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MEASUREMENT OF ENERGY EXPENDITURE UNDER FIELD CONDITIONS
USING DOUBLY LABELED WATER

FINAL REPORT

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FOREWORD

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Dale A. Schwab _____ *8/31/88*
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INTRODUCTION

I. HYPOTHESIS

The doubly labeled water method will be a valid method for measuring energy expenditure and water intake of soldiers under field conditions.

II. OBJECTIVES

- A. Validate the doubly labeled water method for measuring energy expenditure of soldiers under field conditions.
- B. Validate the deuterium oxide method for measuring water intake of soldiers under field conditions.
- C. Determine the amount of change in baseline isotopic abundances in the field and whether the changes are uniform.
- D. Determine changes in total body water and lean fat free mass of soldiers under field conditions.
- E. Test the ability of soldiers to meet their energy expenditure requirement for field exercise using a light weight moderate calorie ration.

III. MILITARY SIGNIFICANCE

Caloric requirements of healthy adults and thus the soldier are equal to energy expenditure. Measurement of energy in the field, however, has been very difficult using traditional techniques. Development and use of the doubly labeled water method for measuring energy expenditure overcomes many of these difficulties. Thus, it should be possible to actually measure a soldier's energy expenditure under field conditions. This will improve estimates of energy requirements, and help to design better military rations, which in turn should improve the soldier's performance.

IV. BACKGROUND

A. Measurement of Energy Expenditure

A reliable and practical method of assessing energy expenditure is needed for a better understanding of energy expenditure and energy requirements in healthy individuals. Current methods for measuring energy expenditure are either inaccurate or not very practical for use in free living subjects especially for periods of more than 24 hours. Thus an investigator is faced with the prospect of measuring energy expenditure accurately under artificial laboratory conditions, or inaccurately under real living conditions. Because of this dilemma, energy expenditure, and hence, the energy requirements are usually not measured. If they are estimated, they are usually estimated by an indirect method and are not infrequently in error.

The most accurate methods for measuring energy expenditure are direct calorimetry, and indirect calorimetry by gas exchange. Direct calorimetry requires that the subject be confined in the

calorimeter and is therefore limited to short times and to activities that can be performed in the small enclosure. Indirect calorimetry based on respiratory gas analysis requires that the subject be fitted with a mask or housed in an enclosure for the measurement of carbon dioxide production and oxygen consumption. Indirect calorimetry is therefore nearly as limited as direct calorimetry except that the measurement device can be placed on a cart and carried to the subject.

In order to avoid these restrictive methods, investigators have turned to activity monitoring and heart rate monitoring. In the former, energy expenditure rates of typical tasks such as standing, sitting, or walking are measured by direct or indirect calorimetry for a short period of time. The subject, or an observer, then keeps a minute by minute log of activity during the study period and the total energy expenditure is calculated from the time spent at each activity. While useful, this approach is tedious, expensive, and subject to errors in excess of 10% as a result of either inaccurate logs (1,2) or a poor match between the types of activities for which the energy expenditure rates were measured and the actually activities performed (3).

Heart rate monitoring is also subject to large errors. If these errors are to be reduced to less than 10%, individual calibrations of heart rate versus energy expenditure rate must be made and these calibrations should be done over a 24 hour period using activities that match the activity to be performed during the study period (4). Thus, heart rate monitoring, if it is to be accurate, is expensive and restrictive (during calibration), and requires cooperative subjects.

The doubly labeled water method avoids these problems and provides a reliable and practical means of measuring average daily expenditure for periods of 4 to 21 days. This method is based on the observation that the oxygens of carbon dioxide are in isotopic equilibrium with the oxygen of body water (5). Thus, after the oral administration of a loading dose of oxygen and hydrogen labeled water, the oxygen isotope will be eliminated as both water and carbon dioxide, while the hydrogen isotope is eliminated only as water. The difference between the two elimination rates will be a measure of oxygen elimination rate via carbon dioxide, which is equal to twice the carbon dioxide production rate (r_{CO_2}). Stated mathematically:

$$r_{CO_2} = \frac{(N/2)(k_O - k_H)}{2}$$
where N is the total body water, k_O is the oxygen isotope elimination rate, and k_H is the hydrogen isotope elimination rate. The energy expenditure is calculated from the carbon dioxide production rate and the energy equivalent per mole of carbon dioxide adjusted for the respiratory quotient as measured or as estimated from the diet.

The advantages of the double labeled water method are that it is noninvasive, convenient for the subject, and not limited by subject compliance. The method does not place restrictions on the activities of the subject and can, therefore, measure energy expenditure under unrestricted (real) living conditions. The only samples needed are urine and saliva which are required at the beginning, middle and end of the study period for the

determinations of the isotope elimination rates (6). Between the two samples the subject is free to engage in typical activities without the time consuming need to fill out logs.

B. Previous Validations

Since developed by Lifson (7), the method has been validated for the measurement of carbon dioxide production in 80 small animals by six independent investigators (8). The mean accuracy is better than 1% and the mean precision is 7%.

We have previously validated the doubly labeled water method in humans (6,9,10,11) and found the method to be accurate to 1% and to have a precision of 4 to 8% which is very similar to the experience in the animal studies. This accuracy and precision is not as good as the 1.5 to 3% generally reported for calorimetry, but is considerably better than that obtained by other less restrictive methods of measuring energy expenditure. In addition, our validation studies, three other laboratories have compared the doubly labeled water method against 24 hour respiratory gas analyses and demonstrated that it was accurate to several percent (12,13).

Although these validations have demonstrated the validity of the doubly labeled water method in controlled settings, there are confounding factors that need to be considered in field studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. This change may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This is similar to the problem of patients beginning total parenteral nutrition in which there was a substantial shift in baseline isotopic abundance (11). This change would have caused energy expenditure to be overestimated by 30%. To avoid this error, measurement of energy expenditure was not initiated until after a ten day equilibration period (11). This delay is not always practical for field studies and other measures can be taken to compensate for baseline changes. In a recent study in infants, equations were developed based on isotopic abundances of initial body water and of ingested solutions to predict baseline abundance changes and these were used to reduce errors in the calculated energy expenditure. (9).

In the present contract, energy expenditure of special operation soldiers consuming either an energy adequate ration or a 2000 kcal/d ration was measured during a 25 day field study. This exercise was part of an evaluation of a newly developed lightweight ration used as the sole source of food for special operations soldiers for 30 consecutive days. The ration was designed to sustain soldiers in the field with minimum loss of fat-free mass or physical performance, and to be light and compact to be conveniently carried in a rucksack. This exercise presented an opportunity to validate doubly labeled water under field conditions. The soldiers began a new dietary regimen and a new water supply at the onset of the study and baseline isotopic enrichments were predicted to decrease. To compensate for this change, a group of soldiers who did not receive heavy water was followed so that baseline corrections could be made for the labeled subjects.

BODY

I. DOUBLY LABELED WATER FOR ENERGY EXPENDITURE UNDER FIELD CONDITIONS

A. METHODS

Subjects

Thirty six special operations soldiers from the 2nd and 3rd Battalions, Ft. Devens, MA began the study. Eighteen soldiers each were assigned to the lightweight ration group and the meal ready-to-eat group. Nine soldiers in each group, matched for fat-free mass, received $^2\text{H}_2^{18}\text{O}$ (Table 1). Two subjects were removed from the study for medical reasons. Subjects gave their informed consent after being briefed on the purpose and procedures involved in this study. The protocol was approved by the USARIEM and USARMRDC/OSTG Human Use Review Committees.

Protocol

The study began with 2 days of pretesting and mission planning at Fort Devens, MA. Both groups then underwent similar 25-day missions involving reconnaissance, surveillance and electronic warfare. The test site was at Camp Ethan Allen Training Center, Jerico, VT, a mixed deciduous and coniferous forest without established trails. Terrain was hilly, ranging from sea level to approximately 4000 feet elevation. Surface water for use as drinking water was abundant during this field training exercise. The study was conducted in late September and October, with temperatures ranging between 30-61 °F. A central base camp was established in the field and the teams were infiltrated and exfiltrated weekly.

Weekly testing was performed at the base camp. Body weights were taken at 6:00 A.M. after an overnight fast with the same uniform (T-shirts, fatigue trousers, pockets empty, and stocking feet). Digital electronic battery powered balances (SECA Model 770), accurate to ± 0.1 lb. were calibrated prior to each use with 100 lb. calibration weights. Blood samples were drawn on days 1, 15, and 30 at 6:00 A.M. following an overnight fast for determination of hemoglobin and hematocrit.

Diets

The calorie adequate control diet was the meal ready-to-eat ration, which provided a maximum of 4020 kcal/d available to take to the field. The new lightweight ration contained a maximum of 1980 kcal/d, all of which was taken to the field at the beginning of the study. The meal ready-to-eat ration provided 15%, 36% and 49%, and the lightweight ration 14%, 47% and 39% of energy as protein, fat, and carbohydrate, respectively.

