUND

The following sections review the currently available understanding of how DISSUB conditions might affect important body functions.

ACTIVITY AND DIETARY EFFECTS ON CO₂ PRODUCTION

Surviving crew aboard a DISSUB who are not required to perform essential tasks should be restricted to their bunks. By avoiding all unnecessary physical activity, the survivors' metabolic rate, and thus their oxygen consumption (VO₂) and VCO₂, will be minimized. However, the DISSUB will, as described above, probably be cold, and survivors will shiver to varying degrees despite the ample clothing and blankets that should be available, at least in the forward compartment of the submarine. In addition, the survivors' diet (both composition and energy content) can also affect their metabolism, RQ, and, consequently, VCO₂. Differences in these modulating factors, due to variations in inspired atmospheric composition, ambient temperature, clothing, and diet, probably contribute to the wide range of VCO₂ measurements reported in different DISSUB experiments, summarized in Table 1. No reported study has adequately replicated the environmental conditions likely to prevail in a USN DISSUB forward compartment. Previous studies have employed an unrealistically warm ambient temperature, unrealistically low CO₂ levels, or an insufficient duration for a steady state to be reached with respect to acclimatization to hypercapnia or diet, both major modulators of VCO₂. In several prior studies, subjects were starved and dehydrated, which is likely to have had a considerable effect on VCO₂ measurements reported. While survivors in some compartments, such as the aft end of a fast attack submarine, will have access to little or no food, in other compartments they should have access to a more substantial quantity of food in the freezers, refrigerators and dry food stores. Thus, VCO₂ measurements reported from studies involving starvation conditions may be applicable for some but not the majority of expected survivors. In this study, VCO₂ measurements were completed under standardized conditions in which subjects were fed a carefully controlled diet derived from the foods likely to be available on a DISSUB. These data should provide a baseline for future studies in which intentional dietary manipulation might serve as a means of lowering the VCO₂ in DISSUB survivors.

THERMOREGULATION

As already mentioned, the DISSUB will probably become cold. Cold exposure elicits two principal physiological responses in humans: peripheral vasoconstriction that tends to retard convective loss of body heat to the environment, and shivering which increases metabolic heat production. If these responses are inadequate to maintain the normal balance between heat loss and heat production, then the body temperature will fall. Besides being exposed to cold, DISSUB survivors will be breathing a mildly hypoxic, moderately hypercapnic atmosphere. Previous studies report that both shivering and peripheral vasoconstrictor responses to cold are affected when the O₂ and CO₂ content of the inspired air is altered from normal sea level values. However, the effects reported are not entirely consistent.

Hypoxia is usually observed to blunt shivering during cold exposure²⁻⁴, but not always^{5,6}. In one of the two studies that failed to show a hypoxia-induced blunting of shivering, the metabolic heat production was lower than in normoxia, although the difference was not quite large enough to achieve statistical significance⁵. In the other⁶, the hypoxia was less severe (F₁O₂ = 15%) than in the studies in which an effect was observed (F₁O₂ = 10-12%). It is also possible that the blunting of shivering, which some have attributed to hypoxia, is secondary to the hypocapnia which normally results from hypoxia-induced increases in ventilation. This could

explain why shivering was not affected by mild hypoxia, since in such conditions the associated hypocapnia may not develop until there have been several days of exposure. Johnston et al. observed a delayed onset of shivering during cold exposure when subjects breathed hypoxic gas supplemented with CO₂ to prevent hypocapnia, compared to breathing normoxic gas⁷. They argued that this demonstrated that hypoxia, not hypocapnia, blunted shivering. However, their protocol was not designed to evaluate steady-state shivering rates. Therefore, whether steady-state shivering thermogenesis would be affected by mild hypoxia (i.e., F_IO₂ = 15-18 %) in the absence of hypocapnia remains to be shown.

There is better agreement on the effect of hypoxia on the vasoconstrictor response to cold. The consistent observation is that hypoxia induced by breathing a hypoxic gas mixture or exposure to high altitude results in a smaller decline in skin temperature during cold exposure^{2, 3, 5, 8}. Furthermore, breathing a hypoxic gas enriched with CO₂ to prevent hypocapnia delayed the onset of vasoconstriction until a lower core temperature had been achieved than when a normoxic, normocapnic gas was breathed⁷. Only in the study using the mildest level of hypoxia (F_IO₂ = 15%) was there no effect on the vasoconstrictor response to cold⁶. That observation again raises the question of whether there is some critical threshold of hypoxia that can be tolerated without affecting vasoconstrictor responses to cold, or whether, perhaps, the factor affecting vasoconstriction is hypocapnia, which is absent during acute exposure to mild hypoxia.

Relatively little research has been directed at the effects of hypercapnia on responses to cold. Johnston et al.⁹ observed that the onset of shivering, but not vasoconstriction, during progressive cooling was delayed until a lower core temperature threshold was achieved when subjects breathed normoxic gas containing 4% CO₂ compared to when they breathed normoxic, normocapnic air. However, as with the hypoxic studies from the same laboratory, the experimental protocol was not designed to evaluate steady-state shivering, and another study from this same group suggested that shivering was only transiently suppressed by hypercapnia, rapidly returning as respiratory acidosis produced by hypercapnic breathing was buffered¹⁰. Fothergill et al.¹¹ found that five minutes of breathing 6% CO₂ lowered forearm blood flow during cold water immersion, but this, again, was not an effect on steady-state responses. In the only reported study that attempted to evaluate the effect of hypercapnia on shivering and vasoconstrictor responses to cold under steady-state conditions, no differences in responses were observed between trials in which normoxic, 4% CO₂ gas was breathed compared to when normoxic, normocapnic gas was breathed¹². An interpretation of the collective findings from all these studies is that the acute effect of breathing hypercapnic gases seems to have little effect on thermoregulatory responses to cold, perhaps because the blood buffering systems rapidly compensate for respiratory acidosis.

The earlier studies described thus far only considered the thermoregulatory effects of relatively short (1-3 hours) alterations of inspired O₂ and CO₂. No study has investigated the potential thermoregulatory effects of breathing elevated levels of CO₂ continuously for several days. Of the few studies investigating how chronic hypoxia affects thermoregulatory responses to cold, only one evaluated thermoregulatory responses elicited during whole-body cold exposure³, while the others^{8, 13} considered responses elicited by localized cold exposure of the hand or finger while the rest of the body was kept warm. Among the adaptations induced by chronic hypoxia is a progressive rise in circulating levels of norepinephrine, which is generally

accepted to indicate a chronic elevation in sympathetic nervous tone¹⁴. A chronic elevation in sympathetic nervous activity might produce a down-regulation of sympathetic receptors, and, therefore, physiological responses to cold mediated by sympathetic stimulation, such as peripheral vasoconstriction, might become less pronounced with chronic hypoxia. Consistent with this speculation are the observations that following six weeks of chronic hypoxia ($F_{I}O_2 = 11\%$), shivering and vasoconstrictor responses elicited by whole-body cold exposure were still blunted compared to normoxic values, but the blunting appeared slightly less pronounced than when observed initially under acute hypoxic conditions³. On the other hand, vasoconstrictor responses elicited in the fingers during local cooling under hypoxic conditions appear to become more pronounced as the duration of hypoxia continues¹³. These studies all considered effects of fairly severe hypoxia and it is not clear whether chronic exposure to less severe hypoxia would induce similar effects. The apparent discrepancy between responses to whole-body cold exposure and those elicited by localized cooling warrants further study.

COGNITIVE FUNCTION

The DISSUB environment may produce symptoms (e.g. headache, nausea, etc.) and/or decrements of cognitive function to a point that the escape procedure could not be undertaken successfully. This is important because mistakes could potentially result in injury or death of the escape trunk occupants and, possibly, disable the trunk for subsequent escapers. The effects of the DISSUB environment on cognitive function and symptomatology have not been previously reported. In a study of prolonged (26 days) exposure to slightly elevated CO_2 levels (0.7% and 1.2%) with normoxia, normal temperatures, and unrestricted activity, some evidence of mild impairment of tracking ability, but not short term memory, was observed¹⁵. In another study, seven days of exposure to cold in an anti-exposure suit (4.4 °C, 99% relative humidity) had no effects on measurements of reaction time or short-term memory, but subjects breathed a normocapnic, normoxic atmosphere which fails to adequately simulate the DISSUB environment¹⁶. The level of CO_2 expected to develop on a DISSUB appears to be the threshold level for CO_2 in the inspired air at which subjects begin to report experiencing headaches¹⁷, but a comprehensive assessment of symptoms experienced and the time course of their development and resolution during a period of hypercapnia in combination with mild hypoxia and cold exposure has not been reported. Prolonged exposure to lower levels of hypercapnia ($F_{I}CO_2 = 0.7\%$ and 1.2%) reportedly has minimal effects on sleep¹⁸ but effects of exposure to the higher levels expected to develop on the DISSUB remain unknown. Thus, a more complete, systematic evaluation of these types of potential performance impairments in DISSUB survivors is required.

HAND GRIP STRENGTH AND ENDURANCE

The manual tasks needed for submarine evacuation include turning valves and supporting body weight with the hands, so whether grip strength might be affected by exposure to the DISSUB atmosphere is an important question. No study has evaluated grip strength changes during prolonged exposure to hypercapnia in combination with mild hypoxia and cold. However, some studies address the effects of these stressors independent of the others. Isometric force testing has been utilized to quantify changes in overall muscle strength and has been used as a marker of strength during acute and chronic exposure to hypoxia. When humans are exposed acutely (48 hours) to hypoxia, maximal upper-torso isometric force is significantly

increased¹⁹. A follow-up study from the same laboratory observed an increase in the maximal voluntary contraction during isometric handgrip exercise following 8 days of altitude exposure²⁰. One mechanism to explain the increase in isometric strength proposed from these experiments was respiratory alkalosis, secondary to hyperventilation. This line of research suggests that, if subjects are exposed to the opposite scenario, i.e., many days of breathing high CO₂, inducing respiratory acidosis, then strength could decrease. This hypothesis is supported by a study²¹ that demonstrated that hypercapnia decreases muscle contractility in the adductor pollicis.

POSTURAL STABILITY

DISSUB survivors must be able to perform the complex task of making an escape after being exposed to the environmental conditions of mild hypoxia, cold, and hypercapnia for several days. Each of these environmental conditions produces singular effects on motor function. Dizziness and fatigue are two characteristics of acute hypoxia that are indicative of impairments in mental and psychomotor function. However, quantitative measurements of dizziness, disorientation, disequilibrium, and non-exercise-induced fatigue are difficult to obtain. One possible approach is the measurement of postural sway or balance. When hypoxia (70% saturation of hemoglobin with oxygen) is induced over several minutes by exposure to an altitude of 19,000-20,000 ft, balance and postural control are significantly impaired²². Hypoxic effects have been noted using the Romberg index, the ratio of body steadiness between an eyes-open and eyes-closed condition, as low as 8,000 ft after descent from 18,000 ft, even when subjects reported no subjective dizziness or unsteadiness²³. Thus, it appears that balance and postural stability are degraded with acute and rapid hypoxia. During relatively longer exposure to moderate and high terrestrial altitudes only anecdotal evidence is available on balance and postural stability, studies that involved trekkers who were probably suffering from high altitude cerebral edema²⁴.

Effects of whole-body cold exposure and/or hypercapnia on postural stability are poorly documented. Magnusson et al.^{25, 26} exposed feet to cold temperatures and found that body-sway velocity increased significantly compared to when the feet were not cooled. The difference in body-sway between conditions was less prominent when the subject's eyes were open. However, cooling of the feet lasted only a few minutes, and there are no published reports of postural stability during more prolonged exposures to the conditions of mild hypoxia, hypercapnia, and cold exposure. Nevertheless, it is quite likely that some component of the postural balance system, whether it is the visual, vestibular, or proprioceptive feedback and/or the reflexive and voluntary muscle responses, will be detrimentally affected. If so, then further studies of potential psychomotor impairments among DISSUB survivors may be necessary.

HYPOTHESES

- a. The VO₂ and VCO₂ of survivors in a DISSUB will be substantially less than those of ambulatory office workers on which current USN planning estimates are based;
- b. The combination of mild hypercapnia and hypoxia will be additive in increasing ventilation throughout the DISSUB exposure duration;

- c. Suppression of the vasoconstrictor responses to cold, previously attributed to the effects of acute hypoxia, is primarily an effect of hypocapnia, and, therefore, will be prevented by hypercapnia under DISSUB conditions;
- d. Chronic exposure to the environmental conditions in a DISSUB will degrade isometric handgrip strength and endurance, psychomotor performance, cognitive function, and postural stability.

METHODS

All experimental procedures described in this protocol were undertaken at USARIEM using the hypobaric chamber facilities and other laboratories there, as required, in support. The protocol was approved by the US Navy and Army via the Committee for the Protection of Human Subjects at NSMRL, the USARIEM Human Use Review Committee, and the US Army Human Subjects Research Review Board.

ENVIRONMENTAL CONDITIONS

The trial was conducted in consecutive atmospheric phases as illustrated in Table 2 and described below:

CONTROL	For two days preceding experimental treatments. <u>Purpose:</u> Baseline normative (normoxic, normocapnic, normothermic) data collection; also, familiarization of subjects with the experimental protocol. Subjects were free to move within the laboratory building.
1 st TRANSITION	Began at 0200 (Hour 0 Elapsed Time) and ended at 0600 (Hour 4) on Day 1. <u>Purpose:</u> Change F _I O ₂ to 16.75%.
ACUTE HYPOXIA	Began at 0600 and ended at 1500 on Day 1 (Hours 4 to 13). <u>Purpose:</u> Collection of physiological/psychometric data during acute normocapnic hypoxic (F _I CO ₂ = 0.04%, F _I O ₂ = 16.75%) conditions similar to those aboard a submarine under normal conditions (i.e., before becoming disabled).
2 nd TRANSITION	Began at 1500 on Day 1 (Hour 13) and ended at 1500 on Day 2 (Hour 37). <u>Purpose:</u> Replication of the development of environmental conditions in a DISSUB. Chamber air temperature was reduced exponentially from 22°C to 4°C, the F _I CO ₂ was increased linearly from 0.04% to 2.5% (surface equivalent) and relative humidity was increased linearly from 50% to ~85%.

