NASA CR-
147498

DEPARTMENT OF PHYSIOLOGY

LOVELACE FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH

ALBUQUERQUE, NEW MEXICO 87108

(NASA-CR-147498) SPECIALIZED PHYSIOLOGICAL STUDIES IN SUPPORT OF MANNED SPACE FLIGHT Annual Research Report, 1 Jan. - 31 Dec. 1975 (Lovelace Foundation for Medical Education and) 87 p HC $5.00 CSCL 06P G3/52

N76-19786

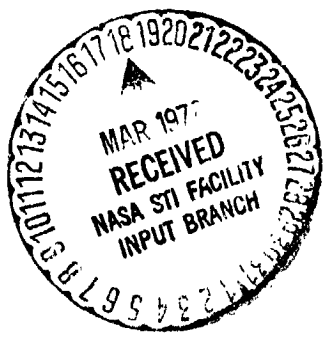
Unclas
18527

RESEARCH REPORT ON:

SPECIALIZED PHYSIOLOGICAL STUDIES IN SUPPORT OF MANNED SPACE FLIGHT

TO: THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

LYNDON B. JOHNSON SPACE CENTER, HOUSTON, TEXAS



CONTRACT: NAS 9-14472

FEBRUARY 1976

Annual Research Report

To: NASA L.B. Johnson Space Center
Biomedical Research Office
Houston, Texas 77058

Contract: NAS 9-14472 Monitor: Dr. John Rummel

Report Period: January 1, 1975 - December 31, 1975

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PART I

A STUDY OF FACTORS AFFECTING TOLERANCE TO GRAVITATIONAL STRESS SIMULATED BY LOWER BODY NEGATIVE PRESSURE

ABSTRACT

Ten subjects, of whom 5 were competitive long distance runners (R) and the other 5 untrained men, were tested for tolerance to lower body negative pressure (LBNP) before (A-I) and after (A-II) acute dehydration by working intermittently for two hours at 50°Cdb, 26°Cwb at 30% $\dot{V}_{O2max}$, without fluid replacement, which led to a water loss of 2.5% body weight and increased rectal temperature by 1°C. This was followed by 8 days of acclimation by working continuously for 100 min at 30% $\dot{V}_{O2max}$ in the hot dry environment with fluid replacement. On the second day thereafter the LBNP tests were repeated before (B-I) and after (B-II) acute dehydration. The LBNP test consisted of 5 min long consecutive stages at -20, -30, -40, -50 and -60 Torr. Tests were terminated when syncope was imminent or the full sequence was completed. Tolerance was expressed in terms of cumulative stress in Torr x min. Measurements of body mass, density, fat fraction and total body water (TBW) were made before and after acclimation. Blood volume and its constituents were determined before and after each of the four LBNP tests. During LBNP, heart rate, blood pressure and changes in calf and forearm volume were recorded every minute. Results showed: Acute dehydration caused a significant loss in average LBNP tolerance on all subjects. Acclimation to heat did not significantly affect LBNP tolerance in hydrated subjects (B-I vs A-I) but significantly improved it on dehydrated subjects (B-II vs A-II). R's had lower LBNP tolerance than NR's under all test conditions. The difference between the two groups was highly significant in tests A-I and A-II. After heat acclimation the difference between the two groups were smaller and not significant. Heart rates were consistently lower before and during the LBNP test in the R's but were higher after dehydration in all subjects due to a higher T_{re}. There was an average loss in plasma volume (PV) of 9% during the initial LBNP test (A-I) an additional loss of 4% during dehydration and another 1% during the following LBNP tests (A-II). After acclimation 11% PV was lost before (B-I), none during dehydration and 4% during the last LBNP test (B-II). Striking was the relatively small loss in PV during dehydration before acclimation and its absence after it. There was a highly significant correlation between the amount of PV lost and the LBNP tolerated in each

test. Total circulating plasma protein remained unchanged during both series. Leg volume (LV) increased and arm volume (AV) decreased progressively with LBNP, but these changes were significantly less after dehydration. There was a highly significant correlation between loss in PV and leg swelling. Limb compliance (ΔLeg vol%/ΔTorr x min) was increased after acute dehydration before acclimation, but this trend was reversed after acclimation. The R's had significantly greater limb compliance in all tests than the NR's. It was concluded that acute dehydration with hyperthermia adversely affects LBNP tolerance because central blood volume is already depleted for thermal regulation and smaller shifts of intravascular and extravasated fluids become critical. Acclimation to heat improves the ability to conserve plasma volume in the heat, but not under LBNP. Acclimation also reduces limb compliance and fluid displacement. The main reason for the greater susceptibility of the runners to LBNP found in this study was their much greater propensity to accommodate fluid in their lower extremities than the other subjects.

INTRODUCTION

One of the most consistent alterations in physiological functions observed in manned space flight has been a reduced orthostatic tolerance on return to earth's gravitational field. Fortunately, this phenomenon is transient and is usually overcome within 48 hours post flight. The adverse effects of weightlessness on the responses to gravitational stress have also been documented in space by simulating orthostasis with lower body negative pressure (LBNP) at regular intervals in the SKYLAB program, where the device was used to counteract cardiovascular deconditioning and changes in quantity and distribution of intravascular and extravascular fluids.

Despite the large number of investigations of the physiological mechanisms that come into play in changes of posture, under acceleration on the centrifuge and under LBNP, a number of questions remain to be answered concerning the effects of superimposed environmental stress such as excessive heat and dehydration and the efficacy of acclimation to heat in alleviating them. Conflicting evidence has been forthcoming on the latter point from studies showing an improvement in orthostatic tolerance after heat acclimation (25) and others (8) who claim that it constitutes a liability. Other important questions are whether or not physical training provides any protection under gravitational stress and whether physical condition and body composition should be essential criteria in the selection of candidates for the Space Shuttle.

The purpose of the studies reported here was threefold: 1. To study the effects of acute dehydration leading to loss of total body water as well as plasma volume and hyperthermia on tolerance to LBNP and the cardiovascular response and shifts in body fluid associated with it. 2. To determine whether acclimation by working in the heat for 9 consecutive days affects LBNP tolerance before and after acute dehydration. 3. To explore possible differences in LBNP tolerance between well-trained and untrained men and the physiological factors involved.

METHODS AND PROCEDURES

LBNP test.

The LBNP box was constructed out of 3/4" plywood 48" long, 26" wide and 16" high with a semicircular opening at one end partially covered by a sliding baffle adjustable to each subject's circumference at the iliac crests and padded with bubble plastic. The entire box was wrapped in a large sheet of clear mylar which was long enough to fit around the subject's waist, where it was secured tightly with a broad velcro belt. An adjustable, well-padded saddle attached to the floor of the box prevented the subject from bracing his feet against the bottom of the box when under negative pressure. Several ports led through the walls of the box and the plastic cover for attaching the pump (domestic vacuum cleaner), the ventilation line, a manometer and a thermometer. With this simple and inexpensive device negative pressures down to -100 Torr could be attained in a few seconds and held at any desired level with a variable leak in the venting line in the form of a large aluminum stopcock. Down to -60 Torr, the lowest pressure employed in this study, the pump still had sufficient power to tolerate considerable leakage through the valve and around the seal, thus providing enough ventilation through the box to prevent an undesirable increase in temperature during the test. The room temperature was maintained at 29°C to minimize thermoregulatory responses on the part of the subjects clad in trunks only.

The LBNP test was conducted in consecutive steps of 5 minutes duration at -20, -30, -40, -50 and -60 Torr. The test was terminated whenever syncope appeared imminent either from objective signs (pulse, blood pressure, aspect) and/or complaints of dizziness or nausea by the subject. Otherwise the sequence was continued up to 5 minutes at -60 Torr. Ambient pressure was re-established immediately by fully opening the valve and shutting off the pump, whereupon all subjects recovered rapidly without fainting. It might have been more appropriate from the point of view of statistics to continue the test beyond -60 Torr in those subjects who tolerated this level until they also approached syncope. However, already at -60 Torr the suction caused considerable discomfort at the crotch and the seal around the abdomen and these painful sensations would have adversely affected the test results. As it turned out only one of the 10 subjects

completed the entire test profile (Fig. 1) down to -60 Torr in all four LBNP tests so that the decision to terminate at this level appeared justified, although the actual LBNP tolerance may have been underestimated in a few instances. In order to specify individual LBNP tolerance in this test profile the duration of the test as well as the levels of negative pressure sustained were taken into account by adding up the products of negative pressure x time for each step to obtain cumulative stress in terms of Torr x minutes, which is a curvilinear function of time (Fig. 1). This parameter appeared more appropriate to correlate concomitant physiological changes with, such as plasma volume, limb volume etc., than time or negative pressure alone. Each subject experienced four LBNP tests in the course of the study, the first (A-I) before working in the heat, the second (A-II) immediately thereafter. The third and fourth tests were performed after 9 days of acclimation, again before (B-I) and after (B-II) acute dehydration.

Monitoring.

Heart rate and blood pressure were taken every minute for 5 minutes before and throughout the duration of LBNP with a cuff placed on the right arm. Changes in circumference of the left calf and forearm were recorded continuously by means of a mercury-in-silastic strain gauge (Model 270 Plethysmograph, Parks Electronic Laboratories) on an oscillograph recorder (Honeywell, Model 1508). The calibration and attachment of the gauges as well as the subsequent calculation of limb volume change closely followed the procedure described by Holling et al. (10). A deflection of approximately 45mm for 1% change in limb circumferences was obtained with this arrangement. After attaching the gauges and entering the box the subject's left knee was supported in slight flexion on a foam rubber cushion and the left hand also rested on a cushion keeping the forearm at an angle of 45^{o} approximately level with the heart.

Blood volume and constituents.

Total hemoglobin and blood volume were determined with a carbon monoxide rebreathing method (18) a few minutes before beginning the first LBNP test after the subject had rested supine in the box for 15 min. An infra-red method (19) was used to measure COHb saturation and total hemoglobin was calculated as follows:

$$(1) \qquad THb = \frac{V_{CO} \times .985 \times 100}{1.34 \times \Delta S_{CO}}$$

Where THb = total Hb. V_{CO} = volume of CO in ml (STPD) introduced into the rebreathing system, 0.985 = average fraction of CO taken up by the blood by the end of the 10th minute of rebreathing. 1.34 = CO capacity of 1 gram Hb.

Blood volume (BV) was then derived from THb and hemoglobin concentration (Hb) as:

$$(2) \qquad BV = \frac{THb}{Hb \times 10}$$

Plasma volume (PV) from BV and hematocrit (Hct) as:

$$(3) \qquad PV = \frac{BV \times (100 - Hct)}{100}$$

Red cell volume (RCV) as:

$$(4) \qquad RCV = BV - PV$$

Since one can assume that THb does not change in the course of LBNP and during dehydration in the heat, PV, BV and RCV were estimated immediately following the first LBNP test and also before and after the second test on the basis of subsequently measured Hb_t and Hct_t as compared to the control values PV_c, Hb_c and Hct_c. Thus:

$$(5) \qquad PV_t = PV_c \frac{Hb_c \times (100 - Hct_t)}{(100 - Hct_c) \times Hb_t} \qquad \text{and}$$

$$(6) \qquad BV_t = \frac{PV_t \times 100}{(100 - Hct_t)} \qquad \text{with}$$

$$(7) \qquad RCV_t = BV_t - PV_t$$

The ratio Hb/Hct gave the Hb content of the red cells. Plasma protein concentration (PP) was determined with an autoanalyzer (SMA-12, Technicon Corp) on samples taken before and after the heat exposure. Multiplied by the PV this gave the total plasma protein:

$$(8) \qquad TPP = PP \times PV$$

Dehydration.

Following the initial LBNP test (A-I) the limb plethysmograph leads were disconnected leaving the gauges in place and the subjects proceeded

to the adjacent hot room (50°Cdb, 26°Cwb) after voiding urine and inserting a rectal thermocouple. Body weight was taken and they commenced two hours of intermittent work on a bicycle ergometer set at a work load corresponding to 30% of the individuals $\dot{V}_{O2max}$ in the following sequence:

Work (min)	Rest (min)
0 - 20	21 - 30
31 - 50	51 - 60
61 - 80	81 - 90
91 - 100	101 - 110
111 - 120	

Heart rate was noted at regular intervals during the heat exposure including the last minute of each work and rest period (Fig 2, 3). Rectal temperature (T_{re}) was recorded continuously on a Honeywell multipoint recorder and checked periodically with a clinical thermometer (Fig 4). Body weight was taken after each work cycle to estimate evaporative fluid loss. No fluid replacement was permitted.

On completing the dehydration procedure and the final weighing the subjects were transferred back into the LBNP box where the limb plethysmographs were reconnected and electrically balanced to compensate for undetermined changes in the base line after exercise in the heat. In less than 20 minutes after leaving the hot room the second LBNP test (A-II) was started following exactly the same protocol as in A-I, again preceeded and followed by blood samples for Hb, Hct and plasma protein. The rectal thermocouple remained in place and T_{re} was recorded until the end of the test.

The subjects reported for these tests at 8 am after their usual breakfast. They had been requested to drink 8-10 oz of water or preferred beverage before retiring the night before to ensure adequate hydration. Total duration of the procedure was approximately 4 1/2 hours.

Ancillary measurements.

Within a week before the main test series total lung capacity was determined by the nitrogen dilution method, a value necessary for the subsequent estimation of fat free weight (FFW) by hydrostatic weighing. Usually on the same day maximal aerobic power ($\dot{V}_{O2max}$) was obtained using a bicycle ergometer test in which the brake load is increased by

75 mkg/min from a base-line of 300mkg/min for 3 minutes until the subject is unable to maintain the pedalling rhythm of 50cpm (see Report Dec. 1971, Fig A-1, Contract NAS 9-7009). The results of this test were used to set the work load for each subject in exercise in the heat and also as a measure of his physical condition.

On the day immediately preceding the LBNP tests the subjects reported at 8 am without breakfast for the determination of fat free weight by hydrostatic weighing (14) and the estimation of total body water (TBW) by an alcohol dilution method described elsewhere in the report (p. 75).

Acclimation to heat.

The day following the first LBNP experiments (A-I and A-II) each subject began a period of heat acclimation consisting of 100 min continuous work per day in the heat for eight consecutive days. The work load and thermal conditions were the same as used in the dehydration exposure between the LBNP tests. Fluid losses were followed by weighing at 15 min intervals and replaced with a 0.1% NaCl solution kept at 37°C. Heart rate was counted every 5 minutes and rectal temperature measured by clinical thermometer at the time of each weighing.

After completing the 8 day heat acclimation making a total of 9 days exercise in the heat including the dehydration exposure, the subjects returned on the next day to repeat the determinations of FFW and TBW as described above. No work in the heat was performed on this day. On the following day the series was completed by repeating the LBNP tests before and after acute dehydration (B-I and B-II) according to exactly the same protocol as in A-I and A-II.

A typical schedule for one of the subjects was as follows:

Day	Time	Item
-2	8:00-10:00	Lung volumes, $\dot{V}_{O2max}$ test, LBNP try-out.
-1	8:00-12:00	Hydrostatic weighing, Total body water
1	8:45- 9:14	LBNP test A-I
	9:35-11:34	Work in hot room
	12:00-12:30	LBNP test A-II
2-9	15:00-16:40	Work in hot room
10	8:00-12:00	Same as day -1
11	8:45-12:30	Same as day 1, LBNP test B-I and B-II

Subjects.

In view of time involved with each subject (12 1/2 days) it was originally planned to engage not more than 6 subjects in this study. However, during preliminary recruiting among members of the staff and personal acquaintances we soon found 5 individuals who expressed a lively interest in participating. But all of them happened to be competitive runners who ran 3-5 miles daily and participated regularly in regional long-distance track events. At this point we became concerned that choosing the majority of our subjects in such excellent physical condition and training might bias the results of the experiments in one way or another; so we decided to expand the group of subjects to include an equal number of men who had not recently engaged regularly in any strenuous physical activities. We finally managed to recruit the additional number of subjects, and this was fortunate because the choice of 5 non-runners (NR) and 5 runners (R) added another unforeseen element of interest to the study in that the two subgroups showed some distinctly different responses not only to exercise in the heat but also to LBNP. As seen in Table 1 the two groups were about equal in stature but the NR's were on the average 15.7kg heavier with 13.5% more fat content than the R's. On the other hand the maximal aerobic power of the R's was 25% greater than the NR's if one relates $\dot{V}_{O2max}$ to fat free weight. All subjects were thoroughly acquainted with the purpose and procedures involved in the study including the discomforts and possible hazards to obtain their informed consent. Each of them was familiarized with the LBNP procedure in a trial run prior to the actual tests.

RESULTS

Before dealing with the main objective of the study, namely the responses to LBNP under the effects of acute dehydration and how these are modified by acclimation to heat, it may be in order to describe the nature and degree of these modifying factors and how they affected the subjects prior to LBNP. Table 1 shows that working in the heat for 2 hours on 9 consecutive days did not alter gross body weight on the average for all subjects. However the NR's lost 0.6kg due to a small reduction in fat while the R's gained 0.5kg mainly in fat free weight. There was a small increase in total body water (TBW) (Table 2) after heat acclimation, slightly more in the NR's (0.9 liters) than in the R's (0.5 liters) and since FFW changed very little in either group the water content of FFW increased from 70.9% to 71.9%.