The lightweight ration group was issued a 30 day supply of rations (30 pounds) which was carried in their rucksacks. Due to space and weight limitations the meal ready-to-eat group could only carry a maximum of a one week supply of rations (21 pounds). They were free to take or leave individual food items as they desired, and actually took only 3600 kcal/d into the field. The

two groups were not permitted to trade food items since they were physically separated during the field exercise. No other food items were permitted during the test and foraging was not permitted. Daily food consumption was recorded by each subject in individual log books. Subjects circled his estimate of degree of consumption, (1/4, 1/2, 3/4, or all) of the food item consumed, on a list of component ration food items. Prior to the study each soldier received training and instruction on food and water recording by a dietitian. At the end of each week the subjects were interviewed by the same dietitian and new log books issued.

Activity Patterns

A daily log of activities was recorded by a member of each group. The daily logs contained a description of each day's activities, ie, "static with local reconnaissance", "infiltration", "exfiltration", "reconnaissance", or "set-up base camp". In addition, the approximate distance traveled and the load transported by the soldiers was recorded. Mean daily energy expenditure was calculated for each group using these activity estimates.

Compact (2.5" x 3.5" x 3/4"), light-weight (3 oz) microprocessor-based activity monitors were also employed. They employ a two-element piezoelectric crystal, sensitive to 0.01 g of force in all three planes of excursion, to transduce motor activity.

Doubly Labeled Water

Due to the length of this study (28 days) subjects had to be redosed with doubly labeled water midway through the study (Figure 1). For the initial dose of heavy water, subjects received 0.29 g H₂¹⁸O/kg TBW (determined by underwater weighing), and 0.14 g ²H₂O/kg TBW. The dose was adjusted to 50 ml with tap water and given orally. The container was washed with another 50 ml of tap water and also given to the subject. The mid-study dose provided 0.22 g H₂¹⁸O/kg TBW and 0.10 g ²H₂O/kg TBW. For the final total body water determination, subjects were given a final dose providing 0.09 g H₂¹⁸O/kg TBW and 0.08 g ²H₂O/kg TBW. The initial doses are about 16% larger than we normally employ, and were chosen because water intake was expected to be 25% greater than in urban settings. Urine and saliva samples were collected throughout the study (Figure 1).

Total body water was calculated using ¹⁸O isotopic enrichments measured predose, and 3 and 4 hours after the dose

$$TBW = \frac{d}{MW_d} \times \frac{APE_d}{100} \times 18.02 \times \frac{1}{R_{std} \times \Delta \delta^{18}O} \times \frac{1}{1.01}$$

where d is the dose given in grams, MW_d is the molecular weight of the dose water, APE_d is the atom percent excess enrichment of the dose water, R_{std} is the ratio of heavy to light isotope of SMOW, i.e., 2.005 x 10⁻³, Δδ¹⁸O is the per mil difference from the predose sample. The final step in the equation, division by 1.01, is necessary since the ¹⁸O dilution space is larger than TBW (13).

The mean daily CO₂ production (rCO₂, mol/day) was calculated according to Schoeller et al (6):

$$rCO_2 = (N/2.078) (1.01k_O - 1.04k_H) - 0.0246r_{Gf}$$
where N is the average of the beginning and end of period total body water and r_{Gf} is the rate of water loss via fractionating gaseous routes, and is estimated to be 1.05N(1.01k_O - 1.04k_H). The ²H and ¹⁸O isotope elimination rates (k_H and k_O) were calculated by the two-point method using the isotopic enrichment relative to predose, and the time difference between collection of the initial and final samples: $k = (\ln APE_f - \ln APE_i) / \Delta t$.

Energy expenditure was calculated by multiplying rCO₂ by the energy equivalent of CO₂ calculated from the macronutrient content of each diet, and body stores of protein and fat used for energy. These averaged 5.72 and 5.99 kcal/liter CO₂ for the meal ready-to-eat group and lightweight ration group, respectively, based on the calculated RQ of 0.84 and 0.79 (15).

Intake/Balance (I/B)

Energy expenditure was also calculated using energy intake and change in body energy stores. The dietary energy intake was calculated from the daily food consumption log books using a computerized nutrient analyses system (16). The change in body-energy stores was calculated from change in fat-free mass and fat mass between days 1 and 28. Fat-free mass changes were assumed to be 27% protein and 73% water, and fat mass to be 100% fat. The energy equivalents used for protein and fat were 4.4 and 9.5 kcal/g, respectively (17).

Isotopic Analyses

The ¹⁸O isotope abundances were measured on a Nuclide 3-60 Isotope ratio Mass Spectrometer (6). Briefly, urine and saliva samples were equilibrated with 1 ml of CO₂ at 25°C for at least 48 h. The CO₂ was then cryogenically purified under vacuum before introduction into the mass spectrometer.

The hydrogen isotope abundances were measured on a Nuclide 3-60 HD Isotope Ratio Mass Spectrometer as previously described (18), except that water samples were reduced over zinc instead of uranium. Analyses were done in triplicate. Urine samples were drawn into disposable 2 uL micro-pipettes and placed in a 10 cm quartz sample tube (9 mm OD). The sample tube was then attached to one side of a two-orifice vacuum transfer line maintained under positive pressure with dry nitrogen gas. The sample tube was isolated from the nitrogen gas and then immersed in liquid nitrogen. Once the sample was frozen, the line was evacuated to remove air. The line was then isolated from vacuum and the sample distilled with heating into 15 cm reduction tubes made from 6 mm OD Vycor tubing (Corning Glass works). The reduction tube, containing 40 mg zinc reagent (Friends of Biogeochemistry, Bloomington, Indiana) was immersed in liquid nitrogen. The reduction tube was then sealed with a hydrogen and oxygen flame and placed in a 500°C oven for 30 minutes to reduce the water to hydrogen gas (19).

Statistical Analysis

Results are presented as means \pm SD. Two-tailed Student t tests were used to test for differences between between groups (all subjects in each group), while paired-t tests were used to test for differences between the matched pairs. Precision of the methods were compared using the variance ratio test (F-test). A minimum P value of 0.05 was required for statistical significance.

B. RESULTS

Baseline Isotope Abundance

The ^2H isotope abundance in the group receiving no isotope decreased from $-50 \pm 7\text{‰}$ to $-67 \pm 7\text{‰}$ vs. Standard Mean Ocean Water (SMOW) by day 27, while the ^{18}O abundance decreased from $-6.2 \pm 1.4\text{‰}$ to $-8.2 \pm 1.5\text{‰}$ vs. SMOW (Figure 2). Mean changes were used to correct isotope abundances in subjects receiving $^2\text{H}_2^{18}\text{O}$. These changes are quite close to the anticipated change. Although actual drinking water samples were not collected, estimates of change in drinking water are -20‰ and -2.5‰ for ^2H and ^{18}O , respectively (20). The decrease in isotope abundance of body water are similar to the estimated decrease in drinking water, but not as great, due to nonaqueous sources of oxygen and hydrogen (21).

Energy Intake

Although the meal ready-to-eat group took rations supplying 3600 kcal/d into the field, they only consumed 2840 ± 280 kcal/d during period 1 and 3080 ± 630 kcal/d during period 2. The lightweight ration group consumed nearly all of their rations and energy intake was quite steady during period 1 and 2, 1900 ± 130 and 1960 ± 120 kcal/d, respectively.

Body Composition Change

Total body water decreased between day 0 and day 14, then tended to increase slightly by day 27 (Table 2). The day 14 data probably reflected dehydration and thus should not be used to calculate fat free mass. This could be demonstrated by the greater loss of TBW during the first period than at the end of the study, as well as hematological measures. Mean hematocrits of the meal ready-to-eat group increased from $43.6 \pm 2.1\%$ at the beginning of the study to $45.9 \pm 1.5\%$ at the end of period 1, while that of the lightweight ration group increased from 44.7 ± 3.0 to 46.6 ± 3.1 . Hemoglobin concentrations followed the same trend, increasing from 14.65 ± 0.61 to 15.29 ± 0.48 in the meal ready-to-eat group and from 19.97 ± 0.82 to 15.51 ± 1.04 in the lightweight ration group.

At day 27, the soldiers were back at Fort Devens and had had a chance to rehydrate. Thus, body composition changes are compared only between days 0 and 27. The meal ready-to-eat group lost 1.1 ± 1.8 kg, of which 1.0 ± 1.1 kg (about 90%) was fat (Table 3). The lightweight ration group, which had a much larger energy deficit, lost 4.3 ± 0.7 kg, with fat comprising 2.9 ± 0.8 kg (about 68%) of the loss.

Activity Patterns

The energy expenditures calculated by the factorial method, using the activity diaries, were similar for the two groups. During the 25-day exercise the lightweight ration group traveled approximate 46 km, and the meal ready-to-eat group traveled 48 km, while carrying loads up to 100 pounds as well as local reconnaissance missions with no loads. The total daily energy expenditure estimated from the activity increased from about 3400 kcal/d during period 1 to 3600 kcal/d for period 2 for both groups.

The activity monitors employed were not sturdy enough to survive the rigors of this field exercise. Due to equipment failures complete data were recorded from only one subject in each group for the last two weeks of the study. Activity levels between the two subjects were within 5% of each other. Therefore, these two measures of activity levels indicate that the two groups did conduct similar operations during the study.