DISSUB	Began at 1500 on Day 2 (Hour 37) and ended at 1900 (Hour 161) on Day 7. <u>Purpose:</u> Collection of physiological/psychometric data under steady-state hypercapnic, hypoxic, humid, and cold conditions simulating the DISSUB environment.
3 rd TRANSITION	Began at 1900 (Hour 161) and ended at 2400 (Hour 166) on Day 7. <u>Purpose:</u> Rapidly restored subjects to normocapnic hypoxia. While maintaining $F_{I}O_2 = 16.75\%$, chamber CO_2 levels were returned to normal, humidity reduced to 50% and ambient temperature increased to 22°C as quickly as possible.
CHRONIC HYPOXIA	Began at 2400 (Hour 166) on Day 7 and ended at 1600 (Hour 206) on Day 9. <u>Purpose:</u> Isolate the effects of several days of hypoxia from those of other environmental abnormalities present in a DISSUB.

The USARIEM Hypobaric Chamber facility consists of a larger chamber, where the subjects bunked and spent most of their time during the study, a smaller chamber where most experimental tests were completed, and an airlock chamber which connected the other two chambers and where sanitary facilities were located (Figure 1). The chambers can be sealed and operated independently when needed, or connected via opening the airlock between the chambers. During chamber operations, personnel entered and left the chamber from the outside via the airlock, thereby allowing the other two chambers to be maintained constant at the desired atmosphere while the airlock was brought to equilibrium. The normoxic, normocapnic conditions required for baseline testing at Hour -36 were achieved by circulating ambient air at normal pressure through the chamber. When hypoxic, hypercapnic conditions were required, the fractional content of nitrogen and carbon dioxide in the atmosphere circulated through the chamber was manipulated using a mixing system to achieve $F_{I}O_2 = 16.75\%$ (Hours 4-206) and $F_{I}CO_2 = 2.5\%$ (Hours 37-161), while the barometric pressure was maintained as near to sea level as possible.

During the control period the subjects' clothing, activity, and food were ad libitum (within the constraints allowed by confinement in the chamber) with the following exceptions: Smoking or chewing tobacco was not permitted within the chamber or within 12 hours of any test. Subjects did not consume food, shower or engage in vigorous physical activities for four hours preceding any cold exposure test. Subjects abstained from alcohol for 24 hours before any testing. They were permitted to read, listen to personal music equipment, watch TV and play games when they were not engaged in testing.

From Hour 0 to 166, the subjects remained resting in their bunks when not involved in testing. There was constant, diffuse lighting in the chamber that was reduced to a low level at night. Television, radios, videos, personnel stereos, smoking, washing, showering, newspapers and telephone calls were not allowed. From Hour 166-206, the subjects were permitted to play

cards and watch a video to improve morale and encourage continued participation through the study's completion.

TEST SUBJECTS

Nine male, volunteer, non-smoking, USN personnel were recruited for the study, and informed consent was obtained in accordance with the Declaration of Helsinki. Two subjects discontinued participation during the study. Data are presented for 7 subjects. Subjects passed a full medical assessment before being allowed to participate in the experiment. The number of test subjects represented the maximum number of subjects that can be accommodated during sustained operations in the USARIEM hypobaric chamber. Despite the constraints, this sample size was considered adequate to accomplish the primary purpose of the study, which was to obtain an estimate of the VCO_2 expected for survivors aboard a DISSUB.

CLOTHING AND NUTRITION

For Hour 0 to 166, except as described later, the subjects wore as much regulation clothing as they wished from the following items: a woolen sweater, heavy cotton jacket, woolen hat, coveralls, long johns, and a pair of sneakers and socks. In addition to a bunk they were provided with a mattress, pillow, pillowslip, two sheets and up to three blankets.

The subjects had unrestricted access to water throughout the entire study. During Hours 0 to 166, the subjects' activity level and food intake were strictly regimented and monitored.

During Hours 0 to 166 the subjects were served meals twice daily (0700 and 2000 hours), prepared by staff according to a menu derived from the operational load-out of a 688-class submarine. The amount of food provided each day was standardized and designed to maintain energy balance. The individual portions for each meal were weighed and measured before and after service by trained staff, to provide an accurate assessment of energy and macronutrient intake. Dietary intake was analyzed using the Nutritionist Five nutrient database²⁷ and specific manufacturers' nutrient information as appropriate.

The amount of food (energy) provided to each subject was based on the expected energy expenditure determined from the resting metabolic rate measurements completed during the control period. These values were compared to resting energy expenditure estimates calculated using the Harris Benedict formula,²⁷ and, in the event of a difference of more than 20% in this comparison, the latter calculated data were used. An additional 10% was added to account for the thermic effect of food²⁷. No adjustments were made for activity since the volunteers were in bed when not testing. For example, using the equation adapted from the Food and Nutrition Board, National Research Council²⁸, for 18-30 year old males with a reference body weight of 79 kg, targeted energy intake was approximately 2100 kcal per day $\{[(15.3 \times 79) + 679] \times 1.1 = 2100\}$. The macronutrient composition of the diet (carbohydrate, protein, and fat) was maintained at a constant ratio of 40%, 20%, and 40%, respectively, to yield a food quotient (FQ) of 0.85. The FQ is the ratio of carbon dioxide produced divided by oxygen used for the biological oxidation of a representative sample of the diet. At energy balance, the FQ is equal to the respiratory quotient (RQ).

BODY WEIGHT, COMPOSITION, AND FLUID BALANCE

Body fat mass and lean body mass were measured using dual x-ray absorptiometry (DEXA; Model DPX-L, Lunar, Madison, WI). This was done once during the control period and again following recovery from the simulated submarine exposure. Duplicate skin-fold measurements used to calculate percentage body fat²⁹ were taken on the right side of each subject at the following sites: chin, chest, abdomen, side, subscapula, biceps, triceps, thigh, knee, calf, and suprailium. Nude body weight was measured at 0700 following the first micturition on each day of the trial.

Total daily water intake was calculated from the records of food and fluid consumption maintained throughout the study for each volunteer. Daily (24-hr) urinary water loss was measured starting at 0700 during the control period (Hour -19).

Weight, body composition, and fluid changes were analyzed with dependent t-tests.

RATES OF CARBON DIOXIDE PRODUCTION AND OXYGEN CONSUMPTION

Three methodologic approaches were used to measure the $\dot{V}CO_2$ of the test subjects. These were:

1. Conventional analyses of mixed expired gas;
2. Doubly labeled water technique;
3. Energy balance calculations based on analyses of dietary energy intake and changes in body composition.

The first method was chosen because it is a “gold standard” method and because it has a relatively high temporal resolution that was useful in determining acute changes in $\dot{V}CO_2$ brought on by whole-body cooling. The second and third methods provided estimates for $\dot{V}CO_2$ and total energy expenditure time-averaged over the course of days of exposure to experimental conditions. Cross-correlations of these three sources of similar data were useful in validating the final estimates of $\dot{V}CO_2$ under DISSUB conditions. Details of these methods follow:

Analyses of mixed expired gas.

Twice each day, at approximately 0600 and 2000, resting ventilation was measured while the volunteers remained as inactive as possible in a recumbent position. During control and acute hypoxic conditions, subjects breathed room air through a low-resistance mouthpiece, valve and breathing circuit connected to a metabolic cart (Model Vmax229, SensorMedics, Yorba Linda, CA). It was discovered at Hour 27 that the metabolic cart was incapable of operating in a CO_2 -rich environment. Consequently, the subsequent readings were taken using a Douglas bag to collect expired air for 7 minutes, which was analyzed using an oxygen analyzer (Model S3A, Applied Electrochemistry, Sunnyvale, CA), a carbon dioxide analyzer (Model LB2, SensorMedics, Yorba Linda, CA), and a Tissot spirometer to measure expired air volume. The

following parameters were measured: minute ventilation, VO_2 , VCO_2 , and respiratory exchange ratio (RER).

Doubly labeled water technique.

The doubly labeled water method (DLW) of measuring total daily energy expenditure is based on the assumption that after an initial oral dose of stable, non-radioactive $^2\text{H}_2^{18}\text{O}$, deuterium (^2H) is eliminated from the body as water, whereas ^{18}O leaves as both water and exhaled carbon dioxide³⁰. At Hour -19, after waking, the volunteers collected a sample of their first morning void and then drank the measured dose of doubly labeled water. The energy expenditure measurement period started with collection of the first morning urine sample at Hour 4. During the energy expenditure period, which lasted until Hour 148, the volunteers collected a sample of their first morning void each day. VCO_2 was calculated from the difference in elimination rates of the two isotopes. Two subjects did not receive doubly labeled water so that they could serve as controls for background changes in the isotope levels.

Energy balance studies.

Dietitians measured food consumption as described above to assess energy intake. Body composition changes measured by DEXA (also see above) were partitioned into lean and fat mass changes from which the net changes in stored energy were calculated. Total energy expenditure equaled energy intake plus the net change in stored energy. Using a metabolic fuel quotient that accounts for both macronutrient intake and body fuel store use, VCO_2 was calculated from total energy expenditure.

METABOLIC RESPONSE TO WHOLE-BODY COOLING

Subjects sat quietly wearing only shorts, socks and woolen glove liners for 20 min with the ambient air temperature maintained at 22°C and relative humidity at 50%. Baseline values of VO_2 and VCO_2 were obtained as described above. The ambient air temperature of the chamber was then reduced by 1°C·min⁻¹ over a ten-minute period, after which air temperature was maintained constant at 12°C for 150 min. VO_2 and VCO_2 were measured every 20 min during this whole-body cold exposure. While exposed to cold, the subjects were not allowed to employ behavioral thermoregulation such as unnecessary physical activity or “huddling”. This test was performed on each subject at approximately Hours -36, 10, and 106.

Results of carbon dioxide production and oxygen consumption were analyzed using one-way (time) repeated measures analysis of variance. The level of significance was set at $P < 0.05$.

THERMOREGULATORY RESPONSES TO COLD

The overall objective of this aspect of the study was to examine how acute and chronic exposure to hypoxia and hypercapnia affect thermoregulatory responses elicited during cold exposure and, consequently, the body's ability to maintain thermal balance and normal temperature. Two approaches to assessing the development of thermoregulatory impairments were used. First, the resting body core temperature was monitored throughout the entire study to determine whether, particularly during DISSUB conditions, any significant decline occurred.

Secondly, we assessed each subject's peripheral temperature responses (cold-induced vasodilation) elicited during acute cold exposure.

Core temperature.

Resting body core temperature was continuously monitored using ingested, disposable, telemetric temperature sensors encapsulated in pill form (CorTemp™, Human Technologies, St. Petersburg, FL). These sensors telemeter temperature measurements at user-defined intervals to a small data logger (BCTM-2, Personal Electronic Devices, Wellesley, MA) worn by the subject. The data logger indicates real-time core temperature on a digital display, and records the temperature measurements and time in a file which can be downloaded to a computer for analysis. The telemetry pill method provides valid measurements of core body temperature during exposure to hot, cold and thermoneutral conditions, in both sedentary and exercising human subjects^{31, 32}. Each volunteer ingested a radio telemetry pill at Hour 16 and subsequently as needed to replace pills eliminated in the feces as noted by loss of the telemetric signal concomitant with test volunteer bowel movement (gastrointestinal transit time averaged 24-48 hr).

Cold-induced vasodilation.

These studies were completed in the small experimental chamber, separate from the large bunk chamber. The subjects performed the study at the same time of day to minimize any potential confounding influence of circadian variation in body temperature. The same clothing was worn during each trial, with only the inspired atmosphere varying. The middle finger of the dominant hand was immersed into warm water, then cold water, while the subject sat quietly with ambient air temperature maintained at 22°C and relative humidity at 50%. Finger volume was estimated by measuring the amount of water displaced using Archimedes' principle. This was checked by calculating the volume from measurements of the length of the middle finger and its width at the distal interphalangeal joint.

First, subjects placed a rectal probe 10 cm past the anal sphincter. Heat flow sensors with integrated thermocouples (Concept Engineering, Old Saybrook, CT) were taped to ten sites on the nondominant side of the body (foot, calf, medial thigh, lateral thigh, chest, triceps, anterior aspect of the forearm, sub-scapular thorax, forehead, and dorsal hand) for calculation of mean-weighted skin temperature. A thermocouple was attached to the nailbed of the middle finger of the dominant hand. Both hands were positioned so that they were at the same height, approximately level with the heart. Subjects wore athletic shorts and socks under a sweat suit (long pants and shirt) for this procedure.

After the subjects were instrumented and had sat quietly with their hands in a comfortable position for a 15-min stabilization period, they immersed their middle finger up to the middle phalanx in warm (42°C) water. Immersion at this temperature is designed to abolish vasoconstriction³³ and standardize the initial finger temperature for the cold immersion tests. After 15 min in warm water, the volunteers immediately transferred the finger to a cold (4°C) water bath for 30 min. During both warm and cold water immersion, body temperatures and heat flow were recorded every 5 sec.

Results of cold-induced vasodilation were analyzed using one-way (time) repeated measures analysis of variance. The level of significance was set at $P < 0.05$.