The evaporative water loss during acute dehydration was 2.0 liters before and 1.9 liters after the acclimation series, i.e. 4.3% and 4.1% of TBW respectively. Figures 2 and 3 illustrate the course of heart rate (HR) during the acclimation runs before and after (B) acclimation. It is noted that the NR's ran approximately 25 beats higher than the R's before acclimation reaching a maximum of 165 and 138 respectively during the last work period. For the same exposure after acclimation a similar pattern is apparent. However the difference between NR's and R's is less and at the end the NR's had the same HR as the R's before acclimation (138) while the R's were now 19 beats lower. Rectal temperature (T_{re}) was consistently higher in NR's than in R's throughout the entire heat exposure (Fig 4) although it rose progressively with each work bout in both groups both before (A) and after (B) acclimation. The difference between A and B was that the increase in T_{re} was significantly less after acclimation when taking the pooled data on all subjects. Table 3 contains the T_{re} values as they were recorded during the LBNP tests under the four experimental conditions.

Of the four LBNP tests performed before acclimation tolerance was consistently lower after working in the heat, with the exception of subject No.4 who completed both tests (1000 Torr min) without adverse effects (Table 4 and 4a, Fig 5). The mean tolerance of all ten subjects was 720 Torr x min before and 495 Torr x min after dehydration, a difference of 31% ($.001 < p < .01$). In the first test after acclimation (B-I) tolerance to LBNP was only slightly (9%) better than before with normal hydration. However the

loss of tolerance after working in the heat (B-II vs B-I) was only 18%, much less than before acclimation to the heat. Comparing the two tests on dehydrated subjects (A-II and B-II) one finds that the mean tolerance is 30% better ($0.02 < p < .01$) after having acclimated to the heat.

In all four LBNP tests the runners (R) tolerated LBNP less well than the others (Fig 6). This difference was most striking in A-I and A-II before acclimation, where the difference of the means was 42% before and 54% after dehydration, both statistically significant. After acclimation to the heat the differences between the two groups were not as great being only 28% in the euhydrated and 26% in the dehydrated state. This was due almost entirely to the fact that the R's improved their performance under LBNP by 25% in B-I as compared to A-I and by 76% comparing B-II with A-II. However these differences were of borderline statistical significance due to large inter-individual variations.

The characteristic tachycardia associated with orthostasis and under LBNP was observed consistently in this study and appeared to be linearly related to the cumulative stress. In order to compare the different HR responses under the four experimental conditions and between the R and NR groups, the HRs during the final minute of each test are tabulated beside the corresponding control values being the average of 5 min before starting LBNP (Table 5).

Control HR after dehydration associated with hyperthermia was on the average 18 bpm higher than before (A-II vs A-I). However the difference was significantly ($p < .01$) less with only 11 bpm after acclimation to the heat. During LBNP, on the other hand, HR increased by 52% before and 68% after dehydration when the subjects were acclimated. As might be expected, the R's had a slower resting HR than the NR's before all four LBNP tests. One runner had a heart rate as low as 38 bpm after acclimation. The response in HR to LBNP was slightly less in the R's than that of the others in the first test (A-I) and somewhat higher after dehydration (A-II). After acclimation, however, the increment in HR with LBNP was greater in the R's than the NR's both before and after dehydration (B-I and B-II).

Pulse pressure (Table 6) dropped as usual during all LBNP tests with the exception of one individual (No. 8) who was a runner and incidentally had the lowest resting heart rates. On the average the reduction in PP was

25% greater after dehydration before acclimation and 10% after it, but these differences were not statistically significant.

Blood volume calculated from total hemoglobin and Hb concentration (eq. 1) was determined on each subject once before (A) and again after acclimation (B) just before starting the LBNP tests. The results of the base-line measurements of blood volume (BV) and all its constituents are contained in Tables 7 - 14 in the first column under A and B. The means on all subjects showed an increase of 3.7% in blood volume (BV), 4.8% in plasma volume (PV) and 2% in red cell volume (RCV) after 9 days acclimation to heat. The increments were slightly greater in the R's than the NR's. Even before acclimation the R's had more BV (10%) than the others ($p < .05$) to begin with and this was attributable to a larger PV (12%) as well as RCV (9%).

Marked changes in BV were observed during the first LBNP test, during dehydration and also during the second LBNP test. These were determined using the base-line THb and subsequently measured Hb and Hct (eq. 5, 6 and 7). Since it is reasonable to assume that these acute changes were primarily due to shifts of fluid into or out of the vascular system, the fluctuations in PV are most important. These are presented on Table 11 and Figures 7 and 8. It can be seen that during the very first LBNP test all subjects lost an average 283ml (8.7%) PV. During the following exercise period in the heat they lost an additional 132ml (4%) and in the second LBNP test only 34ml (1%) with a total loss of 449ml (13.8%). When the same sequence was repeated after acclimation the loss in PV after the first LBNP exposure was even greater than before, namely 373ml (11%). This time, however, there was no significant loss during dehydration in contrast to the unacclimatized tests, in fact there was a small recovery of PV (1%). In the following LBNP test there was a substantial drop in PV again of 149ml (4.4%) making a total of 495ml (14.5%) below the mean of the controls. Fig 8 shows that the fluctuations in PV were similar in the NR's and the R's but differed considerably in magnitude. The R's lost only 7% PV during the first LBNP test, while the NR's lost 12.5%. On the other hand the R's lost additional 5.3% while in the hot room whereas the NR's had only 0.8% less. During the following LBNP test the R's gained (0.7%) rather than lost PV so that their total deficit at the end was 11.5%. At the same time the NR's PV was further reduced for a total of 16.3%. After acclimation the differences

between the two groups were not as great. Nevertheless, while the major loss in PV was during the first LBNP exposure of this series (NR's: 12.2%, R's: 9.9%), the NR's recovered 1.8% during the heat exposure while the R's showed no change. Both groups lost more PV in the final LBNP run (NR's: 5.1%, R's: 3.6%). The R's did not loose as much PV as the NR's in any phase of the tests either before or after acclimation.

The removal of fluid from the intravascular compartment was accompanied by an increase in plasma protein concentration as shown in Table 12. But this was entirely due to hemoconcentration and not to any notable shifts of protein into or out of the blood, because the total amount of protein present was remarkably constant throughout all of the tests (Table 12). Between the first and the last samples of the series before acclimation there was a difference of +7ml and in those after acclimation of -1ml. These differences were not statistically significant.

It was further noted that the red cell volume (RCV, Table 13) tended to become smaller during the cumulative LBNP and heat exposures. This would suggest that some fluid was being extracted from the cells as well as from the plasma, which would lead to a higher concentration of Hb in the red cells. This was indeed the case as can be seen from Table 14 in the differences between the first and last columns both in series A and B where the differences in PV were greatest. The differences between the means on all 10 subjects were statistically significant. On closer scrutiny of this Table it becomes apparent that the major increase in Hb/Hct occurred between the samples after LBNP-I and the ones taken before LBNP-II i.e. the period where the subjects were being dehydrated by exercise in the heat.

Leg volume tended to increase and arm volumes to shrink with progressive LBNP in all tests (Table 15-18). At first glance there appeared to be a direct or inverse relationship respectively to the cumulative stress in terms of total Torr x min. For instance in test A-I the maximum increase in leg volume (Final column, Table 15) was 3.95% on the average for all subjects and the mean exposure was 720 Torr x min (Table 4). After dehydration the same subjects tolerated only 495 Torr x min and their maximum change in leg volume was 2.77%. In both cases this corresponds to an increase of 0.55% per 100 Torr x min. Obviously this is an oversimplification. When one looks at the corresponding figures for the R's alone in the same test (A-I)

one finds that their increase in leg volume was 3.95% and they only reach a mean of 527 Torr x min which amounts to 0.75% per 100 Torr x min. The Tables 15 - 18 also show that limb volumes continue to change not only from one LBNP step to another but also during the time when the pressure was constant. This serves to emphasize the importance of the time factor in assessing the overall impact of LBNP. The decrease in arm volume (Tables 17 and 18) generally followed the same pattern as the increase in leg volume but was not as great. On the average for all subjects the final readings before terminating the test showed a decrement of 1.55% in A-I and 1.27% in A-II after the unacclimated dehydration and 1.97% (B-I) and 1.54 (B-II) after acclimation. Fig 9 combines the simultaneous changes in leg and arm volume representing mean values at the end of the tests for all 10 subjects and for the NR's and R's separately. As mentioned above, there was less change in leg volume in all subjects after dehydration than before and the difference was statistically highly significant ($p < .001$). There was no significant difference between the euhydrated subjects before and after acclimation (B-I and A-I). Comparing NR's with R's the latter increased their leg volume slightly more than the others in the very first LBNP test (A-I) but tolerated much less LBNP. After the first dehydration leg volume increased considerably less in the R's than in the NR's but after acclimation the effect of acute dehydration on changes in leg volume was the same in both groups (B-II). The reduction in arm volume was approximately half as great as the increase in leg volume on the average in all tests. After dehydration arm volumes did not decrease as much as before regardless of the state of acclimation (pooled data: $p < .05$) and arm volumes were affected slightly more after acclimation both before and after dehydration (B-I vs A-I and B-II vs A-II).

DISCUSSION

The experimental design of this investigation encompassed several different factors that might affect tolerance to gravitational stress in one way or the other. One of these was a combination of environmental and physical stress by exercising in a hot, dry environment which not only caused considerable loss of body water but also increased body temperature (+1°C) so that the subjects entered the following LBNP tests both dehydrated and in a state of hyperthermia. The other factor superimposed on the preceding was acclimation of the subjects by exercising in the heat daily for 9 consecutive days. It is readily apparent that this regimen had an adequate acclimating effect from the fact that the subsequent acute heat exposures were tolerated much better, as attested by the significantly lower T_{re} and HR (Fig 2, 3 and 4) as well as less discomfort experienced by the subjects. Finally, an additional variable, introduced by the choice of the subjects, turned out to be of unexpected significance. All of the 5 R's were long-distance runners, a type of athlete whose cardiovascular system is highly adapted for sustaining maximal blood supply to the working muscles, specifically of the lower extremities. The control group (NR's) had not engaged in any unnecessary physical activities for several years but were of normal physical fitness for their age as judged by their maximal aerobic capacity according to reference standards in this laboratory. However, on the average they were fatter than the R's and one of them was particularly obese (subject #3). Besides their superior physical fitness the R's had another advantage over the NR's at least in the dehydration bouts in the hot room. Since the experiments were carried out in the late summer, the R's had been practicing regularly outdoors at temperatures around 30°C and had probably acquired a certain amount of natural acclimation before they entered the experimental series. It is therefore not surprising that their HR and T_{re} stayed well below those of the NR's during the heat exposure before the acclimation runs. Even after completing 9 days of acclimation, where all subjects improved their heat tolerance, the R's had lower HR and T_{re} throughout (Fig 2, 3 and 4) and their evaporative water loss was greater. Contributing to the latter was the fact that although all subjects exercised at 30% of their individual $\dot{V}_{O2max}$, the R's were working at an average workload of 405 mkg/min and the NR's at only 290 mkg/min.

The early observations by Eichna et al. (6) that physical exertion combined with heat stress increase the incidence of postural hypotension have been confirmed many times using the tilt table procedure (4, 13, 23) and Greenleaf et al. have recently (8) demonstrated decreased orthostatic tolerance after combined dehydration plus exercise in the heat. It was therefore not unexpected that these experiments using the LBNP procedure gave similar results that could be expressed in more quantitative terms in this study using a score in cumulative Torr-min described above.

After heat exposure the average LBNP tolerance of all subjects was 31% less than before and the difference was statistically highly significant. A striking difference was found between the R's and NR's both before (A-I) and after dehydration in the heat (A-II). On the average the R's tolerated 42% less LBNP than the NR's in the control test and 54% after the first heat exposure, both differences being significant. Klein et al. (12) compared 12 physically untrained students with 12 highly trained athletes, who had a significantly higher $\dot{V}_{O2max}$, as to their tolerance to tilting as well as to $+G_z$ acceleration on the centrifuge and found no difference. Shvartz and Meyerstein (24) noticed in a tilt-table study that those who fainted tended to have a lower $\dot{V}_{O2max}$ and found a negative correlation between $\dot{V}_{O2max}$ and orthostatic heart rate response. They concluded, however, that tilt tolerance has only a minor dependence on aerobic capacity. In another study on the relationship between physical endurance activities including running, and orthostatic tolerance the same author (22) commented that the greater development of leg muscles does not cause any substantial improvement in orthostatic tolerance. The results of the present study strongly suggest that such a development may well be a handicap rather than an asset under gravitational stress.

The acclimation regimen had no significant effect on LBNP tolerance before the subjects were exposed to heat (B-I), although the average (n = 10) was slightly better than in A-I. However, the loss in LBNP tolerance due to dehydration and hyperthermia (B-II) was 18% although not significant. Compared with the dehydrated state before acclimation (A-II) LBNP tolerance was 30% better and this was statistically significant. The R's appeared to benefit more from the acclimation than the rest. They gained 25% comparing the euhydrated states (B-I vs A-I) and 76% in the dehydrated states (B-II vs A-II) before and after. As a result the differences in tolerance between R's and NR's became

smaller and were no longer statistically significant after acclimation. Previous investigations concerned with heat acclimation and orthostatic tolerance have led to conflicting results. While Shvartz (25) reported recently that the adverse effects of heat stress on orthostasis are markedly alleviated by acclimation, Greenleaf et al. (8) concluded from their study that acclimation appears to be a liability for orthostasis under heat stress due to a less sensitive vasoconstrictive system conditioned for heat dissipation rather than postural requirements. Both these tilt-table studies differ considerably from the present one in their experimental protocol, particularly in that they permitted fluid replacement during the heat stress preceding the orthostatic tests, but Greenleaf et al. also superimposed dehydration by several days of water deprivation.

In the following discussion an attempt will be made to interpret some of the changes observed in cardiovascular response, state of hydration, body temperature, plasma volume and limb volumes in relation to LBNP tolerance under the different experimental conditions.

During the LBNP tests after dehydration T_{re} was about 1°C higher than before and this was closely reflected in the resting HR. Fig 10 shows the correlation between resting HR before LBNP and corresponding T_{re} for all 40 tests ($r = .77$, $p < .001$). However, the increase in resting HR after working in the heat was not as great after acclimation. The percent increase in HR during LBNP on the other hand was of the same magnitude before and after dehydration prior to acclimation (Table 5), but greater after the latter. Resting HR was markedly lower in the R's than the NR's in all tests, but the increment during LBNP was generally greater, particularly after acclimation. Pulse pressure (PP) dropped significantly during LBNP, on the average 38% before and 53% after dehydration almost entirely due to a reduction in systolic pressure. The changes in PP were similar in R's and NR's.

In order to reveal possible relationships between resting cardiovascular status and the response to the following LBNP a multiple classification analysis of variance (20) was performed using resting HR and PP in relation to LBNP tolerance. A strong ($p < .05$) interaction between HR and PP was found which indicated that in those tests, where resting HR and PP were low, LBNP tolerance tended to be less than in the others. This observation is contrary to results obtained on subjects studied on the tilt table after

prolonged bed rest where higher resting heart rates were associated with reduced orthostatic tolerance. Pursuing this point further the difference in T_{re} before and after dehydration was considered as a possible contributing factor, since a strong link between resting HR and T_{re} had already been established. For this purpose the correlation between the change in T_{re} and the difference in LBNP tolerance before and after dehydration as a percent of the tolerance before was calculated. This correlation (Fig 11) was statistically significant in the tests before acclimation (A) ($r = .64$, $p < .05$). After acclimation the variance was much greater so that the significance on the pooled data was equivocal. Nevertheless, the implications are that the loss in LBNP tolerance after dehydration was less, the greater the difference in T_{re}. This is difficult to reconcile with the generally accepted adverse effect of hyperthermia on tolerance of gravitational stress, as recently confirmed in studies on the centrifuge by Alan and Crossley (2). They noted a significant reduction in the grayout threshold under $+G_z$ in subjects with controlled elevation of body temperature by immersion in hot water where no dehydration was involved. A possible explanation for the incongruous finding in our study could be that those individuals who increased T_{re} more during work in the heat did so because they were not evaporating as much fluid and entered the second LBNP test less dehydrated. This was the case in the NR's who had higher temperatures after dehydration and greater tolerance to LBNP than the R's.

It is well known that orthostasis (26) and LBNP (16) lead to a loss of plasma volume (PV) depending upon the intensity and the duration of the stress. Exercise in a hot environment also depleted PV and usually the loss in PV is disproportionately greater than in TBW (1, 9, 21). Apparently PV is replenished rather rapidly after cessation of either forms of stress even if the subjects receive no fluid supplement (16 , Myhre, L.G. unpublished data). Since the protocol of this investigation involved both LBNP and exercise in the heat sequentially, with an intervening period of 15-20 minutes, the interpretation of the PV measurements which were taken immediately before and after the LBNP tests is complex. Retrospectively it might have been better to interpose a longer interval between the LBNP and heat exposures to allow for near complete restoration of PV. But this would have imposed additional hardships on the subjects.