Energy Expenditure

Energy expenditure measured using doubly labeled water was calculated for both periods for each subject (Table 4). Means values compared well with intake/balance (Table 5 and 6). When all 16 subjects are compared, energy expenditure by doubly labeled water was 5% higher than I/B (3400 ± 260 and 3230 ± 520), although the difference was not significant. Energy expenditure measured by doubly labeled water also compared well with that calculated by the factorial method (3500 kcal/d). Comparison of the methods demonstrated that doubly labeled water provided a more precise measure of energy expenditure. The standard deviation of energy expenditure from the I/B method was 2 times that of doubly labeled water ($p < 0.01$).

We compared expenditure in period 1 and 2 because scheduled activities (activity logs) in period 2 were estimated to increase energy expenditure by 200 kcal/d. Although energy expenditure appeared to increase in period 2 (3490 ± 290 vs. 3320 ± 340) the difference did not quite reach statistical significance ($p < 0.08$).

To investigate if the lightweight ration group had a reduced energy expenditure in response to the lower caloric intake, we compared energy expenditure for 7 matched pairs of subjects. These were the remaining pairs from the 9 pairs matched by fat-free mass before the exercise began. Energy expenditure of the fat-free mass matched pairs was not significantly different (Figure 3). However, the energy expenditure of the lightweight ration group was 210 ± 320 kcal/d lower than that of the meal ready-to-eat group. Activity logs indicated no difference between groups.

C. DISCUSSION

Although the use of doubly labeled water for determination of energy expenditure in humans has been extensively validated in controlled settings (9-12), it has not been validated under field conditions. In this field trial doubly labeled water was shown to give accurate estimates of energy expenditure. Doubly labeled water worked in these soldiers who moved to a new location,

changed food and water supplies, underwent rigorous physical activities, half received adequate calories and half had over a 1000 kcal/day energy deficit, and thus lost considerable weight. Under the sum of these conditions doubly labeled water gave accurate estimates of energy expenditure.

The mean energy expenditure by doubly labeled water, 3400 ± 260 kcal/d, compared very well with that estimated by the factorial method, 3500 kcal/d, and compared well with the intake/balance method, 3230 ± 520 . Although energy expenditure by doubly labeled water was not significantly different from the I/B method, it gave an estimate that was 5% higher than I/B. The intake balance method used in this study was not the most accurate, so it is not surprising that the two methods did not agree perfectly. Food items were not weighed but an estimate of the proportion of consumption of each food item was recorded by the soldiers.

The major advantage of the doubly labeled water method was that it was much more precise than the I/B method. Because of this, the doubly labeled water method can provide much tighter estimates of energy expenditure than other field techniques. A second advantage of the doubly labeled water method was that it did not require the burdensome intake logs that required extensive subject cooperation and greater effort by the field personnel.

The precision of the doubly labeled water method in this study is hard to estimate because we do not have a highly precise reference method. Under controlled conditions, we estimate that doubly labeled water, using the two point method, has a coefficient of variation of about 4% in our hands. From a theoretical viewpoint, we predict that the coefficient of variation is at best 5% in this field study based on propagation of error analysis of the uncertainties in isotopic measurements and the energy equivalents of carbon dioxide at different respiratory ratios (22).

There are several factors that might lead to a decrease in precision of the doubly labeled water method in this field study. The primary factor for a potential decrease in precision is the that mean changes in baseline isotope abundance of the unlabeled subjects were applied to the labeled subjects although there was a great deal of individual variation. We could find no clear trends between initial enrichment and decrease in baseline abundance, nor did it appear that all subjects were moving to a similar final enrichment (Figure 2). Therefore, we used the mean decrease in baseline isotope abundance, which would introduce some individual error in the doubly labeled water method. In addition, the soldiers were moved from the test site in Vermont, to Fort Devens, MA, and therefore, changed water supply, the day before the final samples for energy expenditure and total body water determinations were taken. Depending on the amount each soldier drank during this day, this could also introduce a slight error in the doubly labeled water method.

The coefficient of variation of the doubly labeled water method, however, can be no worse than 9% which is the average coefficient of variation for the interindividual variation in energy expenditure. It can be no worse than this because this

includes both random measurement error and true differences in expenditure between the different subjects. Thus, in our hands, the precision of the doubly labeled water method lies between 5 and 9% in field studies. Because interindividual variation is at least 5%, it is more likely the precision lies closer to 5% than 9%.

Use of the doubly labeled water method in the field required some extra precautions and additional steps taken in the present study. We gave a 16% higher than normal isotope dose to these soldiers because water intake was expected to be considerably higher than in urban settings. Because of the length of the study (25 days) subjects had to be redosed in the middle of the study. Also, since these soldiers changed location and food and water supply, the isotopic abundance of a group not receiving isotope had to be followed during the study to correct isotope abundances in subjects receiving isotope.

Energy expenditures calculated with and without baseline correction were quite similar. Without baseline correction, energy expenditure was slightly lower than when baseline correction was applied (3290 ± 300 kcal/d vs. 3400 ± 260 kcal/d). The decrease in baseline abundance did not greatly affect the energy expenditure estimate because the optimum isotope dose for this study was given, that which gave an initial ratio of deuterium: ^{18}O ‰ of 5:1 (22,23). In addition, the deuterium and ^{18}O isotope abundances changed in parallel direction.

The newly developed lightweight ration was designed to sustain soldiers in the field with minimum loss of fat-free mass or physical performance. Since the soldiers receiving this ration had over a 1000 kcal/day energy deficit it was expected that they would lose weight and possibly decrease energy expenditure during the study. The soldiers in the lightweight ration group lost 4.3 ± 0.7 kg body weight with fat-free mass comprising 1.4 ± 1.1 kg of the loss. This is a substantial weight loss but is less than weight losses normally associated with a decrease in physical performance (24). The weight loss in the lightweight ration group was similar to that observed during semi-starvation in the Minnesota experiment (25). In the Minnesota experiment, 32 male subjects similar to those in the present study (26 ± 4 yr., 69.4 ± 5.8 kg, 178.8 ± 5.8 cm) lost 5.1 kg after 4 weeks of semi-starvation (1640 kcal/d vs. 3490 kcal/d during weight maintenance) while maintaining a similar activity level.

To examine whether the lightweight ration group reduced energy expenditure in response to the low energy intake, energy expenditures of matched subjects in each group were compared. Although the energy expenditure of the fat-free mass matched pairs was not significantly different, the energy expenditure of the lightweight ration group was 210 ± 320 kcal/d lower than the meal ready-to-eat group. The decrease in precision of the doubly labeled water method in this trial could have interfered with the detection of this difference, if there was a real difference. In the Minnesota experiment, BMR decreased by about 19% after 5 weeks of semi-starvation (25). A 19% decrease in BMR in the lightweight ration group would have resulted in a decrease of about 350 kcal/d. The energy deficit in the present study was

not as great as in Minnesota experiment, and the "semi-starvation" period in the present study was 25 days, whereas the shortest semi-starvation time point in the Minnesota experiment was 35 days. In addition, even though the meal ready-to-eat group received adequate diet, they did not consume enough calories to meet energy needs, as evidenced by the 1.1 ± 1.8 kg decrease in body weight. Therefore, one would not expect to see as great a decrease in BMR in the lightweight ration group compared to the meal ready-to-eat group, as was seen in the Minnesota experiment.

In summary, the doubly labeled water method works in the field. Even though the precision was not as good as we have had in the past, doubly labeled water gave accurate mean estimates of energy expenditure during this 25-day field trial. When using doubly labeled water, it would be advantageous to have subjects maintain their normal source of food and water intake during an energy expenditure study. If the source of food and water change during or immediately preceding a study, baseline corrections will have to be applied, with a possible concomitant decrease in precision.

II. MEASUREMENT OF WATER INTAKE

A. Methods

Measurement of Water Intake by Deuterium Turnover

Total water influx was calculated from deuterium turnover using a linear growth model (7) with correction for isotope fractionation (see Table 7 for symbols);

$$r_{tI} = (D k_H) (1/\theta) \quad [g/d] \quad (2)$$

During each period, the deuterium dilution space was taken as the average of the space at the start and end of the period. This was calculated from the measurement oxygen space assuming that deuterium space was 3% larger than the oxygen space.

Oxygen dilution spaces at dose 1, 2 and 3 were determined as described in Section V. A.

Fractionation

Deuterium exits the body water via breath and insensible cutaneous routes more slowly than hydrogen (21). Uncorrected, the effect of fractionation is to underestimate total water intake. The correction is based on the portion of total efflux subject to fractionation and is calculated as:

$$\theta = (r_{bE}/r_{tE})F_b + (r_{cE}/r_{tE})F_c + (r_{nfE}/r_{tE}) \quad (3)$$

Factors for isotope fractionation in respiratory (F_b) and transcutaneous (F_c) water efflux are 0.941 and 0.924, respectively (21). Total water efflux was approximated from water influx calculated without the fractionation correction. Under these conditions, r_{bE} was 10% (± 2.5), r_{cE} was 6% ($\pm 0.8\%$) of r_{tE} . In calculating fractionation due to breath water efflux, equality between volume air inhaled and exhaled was assumed. Air volume was taken to be the volume inhaled, and thus exhaled, to handle measured CO_2 production assuming expired air is 3% CO_2 . Water efflux via breath was calculated from air volume and absolute humidity, in g water per cubic meter of air. Absolute humidity was calculated as:

$$A = 216.5 P/T \quad [\text{mg/L}] \quad (4)$$

For expired air, a temperature of 35.6°C and 95% saturation was assumed.