HAND GRIP STRENGTH AND ENDURANCE

Maximal isometric handgrip force and handgrip endurance were measured with a customized hand dynamometer that included a BLH electronic load cell transducer interfaced with a computer³⁴. Studies were performed twice during the control period (Hours -35 and -13) and once at Hours 35, 86, and 134. Grip strength was measured with the subject sitting upright with the dominant arm flexed approximately 90 degrees. Subjects squeezed the dynamometer as hard as possible for 1-2 sec and were provided with visual feedback via a real time display on a screen. Subjects completed the procedure three times during each trial, pausing to permit muscular recovery. Peak force attained was recorded as the maximum voluntary contraction (MVC). At least 15 min after the final grip to measure MVC, handgrip endurance was measured. Grip endurance was determined as the holding time at 60% of the control value of MVC measured at Hour -35³⁴. A screen display of the target force plotted over time gave subjects two lines at 57.5% and 62.5% of baseline MVC. Subjects were instructed to maintain a force within these limits. When grip force fell below 57.5% of MVC for > 2 sec, the endurance test was terminated and the duration of grip recorded.

Results of hand grip strength and endurance were analyzed using one-way (time) repeated measures analysis of variance. The level of significance was set at $P < 0.05$.

SYMPTOMATOLOGY ASSESSMENT

The incidence of hypoxia- and/or hypercapnia-induced symptoms (e.g. dizziness, shortness of breath, headache, etc.) was determined using the Environmental Symptoms Questionnaire (ESQ) reference. The ESQ is a self-reported, 68-question inventory used to document symptoms induced by altitude and other stressful environments³⁵. The ESQ takes approximately five minutes to complete. The ESQ was administered once every morning after arising.

COGNITIVE TEST BATTERY

A battery of six cognitive tests to assess a broad spectrum of cognitive functions ranging from simple tasks to complex skills has been constructed from tests previously used to assess the effects of cold or hypoxia on performance and mood (see below). Generally, the relatively simpler tasks such as the USARIEM Visual Vigilance Task and the Four-Choice Visual Reaction Time Test are more sensitive to subtle changes in performance produced by a wide variety of factors. However, as the magnitude of the changes in cognitive state induced by the experimental treatment increase, more complex tasks in the battery, such as Grammatical Reasoning, may also detect changes in performance³⁶. Included in the battery was a single, brief test of mood state, the Profile of Mood States. This is a widely used standardized test that provides critical insight into the factors underlying any changes in cognitive performance. The cognitive test battery was completed each evening during the experiment. The battery was

completed once before the control period and twice during the control period (Hour -32 and -8), so that the first two trials served as practice sessions to familiarize subjects with all the tests and stabilize their performance.

USARIEM Visual Vigilance Task.

This test is extremely sensitive to a wide variety of environmental conditions, nutritional factors, sleep loss and very low doses of hypnotic drugs and stimulants³⁷. It was designed to simulate various critical military activities that require maintenance of vigilance such as standing radar and sonar watch and communications monitoring. The subject continuously scans a computer screen to detect the occurrence of an infrequent, difficult to detect, faint stimulus that appears randomly on the screen for two seconds. Typically, the stimulus occurred once a minute. Upon detection of the stimulus the volunteer pressed the space bar on the keyboard as rapidly as possible. The computer recorded whether or not a stimulus was detected and the response time for the detection. Responses made before or after stimulus occurrence were recorded as false alarms. Each test session lasted 18 min.

Four-Choice Visual Reaction Time Test.

Tests of visual reaction time were administered using portable laptop computers following procedures previously described³⁸. Volunteers were presented with a series of visual stimuli at one of four different spatial locations on the computer screen. They indicated the correct spatial location of each stimulus by pressing one of four adjacent keys on the computer keyboard. The measurements recorded included correct responses and incorrect responses (hitting the wrong key), the response latency for each trial, premature errors (responding before the presentation of the stimulus), and time-out errors (response latency greater than one second). This test took about five minutes to complete.

Matching to Sample Test.

This test assesses short-term spatial memory (working memory) and pattern recognition skills. The volunteer responded by pressing the down arrow key when the word "READY" appeared on the screen. The volunteer was then presented with an 8 X 8 red and green checkerboard matrix. The matrix was displayed for 4 sec, then the screen was blanked for a variable interval. After the delay, two matrices were presented on the screen: the original sample matrix, and a second matrix that differed slightly in that the color of two of the squares was reversed. In each trial, the original matrix was either displayed on the left side of the screen and the altered matrix on the right, or vice versa; this left-right presentation of the matrices was randomized. The volunteer had to select the original matrix by responding using the left or right arrow key. A comparison response (left or right arrow key) had to be made within 15 sec; otherwise a time-out error was recorded. Correct responses were recorded, as were the response times to choose the matrix. The task consisted of 20 trials and lasted approximately five minutes.

Repeated Acquisition Test.

This test assesses learning and short-term memory. The volunteer was required to learn a sequence of 12 key presses on the four arrow keys of a laptop computer. The outline of a rectangle was presented on the screen at the beginning of a trial. Each correct response filled in a portion (1/12th) of the rectangle from left to right with a solid yellow color. Each incorrect response blanked the screen for 0.05 sec. When the screen returned, the volunteer was at the same point in the sequence as before the incorrect response. The volunteer had to learn the correct sequence by trial and error. When a sequence was correctly completed, the rectangle filled, the screen blanked, and another empty rectangle appeared for the next trial. The session ended when the volunteer completed 15 correct sequences (15 trials). Each session consisted of a sequence of randomly selected keys from a list of 32 different sequences. Incorrect responses and time to complete each trial were recorded. The time to complete this task was approximately 10 min.

Grammatical Reasoning.

This test assesses language-based logical reasoning and has been used to assess the effects of various treatments on cognitive function. It has been adapted from the Baddeley Grammatical Reasoning Test. On each trial a statement was followed by the letters AB or BA. The volunteer decided whether or not each statement correctly described the order of the two letters. The "T" key on the keyboard was pressed for correct (statement is true) and the "F" key was pressed for incorrect (statement is false). Statements could be positive/negative or active/passive, and a given letter could precede/follow the other letter. The session lasted for 32 trials. The time to complete this test was approximately five minutes.

Profile of Mood States (POMS) Questionnaire.

The POMS is a widely-used, standardized, computer or paper-and-pencil administered inventory of subjective mood states which is extremely sensitive to a wide variety of environmental factors, sleep loss, and nutritional manipulations. It takes less than five minutes for volunteers to rate a series of 65 mood-related adjectives on a five-point scale, in response to the question, "How are you feeling right now?" Previous research has shown that the adjectives factor into six mood sub-scales (tension, depression, anger, vigor, fatigue, and confusion). Various sub-scales have been shown to be sensitive to environmental factors and nutritional manipulations.

Results of cognitive performance were analyzed using one-way (time) repeated measures analysis of variance. The level of significance was set at $P < 0.05$.

ACTIVITY AND SLEEP ASSESSMENT

Motion logger Actigraphs (Model AMA-32 or equivalent, Precision Control Devices, Ft. Walton Beach, FL) were employed to assess day-to-day patterns of rest and activity, total physical activity, and to estimate duration and fragmentation of sleep. The use of Actigraphic data to assess and quantify sleep vs. waking states of humans has been demonstrated

previously³⁹, including several USARIEM studies⁴⁰. Currently, the best algorithm to detect sleep vs. wake status is 90% accurate when validated against conventional polysomnographic sleep scoring⁴¹.

The DISSUB environment was expected to substantially disrupt the pattern and quality of sleep. To establish a baseline, the subjects wore the monitors, which are similar to a wristwatch (4 cm x 3.1 cm x 1 cm, 57 g), on the wrist of the non-preferred hand for a control period of one week, one month following the chamber trial. Actigraphs were also worn throughout the chamber trial. Each device contains a microcomputer, 32 kb of memory, an analog-to-digital converter, and a piezoelectric sensor. The monitors sampled total activity counts in one-minute blocks of time. They were powered by standard wristwatch batteries and could record continuously for over a week. Data collected by the Actigraphs were downloaded to a computer for further analysis using the ACTION 3 computer program (Ambulatory Monitoring, Ardsley, NY).

The 24-hr data were sub-divided into two 12-hr time blocks: nighttime sleep (2000 to 0759 hours) and daytime sleep (0800 to 1959 hours). The total amount of sleep per study day was also derived. These data were then regrouped into the classifications of the two main test study periods/conditions: baseline and DISSUB. Complete sleep breakdowns consisted of one daytime and two nighttime periods for control conditions (Hours -42 to -6), and five nighttime and five daytime periods (Hours 37 to 161) for DISSUB conditions. An additional test study period/condition was obtained approximately five weeks after completion of the laboratory portion of the study ("post-chamber period"). The breakdown for this post-study data consisted of seven nighttime and six daytime time blocks.

Descriptive statistics (mean and standard error of the mean) were obtained. Repeated-measures analyses of variance (ANOVA) were conducted on each of the parameters recorded. The 0.05 level of probability was used to determine statistical difference for all analyses. Post-hoc differences were identified using a least significant difference pairwise multiple-comparison test.

POSTURAL STABILITY

Subjects were screened for any significant lower extremity injury or equilibrium dysfunction. Postural stability was assessed on Hours -36, 106, and 168 and immediately after returning to normal sea level environmental conditions using a computer-controlled unstable platform balance system (KAT 2000, OEM Medical, Carlsbad, CA). The balance system consisted of a circular platform (6 inches above the floor) controlled by varying the pressure in a pneumatic bladder around a central pivot point. The bladder was adjusted for ambient pressure and subject weight.

Each postural stability assessment included three, one-minute balance tests which were practiced before the study commenced. The tests were performed in socks with the subjects' arms akimbo and their eyes on a fixed point. For the first and second tests, the subjects stood with both feet equidistant from the pivot point, approximately 10 inches apart, and attempted to keep the platform as level as possible, either with the eyes open or closed. During these two

tests, the subjects received no feedback on their performance. For the third test (dynamic test), the subjects stood as indicated above, but attempted to move the platform in a direction so as to 'chase' a computer-controlled moving object visible to the subject.

A balance index was derived from a tilt sensor, which measured the absolute distance between the tilted position and a reference point. Thus, the balance index is inversely proportional to balancing skill. Handrails were situated about 45° to the left and right from the subject's midline, and a staff member observed the subject to provide assistance and prevent a fall. Data were analyzed using a repeated-measures ANOVA followed by a Tukey post-hoc test for critical differences if a statistically significant result was obtained from the ANOVA.

RESULTS

ENVIRONMENTAL CONDITIONS

Table 2 shows a summary of the environmental conditions in the large chamber during the phases of the study.

TEST SUBJECTS

The anthropometric data for the subjects are shown in Table 3. It can be seen from the table that the subjects lost weight and body fat. The average weight loss from Hour -42 to the end of the experiment (Hour 206) was 1.6 kg ($p < 0.05$) and loss of fat was 0.5% ($p < 0.02$ by paired t-test). Another way of expressing this is that 0.37 kg of the average weight lost was attributable to loss of body fat.

An anthropometric study of 1017 submariners conducted in 1989⁴² found that their mean body weight was 80.49 kg with a standard deviation of 12.46. The body weight of our subjects (Table 3) is not statistically different from this and consequently, with respect to body weight, they appear to represent the submarine force well.

CLOTHING

The subjects selected clothing from the supplied complement of standard USN items as shown in Table 4. Hours 38 and 86 are shown separately. By Hour 86 each subject's ensemble had stabilized and didn't change subsequently. The clo values for each item were kindly provided by Dr. Gonzalez of USARIEM. The combined clo value of each ensemble was calculated using the equation: $\text{ensemble clo} = (0.835 \times \text{ensemble sum}) + 0.86$ ⁴³. The subjects spent most of their time in their bunks, which had a mattress, 2 sheets and 3 blankets, with a calculated insulation value of 2.7 clo⁴⁴. Total clo consisted of ensemble clo plus bedding clo. An item that the subjects all asked for was gloves, and these were provided on Day 3. In addition, some articles of clothing were worn in an unconventional manner to reduce heat loss from the head - the "turban" worn by some subjects was in fact a woolen sweater. For this reason and because the subjects did not spend all their time in their bunks, the total insulation values shown should be considered an approximation. The trend that can be seen is that the total

insulation at Hour 86 was higher than at Hour 38 (4.97 mean clo at Hour 38, 5.26 mean clo at Hour 86) with only one subject choosing to wear less as the trial progressed. When combined with the body core temperature data and subjective reports, it can be seen that, although the subjects avoided hypothermia, they felt cold, and that a considerable amount of insulation is required by survivors in a DISSUB environment.

NUTRITION

As stated in the methods section, it was the goal to give each subject just over 2000 kcal per day, split 40%, 20%, 40% between carbohydrate, protein and fat, respectively. The subjects were not forced to eat the meals provided to them, and, since not all the food presented was consumed, the total intake was slightly less than desired. The failure of the subjects to eat all their food reflects the fact that it was served cold and, inevitably, given the limited availability of palatable items that would be safe to eat in a real DISSUB, not particularly appetizing. Despite this, they did consume, on average, just over 2000 kcal per day as shown in Figure 2. In large part this was due to the remarkable ingenuity of the nutritionists to come up with inventive menus and the highly attractive way in which each meal was presented. The macronutrient breakdown is shown in Figure 3. It shows that the desired split among macronutrients (40% carbohydrate, 20% protein, and 40% fat) was not achieved, with the actual values being approximately 47% carbohydrate, 16% protein, and 37% fat. The effect of this was to increase very slightly the VCO_2 above that which could be achieved with a higher proportion of fat in the diet. Since the metabolism of protein and carbohydrate generates similar amounts of CO_2 , the 4% reduction of protein in favor of carbohydrate made little difference to the RQ of the subjects.