The following table summarizes the changes in PV in percent of the initial PV as a result of LBNP- I, of the acute dehydration and the following LBNP-II test in the series before (A) and after (B) acclimation, showing the net total in the last column. The major loss of PV occurred during

		Initial PV Liters	ΔPV% LBNP-I	Dehydr.	LBNP-II	Total
A	NR	3.062	-12.5	-0.9	-3.0	-16.4
	R	3.431	- 7.0	-5.3	+0.7	-11.5
	All	3.247	- 8.7	-4.1	-1.0	-13.8
B	NR	3.207	-12.2	+1.8	-5.3	-15.7
	R	3.601	- 9.9	0.0	-3.6	-13.5
	All	3.406	-11.0	+0.8	-4.4	-14.6

the first LBNP tests both before and after acclimation when all subjects were well hydrated. Before acclimation (A) the following acute dehydration caused a smaller drop in PV which on the overall average was proportional to the loss of total body water (4.3% TBW). However the R's lost considerably more PV than the NR's at this time. The second LBNP test on the dehydrated subjects had very little effect on PV but the NR's lost a little more here than the others. After acclimation (B) PV was lost during the LBNP tests (I and II) only, while there was a slight gain during dehydration with a similar loss in TBW (4.1%) as before. This implies that the subjects were better able to conserve PV in the face of acute dehydration after acclimation ($p < .001$) but not during LBNP.

Previous experiments in this laboratory using a similar dehydration procedure, but without preceding LBNP exposure on well hydrated subjects have consistently revealed a relatively much greater depletion of plasma volume than fluid loss of the body as a whole. But the PV loss was minimal during dehydration in this study before acclimation and completely absent after it. This was evidently attributable to the significant preceding depletion of PV with a corresponding increase in plasma protein concentration (Table 12) and oncotic pressure. Thus plasma dehydration during exercise in the heat appears to be a self-limiting process as the oncotic pressure

rises favoring water retention in the blood. Obviously this mechanism is not able to cope with the grossly elevated hydrostatic pressure across the capillary walls created by the following LBNP and further loss of PV ensued even in the dehydrated subjects, although not nearly as much was lost as before.

The fact that the R's lose less PV than the NR's in all phases of the series is readily explained by their lower LBNP tolerance and therefore lesser exposure. A high correlation was found between loss of PV during each LBNP test and cumulative stress tolerated (Torr x min) as illustrated by the regression lines in Fig 12. The regression equation and correlation coefficients were as follows, where y = ΔPV(ml) and x = LBNP tolerated:

A-I : $y = -356x - 41$, $r = -.875$, $p < .01$

A-II: $y = -232x + 81$, $r = -.650$, $p < .05$

B-I : $y = -257x - 169$, $r = -.872$, $p < .01$

B-II: $y = -265 + 21$, $r = -.632$, $p < .05$

Why the correlations were higher and more significant in the tests before dehydration (A-I, B-I) than after (A-II, B-II) is not clear, but may be due to the interaction of other factors such as the elevated body temperature in the latter. Nevertheless the main conclusion is that the more LBNP was tolerated, the greater the loss of PV.

Regressions were also computed for the relationship between the deficit in PV after the first LBNP tests and the dehydration on LBNP tolerance in the second tests, but no significant correlation was found. This is in keeping with the observation that the R's had significantly more BV and PV than the NR's to start with, when corrected for body weight. And yet their LBNP tolerance was much less.

The total amount of circulating plasma protein (Table 12) remained remarkably constant throughout both series despite major changes in plasma protein concentration, attesting to the absence of any significant shifts of protein into or out of the blood neither during dehydration nor during repeated LBNP maneuvers. Another interesting finding was a small but significant increase in Hb content of the red cells and a corresponding shrinkage in RCV (Tables 13, 14) most of which occurred during dehydration and less during LBNP. Apparently the blood cells loose some fluid as well.

In 1931, Waterfield (26) reported a fascinating study on the swelling of the legs measured with a water plethysmograph on transition from the recumbent to the erect posture. On the basis of concomitant measurements of BV and PV he observed a close inverse relationship between PV and leg volume in orthostasis and concluded that a substantial part of the leg swelling was attributable to leakage of fluid creating transient edema. Later Brown et al. (5) used an ingenious teeter board device to record fluid shifts with LBNP continuously from the shift in the center of gravity of the body. They estimated that a displacement of about 10ml/kg body weight of blood took place from the upper to the lower part of the body under -70 Torr in the course of 60 sec. Lower body negative pressure and a whole leg plethysmograph was used by Musgrave et al. (17) to measure leg volume changes at -20 and -40 Torr up to 20 minutes. They observed increases of 2.8% at -20, and 3.6% at -40 Torr whereby they attributed the changes persisting beyond 10 minutes entirely to capillary filtration. However their records which show a fast component lasting not more than two minutes and a slow one with a constant slope following it, strongly suggest that capillary filtration predominates after the first minute or two. Another report pertinent to the present study by Murray et al. (16) showed that the increase in leg volume under LBNP (-4 Torr) was not as great after a phlebotomy of 500 ml.

Limb volume measurements were included in this study to ascertain whether acute dehydration and subsequent acclimation to heat had a noticeable effect on fluid shifts within the body and how these related to changes in BV and PV. It was also of interest to find out if the leg volume changes had anything to do with the observed lower tolerance of R's versus NR's.

It is clear from Fig 9 which shows simultaneous changes in leg volume (LV) and arm volume (AV), that the legs did not swell as much after dehydration (A-II and B-II) as before (A-I and B-I) and these differences were highly significant ($p < .001$). One must assume that the heat stress had already induced an appreciable shift of blood into peripheral vessels, so that a smaller shift during LBNP was sufficient to cause a critical depletion of the central blood volume. Unfortunately, we were not able to measure the changes in limb volumes that may have taken place during

dehydration between the LBNP tests. The shrinkage in arm volume was not as great as the swelling of the legs. However it was also significantly less after dehydration when all data were pooled. Loss of AV under these circumstances is commonly attributed to increased venous tone (3, 7) and this is usually diminished when body temperature rises as in our experiment. However Johnson et al. (11) observed in experiments with LBNP at different T_{re} that during heating the skin retains the ability to vasoconstrict, but that this vasoconstriction cannot completely override heat induced vasodilation. Our results on AV changes in a hyperthermic state are compatible with this contention. The changes in AV and LV were apparently not affected by the acclimation process.

Another pertinent finding was a statistically highly significant negative correlation between swelling of the leg (Δ%LV = y) and loss of plasma volume (ΔPV, ml = x) under LBNP:

$y = 2.543 - .0036x \quad (r = .64, p < .01)$

This confirms the early observations by Waterfield in orthostasis (26).

It is also interesting to see that in the very first LBNP test (A-I) the R's showed slightly greater changes in LV and AV than the NR's although they had been exposed to 42% less LBNP stress. This implies that they had reached a critical phase of fluid displacement at a lower level of stress and that their legs had a greater tendency to accommodate fluid under LBNP than the other subjects. This propensity can be quantitated in terms of total limb compliance if one determines the slope b of a linear regression of the form $y = a + bx$ where $y = \Delta$%LV and x = stress in Torr x min during the course of each test. A statistical analysis on all 40 LBNP tests where LBNP tolerance was plotted against leg and arm compliance is shown in Fig 13. There was a highly significant negative correlation confirming the hypothesis that high compliance is associated with low tolerance. The mean values for compliance presented in Table 19 give several interesting clues. On the average leg compliance was significantly greater in all subjects after the first dehydration unacclimated. After acclimation, however, compliance was significantly lower in the dehydrated LBNP tests (B-II) than under the same conditions before (A-II). As mentioned above (Table 4) the greatest improvement in tolerance was seen between these two tests, particularly in the R group. Furthermore, leg

and arm compliance were uniformly significantly greater in the R's than the NR's ($p < .01$). It seems reasonable to conclude that the higher leg compliance of the R's is causally related to their greater susceptibility to LBNP. Limb compliance as defined here reflects the capacity to accommodate both intravascular and extravascular fluid by capillary filtration which leads to edema. An attempt was made to estimate the relative magnitude of the latter by measuring the change in leg volume which persisted 45-60 sec after releasing the LBNP. At first there was a rapid drop in volume, reflecting depletion of the capacity vessels, followed by a plateau still considerably higher than the baseline. This deflection was used to estimate the residual swelling due to edema and expressed as a fraction of the maximal deflection observed during LBNP (edema index). The residual volume change amounted to an average 35% of the maximal volume change in the tests before dehydration, but was only half as great (18%) in the dehydrated subjects. The difference was also reflected in correspondingly smaller changes in PV. Finally, when the results of all the tests before and after acclimation were pooled and analyzed, changes in plasma volume correlated closely and inversely with the edema index ($r = -.66$, $p < .01$).

We owe a vote of thanks and appreciation to our indomitable subjects including Captain P.R. Elliott (USAF) who was also a great help in conducting the experiments.

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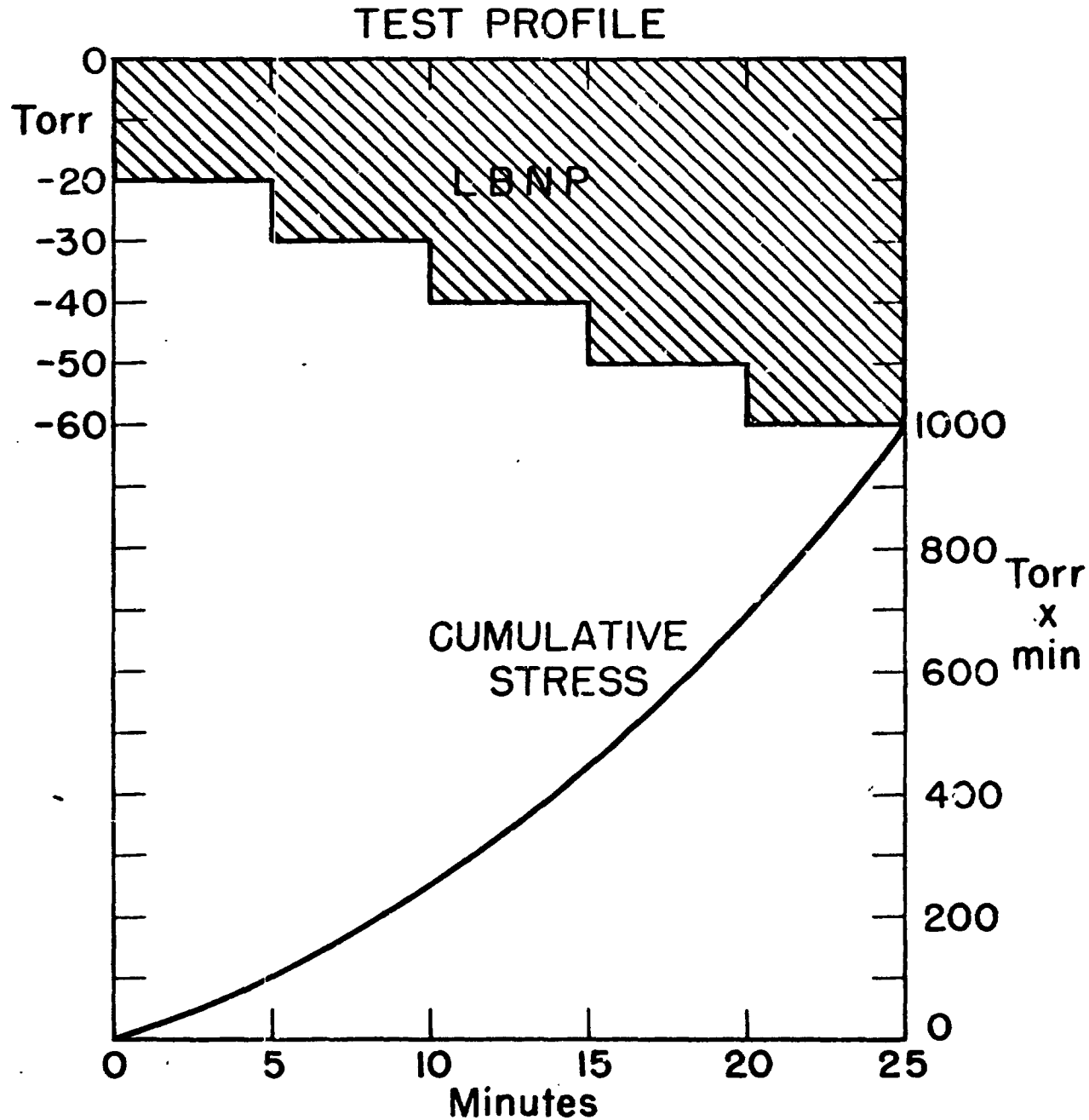
TEST PROFILE
0
Torr
-20
-30
-40
-50
-60
LBNP
1000
800
600
400
200
0
Torr
x
min
CUMULATIVE
STRESS
0
5
10
15
20
25
Minutes