There is no data on the unidirectional efflux of water through the skin of men under field conditions. It was assumed that unidirectional efflux was the same as under laboratory conditions. Results from several studies suggest the adult rate of transcutaneous water efflux from exposed skin at room temperature and rest is 0.14g/min.m² (26). Insensible cutaneous water efflux was estimated with this and body surface area (m²), calculated as $(3.2 W^{0.7285} - 0.0188 \log W_H^{0.3}) \div 10^4$ where W is weight in g, H is height in cm (27). Clothing probably presents a barrier to air flow across the skin and a barrier reduces the rate of evaporation (27). We therefore assumed that the rate of transcutaneous water efflux was only 0.07g/min.m² in areas covered by clothing. Because subjects in this study wore clothing over about 75% of their bodies, we used an effective rate of transcutaneous water efflux of 0.12g/min.m². Under conditions of this field exercise, θ was calculated to be 0.99.

Preformed Water Intake

Water influx measured by deuterium elimination is the sum of water influx from all routes. These include atmospheric water absorbed transcutaneously and through the respiratory route, preformed dietary water, and metabolic water.

Respiratory water influx was calculated as the product of inspired air volume and absolute humidity. Absolute humidity was calculated as described for estimation of breath water efflux except relative humidities and ambient air temperatures were determined from wet and dry bulb thermometers; saturation vapor pressure over water at ambient temperature was from the Smithsonian Meteorological Tables (29). The median absolute humidity of 14mg/L humidity was used (range 9.6 to 18 mg/L).

Transcutaneous water influx was calculated using a value for transcutaneous absorption of 180 mg/m² body surface area per minute as determined by Pinson in adults in an atmosphere saturated with water vapor (21.7mg/L) at 24°C (32).

$$\text{Transcutaneous water influx (g/min)} = 0.18 (A/21.7) (\text{BSA}) \quad (5)$$

The mean absolute humidity (14mg/L) was used in calculating transcutaneous water influx. We assumed a 25% reduction in transcutaneous water influx because half the body was clothed.

Metabolic water was calculated from estimates of energy expenditure and substrate oxidation. Total energy expenditure was estimated from doubly labeled water. Substrate oxidation and thus metabolic water production were calculated assuming: 1) protein oxidation equal intake plus 0.27 times change in fat free mass, and 4.75 kcal/g and 0.41 g water/g protein; 2) all dietary carbohydrates was oxidized and carbohydrate oxidized yielded 4.18 kcal/g and 0.6 g water/g; 3) the caloric difference between total energy expenditure and the sum of calories from carbohydrate and protein oxidation were from fat oxidation which yielded 9.4 kcal and 1.07 g water/g (15).

B. Results

Water Influx

Total water influx was calculated for each subject (Tables 8 and 9). Values ranged from about 2 kg/d to almost 5 kg/d. Influx was relatively consistent for each subject between period 1 and 2, but did tend to increase by 300 g/d in period 2. Average influx was 800 g/d greater for the RLW group compared to the MRE group (3720 ± 1174 vs. 2910 ± 680 g/d), but the difference was not statistically significant.

Preformed Water Intake

Preformed water intake was calculated from total influx by subtracting the sum of the calculated respiratory water influx, transcutaneous water input, and metabolic water (Tables 10 and 11). Preformed water intake tended to increase between periods 1 and 2 by about 400 g/d. Preformed water intake also tended to be 870 g/d greater in the RLW group than in the MRE group (3150 ± 1180 vs. 2270 ± 700 g/d), but again was not statistically significant because of the large interindividual variation.

Comparison With Recorded Intake

Preformed water intake did not compare well with recorded intake. Recorded intake average $60 \pm 86\%$ greater than preformed water intake calculated from deuterium turnover (Tables 10 and 11 and Figure 4). This difference is statistically significant ($p < 0.05$). Even if the one gross outlier (+334%) is dropped, recorded intake averaged $41 \pm 47\%$ ($p < 0.01$).

Comparison With Physiologic Measures

To help determine which of the two estimates of preformed water intake (deuterium or record) are more correct, we compared average daily preformed water intake during period 1 with the change in hematocrit and the change in urine specific gravity. The rationale was that true low intake should be associated with dehydration and increased hematocrit and urine specific gravity (Figures 5-8). Neither preformed water intake by deuterium or recorded water intake correlated well with either of these measures of dehydration.

C. Discussion

The poor comparison of deuterium turnover with recorded intake is hard to explain. In a previous study, we validated the measurement of preformed water intake by deuterium against weighed intake in infants (30). We found that deuterium was accurate to 2% with a 3% coefficient of variation. Zoologists have also validated the labeled water method using tritiated water (31). They have observed similar results under dry conditions. At high humidity, the labeled water method overestimates true intake, but our methods of correcting for respiratory and transcutaneous water influx reduce this overestimate to 10 to 15% (30). No one has reported a large underestimate of water intake as found in this field study.

The difference between preformed water intake from deuterium and from record is not likely to be due to an error in the deuterium measurements. An error of this magnitude would have produced an underestimate of energy expenditure of thousands of kcal/d. This was not the case. Thus, it is unlikely that the deuterium method was greatly in error.

A significant random error in the recorded intake is likely because records were kept in terms of quarters of a canteen, based on the subjects perception of fullness of the opaque canteen. The random error, however, cannot account for the systematic error of almost 1500 g/d. In one individual, (#21) the difference is quite probable due to a recording error. Subject 21 recorded an intake in excess of 10 L/d which is not within the normal physiologic of water intake. In the other 15 subjects, the cause of the offset is not obvious. The subjects were quite precise in recording food intake as evidenced by the comparison of energy expenditure by intake/balance and doubly labeled water, thus it is unlikely they were careless with water intake records. The only two plausible explanation are that intake records include water used for purposes other than ingestion or that there was an error in converting canteen measures to units of g/d.

In summary, the doubly labeled water method was not validated against recorded intake in this field trial. The cause of the problem was not identified, but it is suggested that much of the error must be associated with the intake records.

CONCLUSIONS AND RECOMMENDATIONS

The doubly labeled water method was validated for use in field trials by soldiers. Problems with a change in baseline due to movement to a new local with a change in water source were identified, but can be minimized by the inclusion of subjects who do not receive doubly labeled water. At four to eight such individuals should be included to provide an estimate of average change that can be used to correct the data from the group receiving doubly labeled water. Because this correction can never be exact, it is recommended that studies use the larger isotope dose as used herein and that subjects not be followed for more than two biologic half-lives of those stable isotopes. Smaller doses or longer metabolic periods will lead to smaller final isotope enrichments which will amplify any errors in the baseline change.

The deuterium method for measuring preformed water intake was not validated. The cause of the failure to validate was not irrelevant. Because the method has been validated in small animals and because of the importance of water intake for maintaining hydration and optimal physical performance; it is suggested that further studies be performed to validate this technique for use in soldiers in the field. These studies should be done with much greater control on the measurement of water intake.

TABLE 1. Physical characteristics and body composition of subjects

Subject #	age y	Initial Weight kg	Height cm	Total Body Water, * kg	Lean Body Mass,	
					Isotope Dilution† kg	Under-Water Weighing kg
<u>Meal Ready To Eat Group</u>						
1	23	82.3	185.3	50.0	68.4	70.0
2	26	73.7	172.7	43.8	60.0	61.7
3	24	80.7	190.5	50.5	69.2	67.2
4‡	20	62.8	170.2	38.4	52.6	53.4
9	22	73.5	172.7	42.7	58.4	58.6
10	27	67.6	172.7	40.8	56.0	56.0
13	36	70.1	180.3	42.7	58.5	60.0
15	21	67.0	167.6	42.6	58.3	59.9
Mean	25	72.2	176.5	43.9	60.2	60.9
SD	5	6.8	8.0	4.2	5.8	5.5
<u>Lightweight Ration Group</u>						
21	27	83.2	177.8	49.9	68.3	70.5
23	27	81.7	182.9	46.1	63.1	62.3
24	32	77.5	182.9	46.8	64.1	66.5
31	22	67.4	165.1	40.3	55.2	57.2
32	29	74.2	172.7	44.1	60.5	61.8
33	39	79.2	175.3	44.1	60.4	58.3
35‡	25	79.3	188	46.8	64.0	63.2
36	24	74.2	177.8	45.4	62.3	62.1
Mean	28	77.1	177.8	45.4	62.2	62.7
SD	5	5.1	7.1	2.8	3.8	4.3

*From ^{18}O dilution space/1.01.

†Total body water/0.73.