BODY WEIGHT, COMPOSITION, AND FLUID BALANCE

Cumulative weight loss over the course of Hours 4 to 148 was 2.54 ± 0.46 kg (as mentioned previously, cumulative weight loss over the course of Hours -42 to 206 was 1.6 kg). Total body water (Table 5) decreased 0.4 kg during the period ($n = 5$). The caloric deficit (from measured food intake and daily energy expenditure) was calculated to be 15475 ± 2679 kilocalories causing a theoretical weight loss of 2.01 ± 0.35 kg. Thus, the calculated weight loss from both water loss and the caloric deficit was 2.41 ± 0.97 kg and was similar ($P > 0.05$) to actual weight loss. The subjects lost ~ 0.6 kg fat mass and 0.9 kg of non-fat body mass. These data suggest that after 144 hours of a simulated DISSUB, water loss and caloric deficit accounted for 17% and 83%, respectively, of the total weight loss incurred.

RATES OF CARBON DIOXIDE PRODUCTION AND OXYGEN CONSUMPTION

The results of the resting respiratory studies are summarized in Table 6 and Figures 4-8. The resting VO_2 (Figure 4) during the steady-state DISSUB conditions (Hours 37 to 161) was $0.30 \text{ l}\cdot\text{min}^{-1}$, which was significantly less than in the control period (Hours -24 to 0; $0.39 \text{ l}\cdot\text{min}^{-1}$, $p < 0.003$). This value (equivalent to $0.64 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F ; $0.69 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F) is also much lower than the $1 \text{ ft}^3\cdot\text{hr}^{-1}$ that is used in the Atmosphere Control Manual¹, although the latter datum represents VO_2 averaged over the course of a day's activity (as opposed to strictly resting conditions). Equally, the resting VCO_2 (Figure 5) fell from $0.34 \text{ l}\cdot\text{min}^{-1}$ to $0.29 \text{ l}\cdot\text{min}^{-1}$, which is equivalent to $0.08 \text{ lb}\cdot\text{hr}^{-1}$, or about 80% of the day-averaged value used in the Atmosphere

Control Manual. As expected, the combined effects of mild hypoxia and hypercapnia caused the subjects' minute ventilation to increase as is shown in Figure 6. The standard deviations of the VO_2 and VCO_2 data are quite large because of two factors: these figures vary with body mass, i.e., large people generally produce more carbon dioxide than small people at a similar level of activity; there is also a diurnal effect - the values in the morning tend to be lower than in the evening. It can be seen in Figures 7 and 8 that, with these factors taken into account, the variability of the data is reduced.

Values of average VO_2 and VCO_2 obtained over the course of Hours 4 to 148 from the DLW method are presented in Table 7. These data are higher than values made under resting conditions (Figures 4, 5, 7, and 8) because the DLW data include the metabolic activity of the subjects when they were out of their bunks as well as the specific dynamic action of the food they consumed. Estimated energy expenditure from DLW data obtained from Hours 4 to 148 was 4509 ± 649 kilocalories.

Table 6 summarizes the VO_2 and VCO_2 values obtained by the two methods of measurement (DLW and analyses of mixed expired gas) and covering the three periods of activity during the DISSUB exposure (total activity, semi-nude cold air exposure and bed rest). The lowest resting VO_2 and VCO_2 of DISSUB survivors, in similar circumstances to those generated in this experiment, will occur during bed rest, wearing the maximum clothing insulation available to them. By comparison, the values of VO_2 and VCO_2 measured throughout the DISSUB period by the DLW method, or during the final 20 min of a 150 min, seated, seminude exposure to cold air were significantly higher ($p < 0.003$) than the bed rest values (Tables 6, 7, 8). These latter values of VO_2 and VCO_2 measured during the experimental DISSUB scenario also exceed the average metabolic data assumed by the Atmosphere Control Manual.

THERMOREGULATORY RESPONSE TO COLD

Core temperature.

Although the core temperature of the subjects was logged continuously, two of the loggers developed faults that corrupted the data. During the DISSUB phase of the trial the core temperature data from the thermistor pills were manually logged for safety reasons, and the data presented in Figure 9 are from these manually collected readings. It can be seen that the normal diurnal pattern of core temperature was preserved in the DISSUB conditions. Despite many of the subjects reporting that they felt cold and some of them shivering intermittently, none of the subjects became overtly hypothermic (core temperature below 35°C).

Cold-induced vasodilation.

Results of cold-induced vasodilation experiments are presented in Table 9. Mean skin temperature was lower at Hour 104 compared to Hours -28, 8, and 176. Concomitantly, there was a lower mean finger temperature and a larger time to the first nadir at Hour 104. The blunted cold-induced vasodilation response at Hour 104 is likely due to the lower skin temperatures on that day.

HAND GRIP STRENGTH AND ENDURANCE

The results of the hand-grip testing are shown in Tables 10 and 11. It is notable that there was considerable inter-subject variability. Nonetheless, it can be seen that there was no significant change in either grip strength or endurance over the period of DISSUB survival.

SYMPTOMATOLOGY ASSESSMENT

The 68-item ESQ is divided into nine different factors: acute mountain sickness (AMS) – cerebral; AMS – respiratory; ears-nose-throat; colds stress; distress; alertness; exertion; muscle discomfort; and fatigue. A 10th factor of total symptom score is included (all 68 items). The only significant finding is that on Hours 76, 124, and 148 the subjects felt significantly colder than they did at other times of the study (Table 12).

COGNITIVE TEST BATTERY

USARIEM Visual Vigilance Task.

No significant differences were observed for stimulus detection rate between the study periods, with the subjects averaging 8.7 ± 1.2 correct hits over the entire study. Similarly, there were no significant differences found in the number of false alarms across the study periods (mean = 8.3 ± 3.3). The subjects' reaction time averaged 1.2 ± 0.1 sec during control testing, not significantly different than in DISSUB conditions (1.3 ± 0.1 sec).

Four-Choice Visual Reaction Time Test.

No significant differences were observed between study periods for the number of correct responses or for the total number of errors (premature hits and time-out errors) committed by the subjects. The average correct hits for the entire study were 384.7 ± 4.6 out of a possible 400 maximum, and the mean number of errors was 0.2 ± 0.2 . There was no significant difference in reaction time between the control and DISSUB periods: control 504.2 ± 16.3 milliseconds (ms) vs. 490.1 ± 16.6 ms, respectively.

Matching to Sample Test.

Across the study periods, subjects averaged 8.9 ± 0.2 correct matches, 0.1 ± 0.1 time-out errors, and a response time of 3.3 ± 0.3 sec per pattern. None of these parameters was found to be significantly different.

Repeated Acquisition Test.

There was no significant difference in the number of incorrect responses recorded between control and DISSUB conditions: 5.0 ± 1.1 incorrect keystrokes and 3.7 ± 0.4 , respectively.

Grammatical Reasoning.

There were no significant differences found in the subjects' correct responses to the statements presented to them, averaging 27.6 ± 2.1 across all study periods. Additionally, the number of time-out errors that occurred was not significantly different, with the mean across all study periods being 0.1 ± 0.1 .

Profile of Mood States (POMS) Questionnaire.

No significant differences were found across the study periods in scores for tension, anger, fatigue and confusion. Significant differences were found for the sub-scales of depression and vigor. Reported depression scores averaged 0.3 ± 0.3 during the control period and 1.6 ± 0.6 during DISSUB conditions ($p = 0.03$). Mean scores for vigor were: control: 17.4 ± 1.6 ; DISSUB: 14.5 ± 2.6 ($p < 0.05$). In practical terms these effects are small.

ACTIVITY AND SLEEP ASSESSMENT

One subject was removed from the Actigraph analyses due to substantial missing data discovered within the post-week assessment. Examples of the data collected by the Actigraphs can be found in Figures 10a and 10b.

Activity.

Significant differences were found to exist between the test study periods/conditions for total levels of activity ($p = 0.01$), as measured by activity counts per minute (cpm). During the study, the subjects were most active during the control (145.9 ± 8.4 cpm) and post-chamber periods (142.9 ± 7.3), and least active in DISSUB conditions (118.9 ± 7.7 cpm). Post-hoc testing detected a significant difference between control and DISSUB data ($p = 0.03$).

Daytime cpm were also found to be significantly different between the test study periods/conditions ($p = 0.01$). The subjects averaged 201.9 ± 14.5 cpm during the control period, 159.7 ± 10.7 cpm in the DISSUB condition, and 210.3 ± 8.3 cpm in the post-chamber period. Significant post-hoc testing differences were observed between control and DISSUB ($p = 0.02$) and DISSUB and post-chamber ($p = 0.02$) data.

There was no significant difference discovered across time periods for nighttime activity levels. The subjects recorded a mean of 87.2 ± 3.1 cpm during the study and a mean of 84.5 ± 10.2 cpm in the post-chamber conditions.

Sleep quality.

No significant difference was found in the total amount of sleep the subjects received across the three test periods. During the control and DISSUB periods the subjects averaged 7.1 ± 0.4 hr of sleep per 24 hr. Over the post-chamber period the mean was 7.1 ± 0.5 hr. There was no significant difference in the amount of nighttime sleep (Hours 13-161: 5.8 ± 0.2 hr; post-chamber period: 6.7 ± 0.5 hr), but a significant difference was observed ($p = 0.009$) in the

amount of daytime sleep: the subjects averaged 0.8 ± 0.4 hr during the control period, 1.9 ± 0.3 hr during DISSUB conditions, and 0.5 ± 0.2 hr per day in the post-chamber period. On post-hoc testing, significant differences were observed in daytime sleep between control and DISSUB ($p = 0.02$) and between DISSUB and post-chamber periods ($p = 0.003$).

Fragmentation of sleep, as measured by the number of awakenings per sleep period, was found to be significantly different between test study periods/conditions for all of the sleep assessment breakdowns (Table 13): nighttime ($p = 0.001$), daytime, ($p = 0.01$), and total ($p < 0.001$).

The average duration of the sleep fragmentation occurrences, as measured by the mean awakening minutes per awakening (MAM) during a sleep event, across all test study periods/conditions, was not found to be significantly different. The subjects experienced mean fragmentation occurrence durations of 6.8 ± 0.7 min during the periods/conditions of the study proper, and 4.0 ± 0.5 min over the post-chamber period. Similarly, the mean duration during nighttime sleep was also found not to be significantly different across test study periods/conditions (Hours 13-161: 7.9 ± 1.0 min; post-chamber: 4.6 ± 0.5 min).

A significant difference was discovered between the test study periods/conditions ($p = 0.03$) for the duration of fragmentation occurrences during daytime sleep. Subjects experienced baseline MAMs (Hour -24) of 3.5 ± 1.7 min; in DISSUB conditions (Hours 37-161), of 9.4 ± 1.7 min; in recovery (Hour 168), of 6.8 ± 2.1 min; and over post-chamber, of 3.4 ± 1.1 min. Post-hoc testing determined the differences existed between baseline and DISSUB ($p = 0.02$), and DISSUB and post-chamber ($p = 0.006$) periods.

POSTURAL STABILITY

The results of the postural stability testing are presented in Table 14. Performance in DISSUB conditions (Hour 110) are only compared with Hour 172 (after the DISSUB conditions) because there was insufficient time at the start of the trial to train the subjects sufficiently in the test technique. It can be seen that the subjects performed statistically worse in the DISSUB conditions (Hour 110) compared with Hour 172.

DISCUSSION

We set out to create, as best as possible, the environmental conditions that might be expected to occur in a disabled submarine. With respect to temperature, a realistic worst-case scenario of 4°C (39°F) was closely approximated during the DISSUB phase of the experiment. In a real DISSUB, the relative humidity could be expected to approach 100%. To avoid damage to the electrical monitoring equipment in the living chamber, the humidity was kept just below the dew point, and this averaged about 81%. This was sufficient to cause the subjects' clothing and bedding to become damp, a situation that would not be significantly worse in a real DISSUB. Although mildly hyperbaric conditions are likely to be present in a DISSUB, these were not simulated in this study because the environmental chamber used does not have hyperbaric capability.

The aspect of the atmosphere that was deliberately maintained in a more benign state than might be expected in a DISSUB was the atmospheric carbon dioxide level: for the subjects' safety, this parameter was limited to 2.5%. In a real DISSUB it can be anticipated that, with the existing scrubbing equipment in a well-populated compartment (e.g. forward compartment in a 688-class boat), the level might be maintained at about 3% until the available LiOH is exhausted. At that point the level would climb towards 6%, forcing the survivors to escape. Although the 2.5% level was well tolerated by the subjects in this study, they did complain of, and were treated with analgesics for, headaches. Another symptom they noticed that may have been related in part to elevated atmospheric CO₂ was an inability to concentrate. A number of the subjects brought large quantities of reading material with them. Most of this went unread because of the difficulty they had concentrating. Another effect that was measured was the effect of raised carbon dioxide on the subjects' ventilation rate: in the DISSUB phase minute ventilation was just about 30% greater than in the control phase (Figure 6). Interestingly, this increase was barely noticed by the subjects who did not complain of shortness of breath. It can be anticipated that, in the terminal phase of a real DISSUB scenario, these effects of hypercapnia would be considerably worse and would be accompanied by other unpleasant toxic effects of carbon dioxide including narcosis, anxiety, nausea and stinging eyes.

The subjects were provided with a large amount of clothing and bedding at the beginning of the hypoxia, and, by Hour 86, they had amassed even more (Table 4). One subject was cocooned in just over 6 clo of insulation (for comparison, a business suit with shirt and underwear provides about 1 clo of insulation). The average value of the subjects' insulation was about 5.3 clo. This proved to be adequate to permit the subjects to maintain their core temperature (Figure 9), although one subject did approach hypothermia (core temperature of 35°C) around Hour 72 (data not shown). Although able to avoid hypothermia, the environmental symptoms questionnaire (Table 12) showed that the subjects felt cold. This is supported by the metabolic findings. Despite being provided with 2000 kcal in food per day, the subjects lost weight (Tables 3, 5). While some of this was water (17%), the subjects lost both fat (about 0.6 kg) and non-fat body mass (about 0.9 kg). The subjects were, therefore, in a substantial negative calorie balance, with their diet providing only about half of the energy that they expended. This leads to the first finding of this study: while DISSUB survivors with access to adequate amounts of clothing and about 2000 kcal of food per day can be expected to survive for a week without becoming hypothermic, those in compartments where there is either no access to food or additional insulation may not. They could be vulnerable to whole-body hypothermia - as was seen in a similar trial conducted in the UK with supplies of food and clothing that more closely matched those that are likely to prevail in the aft compartment of a USN DISSUB^{45, 46}. Consideration needs to be given to providing both adequate insulation and food in these compartments.