Figure 1

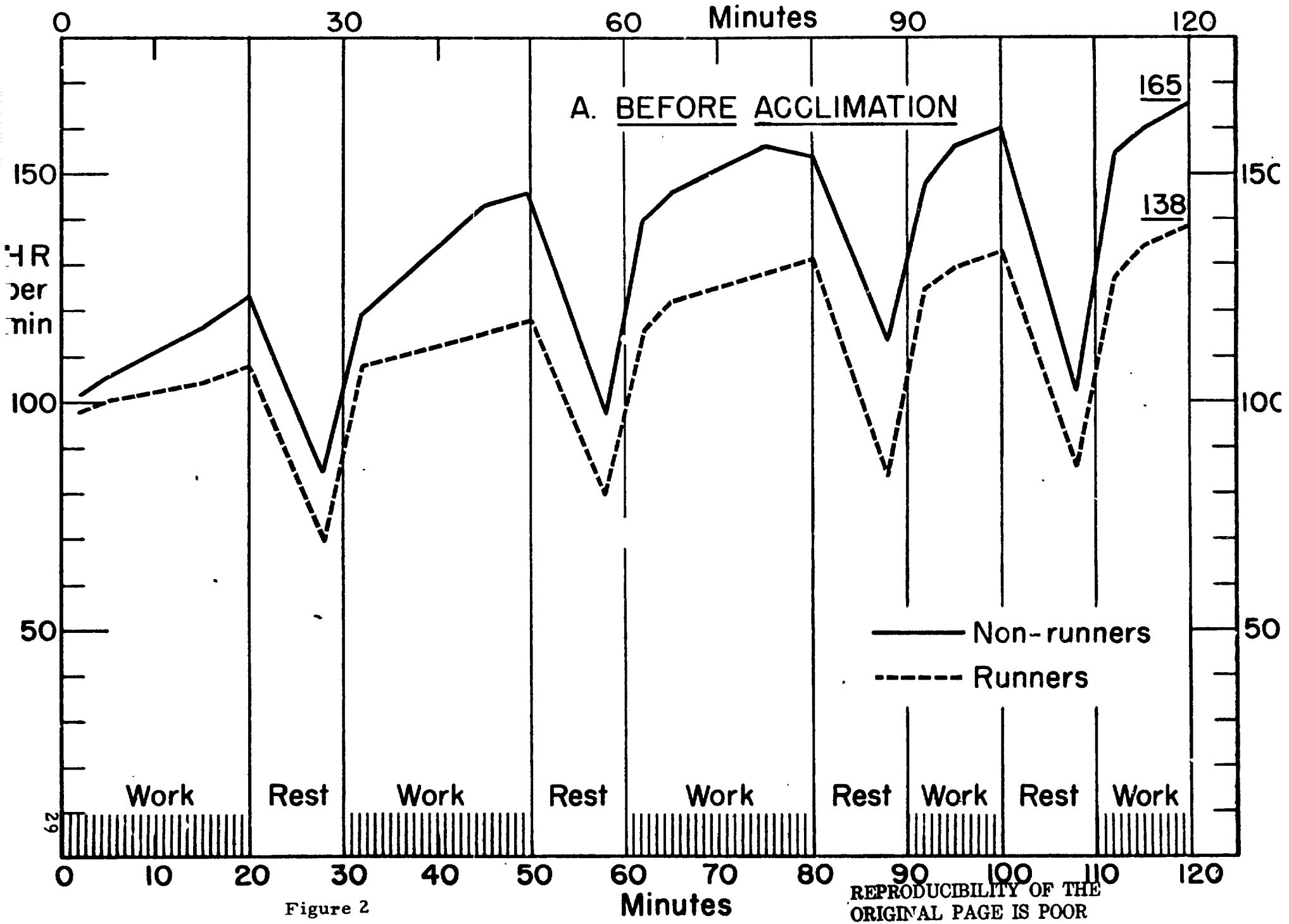

Figure 2

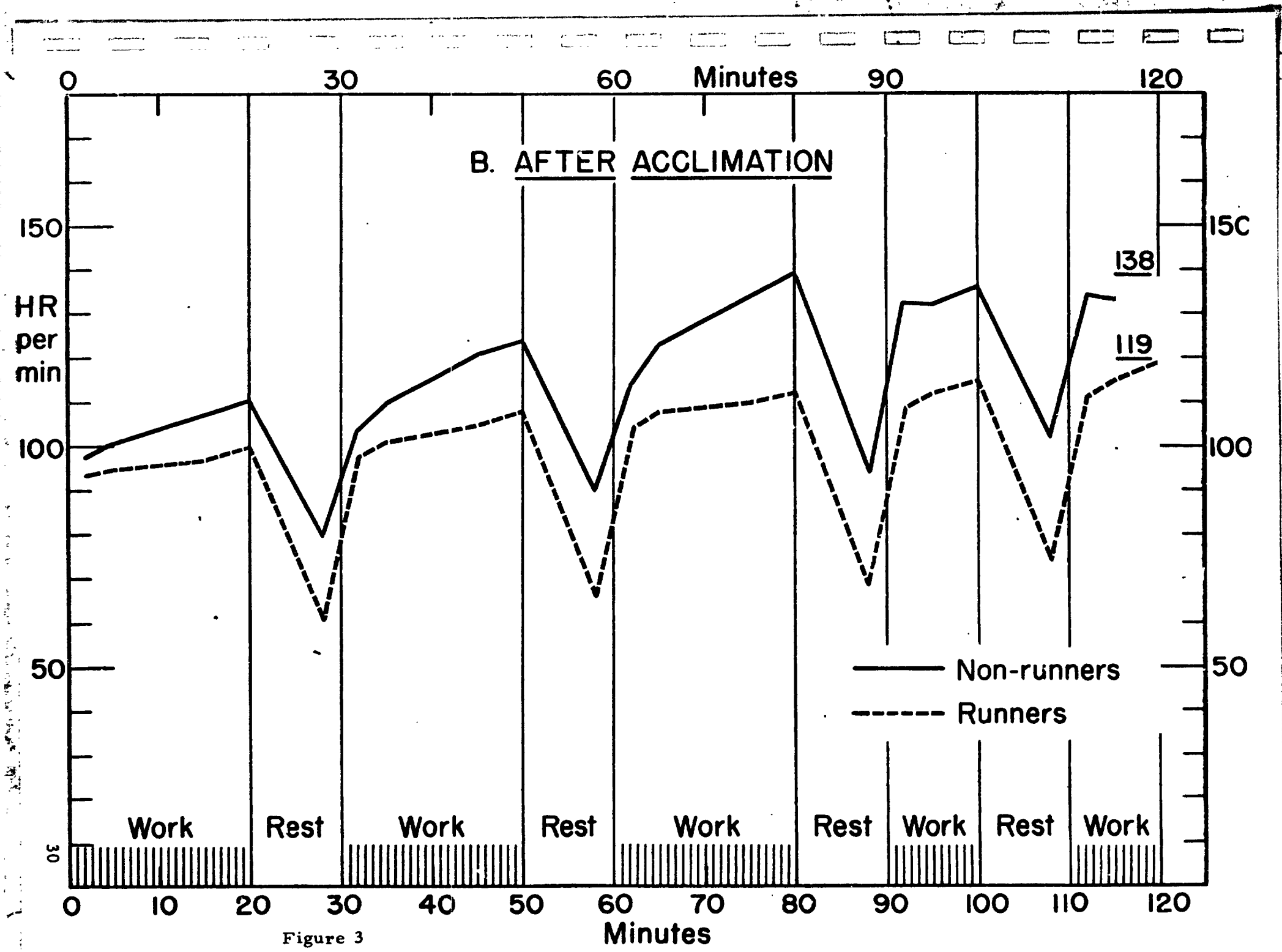
0
30
60
Minutes
90
120
B. AFTER ACCLIMATION
150
HR per min
100
50
30
138
119
Non-runners
Runners
Work
Rest
Work
Rest
Work
Rest
Work
Rest
Work
0
10
20
30
40
50
60
70
80
90
100
110
120
Minutes

Figure 3

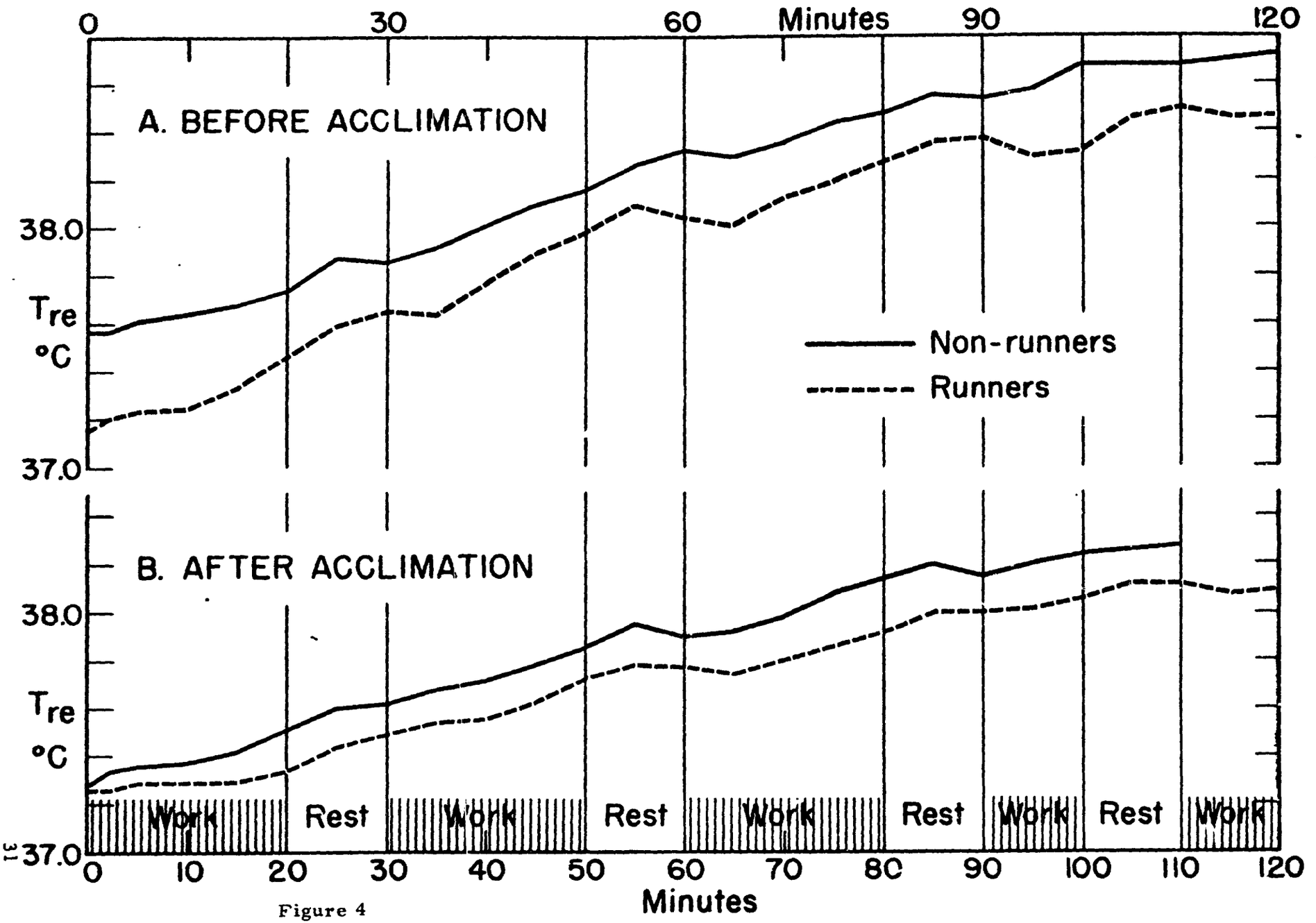
0
30
60
Minutes
90
120
A. BEFORE ACCLIMATION
38.0
Tre
°C
37.0
Non-runners
Runners
B. AFTER ACCLIMATION
38.0
Tre
°C
37.0
Work
Rest
Work
Rest
Work
Rest
Work
Rest
Work
0
10
20
30
40
50
60
70
80
90
100
110
120
Minutes

Figure 4

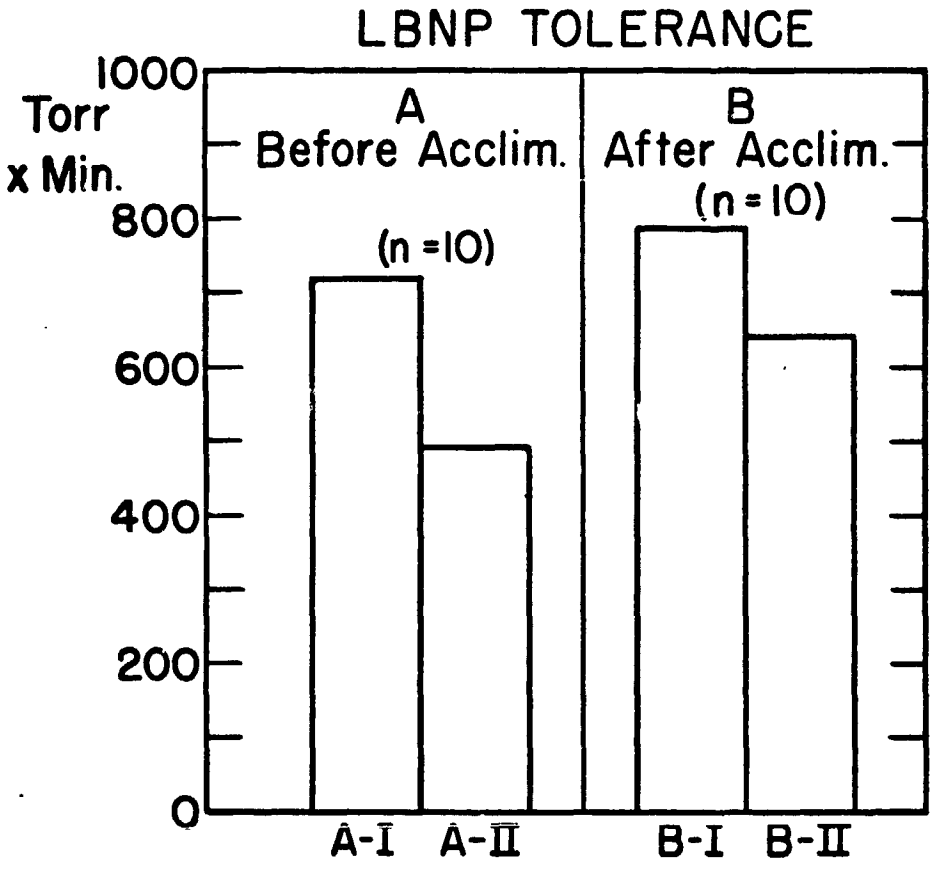

Figure 5. Mean LBNP tolerance before (A-I and B-I) and after (A-II and B-II) dehydration for all subjects.

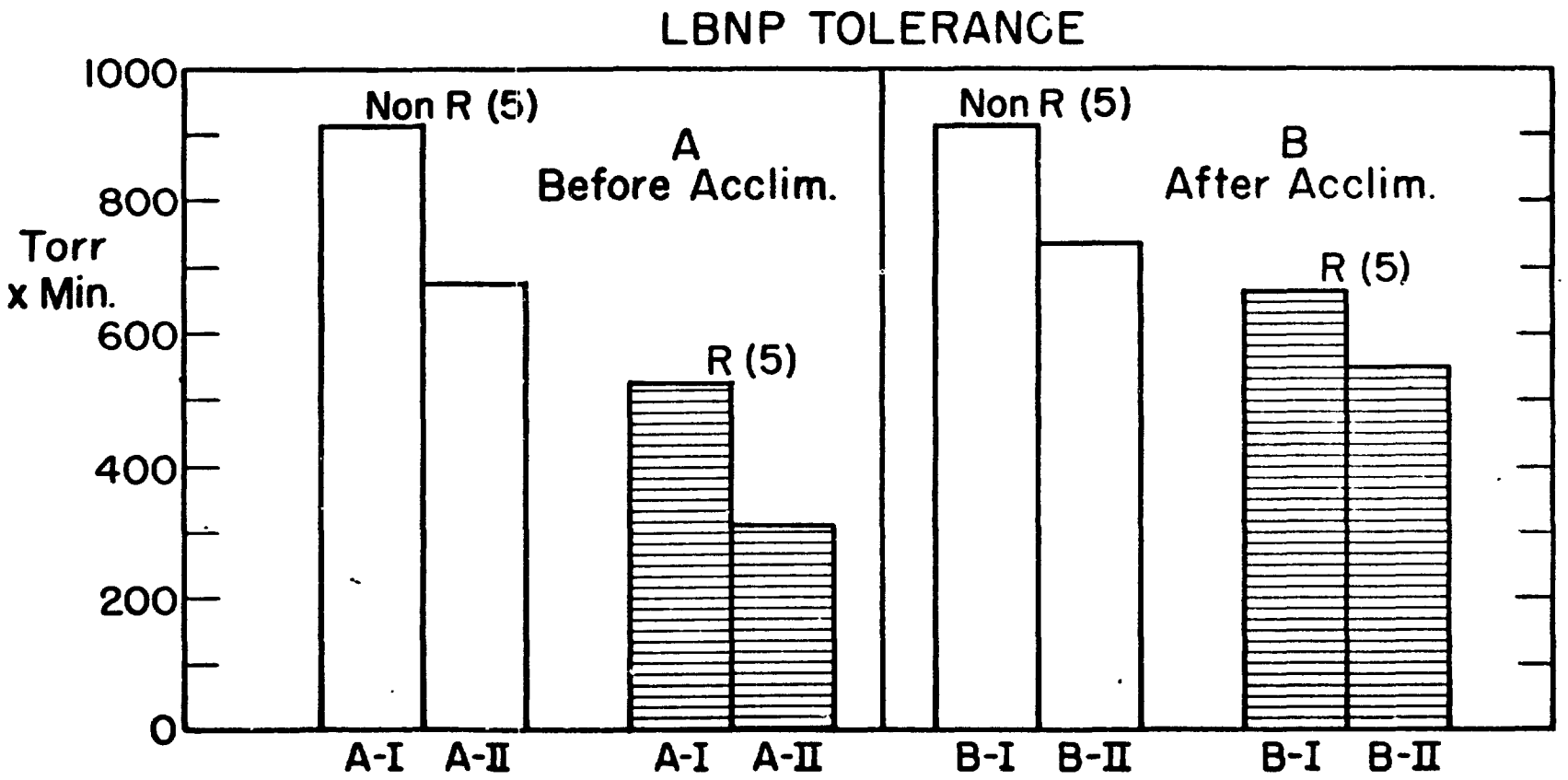

Figure 6. Mean LBNP tolerance before (A-I and B-I) and after (A-II and B-II) dehydration for non-runners (NR) and runners (R).

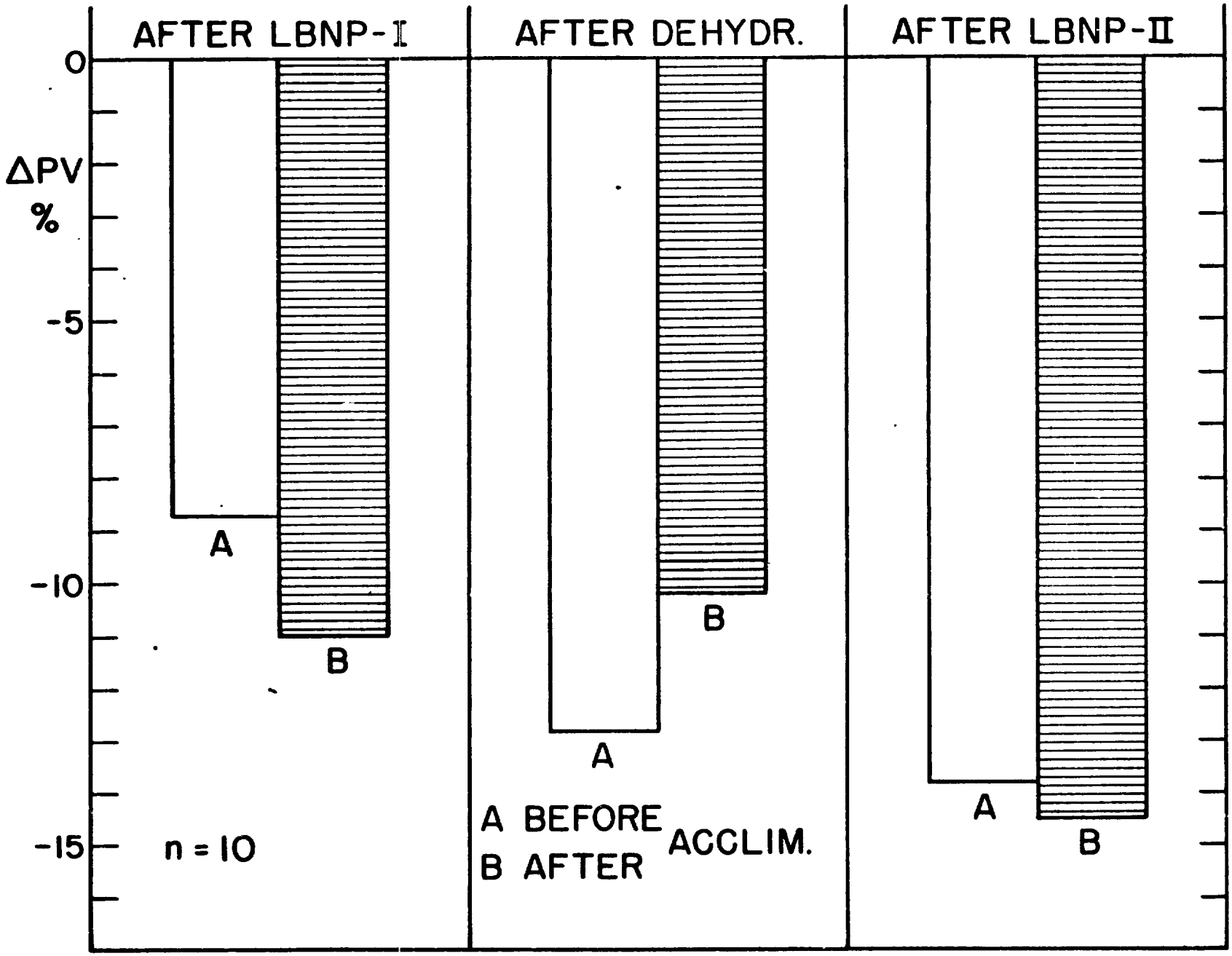

Figure 7. Loss of plasma volume from baseline after LBNP-I, after dehydration and after LBNP-II before (A) and after (B) acclimation.