‡Matching subject dropped from study

TABLE 2. Changes in Total Body Water During Field Exercise

Subject	Total Body Water		
	Day 0	Day 14	Day 27
	kg	kg	kg
1	49.95	47.80	49.55
2	43.83	43.01	43.59
3	50.54	48.33	49.65
4	38.41	37.06	40.28
9	42.66	41.24	40.64
10	40.85	39.85	41.32
13	42.74	41.36	42.74
15	42.55	42.83	43.17
21	49.88	48.38	48.48
23	46.06	44.68	45.37
24	46.78	45.74	45.47
31	40.28	39.44	38.41
32	44.14	42.81	43.94
33	44.12	42.58	43.88
35	46.75	44.51	44.66
36	45.44	44.03	45.28
average	44.69	43.35	44.15
SD	3.53	3.22	3.21

TABLE 3

Body weight loss

	<u>Meal ready to eat</u>		<u>Lightweight ration</u>	
	<u>Period</u>		<u>Period</u>	
	1	2	1	2
Total, kg	-0.8±1.2	-1.1±1.8	-2.8±0.7	-4.3±0.7
TBW*, kg	-1.3±0.8	-0.1±1.1	-1.4±0.4	-1.0±0.8
FFM†, kg	-----	-0.1±1.6	-----	-1.4±1.1
FAT‡, kg	-----	-1.0±1.1	-----	-2.9±0.8

* TBW, total body water based on O^{18} dilution space/1.01. † FFM, fat-free mass calculated as TBW/0.73. ‡ Fat loss calculated as total weight loss - FFM loss.

TABLE 4. Energy Expenditure During Field Exercise

Subject		Total Energy Expenditure			
		kd d-1	ko d-1	DLW kcal/d	I/B kcal/d
1	1	0.0383	0.0615	3542	
	2	0.0449	0.0704	3856	
	avg	0.0416	0.0660	3699	2890
2	1	0.0625	0.0910	3786	
		0.0675	0.0945	3549	
	avg	0.0650	0.0928	3667	3364
3	1	0.0589	0.0811	3311	
	2	0.0741	0.1006	3907	
	avg	0.0665	0.0909	3609	2586
4	1	0.0762	0.1021	2911	
	2	0.0802	0.1088	3320	
	avg	0.0782	0.1055	3116	2938
9	1	0.0662	0.0901	3027	
	2	0.0596	0.0868	3402	
	avg	0.0629	0.0885	3215	2758
10	1	0.0514	0.0789	3432	
	2	0.0575	0.0838	3268	
	avg	0.0545	0.0814	3350	4451
13	1	0.0596	0.0880	3682	
	2	0.0687	0.0968	3592	
	avg	0.0642	0.0924	3637	4101
15	1	0.0853	0.1139	3659	
	2	0.1112	0.1393	3484	
	avg	0.0983	0.1266	3571	3755
21	1	0.0569	0.0794	3497	
	2	0.0662	0.0911	3791	
	avg	0.0616	0.0853	3644	3212
23	1	0.1241	0.1484	3177	
		0.1162	0.1398	3082	
	avg	0.1202	0.1441	3129	3532
24	1	0.1014	0.1210	2622	
	2	0.1163	0.1480	3382	
	avg	0.1089	0.1345	3002	3216
31	1	0.0602	0.0847	3092	
	2	0.0675	0.0947	3351	
	avg	0.0639	0.0897	3222	2639
32	1	0.0969	0.1252	3780	
	2	0.1114	0.1385	3512	
	avg	0.1042	0.1318	3646	3263
33	1	0.0578	0.0794	2968	
	2	0.0633	0.0863	3121	
	avg	0.0606	0.0829	3045	3015
35	1	0.0674	0.0908	3340	
	2	0.0770	0.1000	3150	
	avg	0.0722	0.0954	3245	2807
36	1	0.0577	0.0809	3280	
	2	0.0699	0.0984	4024	
	avg	0.0638	0.0897	3652	3135

**TABLE 5. Energy Intake and Change in Body Composition
During 28 Day Field Exercise**

Subject	Intake kcal/d	Change in FFM kg	Change in FM kg	Change in Body Stores kcal
1	2332	-0.55	-1.40	-13958
2	2716	-0.33	-1.67	-16201
3	2718	-1.21	0.51	3289
4	3209	2.58	0.37	6765
9	2555	-2.77	-0.18	-5066
10	3481	0.63	-2.63	-24245
13	3379	0.01	-1.92	-18060
15	3268	0.85	-1.40	-12184
21	1992	-1.92	-2.98	-30494
23	1953	-0.95	-4.05	-39483
24	1918	-1.79	-3.21	-32459
31	1821	-2.56	-1.84	-20439
32	1992	-0.27	-3.33	-31790
33	1686	-0.33	-3.47	-33229
35	2013	-2.88	-1.72	-19839
36	2035	-0.22	-2.88	-27513
average	2442	-0.73	-1.99	-19682
SD	613	1.43	1.36	13240

TABLE 6
Elimination rates, total body water and energy expenditure

	$\frac{k_D}{d^{-1}}$	$\frac{k_O}{d^{-1}}$	TBW kg	Energy Expenditure	
				DLW kcal/d	I/B kcal/d
Period					
<u>Meal ready-to-eat group</u>					
1	0.0623±0.0144	0.0883±0.0157	43.3±4.0	3420±320	3360±680
2	0.0705±0.0197	0.0976±0.0205	43.3±3.7	3550±230	
<u>Lightweight ration group</u>					
1	0.0778±0.0260	0.1012±0.0266	44.7±2.7	3220±350	3100±280
2	0.0860±0.0241	0.1121±0.0253	44.2±2.7	3430±340	

k_D , ^2H elimination rate constant, corrected for change in baseline isotope abundance; k_O , ^{18}O elimination rate constant, corrected for change in baseline isotope abundance; TBW, average total body water for each period; DLW, doubly labeled water, energy expenditure by doubly labeled water; I/B, energy expenditure for 27 day period, calculated from intake and change in body energy stores.

TABLE 7. Symbols

A	absolute humidity (mg/L)
APE _d	atom percent excess of deuterium oxide
F _b	fractionation factor for breath water
F _c	fractionation factor for cutaneous water
k _H	deuterium elimination rate, $[\ln(i/f)]/t$
MW _{bw}	molecular weight of body water, 18.02
P	water vapor pressure - relative humidity ($P_s/100$)
P _s	saturation water vapor pressure at ambient temperature (mbar)
r _{bE}	rate of respiratory water efflux (g/d)
r _{bI}	rate of respiratory water influx (g/d)
r _{cE}	rate of tranacutaneous atmospheric water efflux (g/d)
r _{cI}	rate of transcutaneous atmospheric water influx (g/d)
r _m	rate of metabolic water production (g/d)
r _{nfE}	rate of nonfractionated water efflux (g/d)
r _{tE}	rate of total efflux (g/d)
r _{tI}	rate of total water influx (g/d)
SMOW	standard mean ocean water
T	temperature (°K)
TBW	total body water
1/θ	correction for effective net fractionation
W _{pd}	post-dose water - preformed water (g)

TABLE 8. Water Turnover for the 28 Day Field Exercise

		MRE Group		
		TBW	k2	water influx
		kg	d-1	g/d
1	1	48.88	0.0383	1932
	2	48.68	0.0449	2248
	avg	48.78	0.0416	2090
2	1	43.41	0.0625	2773
	2	43.30	0.0675	2979
	avg	43.36	0.0650	2876
3	1	49.44	0.0589	2969
	2	48.99	0.0741	3693
	avg	49.22	0.0665	3331
4	1	37.74	0.0763	2927
	2	38.67	0.0802	3153
	avg	38.21	0.0782	3040
9	1	41.95	0.0662	2827
	2	40.94	0.0597	2497
	avg	41.45	0.0629	2662
10	1	40.35	0.0514	2129
	2	40.59	0.0575	2387
	avg	40.47	0.0545	2258
13	1	42.09	0.0596	2567
	2	42.09	0.0687	2949
	avg	42.09	0.0642	2758
15	1	42.84	0.0853	3711
	2	43.07	0.1112	4844
	avg	42.96	0.0983	4277
Period 1 avg		43.34	0.0623	2729
Period 2 avg		43.29	0.0705	3094
Overall avg		43.31	0.0664	2912

TABLE 9. Water Turnover for the 28 Day Field Exercise

		RLW Group		
		TBW	k2	water influx
		kg	d-1	g/d
21	1	49.13	0.0569	2854
	2	48.46	0.0662	3269
	avg	48.80	0.0616	3062
23	1	45.37	0.1241	5686
	2	44.65	0.1162	5242
	avg	45.01	0.1202	5464
24	1	46.26	0.1014	4738
	2	45.61	0.1226	5648
	avg	45.94	0.1120	5193
31	1	39.86	0.0602	2451
	2	38.76	0.0675	2670
	avg	39.31	0.0639	2560
32	1	43.48	0.0969	4273
	2	43.38	0.1114	4889
	avg	43.43	0.1042	4581
33	1	43.35	0.0578	2558
	2	43.23	0.0633	2790
	avg	43.29	0.0606	2674
35	1	45.63	0.0674	3133
	2	44.59	0.0770	3488
	avg	45.11	0.0722	3311
36	1	44.74	0.0577	2637
	2	44.66	0.0699	3184
	avg	44.70	0.0638	2911
Period 1 avg		44.73	0.0778	3541
Period 2 avg		44.17	0.0868	3897
Overall avg		44.45	0.0823	3719