The Actigraph data (Figure 10 and associated text) show that the level of activity of the survivors was significantly reduced during the DISSUB phase of the trial compared with the control periods. However, there was still a remarkable level of activity even when the subjects remained in their bunks. This is probably because, although resting, they were not sleeping for much of the time – indeed the total amount of sleep the subjects got during the DISSUB phase of the trial was no different than during the control phases. It was frequently reported to

investigators that the subjects moved around in bed more than usual to avoid cramps and keep warm. Thus, the subjects largely complied with the instruction to remain in their bunks when not involved in an experiment. It can be predicted with confidence that, with less insulation, the level of activity of the subjects, including shivering, would have been even greater. The degree to which survivors in an actual DISSUB situation would actually be able to reduce their activity levels and associated metabolic rate to or below that of the subjects in this study, and to maintain activity at those low levels, remains unknown.

When measured at rest, in steady state DISSUB conditions, the mean VO_2 was $0.30 \text{ l}\cdot\text{min}^{-1}$ which is equivalent to $0.64 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F or $0.69 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F . Even in the control period the VO_2 ($0.39 \text{ l}\cdot\text{min}^{-1}$; $0.82 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F ; $0.88 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F) was significantly less than the value of $1 \text{ ft}^3\cdot\text{hr}^{-1}$ that is used as an estimate of VO_2 in the Atmosphere Control Manual¹. The mean VCO_2 in resting, steady state DISSUB conditions was $0.29 \text{ l}\cdot\text{min}^{-1}$ which is equivalent to $0.08 \text{ lb}\cdot\text{hr}^{-1}$ or about 80% of the value used in the Atmosphere Control Manual. These values represent the lowest that can be expected in such conditions. These readings were taken when the subjects were lying in bed and not shivering maximally. The data from the cooling experiments and DLW show that the VO_2 and VCO_2 maintained over the course of daily activities were greater ($\text{VO}_2 = 0.56 \text{ l}\cdot\text{min}^{-1}$ or $1.18 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F , $1.27 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F ; $\text{VCO}_2 = 0.46 \text{ l}\cdot\text{min}^{-1}$ or $0.12 \text{ lb}\cdot\text{hr}^{-1}$). The differences between the various measurements of VO_2 and VCO_2 in our subjects clearly reflect activity (shivering or other physical effort). Predicting the level of activity that would be sustained by actual DISSUB survivors becomes a matter of judgment. In the opinion of the authors the VO_2 is likely to fall in the range of $0.64\text{-}1.18 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F ($0.69\text{-}1.27 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F ; $0.30\text{-}0.56 \text{ l}\cdot\text{min}^{-1}$) and the VCO_2 in the range of $0.08\text{-}0.12 \text{ lb}\cdot\text{hr}^{-1}$ ($0.29\text{-}0.46 \text{ l}\cdot\text{min}^{-1}$). The higher values can be expected if there is a sustained requirement for physical activity such as damage control or operating a man-powered carbon dioxide removal system, or if there is increased shivering because inadequate thermal insulation is available.

A somewhat surprising outcome of the trial was the large number of measured variables for which no significant effect of DISSUB conditions was found. These include cognitive function, cold-induced vasodilatation, and hand-grip strength. Even though postural stability was statistically different during the DISSUB period, there is no practical significance to these findings for DISSUB survivors. For ethical reasons it was not possible to simulate the worst credible DISSUB scenario, and, even with the conditions that were provided, the subjects were well aware that they could leave the trial at any point – a situation that would not apply in a real DISSUB. These factors should be borne in mind when studying the findings of the environmental symptoms questionnaire in particular. Nonetheless, from a physiological point of view, we have shown that, if provided with adequate food, clothing and water, naval personnel should be able to survive in these conditions to the point of rescue or making an escape without serious or permanent injury.

CONCLUSIONS

The conditions under which the volunteers survived in this experiment were amongst the best that can reasonably be expected to prevail in a DISSUB. Although cold and humid, the atmospheric pressure was not raised and there were no toxic or radioactive pollutants. The level

of oxygen selected would be easily achievable in most scenarios and the level of carbon dioxide was set at about the lowest that can be maintained with the majority of the crew surviving and using current emergency scrubbing equipment. Furthermore, the volunteers were uninjured and had access to adequate amounts of clothing, bedding, and food, and were fully aware that they were part of an experiment that they could choose to leave at any point, unlike survivors in a real DISSUB. The findings of this study, therefore, need to be interpreted accordingly.

1. The lowest rate of carbon dioxide that a DISSUB survivor is likely to generate is $0.076 \text{ lb}\cdot\text{hr}^{-1}$. This will be achieved when survivors are at rest, lying in their bunks. Any additional activity, such as sitting, standing, walking, eating or shivering will increase VCO_2 . In this experiment the survivors spent about 16 hours of each day in their bunks; for the remainder they were ambulatory, eating or taking part in experiments that induced shivering. The overall VCO_2 as measured by DLW was $0.12 \text{ lb}\cdot\text{hr}^{-1}$. It can be concluded that the likely VCO_2 of DISSUB survivors will fall within a range of $0.08\text{-}0.12 \text{ lb}\cdot\text{hr}^{-1}$ ($0.29\text{-}0.46 \text{ l}\cdot\text{min}^{-1}$). The corresponding range of oxygen consumption values is $0.64\text{-}1.18 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 32°F ($0.69\text{-}1.27 \text{ ft}^3\cdot\text{hr}^{-1}$ @ 70°F ; $0.30\text{-}0.56 \text{ l}\cdot\text{min}^{-1}$).
2. A diet of 2000 kcal per day, although resulting in a negative energy balance, is adequate for the limited period that DISSUB survivors will have to tolerate environmental conditions similar to those in this experiment.
3. The volunteers required a considerable amount of insulation in order to keep warm. They had an average of 5 clo of insulation split almost evenly between clothing and bedding and this was adequate to allow them to maintain their core temperature throughout the day. The volunteers asked for, or improvised, headgear and gloves.
4. There were no physiologically significant effects of this trial on the other variables measured.

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Table 1. Summary of the conditions and carbon dioxide production rates during various DISSUB simulation trials.

Trial	VCO ₂ (l·min ⁻¹)	F _I CO ₂ (%)	Temp (°C)	RH (%)	Diet	Duration	Comments
Consolazio et al., 1944 ⁴⁷	0.20- 0.40	6.75 ^a	30	75	Normal	34-72 hr	Series of 4 laboratory trials
Consolazio et al., 1944 ⁴⁷	0.32	5.2 ^a	16	90	Normal	60 hr	At sea trial
Harrison et al., 1978 ⁴⁸	0.36- 0.43 ^b	1.0-2.75	21-22	81-90	100g glucose no water	18.5 hr	At sea trial
Harrison and Jolly, 1979 ⁴⁹	0.28 ^c	0.75-2.5	15-18 ^d	82-91	100g glucose per 24h no water	31 hr	At sea trial
Harrison and Smith, 1980 ⁵⁰ - Trial 1	0.20- 0.25 ^e	0.65-2.5	20	>90	100g glucose per 24h restricted water ^f	4 d	Laboratory trial
Harrison and Smith, 1980 ⁵⁰ - Trial 2	0.19- 0.25 ^e	0.65-2.5	20	>90	100g glucose per 24h restricted water ^f	7 d	Laboratory trial
Harrison et al., 1984 ⁵¹	0.26 ^g	1.25-2.75	24-27 ^h	N/A	100g glucose per 24h restricted water ^f	48 hr	At sea trial
Windle, 1997 ⁴⁵	0.21 ⁱ	0.04	4-23	99	100g glucose per 24h restricted water ^f	7 d	Laboratory trial

Notes:

- a. In these experiments the CO₂ level was not held constant but was allowed to rise from normal air to the level shown.
- b. Results considered unreliable by the authors. The 25 subjects spent most of the time asleep.
- c. Calculated from a measured VO₂ (0.66 ft³·hr⁻¹) and an assumed RQ of 0.88.
- d. The “survivors” occasionally felt chilly but not cold.
- e. This varied more with the level of activity (which was not controlled) than the inspired CO₂. A maximum likely VCO₂ of 0.26 l·min⁻¹ was estimated, although the authors acknowledged that fear and cold may raise this in a real situation.
- f. Water restricted to nil in the first 24 hours and then 1 pint per man per day thereafter.
- g. Average for 32 men. One “survivor” was operating a bellows CO₂ scrubbing unit. There was poor agreement between measurement techniques.
- h. Many personnel were sweating profusely.
- i. Calculated from the mean VO₂ of the four subjects who lasted 7 days (0.26 l·min⁻¹) and a mean RQ of 0.8.

Table 2. Environmental conditions in the large chamber during the experimental protocol. Elapsed time is expressed relative to sealing the chamber at the onset of the 1st Transition. Conditions for Control, Acute Hypoxia, DISSUB, and Chronic Hypoxia are mean \pm SD (environmental conditions were measured every 10 sec).

	CONTROL	1 st TRANSITION	ACUTE HYPOXIA	2 nd TRANSITION					DISSUB	3 rd TRANSITION	CHRONIC HYPOXIA
ELAPSED TIME (hr)	-48-0	0-4	4-13	13-18	18-22	22-30	30-34	34-37	37-161	161-166	166-206
F _I O ₂ (%)	20.93	21-16.75	16.77 \pm 0.03	16.75	16.75	16.75	16.75	16.75	16.73 \pm 0.06	16.75	16.76 \pm 0.02
F _I CO ₂ (%)	0.04	0.04	0.44 \pm 0.04	0.04-0.5	0.5-1.0	1.0-1.5	1.5-2.0	2.0-2.5	2.49 \pm 0.04	2.5-0.04	0.18 \pm 0.08
RH (%)	50.42 \pm 5.18	50	50.69 \pm 4.79	50-57	57-64	64-71	71-78	78-85	80.48 \pm 5.27	85-50	50.74 \pm 2.94
T _{air} (°C)	22.2 \pm 1.1	22	19.49 \pm 0.76	22-18	18-15	15-11	11-8	8-4	4.51 \pm 0.56	4-22	21.2 \pm 0.82

Table 3. Study subjects' anthropometric data pre- and post-exposure to DISSUB conditions. Body fat was determined by dual x-ray absorptiometry measurements.

Subject	Weight (kg)		Body Fat (%)		Height (cm)	Age (yr)
	Pre	Post	Pre	Post		
1	93.7	91.1	23.6	23.5	175	43
3	70.7	69.6	10.4	9.60	166	26
4	86.4	85.3	24.2	24.3	176	28
5	81.3	80.8	24.8	24.3	174	30
7	94.6	92.9	22.2	21.5	179	37
8	82.1	79.5	7.80	7.10	176	37
9	74.2	72.9	21.3	20.9	176	27
Mean	83.3	81.7*	19.2	18.7*	175	32.6
SD	9.06	8.72	7.03	7.25	4.17	6.5

* significantly different (P<0.05) from corresponding Pre value.

Table 4. Summary of the quantity insulative value of the clothing and bedding used by the subjects at Hours 38 and 86.

ITEM	Clo	Subject 1		Subject 9		Subject 3		Subject 4		Subject 5		Subject 7		Subject 8	
		38	86	38	86	38	86	38	86	38	86	38	86	38	86
Underwear	0.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1
T-Shirt	0.09			1		1	1	1	1	1	1	1	1	1	1
Thermal Top	0.22	1	1	1	1	1	1	1	1	1	1	1	1		
Thermal Bottom	0.18	1	1	1	1	1	1	1	1	1	1	1	1	1	
Sweat Pants	0.30			1	1				1	1	1			1	1
Sweat Shirt	0.29							1						1	1
Shorts	0.04														1
Overalls	0.49	1	1	1	1	1	1	1	1	1	1	1	1	1	
Sweater	0.36	1	1	1	2					2	2	1	2	1	1
Jacket	0.48				1										
Socks-cotton	0.04			1	1			1	1	1	1			1	1
Socks-Army	0.13	1	1	1	1	1	1	1	1	1	1	1	1	1	1
"Turban"	0.20			1	1					1	1				
Watch Cap	0.20	1	1	1	1	1	1	1	1	1	1	1	1	1	
Gloves	0.13		1		1		1		1		1		1		1
ENSEMBLE (clo)		2.21	2.31	2.74	3.47	1.99	2.1	2.26	2.34	2.87	2.98	2.29	2.7	2.51	2.05
BEDDING (clo)		2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
TOTAL (clo)		4.91	5.01	5.44	6.17	4.69	4.8	4.96	5.04	4.57	5.68	4.99	5.4	5.21	4.75

Table 5. Changes in total body water, body fat and fat-free mass in 5 subjects using the DEXA (fat and fat-free mass) and doubly-labeled water (body water and water turnover) techniques. Subjects 7 and 9 did not receive doubly labeled water so that they could serve as controls for background changes in the isotope levels.