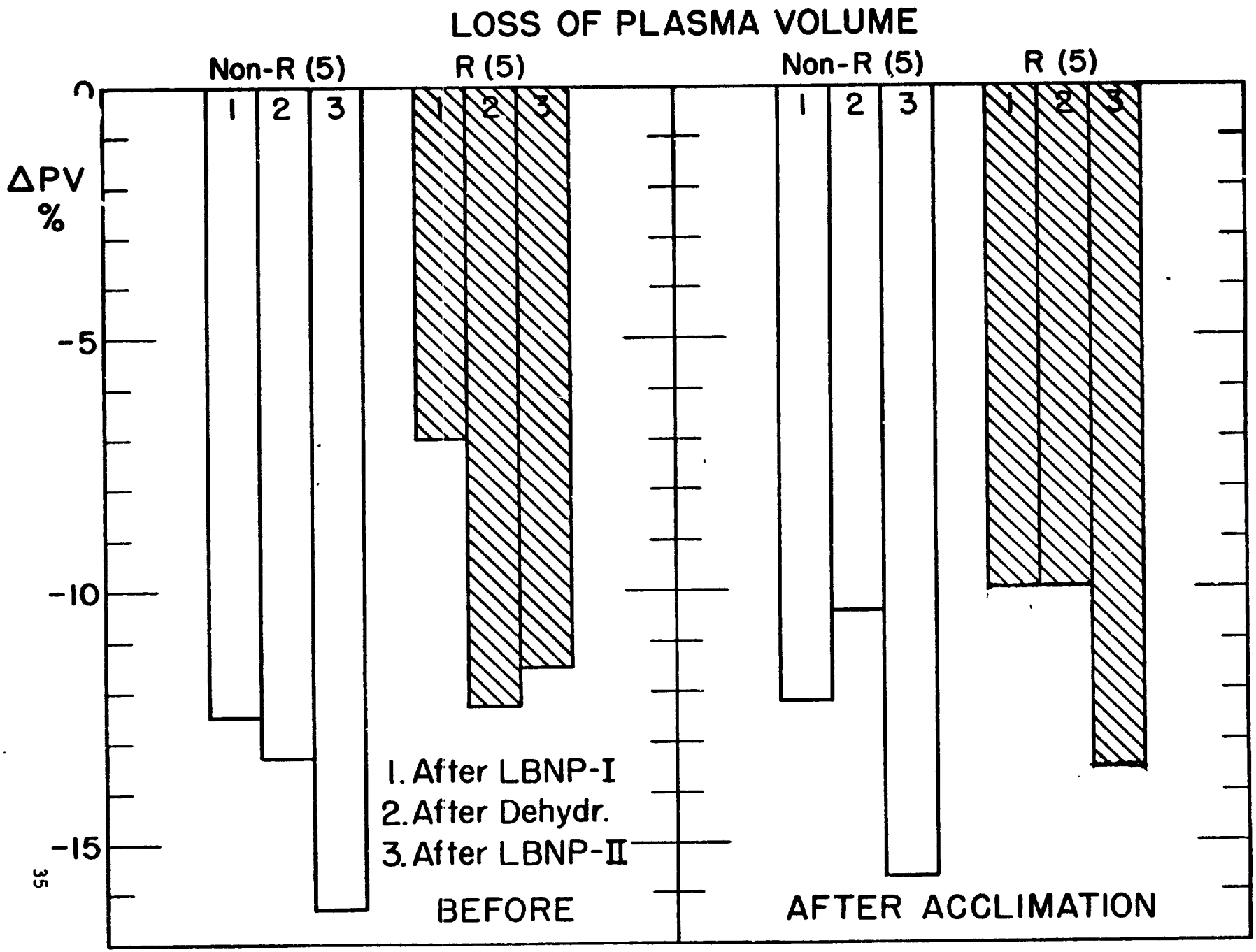
LOSS OF PLASMA VOLUME
Non-R (5)
R (5)
Non-R (5)
R (5)
1
2
3
ΔPV
%
0
-5
-10
-15
1. After LBNP-I
2. After Dehydr.
3. After LBNP-II
BEFORE
AFTER ACCLIMATION

Figure 8

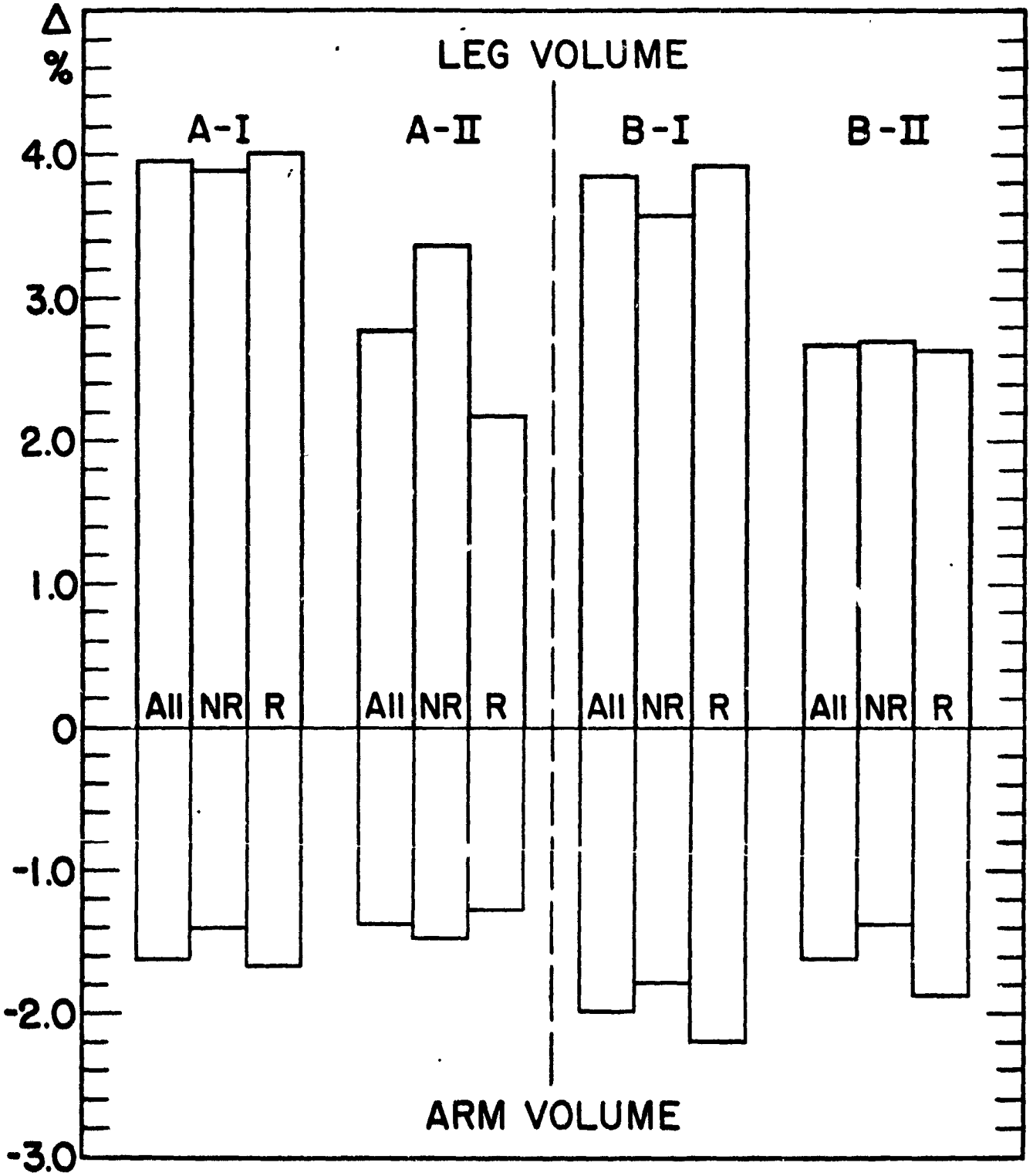

Figure 9. Increase in leg volume and decrease of arm volume at end of LBNP test before (I) and after (II) dehydration. A: before, B: after acclimation.

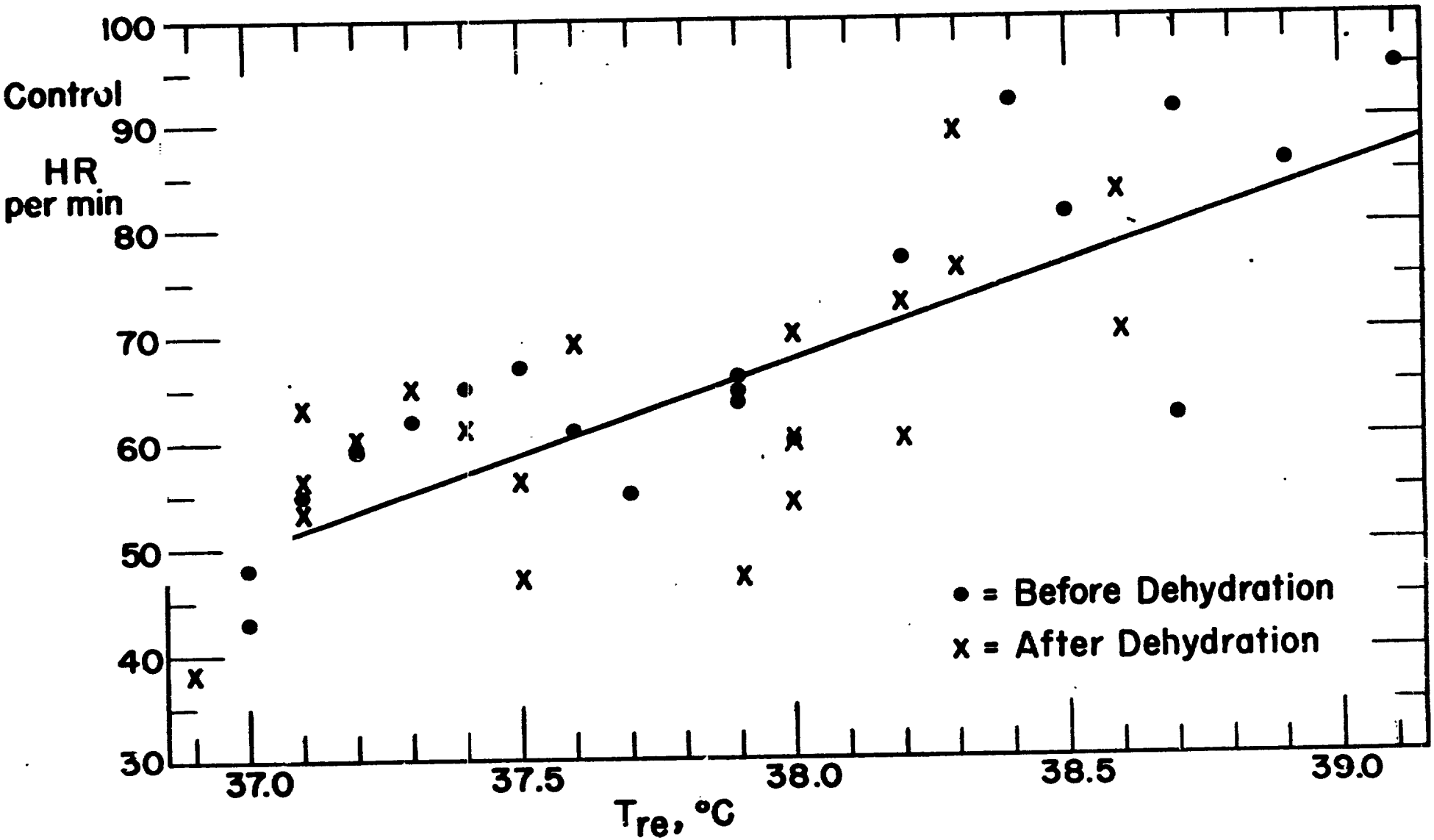

Figure 10. Correlation between heart rate (HR) on entering LBNP test before (•) and after (x) dehydration and rectal temperature (T_{re}). Includes all tests. Correlation coefficient, r = .77.

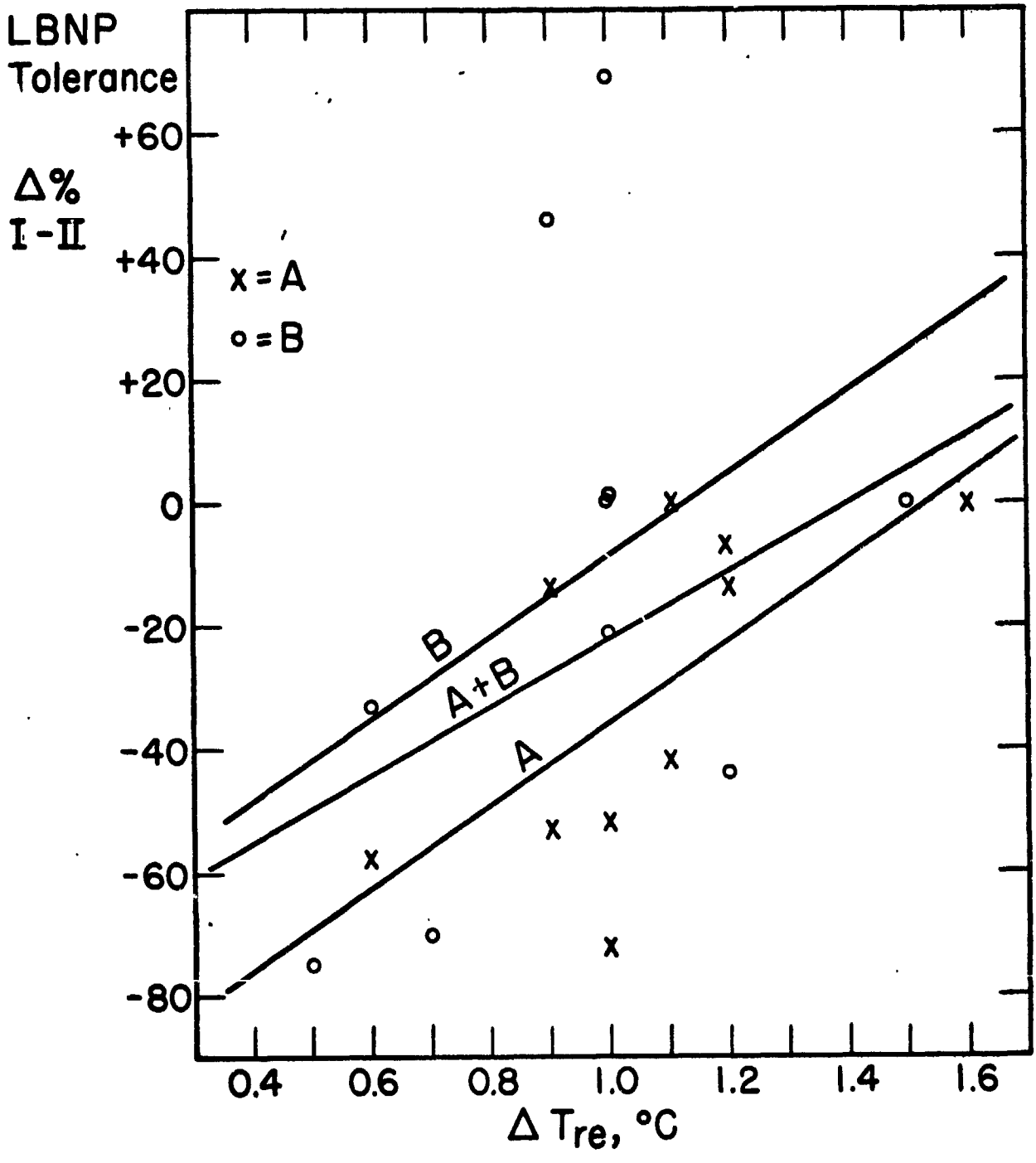

Figure 11. Correlation between the difference in LBNP tolerance before (I) and after (II) dehydration in percent of initial tolerance and the difference in rectal temperature (T_{re}) between tests I and II. A: before, B: after acclimation. All three correlations are positive but significant only in A ($p < .05$).