TABLE 10. Performed Water Intake From Beverages and Food
For the 28 Day Field Exercise

MRE Group		water influx g/d	rbI g/d	rcI g/d	rM g/d	Performed Water g/d	Recorded Water g/d	Differen %
1	1	1932	111	173	368	1281		
	2	2248	83	117	355	1693		
	avg	2090	97	145	362	1487	1443	-3.0%
2	1	2773	118	156	415	2083		
	2	2979	76	106	428	2369		
	avg	2876	97	131	422	2226	5156	131.6%
3	1	2969	104	175	245	2446		
	2	3693	84	121	239	3250		
	avg	3331	94	148	242	2848	5637	97.9%
4	1	2927	91	145	394	2297		
	2	3153	71	101	397	2583		
	avg	3040	81	123	396	2440	2091	-14.3%
9	1	2827	95	156	364	2212		
	2	2497	71	105	334	1986		
	avg	2662	83	131	349	2099	2403	14.5%
10	1	2129	108	151	490	1381		
	2	2387	70	102	622	1593		
	avg	2258	89	126	556	1487	3087	107.6%
13	1	2567	115	158	489	1805		
	2	2949	77	107	544	2221		
	avg	2758	96	133	517	2013	3634	80.5%
15	1	3711	115	147	428	3021		
	2	4844	75	100	530	4139		
	avg	4277	95	124	479	3580	4305	20.3%
Period 1 avg		2729	107	158	399	2066		
Period 2 avg		3094	76	108	431	2479		
Overall avg		2912	91	133	415	2272	3470	54.4%

TABLE 11. Preformed Water Intake From Beverages and Food
For the 28 Day Field Exercise

		RLW Group						
Subject		water influx g/d	rbI g/d	rcI g/d	rM g/d	Preformed Water g/d	Recorded Water g/d	Differen %
21	1	2854	111	168	378	2197		
	2	3269	83	112	402	2672		
	avg	3062	97	140	390	2434	10564	333.9%
23	1	5686	101	170	310	5104		
	2	5242	67	114	322	4739		
	avg	5464	84	142	316	4922	8225	67.1%
24	1	4738	83	167	366	4122		
	2	5648	74	111	402	5061		
	avg	5193	78	139	384	4591	6951	51.4%
31	1	2451	98	146	369	1838		
	2	2670	73	97	376	2124		
	avg	2560	85	121	373	1981	2281	15.1%
32	1	4273	120	157	342	3654		
	2	4889	76	105	358	4349		
	avg	4581	98	131	350	4001	4157	3.9%
33	1	2558	94	163	335	1966		
	2	2790	68	109	330	2283		
	avg	2674	81	136	333	2124	3161	48.8%
35	1	3133	106	172	262	2593		
	2	3488	69	115	261	3044		
	avg	3311	87	143	262	2819	2178	-22.7%
36	1	2637	104	160	376	1997		
	2	3184	88	108	380	2609		
	avg	2911	96	134	378	2303	2847	23.6%
Period 1 avg		3541	102	163	342	2934		
Period 2 avg		3897	75	109	354	3360		
Overall avg		3719	88	136	348	3147	5046	65.1%

DEUTERIUM BASELINE

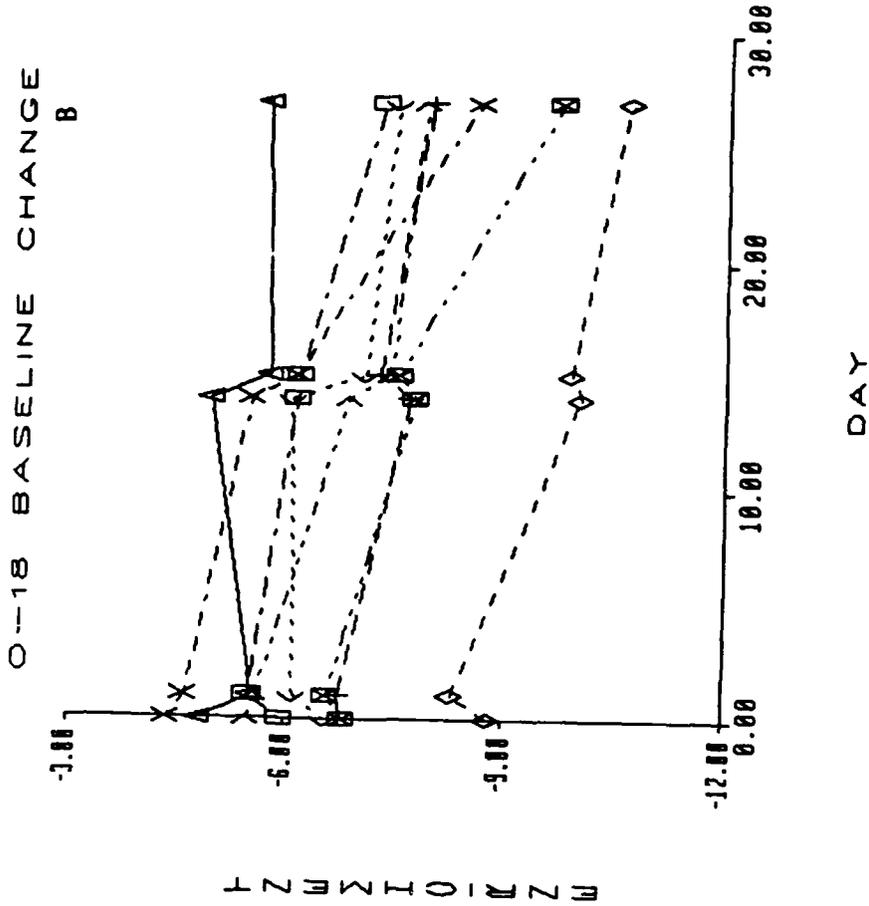
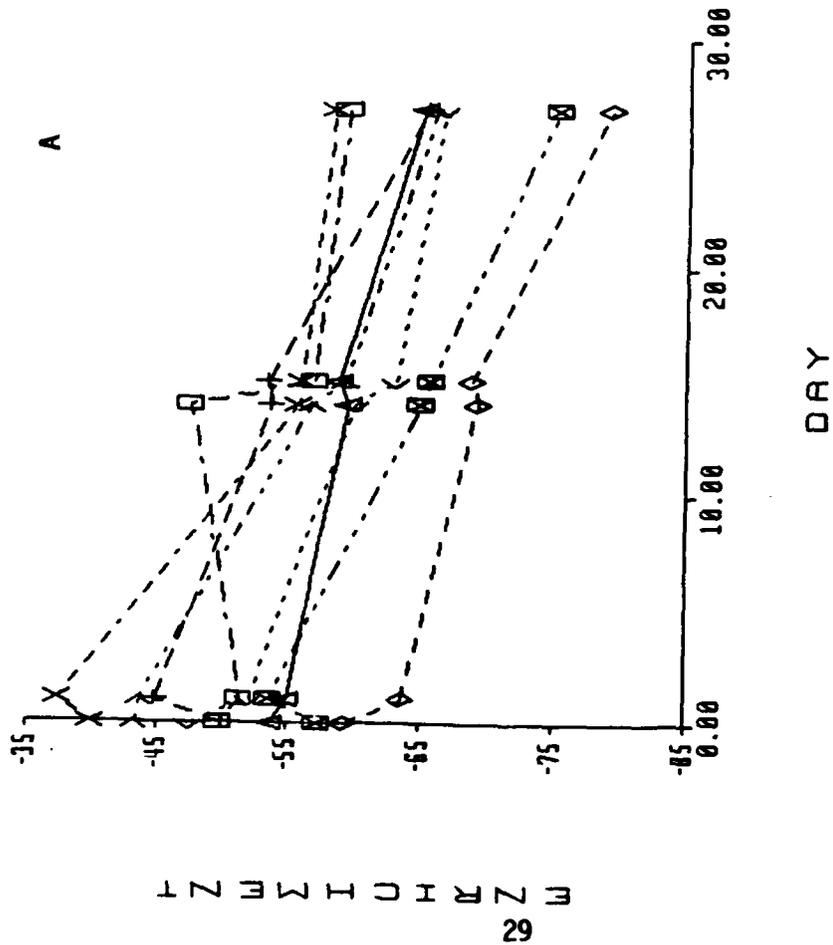
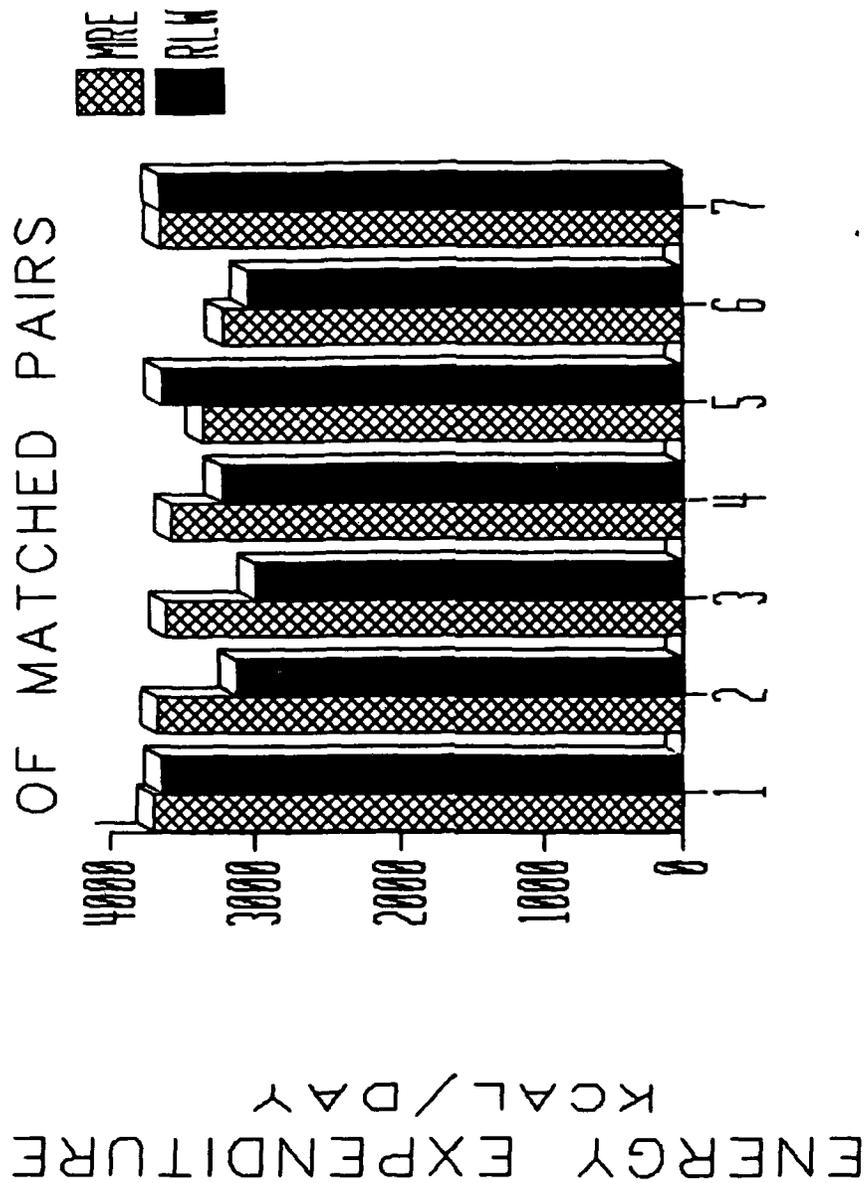