Subject	Total Body Water (kg)			Water Turnover (l/day) Hour -20 to 138	Fat-free Mass (kg)			Fat Mass (kg)		
	Initial	Final	Change		Initial	Final	Change	Initial	Final	Change
1	49.0	47.0	-2.0	3.0	71.6	69.7	-1.9	22.1	21.4	-0.70
3	44.0	43.8	-0.2	2.9	63.4	62.9	-0.5	7.4	6.7	-0.67
4	44.1	43.9	-0.2	2.7	65.5	64.6	-0.9	20.9	20.7	-0.18
5	42.0	42.4	0.4	2.5	61.1	61.2	0.1	20.2	19.6	-0.53
7					73.6	72.9	-0.7	21.0	20.0	-1.03
8	51.7	51.7	0.0	3.8	75.7	73.9	-1.8	6.4	5.6	-0.76
9					58.4	57.7	-0.7	15.8	15.2	-0.57
Mean	46.2	45.8	-0.4	2.98	67.0	66.1	-0.9	16.3	15.6	-0.63
SD	4.03	3.72	0.93	0.5	6.64	6.16	0.71	6.71	6.76	0.26

Table 6. Values for VO₂ and VCO₂ (mean and 95% confidence interval) measured continuously by the doubly labeled water method and periodically by analyses of mixed expired gas (150 min acute cold air exposure test and bed rest periods) during DISSUB simulation.

	Doubly Labeled Water Measurement (Hours 4 to 148)	Acute Cold Air Exposure (Hour 106)	Bed Rest Periods (0600 & 2000 hr local) (Hours 44 to 150)
VO ₂ (l·min ⁻¹)	0.56 (0.43-0.69)	0.51 (0.38-0.63)	0.30 (0.29-0.31)
VCO ₂ (l·min ⁻¹)	0.46 (0.36-0.56)	0.40 (0.30-0.50)	0.29 (0.28-0.31)
VO ₂ (ft ³ ·hr ⁻¹ @ 32°F)	1.18 (0.92-1.46)	1.07 (0.81-1.34)	0.64 (0.61-0.67)
VO ₂ (ft ³ ·hr ⁻¹ @ 70°F)	1.27 (0.99-1.57)	1.15 (0.87-1.44)	0.70 (0.66-0.72)
VCO ₂ (lb·hr ⁻¹)	0.12 (0.09-0.15)	0.10 (0.08-0.13)	0.08 (0.07-0.08)

Table 7. Values of VO₂ and VCO₂ measured by the doubly labeled water technique; subjects 7 and 9 did not receive doubly labeled water so that they could serve as controls for background changes in the isotope levels.

Subject	HOURS 4 to 148				
	VO ₂			VCO ₂	
	l·min ⁻¹	ft ³ ·hr ⁻¹ @ 32°F	ft ³ ·hr ⁻¹ @ 70°F	l·min ⁻¹	lb·hr ⁻¹
1	0.54	1.15	1.24	0.44	0.11
9	-	-	-	-	-
3	0.46	0.98	1.06	0.38	0.10
4	0.55	1.17	1.26	0.45	0.12
5	0.51	1.09	1.17	0.42	0.11
7	-	-	-	-	-
8	0.73	1.55	1.67	0.60	0.16
Mean	0.56	1.18	1.27	0.46	0.12
Standard Deviation	0.10	0.22	0.24	0.08	0.02

Table 8. VO₂ and VCO₂ (l·min⁻¹) measured by analyses of mixed expired gas during the acute cold exposure experiment.

Subject	VO ₂ / VCO ₂		
	Hour -36	Hour 10	Hour 106
1	0.38 / 0.33	0.41 / 0.33	0.50 / 0.38
9	0.44 / 0.44	0.46 / 0.41	0.46 / 0.39
3	0.38 / 0.38	0.41 / 0.30	0.43 / 0.33
4	0.40 / 0.40	0.41 / 0.36	0.39 / 0.28
5	0.42 / 0.32	0.44 / 0.32	0.39 / 0.34
7	0.44 / 0.36	0.45 / 0.37	0.63 / 0.51
8	0.64 / 0.58	0.54 / 0.60	0.75 / 0.57
Mean	0.44 / 0.40	0.45 / 0.38	0.51 / 0.40
Standard Deviation	0.09 / 0.09	0.05 / 0.10	0.13 / 0.10

Table 9. Temperature responses (n = 7, mean ± SE) throughout 30-min cold-induced vasodilation experiments.

Hour	T _{re}	T _{sk}	T _f	T _{min}	t _{min}	T _{max}	t _{max}
-28	37.3 ± 0.2	33.0 ± 0.2	8.1 ± 0.5	5.1 ± 0.2	5.1 ± 0.3	8.8 ± 0.6	13.4 ± 1.7
8	37.2 ± 0.1	33.1 ± 0.2	7.8 ± 0.4	4.9 ± 0.1	5.2 ± 0.4	8.6 ± 0.5	12.4 ± 1.1
104	37.3 ± 0.1	32.1 ± 0.2*	6.7 ± 0.4*	4.7 ± 0.1	6.9 ± 0.8*	7.5 ± 0.8#	12.4 ± 1.3#
176	37.3 ± 0.1	32.6 ± 0.3	7.3 ± 0.2	5.0 ± 0.1	5.2 ± 0.3	8.5 ± 0.6	10.8 ± 1.0

T_{re}, rectal temperature; T_{sk}, mean skin temperature; T_f, mean finger temperature; T_{min}, minimum finger temperature at first nadir; t_{min}, time to first temperature nadir; T_{max}, maximum temperature at first apex; t_{max}, time to first temperature apex

denotes n =5

*, denotes significant difference (P<0.05) at Hour 104 compared to Hours -28, 8, and 176.

Table 10. Maximum voluntary contractile grip strength (kg force) of the subjects in control period (Hours -35 and -13) and in DISSUB conditions.

Subject	Elapsed Time (Hours)				
	-35	-13	35	86	134
1	63.6	59.4	61.8	61.5	60.9
9	41.9	43.9	44.0	45.9	43.0
3	60.8	61.9	56.1	56.8	60.1
4	54.5	53.6	48.2	50.2	46.5
5	57.6	59.6	64.3	63.4	69.8
7	65.2	56.5	57.3	60.7	57.6
8	77.5	77.8	79.2	74.0	75.7
Mean	60.16	58.96	58.70	58.93	59.09
Standard Deviation	10.88	11.52	11.52	9.20	11.65

Table 11. Grip endurance (at 60% of control maximum) in seconds of the subjects in control period (Hours - 35 and -13) and in DISSUB conditions.

Subject	Elapsed Time (Hours)				
	-35	-13	35	86	134
1	91.53	86.56	89.44	95.59	75.78
9	97.47	101.09	123.16	135.75	98.56
3	90.63	76.53	58.84	51.62	65.34
4	101.13	79.50	91.35	89.78	93.56
5	91.22	91.00	108.78	93.00	100.91
7	88.34	116.09	107.75	97.25	103.94
8	80.53	91.60	49.87	74.53	80.22
Mean	91.55	91.77	89.88	91.07	88.33
Standard Deviation	6.58	13.47	26.93	25.46	14.64

Table 12. Subjective Environmental Symptoms Questionnaire responses during control and DISSUB conditions. Shaded areas highlight periods of significant difference (* p<0.001).

	Elapsed Time (Hours)								
	-44	-20	4	28	52	76	100	124	148
Total Score	8.43±9.25	9.00±7.48	11.43±11.41	6.86±6.12	10.43±6.55	21.29±14.23	16.57±10.92	19.71±16.63	17.57±16.48
AMS-C	0.08±0.14	0.07±0.09	0.05±0.13	0.04±0.09	0.08±0.11	0.40±0.91	0.15±0.14	0.18±0.18	0.07±0.10
AMS-R	0.10±0.11	0.15±0.14	0.20±0.21	0.16±0.24	0.12±0.12	0.25±0.20	0.22±0.21	0.19±0.22	0.16±0.16
ENT	0.13±0.26	0.30±0.33	0.33±0.39	0.13±0.13	0.12±0.18	0.22±0.20	0.41±0.36	0.29±0.40	0.34±0.51
COLD	0.11±0.16	0.00±0.00	0.01±0.03	0.02±0.06	0.58±0.34	1.20±0.54*	0.54±0.37	0.84±0.55*	0.76±0.63*
Distress	0.17±0.29	0.17±0.19	0.16±0.22	0.09±0.12	0.14±0.14	0.19±0.17	0.21±0.21	0.32±0.43	0.24±0.32
Alertness	2.05±0.35	1.55±1.14	1.93±0.93	1.96±0.37	1.67±0.76	1.77±0.86	1.86±0.49	1.93±0.46	1.84±0.54
Exertion	0.11±0.16	0.05±0.14	0.03±0.08	0.05±0.10	0.10±0.17	0.23±0.37	0.24±0.29	0.21±0.27	0.19±0.29
Muscle Discomfort	0.12±0.16	0.16±0.17	0.23±0.29	0.15±0.20	0.16±0.26	0.30±0.31	0.37±0.37	0.31±0.37	0.35±0.44
Fatigue	0.18±0.16	0.24±0.24	0.37±0.30	0.15±0.16	0.22±0.22	0.34±0.44	0.32±0.29	0.41±0.55	0.34±0.35

Table 13. Sleep fragmentation (number of awakenings per sleep period) by test study period. Data presented are mean +/- SEM. For nighttime sleep, post-hoc test revealed differences between control and DISSUB ($p = 0.01$) and DISSUB and post-chamber periods ($p = 0.02$). Post-hoc differences for daytime sleep were observed between control and DISSUB ($p = 0.006$), and DISSUB and post-chamber periods ($p = 0.03$). Total sleep post-hoc differences were determined between control and DISSUB ($p < 0.0001$) and DISSUB and post-chamber periods ($p = 0.001$).

	Study Period/Condition		
	Baseline	DISSUB	Post-chamber
Nighttime	14.8 ± 1.3	22.4 ± 2.2	14.8 ± 2.0
Daytime	1.7 ± 1.0	9.3 ± 2.4	1.8 ± 0.9
Total	8.2 ± 0.8	15.9 ± 0.4	8.3 ± 0.9

Table 14. Postural balance indexes before (Hour -36), during (Hour 110), and following (Hour 172) DISSUB conditions. Mean ± SD, * = $p < 0.001$, ‡ = $p < 0.01$, n= 7 subjects.

Test	Hour -36	Hour 110	Hour 172
Eyes Open	30.858±9.098	39.090±12.761*	20.843±9.127*
Eyes Closed	186.956±46.288	226.395±46.102‡	179.644±50.698‡
Dynamic	88.253±20.073	106.378±48.614	86.486±35.927

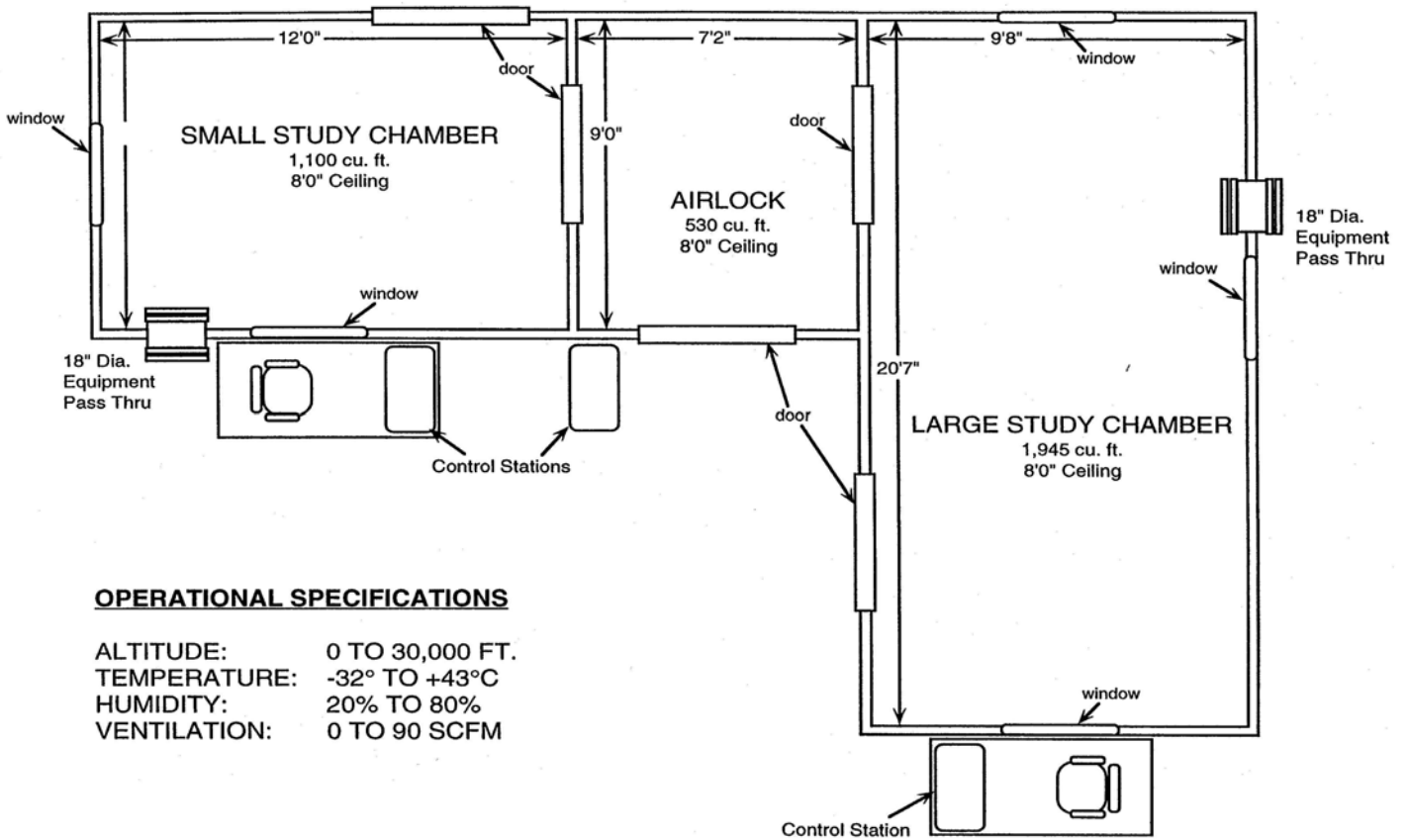


Figure 1. USARIEM Altitude Chamber Complex.