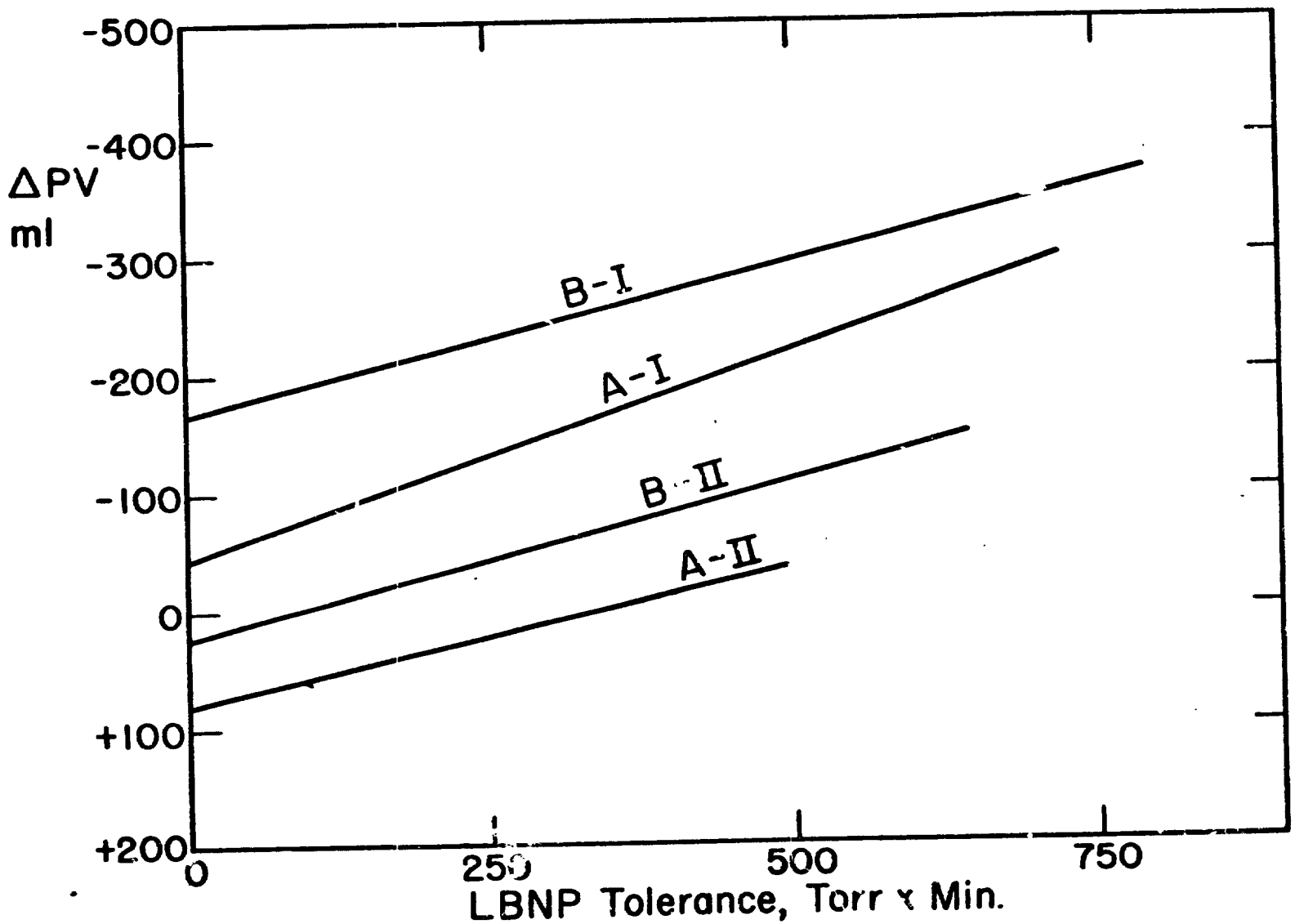

Figure 12. Correlation between loss of plasma volume in the course of LBNP before (I) and after (II) dehydration and (A) before and (B) after acclimation and LBNP tolerance. PV at the beginning of the test was in A-I: 3.247L, A-II: 2.832L, B-I: 3.404L and B-II: 3.058. All correlations are positive and significant.

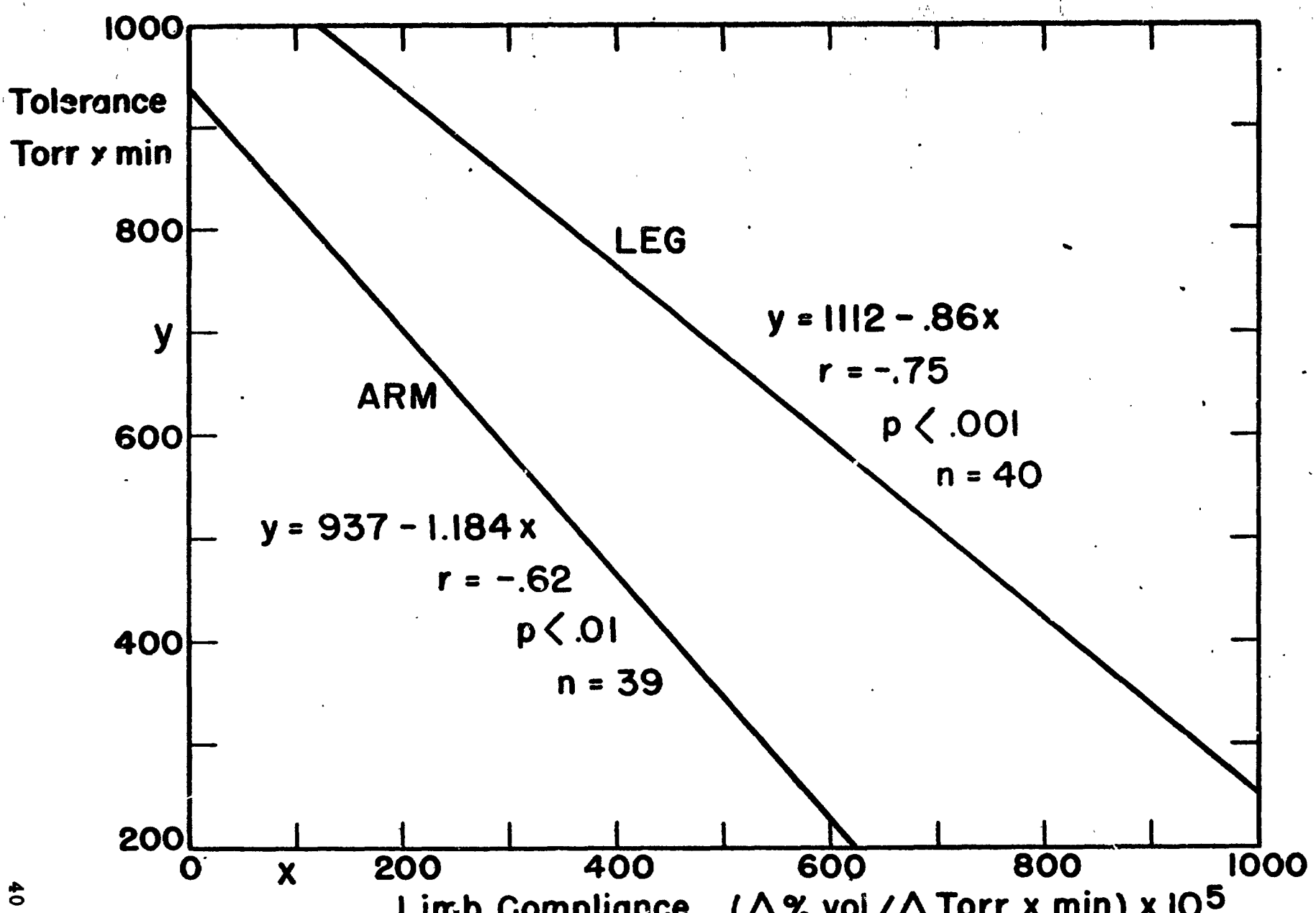

Figure 13. Correlation between LBNP tolerance and limb compliance (percent change in volume per unit LBNP x time).

TABLE 1: PHYSICAL CHARACTERISTICS AND CONDITION OF SUBJECTS BEFORE AND AFTER HEAT ACCLIMATION.

No.	Age y	Ht cm	Weight kg		FFW kg		%Fat		$\dot{V}_{O2max}$		
			before	after	before	after	before	after	L/min	ml/min / kgWt	ml/min / kgFFW
NR: 1	37	185	71.5	70.8	60.1	61.2	15.6	13.5	2.89	40.4	48.0
2	30	168	77.8	76.8	62.6	61.7	19.6	19.7	2.39	30.8	38.3
3	22	184	126.3	126.0	86.1	85.7	31.8	32.0	3.39	26.8	39.4
4	30	170	75.1	74.3	56.1	56.0	25.3	24.6	2.64	35.0	47.0
5	23	183	82.0	81.6	64.7	65.4	21.1	19.8	3.03	36.9	46.9
M 1-5	28	178	86.5	85.9	65.9	66.0	22.7	21.9	2.87	34.0	43.9
R: 6	40	166	57.0	57.4	54.3	55.0	4.8	4.2	3.27	57.4	60.3
7	39	179	70.9	70.6	62.1	61.4	12.3	13.0	3.42	48.2	55.0
8	28	182	70.5	73.5	66.6	69.7	5.5	5.2	3.66	51.8	54.9
9	38	186	78.9	77.7	70.7	69.5	10.4	10.6	3.76	47.7	53.2
10	26	183	76.5	77.2	66.6	66.7	13.0	13.7	3.36	45.2	50.5
M 6-10	34	179	70.8	71.3	64.1	64.5	9.2	9.3	3.50	50.1	54.8
M 1-10	31	179	78.6	78.6	65.0	65.2	15.9	15.6	3.18	45.0	49.4

NR: Non-runners (1-5). R: Runners (6-10). M: Mean. FFW: Fat free weight.
$\dot{V}_{O2max}$: Maximal aerobic power.

TABLE 2: TOTAL BODY WATER, Liters

		Before Acclimation (A)				After Acclimation (B)			
	Subj.	I	II	Δ	Δ%	I	II	Δ	Δ%
NR	1.	44.4	42.6	1.8	4.1	46.6	44.9	1.7	3.6
	2.	44.8	43.1	1.7	3.8	45.0	43.1	1.9	4.2
	3.	59.8	57.5	2.3	3.8	62.3	60.1	2.2	3.5
	4.	39.6	37.8	1.8	4.5	39.4	37.6	1.8	4.6
	5.	46.6	44.7	1.9	4.1	46.2	44.4	1.8	3.9
M 1-5		47.0	45.1	1.9	4.1	47.9	46.0	1.9	4.0
R	6.	41.5	39.4	2.1	5.1	40.3	38.4	1.9	4.7
	7.	47.8	45.7	2.1	4.4	48.6	46.7	1.9	3.9
	8.	41.5	39.5	2.0	4.8	--	--	--	--
	9.	49.3	47.4	1.9	3.9	48.0	46.0	2.0	4.2
	10.	45.8	43.9	1.9	4.1	45.8	43.9	1.9	4.1
M 6-10		45.2	43.2	2.0	4.5	45.7	43.8	1.9	4.2
M 1-10		46.1	44.2	2.0	4.3	46.9	45.0	1.9	4.1
SD		5.7	5.6	0.2	0.4	6.6	6.5	0.1	0.4

I: Before dehydration; II: After dehydration; NR: Non-runners; R: Runners; M: Mean

TABLE 3: RECTAL TEMPERATURE (°C) DURING LBNP

			LBNP - I	Heat exposure	LBNP - II	
		Subj.	End		Begin	End
A	NR	1.	37.7		38.9	38.9
		2.	37.5		38.4	38.3
		3.	37.9		39.1	38.7
		4.	37.6		38.7	38.4
		5.	37.2		38.2	38.1
		M 1-5	37.6		38.7	38.5
	R	6.	37.0		38.0	37.9
		7.	37.3		37.9	37.9
		8.	37.0		37.9	37.9
		9.	37.1		38.7	38.5
		10.	37.4		38.5	38.2
		M 6-10	37.2		38.2	38.1
		M 1-10	37.4		38.4	38.3
		SD	.3		.4	.4
B	NR	1.	37.1		38.6	38.4
		2.	37.1		38.3	38.2
		3.	37.6		38.6	38.3
		4.	37.2		38.2	37.9
		5.	37.4		38.0	37.9
		M 1-5	37.3		38.3	38.1
	R	6.	37.5		38.0	37.9
		7.	37.5		38.2	38.2
		8.	36.9		37.9	37.6
		9.	37.1		38.0	37.9
		10.	37.3		38.3	38.1
		M 6-10	37.3		38.1	37.9
		M 1-10	37.3		38.2	38.0
		SD	.2		.2	.2

NR: Non-runners; R: Runners; A: Before acclumation; B: After acclimation.

TABLE 4: LOWER BODY NEGATIVE PRESSURE TOLERANCE

		BEFORE ACCLIMATION		AFTER ACCLIMATION	
	Subj.	A-I	A-II	B-I	B-II
NR	1	1000	860	1000	1000
	2	865	403	1000	563
	3	700	650	579	459
	4	1000	1000	1000	1000
	5	1000	477	1000	675
	M 1-5	913	678	916	739
R	6	617	175	850	213
	7	397	168	575	173
	8	397	340	330	559
	9	393	393	550	805
	10	830	484	1000	1000
	M 6-10	527	312	661	550
	M 1-10	720	495	788	645

Units are cummulative Torr-minutes. NR: Non-runners; R: Runners; I: Before acute dehydration; II: After dehydration; A: Before acclimation; B: After acclimation; M: Mean.

TABLE 4a: Statistical analyses of data on table 4

Items compared		Differences %	Significant?	Level
All	A-I and A-II	-31%	Yes	$.001 < p < .01$
	A-I and B-I	+ 9%	No	$.1 < p < .2$
	A-II and B-II	+30%	Yes	$.02 < p < .05$
	B-I and B-II	-18%	No	$.1 < p < .2$
NR and R	A-I	-42%	Yes	$.001 < p < .01$
	A-II	-54%	Yes	$.02 < p < .05$
	B-I	-28%	No	$.10 < p < .2$
	B-II	-26%	No	$.10 < p < .2$
NR	A-I and B-I	+.3%	No	$p > .2$
	A-II and B-II	+ 9%	No	$p > .2$
R	A-I and B-I	+25%	No	$.05 < p < .10$
	A-II and B-II	+76%	No	$.05 < p < .10$

TABLE 5: HEART RATE RESPONSE TO LBNP

			LBNP - I			LBNP - II		
		Subj.	Control	Final	Δ%	Control	Final	Δ%
A	NR	1.	55	90	64	86	128	49
		2.	67	108	61	92	130	41
		3.	66	82	24	95	117	23
		4.	61	89	46	91	136	49
		5.	59	86	46	77	122	58
		M 1-5	62	91	48	80	127	44
	R	6.	48	66	38	60	90	50
		7.	62	70	13	65	100	54
		8.	43	48	12	64	79	23
		9.	55	72	31	62	100	61
		10.	65	120	85	81	125	54
		M 6-10	55	75	36	66	99	48
		M 1-10	59	83	42	77	113	46
B	NR	1.	53	83	57	70	104	49
		2.	63	97	54	89	125	40
		3.	69	90	30	83	114	37
		4.	60	89	48	73	134	84
		5.	61	91	49	70	130	86
		M 1-5	61	90	48	77	121	59
	R	6.	47	82	74	54	82	52
		7.	56	100	79	60	97	62
		8.	38	41	8	47	82	74
		9.	56	87	55	60	135	125
		10.	65	105	62	76	131	72
		M 6-10	52	83	56	59	105	77
		M 1-10	57	87	52	68	113	68

A: Before acclimation; B: After acclimation; LBNP - I: Before dehydration; II: After dehydration; NR: Non-runners; R: Runners.