FIGURE 2. Changes in deuterium and ¹⁸O natural abundance during the field exercise. Abundance is expressed as the per mil difference versus Standard Mean Ocean Water.

ENERGY EXPENDITURE OF MATCHED PAIRS



PAIR NUMBER

FIGURE 3. Comparison of energy expenditures of matched pairs of soldiers receiving the MRE or RML ration.

Preformed Average Water Intake

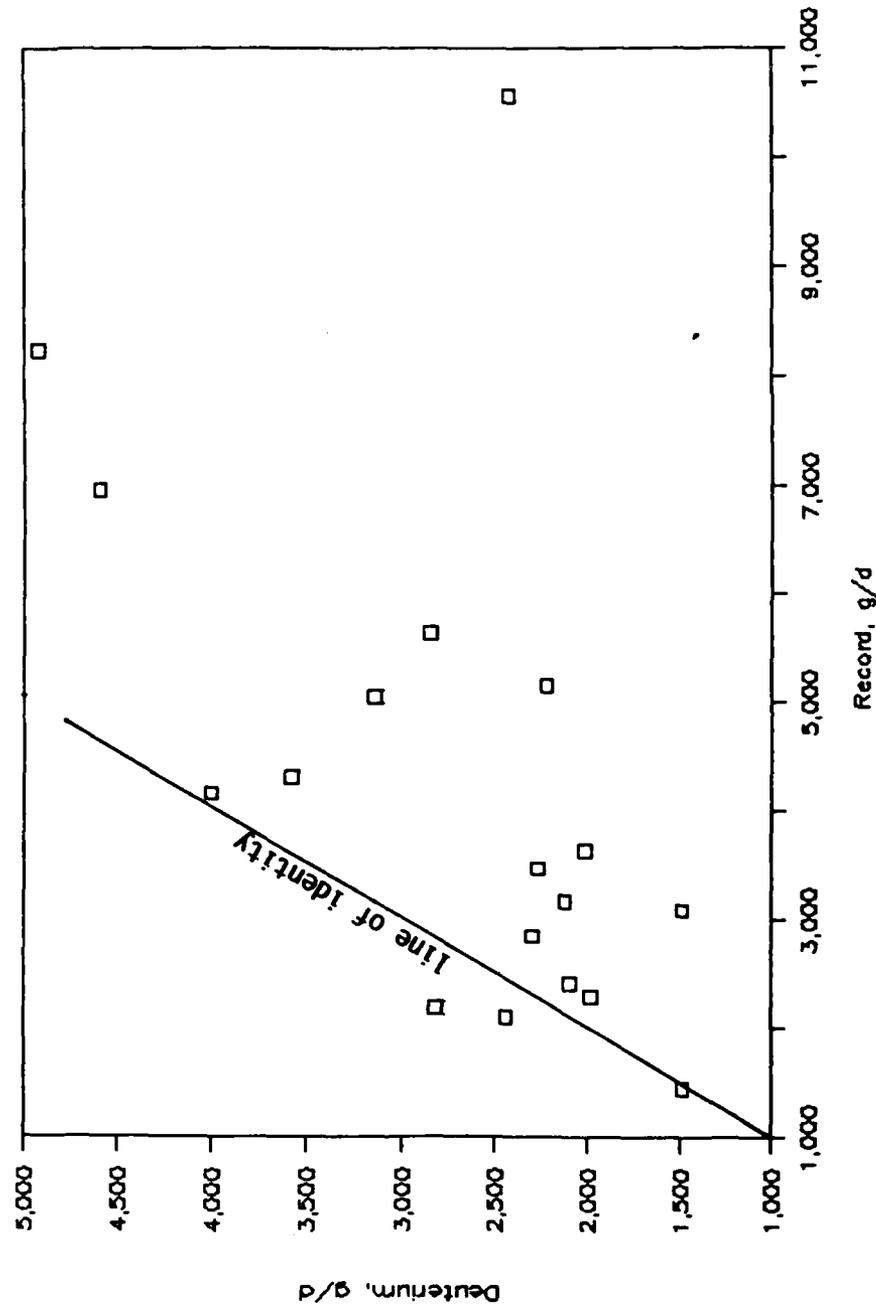


Figure 4. Comparison of preformed water intake by self-report and deuterium.

Water Intake vs. Change in Hematocrit

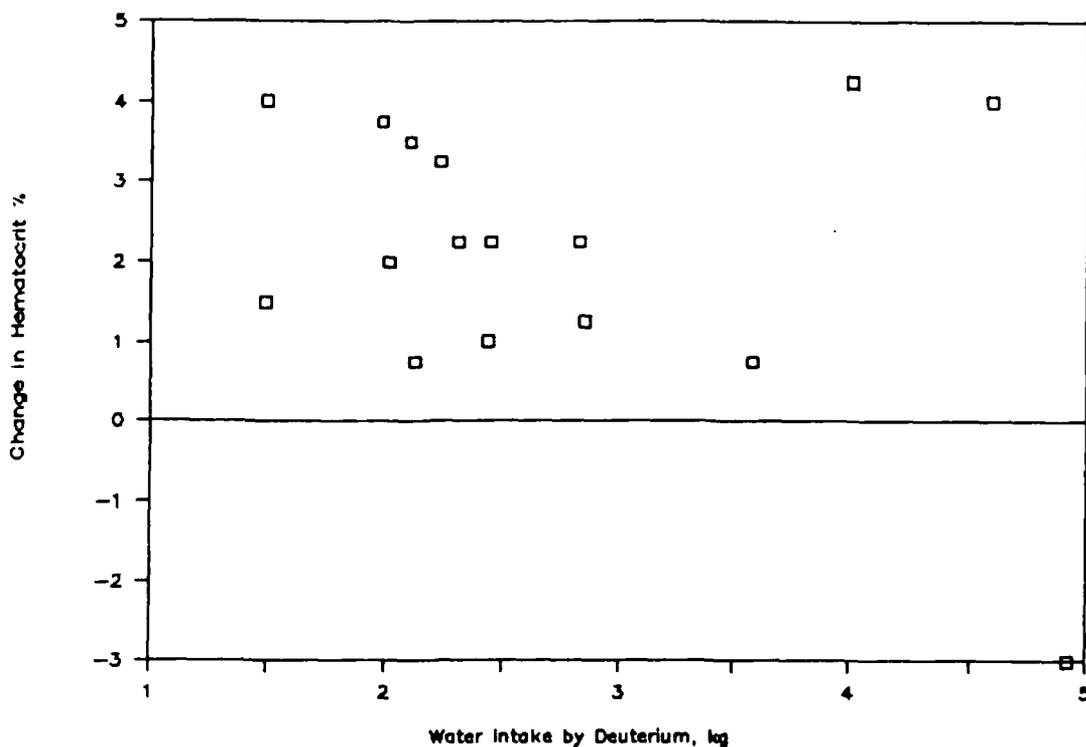


FIGURE 5. Relationship between preformed water intake by deuterium with change in hematocrit during the first two weeks in the field.