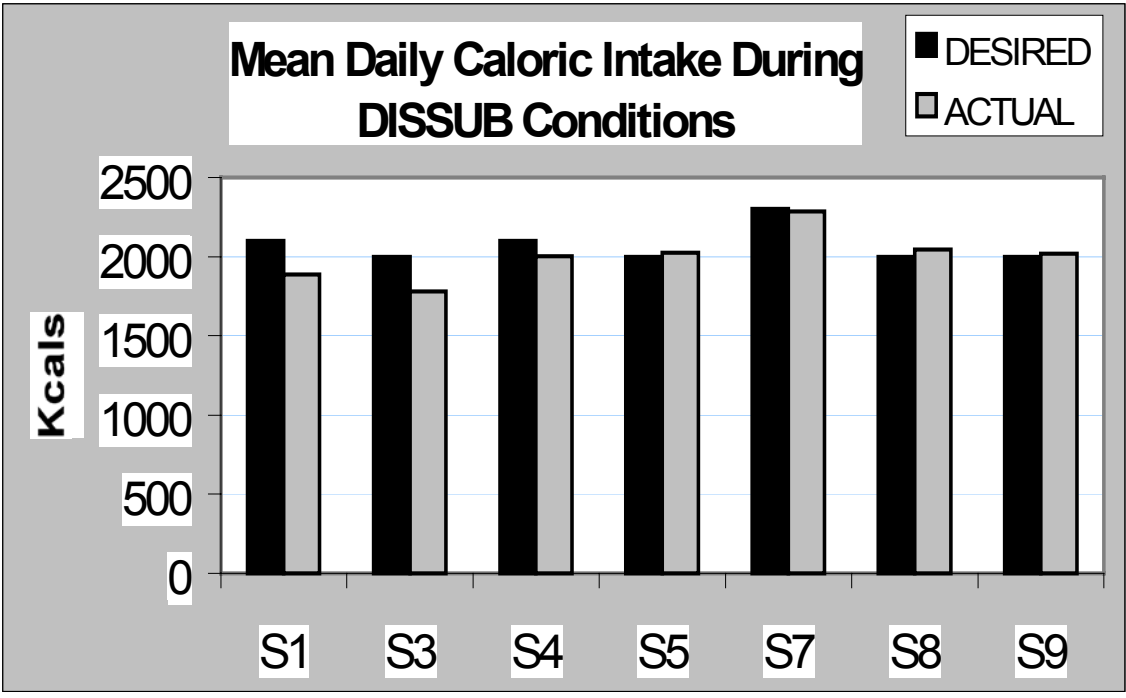


Figure 2. Average daily caloric intake during DISSUB conditions for each subject.

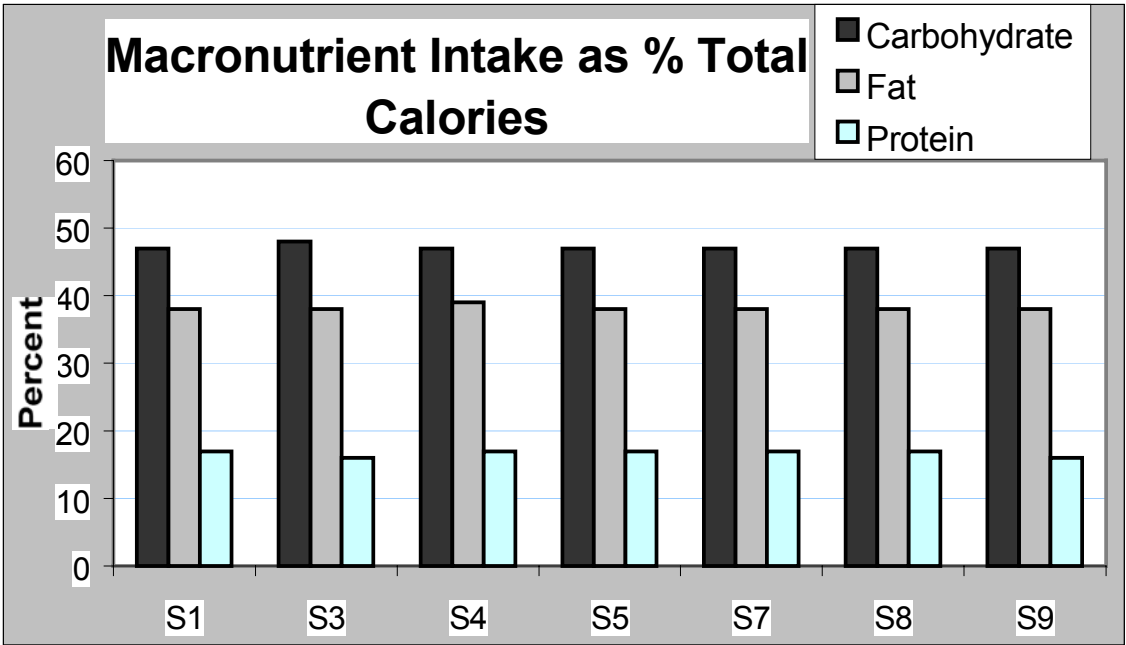


Figure 3. Average daily macronutrient intake during DISSUB conditions for each subject.

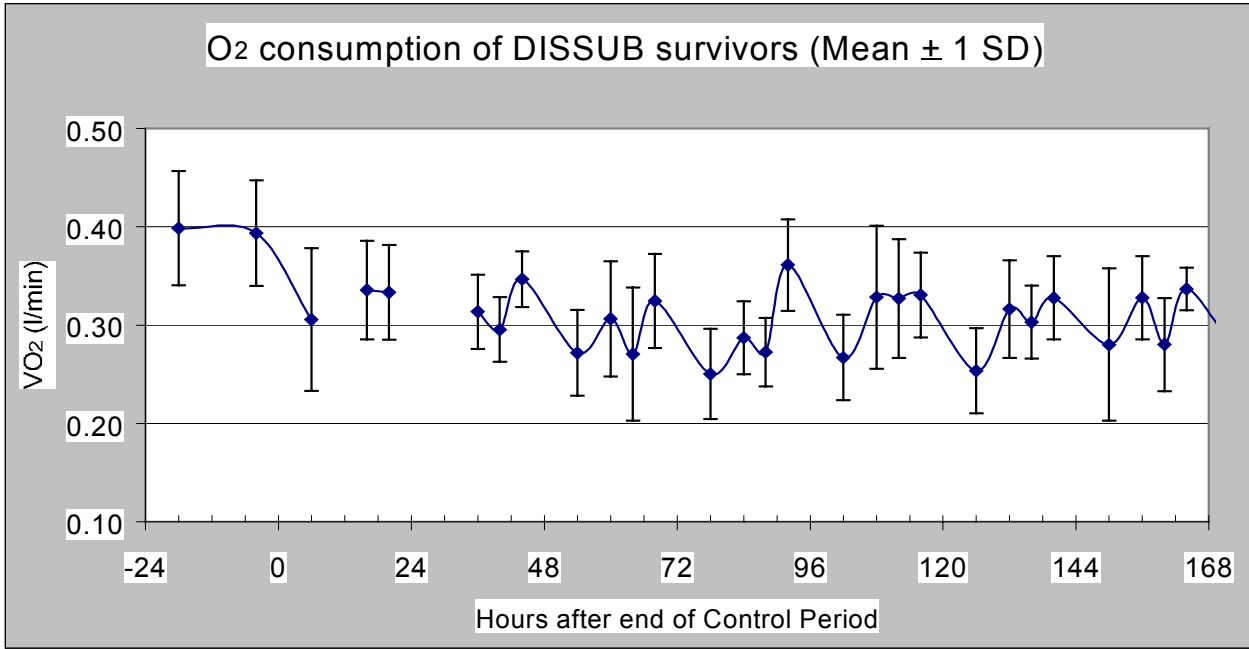


Figure 4. Resting oxygen consumption ($L \cdot \text{min}^{-1}$) using indirect calorimetry. First three measurements were obtained with automated metabolic cart, subsequent measures with Douglas Bags. Hour 0 represents beginning of initial hypoxic exposure.

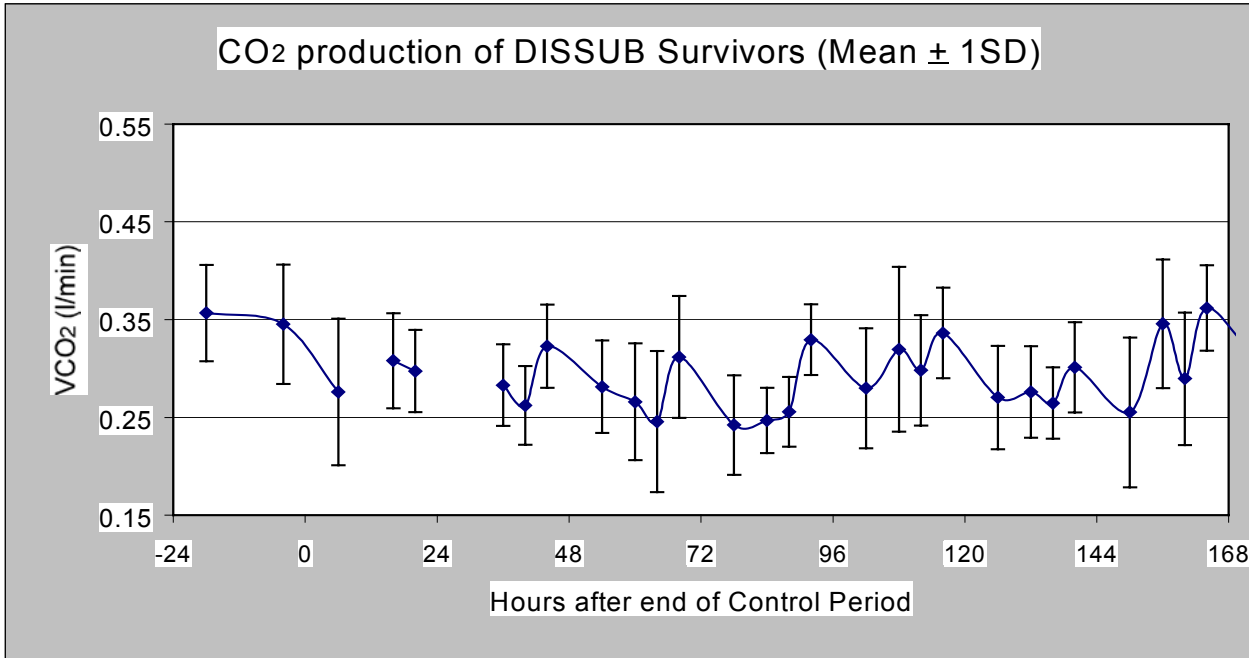


Figure 5. Resting carbon dioxide production ($L \cdot \text{min}^{-1}$) using indirect calorimetry. First three measurements were obtained with automated metabolic cart, subsequent measures with Douglas Bags. Hour 0 represents beginning of initial hypoxic exposure.

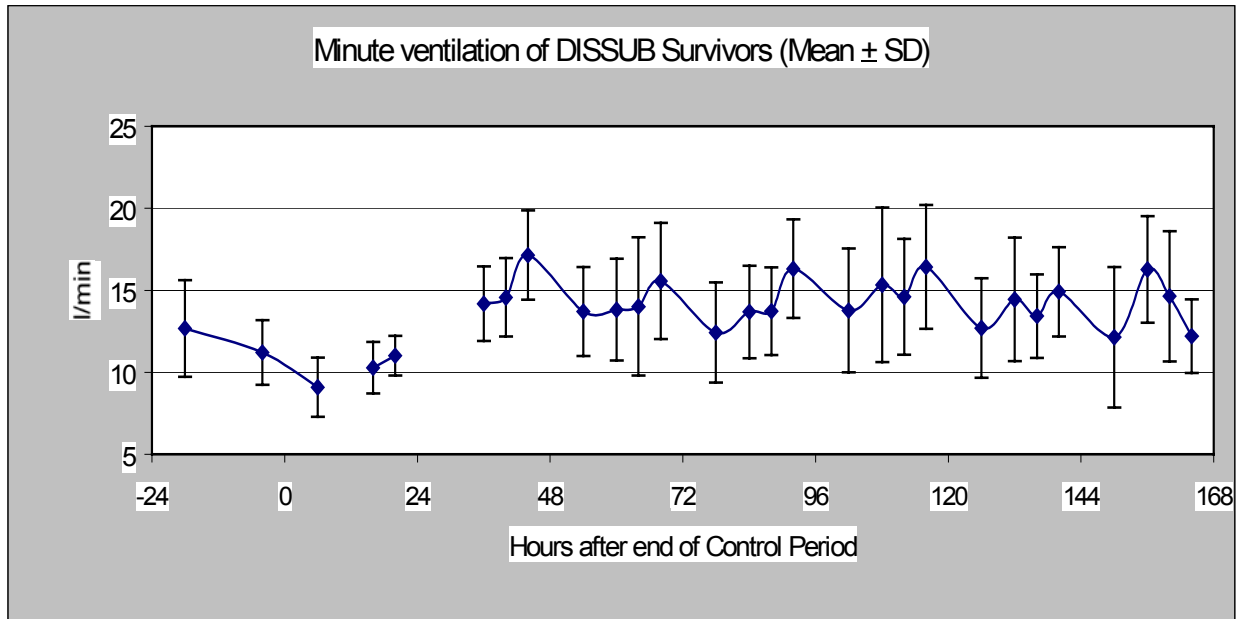


Figure 6. Resting minute ventilation ($L \cdot \text{min}^{-1}$). First three measurements were obtained with automated metabolic cart, subsequent measures with Douglas Bags. Hour 0 represents beginning of initial hypoxic exposure.