TABLE: 6 PULSE PRESSURE UNDER LBNP

			LBNP - I			LBNP - II		
		Subj.	Control	Final	Δ%	Control	Final	Δ%
A	NR	1.	39	22	-44	32	18	-44
		2.	36	12	-67	27	16	-41
		3.	49	24	-51	52	26	-50
		4.	38	20	-47	48	16	-67
		5.	52	36	-31	46	18	-61
		M 1-5	43	23	-48	41	19	-53
	R	6.	31	28	-10	38	22	-42
		7.	38	28	-26	39	14	-64
		8.	34	40	+18	46	32	-30
		9.	41	20	-58	39	8	-79
		10.	49	18	-63	44	20	-55
		M 6-10	40	27	-28	41	19	-54
		M 1-10	41	25	-38	41	19	-53
B	NR	1.	38	24	-37	37	22	-41
		2.	29	18	-38	25	14	-44
		3.	42	32	-24	33	22	-33
		4.	34	20	-41	38	20	-47
		5.	56	38	-32	52	28	-46
		M 1-5	40	26	-34	37	21	-42
	R	6.	33	16	-52	36	30	-17
		7.	30	22	-27	36	20	-44
		8.	32	38	+19	49	30	-39
		9.	41	22	-46	38	13	-66
		10.	51	18	-65	34	12	-65
		M 6-10	37	23	-34	39	21	-46
		M 1-10	39	25	-34	38	21	-44

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 7: TOTAL HEMOGLOBIN, grams

		A	B
NR	1.	844	814
	2.	892	896
	3.	929	976
	4.	699	699
	5.	700	735
	M 1-5	813	824
R	6.	849	874
	7.	961	938
	8.	838	909
	9.	880	909
	10.	928	911
	M 6-10	891	908
	M 1-10	852	866
	SD	90	89

A: Before acclimation; B: After acclimation;
NR: Non-runners; R: Runners.

TABLE 8: HEMOGLOBIN, g/100ml

		Subj.	LBNP - I Before	LBNP - I After	LBNP - II Before	LBNP - II After
A	NR	1.	15.7	16.6	16.5	17.4
		2.	15.5	--	16.7	17.0
		3.	15.0	15.6	16.2	16.4
		4.	14.1	15.3	15.8	16.2
		5.	15.3	16.9	16.9	17.0
		M 1-5	15.1	16.1	16.4	16.8
	R	6.	15.1	15.7	16.1	16.2
		7.	15.3	15.8	16.6	16.3
		8.	14.0	14.4	15.4	15.4
		9.	14.6	15.3	16.0	16.2
		10.	15.8	16.7	17.2	16.8
		M 6-10	15.0	15.6	16.3	16.2
		M 1-10	15.0	15.8	16.3	16.5
		SD	0.6	0.8	0.5	0.6
B	NR	1.	14.2	15.5	15.3	16.0
		2.	15.7	16.9	16.8	17.1
		3.	15.0	15.8	15.9	16.7
		4.	14.2	15.5	15.8	16.2
		5.	15.0	16.3	16.0	16.7
		M 1-5	14.8	16.0	16.0	16.5
	R	6.	14.6	15.6	15.6	15.6
		7.	14.5	15.4	15.6	15.5
		8.	14.3	14.9	15.3	15.6
		9.	14.7	15.5	15.7	16.7
		10.	15.3	16.6	16.4	17.1
		M 6-10	14.7	15.6	15.7	16.1
		M 1-10	14.8	15.8	15.8	16.3
		SD	0.5	0.6	0.5	0.6

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 9: BLOOD VOLUME, Liters

		Subj.	LBNP - I Before	LBNP - I After	LBNP - II Before	LBNP - II After
A	NR	1.	5.380	5.098	5.110	4.837
		2.	5.767	--	5.326	5.239
		3.	6.181	5.974	5.724	5.661
		4.	4.968	4.568	4.435	4.320
		5.	4.574	4.153	4.153	4.112
		M 1-5	5.374	4.948	4.950	4.834
	R	6.	5.620	5.419	5.261	5.232
		7.	6.272	6.081	5.809	5.906
		8.	5.984	5.809	5.457	5.426
		9.	6.032	5.752	5.483	5.436
		10.	5.889	5.541	5.405	5.524
		M 6-10	5.959	5.720	5.483	5.505
		M 1-10	5.667	5.377	5.216	5.169
		SD	.547	.659	.531	.585
B	NR	1.	5.731	5.244	5.340	5.090
		2.	5.692	5.307	5.323	5.248
		3.	6.500	6.191	6.156	5.864
		4.	4.931	4.520	4.412	4.319
		5.	4.895	4.519	4.592	4.397
		M 1-5	5.551	5.156	5.165	4.984
	R	6.	5.995	5.595	5.610	5.610
		7.	6.468	6.114	6.008	6.051
		8.	6.358	6.090	5.962	5.836
		9.	6.202	5.877	5.799	5.448
		10.	5.968	5.503	5.540	5.317
		M 6-10	6.198	5.836	5.784	5.652
		M 1-10	5.874	5.496	5.474	5.318
		SD	.578	.613	.583	.588

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 10: HEMATOCRIT, %

		Subj.	LBNP - I Before	LBNP - I After	LBNP - II Before	LBNP - II After
A	NR	1.	43.8	48.3	47.2	48.4
		2.	45.2	--	48.1	48.1
		3.	42.5	43.9	45.7	45.6
		4.	40.4	43.7	44.0	45.5
		5.	42.9	47.7	46.5	47.6
		M 1-5	43.0	45.9	46.3	47.0
	R	6.	43.1	44.7	45.1	45.3
		7.	44.1	45.5	46.8	45.8
		8.	40.0	41.0	41.9	42.3
		9.	40.3	41.9	43.6	44.0
		10.	44.7	48.3	48.1	46.9
		M 6-10	42.4	44.3	45.1	44.9
		M 1-10	42.7	45.0	45.7	46.0
		SD	1.9	2.7	2.0	1.9
B	NR	1.	41.3	45.1	43.3	45.9
		2.	45.1	48.5	47.0	47.3
		3.	42.9	44.5	44.3	46.0
		4.	39.9	43.4	43.2	44.0
		5.	41.3	45.3	43.7	45.2
		M 1-5	42.1	45.4	44.3	45.7
	R	6.	42.2	45.0	44.1	43.7
		7.	42.4	45.0	44.4	44.0
		8	41.8	43.0	43.0	44.4
		9.	40.0	42.6	41.9	44.6
		10.	43.3	46.6	46.4	48.1
		M 6-10	41.9	44.4	44.0	45.0
		M 1-10	42.0	44.9	44.1	45.3
		SD	1.6	1.7	1.5	1.5

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 11: PLASMA VOLUME, Liters

		Subj.	LBNP - I Before	LBNP - I After	LBNP - II Before	LBNP - II After
A	NR	1.	3.022	2.636	2.696	2.497
		2.	3.163	--	2.763	2.719
		3.	3.554	3.351	3.108	3.080
		4.	2.959	2.570	2.482	2.356
		5.	2.613	2.170	2.222	2.156
	M 1-5		3.062	2.681	2.654	2.563
	R	6.	3.200	2.997	2.888	2.862
		7.	3.507	3.315	3.092	3.202
		8.	3.593	3.429	3.171	3.133
		9.	3.599	3.344	3.091	3.046
		10.	3.258	2.866	2.805	2.932
	M 6-10		3.431	3.190	3.009	3.035
	M 1-10		3.247	2.964	2.832	2.798
	SD		.325	.442	.309	.363
B	NR	1.	3.363	2.879	3.028	2.756
		2.	3.123	2.734	2.819	2.766
		3.	3.710	3.438	3.427	3.167
		4.	2.965	2.559	2.507	2.417
		5.	2.876	2.472	2.584	2.412
	M 1-5		3.207	2.816	2.873	2.704
	R	6.	3.468	3.076	3.139	3.156
		7.	3.728	3.364	3.340	3.387
		8.	3.703	3.470	3.398	3.247
		9.	3.724	3.376	3.372	3.019
		10.	3.383	2.938	2.969	2.760
	M 6-10		3.601	3.245	3.244	3.114
	M 1-10		3.404	3.031	3.058	2.909
	SD		.324	.372	.338	.340

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 12

			Plasma Protein g/100ml		Total Plasma Protein g	
		Subj.	LBNP-I	LBNP-II	LBNP-I	LBNP-II
A	NR	1.	7.2	9.6	218	259
		2.	7.2	8.1	228	224
		3.	6.7	7.6	238	236
		4.	6.9	7.9	204	196
		5.	7.2	8.3	188	184
	M 1-5		7.0	8.3	215	220
	R	6.	7.0	9.3	224	269
		7.	6.9	7.9	242	244
		8.	6.3	7.3	226	231
		9.	6.6	7.6	238	235
		10.	8.0	8.8	261	247
	M 6-10		7.0	8.2	238	245
	M 1-10		7.0	8.2	227	233
	SD		0.5	0.8	21	28
B	NR	1.	6.8	7.6	229	230
		2.	7.1	7.8	222	220
		3.	6.9	7.6	256	266
		4.	6.8	7.8	202	196
		5.	7.4	7.5	213	194
	M 1-5		7.0	7.7	224	221
	R	6.	6.8	--	236	--
		7.	6.9	7.6	257	254
		8.	6.4	7.2	237	245
		9.	6.6	7.3	246	246
		10.	7.4	8.5	250	252
	M 6-10		6.8	7.7	245	249
	M 1-10		6.9	7.7	235	234
	SD		0.3	0.4	18	26

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 13: RED CELL VOLUME, Liters

		Subj.	LBNP - I Before	LBNP - I After	LBNP - II Before	LBNP - II After
A	NR	1.	2.358	2.462	2.414	2.340
		2.	2.604	--	2.563	2.520
		3.	2.627	2.623	2.616	2.581
		4.	2.009	1.998	1.953	1.964
		5.	1.961	1.983	1.93[illegible]	1.956
		M 1-5	2.312	2.267	2.295	2.272
	R	6.	2.426	2.422	2.373	2.370
		7.	2.765	2.766	2.717	2.703
		8.	2.391	2.380	2.286	2.293
		9.	2.433	2.408	2.392	2.390
		10.	2.631	2.675	2.600	2.592
		M 6-10	2.529	2.530	2.474	2.470
		M 1-10	2.421	2.413	2.385	2.371
		SD	.269	.280	.271	.255
B	NR	1.	2.368	2.365	2.312	2.334
		2.	2.569	2.573	2.504	2.482
		3.	2.790	2.753	2.729	2.697
		4.	1.970	1.961	1.905	1.902
		5.	2.019	2.047	2.008	1.985
		M 1-5	2.343	2.340	2.292	2.280
	R	6.	2.527	2.519	2.471	2.454
		7.	2.740	2 50	2.668	2.664
		8.	2.655	2.620	2.564	2.589
		9.	2.478	2.501	2.472	2.429
		10.	2.585	2.565	2.571	2.557
		M 6-10	2.597	2.591	2.540	2.539
		M 1-10	2.470	2.465	2.416	2.409
		SD	.279	.269	.270	.269

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 14: HEMOGLOBIN CONTENT OF RED CELLS, g/ml

			LBNP - I		LBNP - II	
		Subj.	Before	After	Before	After
A	NR	1.	.357	.343	.349	.361
		2.	.341	--	.348	.354
		3.	.354	.355	.355	.360
		4.	.346	.350	.358	.356
		5.	.356	.353	.362	.358
		M 1-5	.351	.350	.354	.359
	R	6.	.350	.351	.358	.358
		7.	.349	.348	.354	.356
		8.	.352	.352	.362	.365
		9.	.362	.365	.368	.368
		10.	.355	.347	.357	.358
		M 6-10	.354	.353	.360	.361
		M 1-10	.352	.352	.357	.359
		SD	.006	.006	.006	.004
B	NR	1.	.345	.344	.352	.349
		2.	.349	.348	.358	.361
		3.	.353	.355	.358	.362
		4.	.354	.356	.367	.367
		5.	.360	.359	.366	.370
		M 1-5	.352	.352	.360	.362
	R	6.	.343	.347	.354	.356
		7.	.344	.341	.352	.352
		8.	.344	.347	.355	.351
		9.	.368	.363	.375	.375
		10.	.353	.355	.354	.356
		M 6-10	.350	.351	.358	.358
		M 1-10	.351	.352	.359	.360
		SD	.008	.007	.008	.009

A: Before acclimation; B: After acclimation; LBNP-I: Before dehydration; LBNP-II: After dehydration; NR: Non-runners; R: Runners.

TABLE 15: INCREASE IN LEG VOLUME (%) UNDER LBNP

A - I

	Subj.	Torr: -20 Min: 2'	5'	-30 2'	5'	-40 2'	5'	-50 2'	5'	-60 2'	5'	Final
NR	1.	1.07	1.37	1.66	1.87	2.28	2.63	2.90	3.05	3.39	3.79	3.92
	2.	0.58	1.10	1.58	1.23	1.89	2.35	2.68	3.31	3.69		3.82
	3.	0.53	0.71	1.22	1.64	2.12	2.53	3.02	3.48			3.71
	4.	0.58	0.61	0.96	1.31	1.66	2.01	2.38	2.79	3.28	3.76	3.83
	5.	0.52	0.56	1.08	1.41	1.86	2.29	2.72	3.15	3.55	4.02	4.13
M 1-5		0.66	0.87	1.30	1.49	1.96	2.36	2.74	3.16	3.48	3.86	3.88
R	6.	1.81	2.11	2.57	3.38	3.60	4.30	4.81				4.94
	7.	2.47	2.57	2.89	3.49	3.49						3.93
	8.	0.74	0.96	1.86	2.37	2.93						3.45
	9.	0.86	0.95	1.56	2.00	2.55						2.98
	10.	0.95	1.35	1.92	2.24	2.85	3.08	3.63	4.04	4.62		4.74
M 6-10		1.37	1.59	2.16	2.70	3.17	3.69	4.22	4.04	4.62		4.01
M 1-10		1.11	1.23	1.73	2.10	2.57	3.03	3.48	3.60	4.05		3.95

A - II

NR	1.	1.20	1.52	1.95	2.09	2.30	2.62	2.89	2.98	3.48		3.58
	2.	1.31	3.45	1.89	2.57	3.16						3.47
	3.	1.41	1.59	2.39	2.49	3.29	3.43					3.64
	4.	0.39	0.46	0.66	0.69	0.99	1.16	1.39	2.17	2.81	2.49	2.60
	5.	1.53	1.92	2.63	2.87	2.89	3.40					3.51
M 1-5		1.17	1.39	1.90	2.14	2.53	2.65	2.14	2.58	3.40	2.49	3.36
R	6.	1.76	1.76	2.53								2.62
	7.	1.30	1.65	2.18								2.28
	8.	0.14	0.14	1.04	1.04	1.81						1.88
	9.	0.25	0.26	0.58	0.68	0.98						1.22
	10.	1.14	1.13	1.71	1.79	2.16	2.36	2.87				2.87
M 6-10		0.92	0.99	1.61	1.17	1.64	2.36	2.87				2.17
M 1-10		1.05	1.19	1.76	1.67	2.09	2.51	2.51	2.58	3.40		2.77

A: Before acclimation; B: After acclimation; LBNP - I: Before dehydration; LBNP - II: After dehydration; NR: Non-runners; R: Runners.

TABLE 16: INCREASE IN LEG VOLUME (%) UNDER LBNP

B - I

	Subj.	Torr: -20 Min: 2'	5'	-30 2'	5'	-40 2'	5'	-50 2'	5'	-60 2'	5'	Final
NR	1.	0.52	0.62	1.39	1.76	1.92	2.28	2.54	2.88	3.23	3.66	3.78
	2.	0.67	0.76	1.23	1.61	2.24	2.85	3.91	3.83	3.86	4.18	4.09
	3.	0.70	0.80	1.50	1.80	2.40	2.62	2.91				3.20
	4.	0.54	0.61	1.18	1.40	1.72	2.14	2.63	3.12	3.44	3.91	4.12
	5.	0.70	1.07	0.96	1.26	1.47	2.00	2.22	2.64	3.01	3.50	3.67
	M 1-5	0.63	0.77	1.25	1.57	1.95	2.38	2.84	3.12	3.39	3.81	3.77
R	6.	1.41	1.66	2.49	2.70	3.40	3.52	4.15	4.51	4.80		4.99
	7.	0.88	1.09	1.65	1.97	2.49	2.62	3.20				3.52
	8.	0.70	0.89	1.74	2.18	2.68						2.93
	9.	0.51	0.68	1.37	1.74	2.27	2.70	3.03				3.08
	10.	0.92	1.14	1.95	2.15	2.65	2.94	3.62	4.13	4.67	5.20	5.24
	M 6-10	0.88	1.09	1.84	2.15	2.70	2.95	3.50	4.32	4.74	5.20	3.95
	M 1-10	0.76	0.93	1.55	1.86	2.33	2.67	3.54	3.72	3.84	4.51	3.86

B - II

NR	1.	0.46	0.54	0.83	1.17	1.57	1.83	2.05	2.05	2.56	2.87	2.97
	2.	1.16	1.07	1.86	2.14	2.05	2.19	2.22				2.22
	3.	0.97	1.07	1.61	1.88	1.60	2.12					2.12
	4.	0.32	0.46	0.94	1.00	1.93	1.77	2.09	2.44	2.99	3.58	3.75
	5.	0.69	0.72	1.11	1.50	1.64	2.00	2.14				2.49
	M 1-5	0.72	0.77	1.27	1.54	1.76	1.98	2.13	2.30	2.78	3.23	2.71
R	6.	1.07	1.30	2.36								2.56
	7.	0.57	0.80	1.24								1.36
	8.	0.17	0.22	0.82	0.96	1.52	1.61	2.17				2.52
	9.	0.73	0.81	1.18	1.28	1.81	2.15	2.45	2.68	3.03		3.09
	10.	0.78	0.65	1.24	1.34	1.79	2.09	2.44	2.71	3.20	3.64	3.69
	M 6-10	0.66	0.76	1.37	1.19	1.71	1.95	2.35	2.70	3.12	3.64	2.64
	M 1-10	0.69	0.77	1.32	1.37	1.74	1.97	2.24	2.50	2.95	3.44	2.68

A: Before acclimation; B: After acclimation; LBNP - I: Before dehydration; LBNP - II: After dehydration; NR: Non-runners; R: Runners.