Water Intake vs. Change in Hematocrit

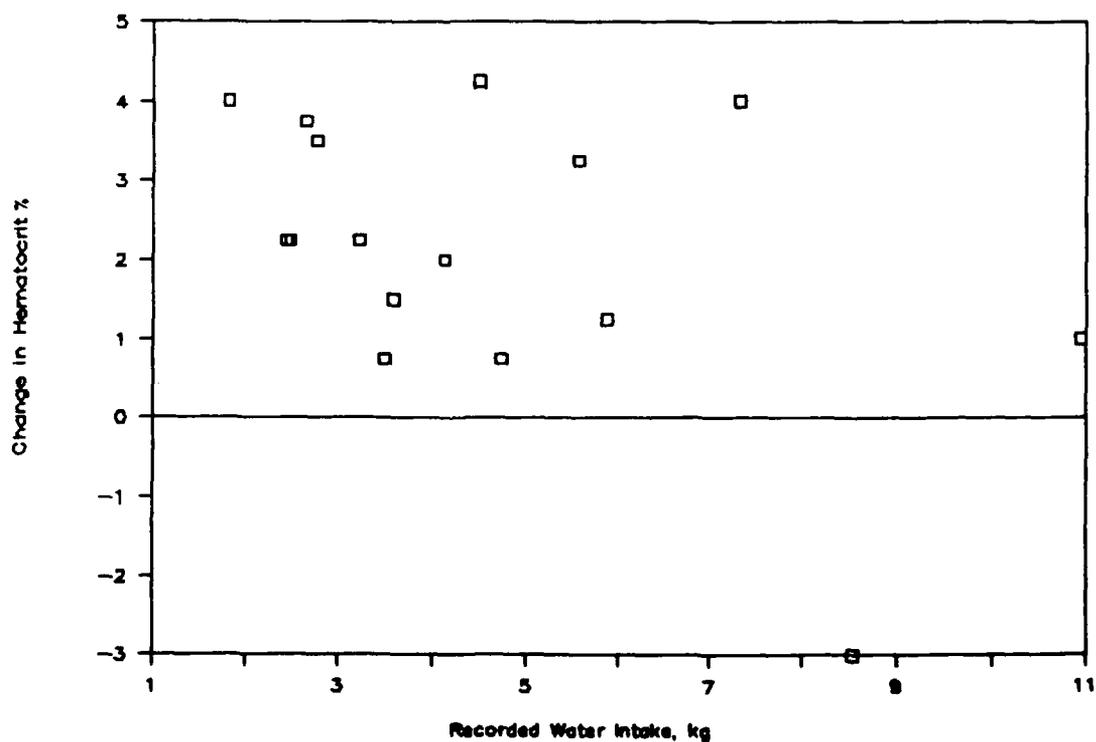


FIGURE 6. Relationship between preformed water intake from self-reported record and change in hematocrit during the first two weeks in the field.

Water Intake vs Specific Gravity Change

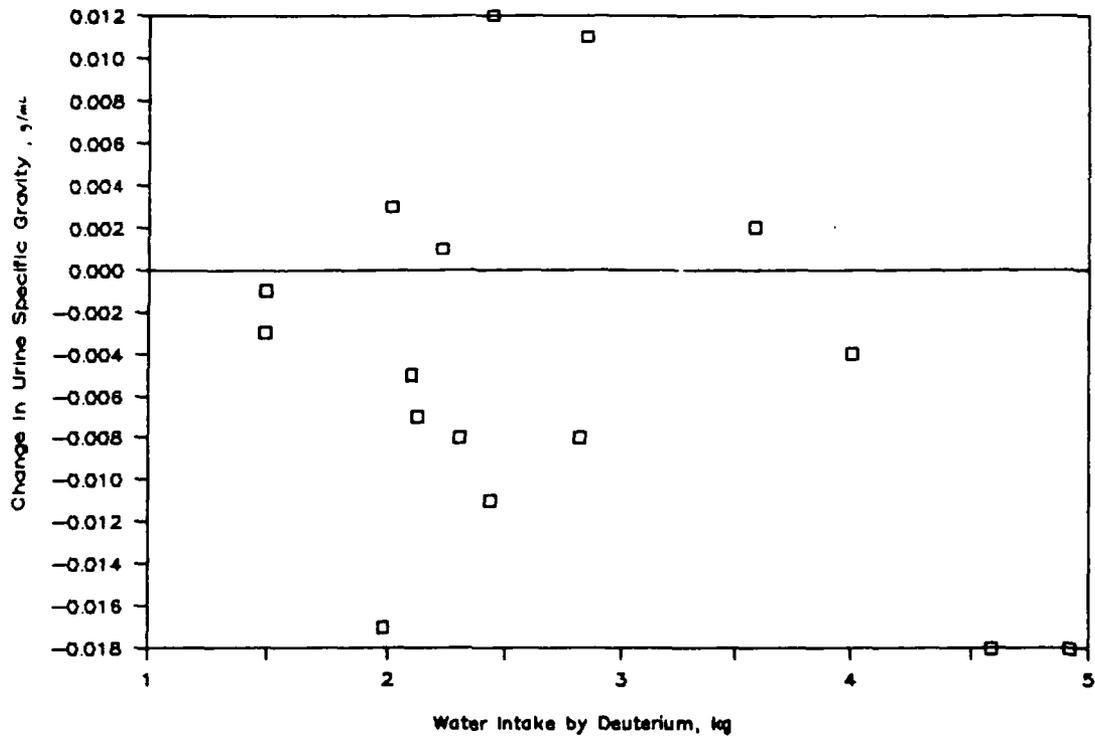


FIGURE 7. Relationship between preformed water intake by deuterium with change in urine specific gravity during the first two weeks in the field.

Water Intake vs Specific Gravity Change

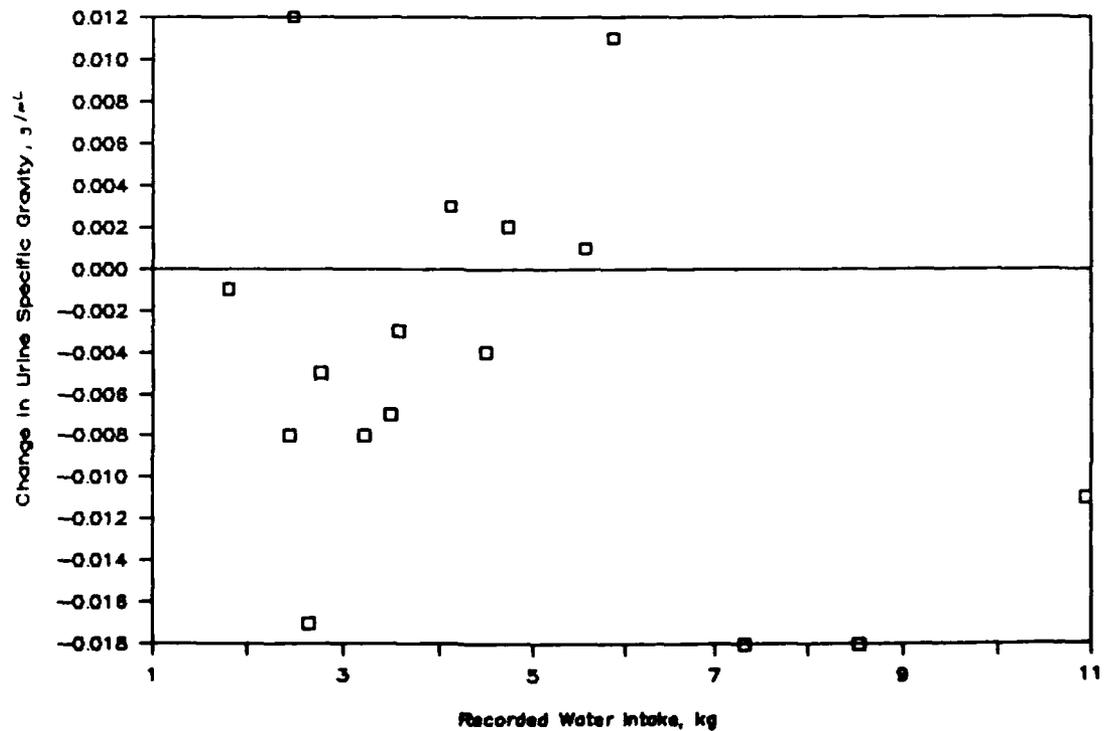


FIGURE 8. Relationship between preformed water intake by self-report and change in urine specific gravity during the first two weeks in the field.

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ANCILLARY INFORMATION

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Personnel receiving pay:
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Graduate degrees resulting:
none