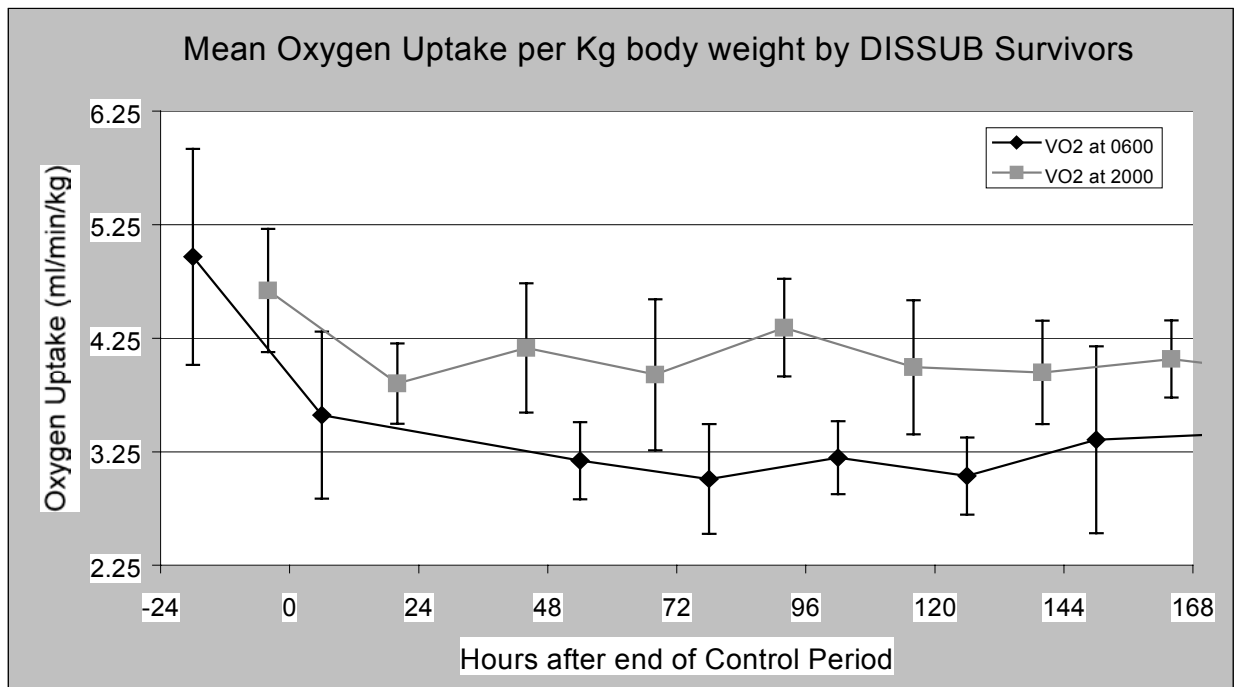


Figure 7. Resting oxygen uptake ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) at two times of the day using indirect calorimetry. First three measurements were obtained with automated metabolic cart, subsequent measures with Douglas Bags. Hour 0 represents beginning of initial hypoxic exposure. Data are means \pm 1 SD.

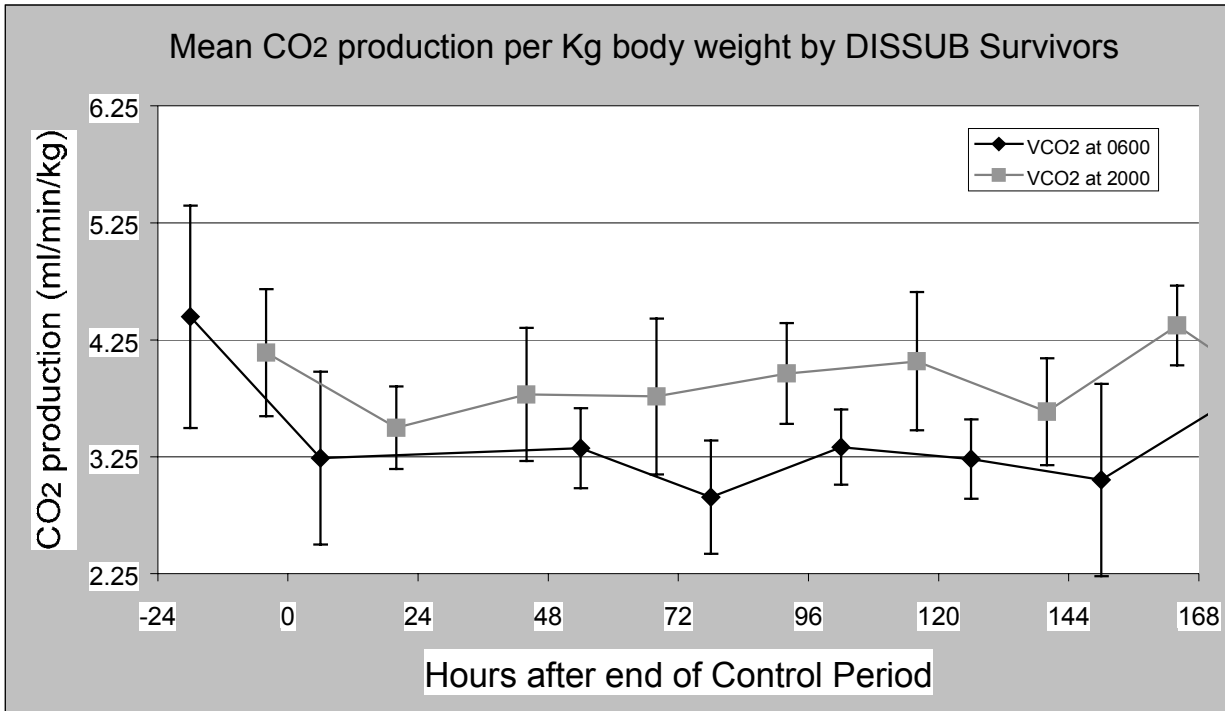


Figure 8. Resting carbon dioxide production ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) at two times of the day using indirect calorimetry. First three measurements were obtained with automated metabolic cart, subsequent measures with Douglas Bags. Hour 0 represents beginning of initial hypoxic exposure. Data are means \pm 1 SD.

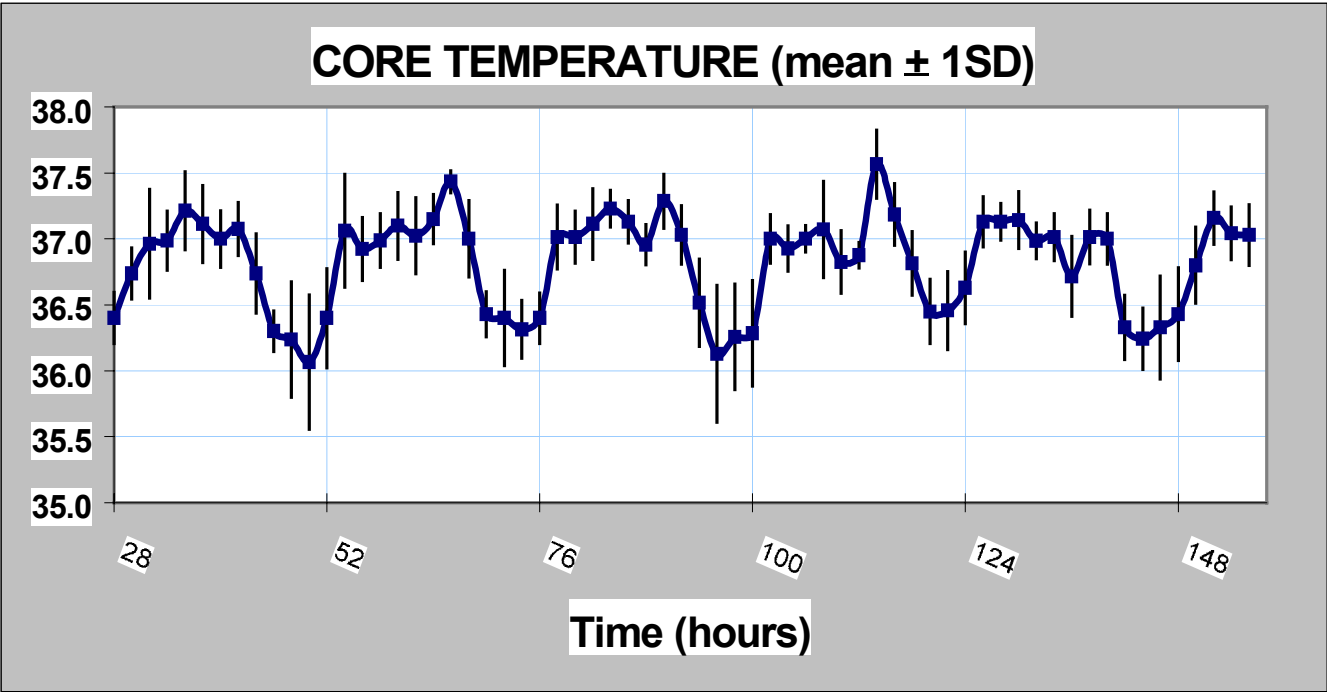


Figure 9. Subjects' core temperature vs. exposure hour while under DISSUB conditions.

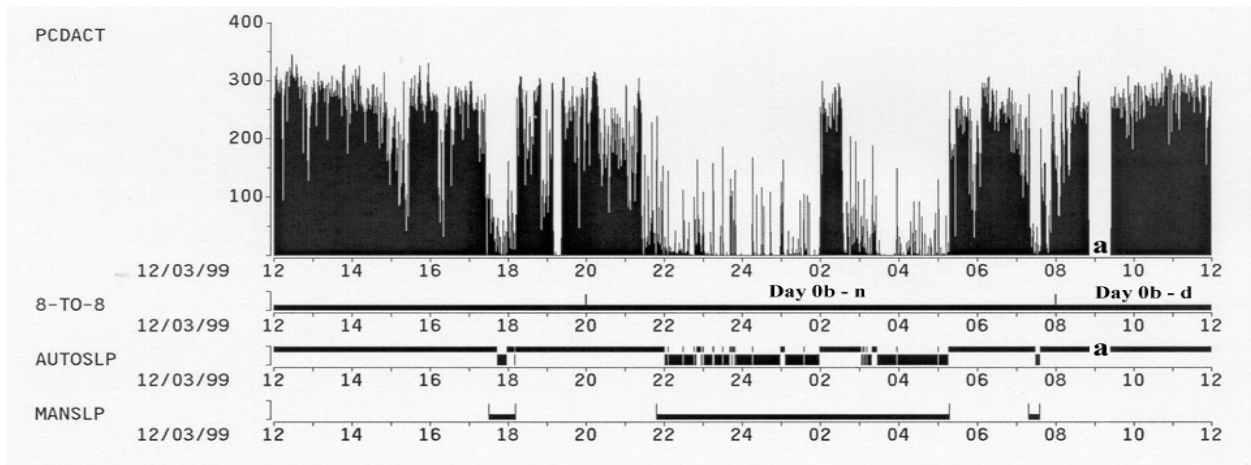


Figure 10a. Twenty-four hour activity plot of one subject from Hour -38 to Hour -14. Each vertical line represents a three-minute mean movement count. The marks on the 8-8 axis show 2000 and 0800 local time. The AUTOSLP axis records periods of sleep derived by algorithm and the MANSLP, a post-hoc, manual determination of sleep periods. 'a' shows missing data.

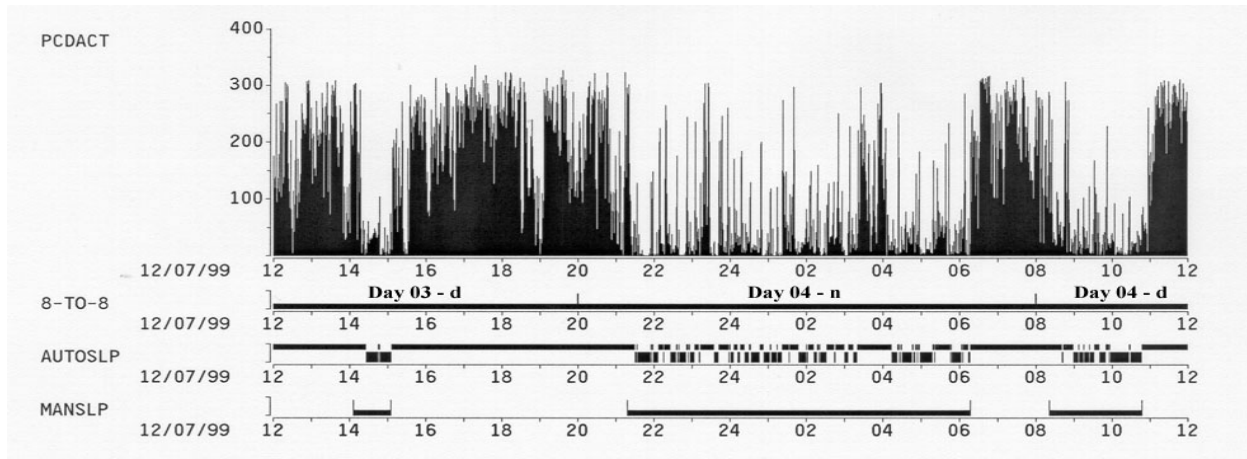


Figure 10b. Actigraph data from one subject (the same subject as in the above figure) during the DISSUB phase (Hour 58 to Hour 82). It can be seen that a greater period was spent sleeping, but the sleep was more fragmented than in the control period.