TABLE 17: DECREASE IN ARM VOLUME (%) UNDER LBNP

A - I

	Subj.	Torr: -20 Min: 2'	5'	-30 2'	5'	-40 2'	5'	-50 2'	5'	-60 2'	5'	Final
NR	1.	1.28	1.05	1.44	1.32	1.57	1.73	1.94	1.72	1.87	1.71	1.71
	2.	0.82	0.68	0.73	0.77	1.07	1.67	1.61	1.58	1.40		1.40
	3.	0.14	0.21	0.23	0.26	0.29	0.30	0.34	0.41			0.41
	4.	0.95	0.65	0.90	0.83	0.96	1.23	1.33	1.37	1.53	1.49	1.49
	5.	1.13	0.88	1.18	1.26	1.67	1.61	1.91	1.99	2.01	2.08	2.08
	M 1-5	0.86	0.69	0.90	0.89	1.11	1.31	1.43	1.41	1.41	1.76	1.42
R	6.	1.36	1.29	1.51	1.58	1.58	2.03	2.06				2.06
	7.	1.43	1.47	1.58	1.31	0.88						0.88
	8.	1.09	1.00	1.53	1.57	1.93						1.93
	9.	0.94	0.86	1.23	1.23	1.72						1.72
	10.	1.03	0.71	0.89	0.89	1.02	1.12	1.49	1.57	1.80		1.80
	M 6-10	1.17	1.07	1.35	1.32	1.43	1.58	1.78	1.57	1.80		1.68
	M 1-10	1.02	0.88	1.13	1.11	1.27	1.44					1.55

A - II

NR	1.	0.46	0.53	0.77	0.62	0.79	1.05	1.33	1.07	1.75		1.75
	2.	0.76	0.83	1.16	1.11	1.12						1.12
	3.	0.14	0.17	0.30	0.38	0.62	0.90					0.90
	4.	0.51	0.64	0.89	0.99	1.11	1.04	1.23	1.45	1.52	1.74	1.74
	5.	0.67	0.60	0.88	0.97	1.00	1.07					1.07
	M 1-5	0.51	0.55	0.80	0.81	0.93	1.02	1.28	1.26	1.64	1.74	1.32
R	6.	0.52	0.37	0.28								0.28
	7.	0.61	0.71	1.23								1.23
	8.	0.70	0.79	1.23	1.37	1.71						1.71
	9.	0.98	1.02	1.28	1.43	1.67						1.67
	10.	0.46	0.56	0.71	1.03	0.99	1.19					1.19
	M 6-10	0.65	0.69	0.95	1.28	1.39	1.19					1.22
	M 1-10	0.58	0.62	0.88	0.99	1.13						1.27

A: Before acclimation; B: After acclimation; LBNP - I: Before dehydration; LBNP - II: After dehydration; NR: Non-runners; R: Runners.

TABLE 18: DECREASE IN ARM VOLUME (%) UNDER LBNP

B - I

	Subj.	Torr: -20 Min: 2'	5'	-30 2'	5'	-40 2'	5'	-50 2'	5'	-60 2'	5'	Final
NR	1.	1.08	0.83	0.86	0.94	0.53	0.54	0.45	0.48	0.42	0.42	0.42
	2.	0.75	0.84	1.01	1.15	1.57	1.68	1.87	1.80	2.21	2.52	2.52
	3.	0.16	0.24	0.39	0.46	0.46	0.60	0.90				0.90
	4.	1.15	0.98	1.13	1.16	1.48	1.40	1.48	1.40	1.62	1.81	1.81
	5.	1.42	1.15	1.53	1.82	1.96	2.17	2.48	2.61	2.90	3.07	3.07
	M 1-5	0.91	0.81	0.98	1.11	1.20	1.28	1.44	1.57	1.79	2.06	1.74
R	6.	1.32	1.39	1.63	1.43	1.38	1.70	1.72	1.85			1.85
	7.	1.78	1.83	2.12	2.00	2.26	2.36	2.62				2.62
	8.	1.25	1.18	1.70	1.72	2.15						2.15
	9.	0.86	0.81	1.40	1.22	1.43	1.78	2.06				2.06
	10.	0.82	0.75	1.12	1.04	1.19	1.23	1.67	1.55	2.07	2.29	2.29
	M 6-10	1.21	1.19	1.59	1.48	1.68	1.77	2.02	1.70	2.07	2.29	2.19
	M 1-10	1.06	1.00	1.29	1.30	1.44	1.52	1.69				1.97

B - II

NR	1.	0.38	0.33	0.60	0.73	0.91	1.00	1.19	1.25	1.02	1.21	1.21
	2.	0.56	0.75	1.10	1.24	1.24	1.50	1.43				1.43
	3.	0.11	0.41	0.57	0.70	0.62						0.62
	4.	0.87	0.76	1.05	1.07	1.23	1.27	1.34	1.40	1.60	1.67	1.67
	5.	0.88	1.02	1.30	1.33	1.48	1.58	1.72	1.81			1.81
	M 1-5	0.56	0.65	0.92	1.01	1.10	1.34	1.42	1.49	1.31	1.44	1.35
R	6.	0.75	0.42	0.53								0.53
	7.	0.71	0.77	1.25								1.25
	8.	1.00	1.18	1.52	1.64	1.65	2.13	2.34				2.34
	9.	1.08	1.14	1.50	1.83	1.81	2.09	2.18	2.34	2.34		2.34
	10.	0.94	0.80	1.03	1.13	1.43	1.67	1.64	1.85	1.96	2.19	2.19
	M 6-10	0.90	0.86	1.17	1.53	1.63	1.96	2.05	2.10	2.15	2.19	1.73
	M 1-10	0.73	0.76	1.05	1.27	1.30						1.54

A: Before acclimation; B: After acclimation: LBNP - I: Before dehydration; LBNP - II: After dehydration; NR: Non-runners; R: Runners.

TABLE 19: LIMB COMPLIANCE UNDER LBNP

Slope of regression: Δ%Vol/ΔTorr x min. *

	Subj.	A-I	A-II	B-I	B-II	Total Average
			LEG			
NR	1.	325	316	348	274	
	2.	399	769	429	292	
	3.	508	495	535	354	
	4.	362	273	391	347	
	5.	401	570	322	329	
	M 1-5	399	485	405	319	402•
R	6.	678	1260	484	1162	
	7.	725	1181	516	719	
	8.	830	563	842	417	
	9.	691	286	551	343	
	10.	490	453	483	335	
	M 6-10	683	749	575	595	650•
	M 1-10	541	617+	490	457+	526
			ARM			
NR	1.	117	160	-32	108	
	2.	159	271	207	227	
	3.	45	194	136	205	
	4.	113	144	111	118	
	5.	175	192	245	200	
	M 1-5	122	192	133	172	155••
R	6.	260	101	153	177	
	7.	121	687	303	685	
	8.	442	437	511	362	
	9.	373	351	296	226	
	10.	158	223	179	173	
	M 6-10	271	360	288	325	311••
	M 1-10	196	276	211	248	233

*Slope values are multiplied by 10^5 for convenience.
• and • = Difference between these two values is significant ($p < .01$)
•• and •• = Difference between these two values is significant ($p < .01$)
+ and + = Difference between these two values is significant ($p < .02$)
A: Before acclimation; B: After acclimation; LBNP - I: Before dehydration; LBNP - II: After dehydration; NR: Non-runners; R: Runners.

PART II

TOTAL RESPIRATORY CONDUCTANCE BY THE FORCED OSCILLATION METHOD USING AIR AND HELIOX AS A SCREENING TEST. A PILOT STUDY.

ABSTRACT

Hypothesis: When a gas mixture of lower density than air such as Heliox (20% O_2, 80% He) is breathed, total respiratory conductance (TRC) will increase because respiratory flow is predominantly turbulent in the larger airways which offer most of the resistance. In patients with obstructive airway disease the increase in TRC will be less or absent if the main site of the obstruction is located in the small airways (< 2mm) where flow is laminar. To test the hypothesis 30 subjects were tested for TRC breathing air and Heliox using the forced oscillation (FO) method. Of these 24 were normal according to standard pulmonary function tests and 6 had a maximal midexpiratory flow (MMEF) below the normal range.

In the 24 normals TRC increased by 69% on transition from air to Heliox and in the abnormals only by 57% and the difference was statistically significant. In the normals and abnormals the correlation between MMEF/VC and TRC/FRC was statistically highly significant. The FO method using air and Heliox may be a sensitive and convenient method for the early detection of airway disease. Further studies on patients are necessary to explore its possibilities.

INTRODUCTION

The flow of gas in the respiratory passages is both turbulent and laminar in character with the latter predominating in the smaller airways (diameter $<$2mm). In healthy individuals the major fraction of airway resistance is located in the larger airways. Therefore flow rate increases for a given pressure gradient when a gas of low density is breathed instead of air. If the main site of resistance resides in the smaller airways, as is usually the case in emphysema, chronic bronchitis and some forms of asthma, the effect of low density gas is less pronounced or absent. This phenomenon has been utilized to determine the site of obstruction in cases with manifest obstructive disease (1) and also for detecting early small airway affliction in non-symptomatic smokers (2). This report presents preliminary results of an attempt to apply this concept to the forced oscillation (FO) method for measuring total respiratory conductance (TRC) in the hope that it might prove to be a more sensitive screening procedure for early pulmonary disorders than the FO method on air alone. Previous reports from this laboratory (Reports: February, 1970, Contract NAS 9-7009; February, 1973, Contract NAS 9-12572 and February, 1974, Contract NAS 9-12572) and elsewhere (3) have described the method and shown that it compares favorably with established methods for the evaluation of airflow such as the maximal midexpiratory flow (MMEF) from the flow-volume loop and the forced expired volume in one second (FEV_1). Moreover, it has the advantage of not requiring any special breathing maneuvers or effort on the part of the examinee. In 1974 (NAS 9-12572) we reported the results of a study on the effects of 100% O_2 on TRC as compared to air in 12 normal subjects. There was only a small increase in mean TRC (11%) but the difference was statistically highly significant attesting to the good discriminating power of the method. The studies performed so far using Heliox were directed toward establishing the order and magnitude of the change in TRC with Heliox and the reproducibility of the measurement in healthy subjects. A comparison was also made between TRC by FO with air and Heliox and the MMEF. Data on only a few abnormal cases are included to demonstrate some of the changes encountered in obstructive disease.

METHODS

The equipment has been modified since the previous report as follows. The low-frequency loudspeaker (woofer) used originally has been replaced by a dual piston, sliding diaphragm pump which provides a constant but adjustable stroke volume at variable frequencies (1-20cps). A stroke volume of 210ml was used in this study on adults. The orifice of the pump is sealed to a lucite tube 5 inches long and 1 inch ID. The other end is tapered to receive a disposable mouthpiece. Interposed in the tube is a Fleisch (No.2) pneumotachograph (heated) with a pressure transducer (Validyne, Model MP45, range ± 2cm H_2O). Another transducer of the same manufacturer (range ± 10cm H_2O) measures the differential pressure inside the tube close to the mouthpiece against ambient. The pressure signal is displayed on the x-axis and the flow on the y-axis of a Tektronix 502 oscilloscope at a sensitivity of 10mm per cmH_2O for the pressure and 5cm per L/sec for the flow. Filter circuits incorporated in the Validyne CD12 transducer indicators were used to eliminate frequencies above 10cps. Side ports in the breathing tube up and down stream of the pneumotachograph gave access to a bias flow of 0.5L/sec to minimize rebreathing. The length and diameter of the attached tubing was so chosen, that it provided adequate impedance to the higher frequency FO pulses without creating excessive resistance to breathing. The flow and pressure signals were also fed into an oscillograph recorder (Honeywell 906B) in parallel with the oscilloscope. From the time-based records synchronous flow and pressure deflections were measured at six intervals close to the endtidal level of a cycle or during the endexpiratory pause, if present. They were averaged from two breathes on air as well as on Heliox and then compared with the results obtained from the angle on the oscilloscope.

PROCEDURE

The subject wearing a noseclip breathes quietly through the device holding his hands to his cheeks to avoid flutter. The frequency of the pump is then adjusted to close the $\dot{V}/P$ loop on the screen at the resonant frequency of the individual's respiratory system, where the phase difference between pressure and flow approaches zero. The $\dot{V}/P$ angle is then noted with a rotating transparent overlay and TRC calculated using the appropriate

calibration factors. The measurement takes a few seconds and in this study was repeated three times after randomly altering the pump frequency before resetting. While the subject remains on the mouthpiece Heliox is admitted by turning a stopcock to a large breathing bag filled with the mixture. The subject is required to perform three full vital capacity maneuvers to flush the lungs with Heliox (20% O_2 - 80% He), where upon he breathes normally and the measurements are repeated. Since all measurements were taken at or close to the endtidal point the functional residual capacity was used as reference volume to obtain the specific respiratory conductance (SRC). The FRC was measured previously by the N_2 dilution method as well as the MMEF from repeated flow-volume loops.

RESULTS AND COMMENTS

Table 1 contains physical information on each subject with standard pulmonary function data as well as specific respiratory conductance (SRC) breathing air and Heliox with the difference in absolute figures and in percent on the right. Numbers 1 - 24 were normal volunteers. At the bottom are values on 6 abnormal individuals. However, only 4 - 6 were patients with a clinical diagnosis of chronic obstructive disease while 1 - 3 were also volunteers who were non-symptomatic but were found to have a MMEF/VC value of less than 0.50 which is considered to be the lower limit of the normal range (95 confidence level). Tables 2 and 3 give the three separate measurements of TRC with two methods: from the oscillograph record (Record) and read from the angle on the oscilloscope (Slope) to illustrate the reproducibility of the method within individuals. The mean difference between TRC on Heliox and on air shown on the right is the mean for each subject from all six measurements. This averaging appeared justified because there was no systematic difference between the two methods. The overall mean from the record was .444 and from the "slope" .435, a difference of less than 2% which is negligible. In the future measurements will be made by the "slope" only which is much less time consuming.

On the average for the 24 normals TRC (Table 2) increased by .230 or 69% on transition from air to Heliox with the individual values ranging from 30-114%. The large scatter may be attributable in part to incomplete

flushing of the lungs with three vital capacities. However, Hutcheon et al. (4) have shown after comparing the effect of Heliox on the flow-volume loop after having the subjects breathe the mixture for 10 min as compared to only three vital capacities that the latter gave very similar results. The shorter procedure makes this method much more attractive for screening purposes and less expensive. Incidentally one should remember that while the density of pure helium is only 13.8% of that in air, the mixture used here with 20% has a density of 31% compared to air.

In the "abnormal" group of 6 people the increase in TRC was .177 or 57% as compared to 69% in the normals. But this relatively small difference was statistically significant ($.02 < p < .05$). Although the difference in numbers makes the comparison rather lopsided. The values for SRC were .085 and .050 in the normals and "abnormals" respectively, this difference being more highly significant ($.001 < p < .01$) than that for TRC.

A number of regressions were tested with the MMEF as reference standard versus the change in TRC with either or both of these variables corrected for volume which cancels out some variance unrelated to the calibre of the airways.

1. $y = MMEF/VC$ $x = \Delta SRC$ $r = .641$, $p < .001$
2. $y = MMEF/VC$ $x = \Delta TRC$ $r = .538$, $p < .01$
3. $y = MMEF$ $x = \Delta SRC$ $r = .515$, $p < .01$

where Δ signifies increase with Heliox over air breathing and SRC = TRC/FRC. The correlation coefficients of all three regressions are statistically highly significant. But the first one, where both variables are corrected for volume appears to be the best, not only because it has the highest r value but also the highest confidence level. The regression line MMEF/VC (y), ΔSRC (x):

$$y = 0.14 + 7.76x$$

is plotted in Fig 1 with the points for all 30 subjects and one standard error (SE). It is noted the "abnormals" designated by x are bunched in the lower left corner of the graph. The lowest point on both coordinates belongs to a patient with severe chronic asthma and bronchitis. The fact that his SRC increased at all may signify that part of his obstruction is in the larger airways. However much more experience with this method is needed on patients and the normal group should be expanded to include children.

The most encouraging result emerging from this pilot study with the new method is the magnitude of the increase in TRC with Heliox. Previous studies (1) using helium with the flow-volume method and measuring MMEF resulted in an average difference of +48% in normal subjects, whereas the mean difference in our normal group was 69%. This suggests that the FO method is more sensitive to changes in gas density than the MMEF and therefore may have greater discriminating power in patients with different types of airway obstruction.

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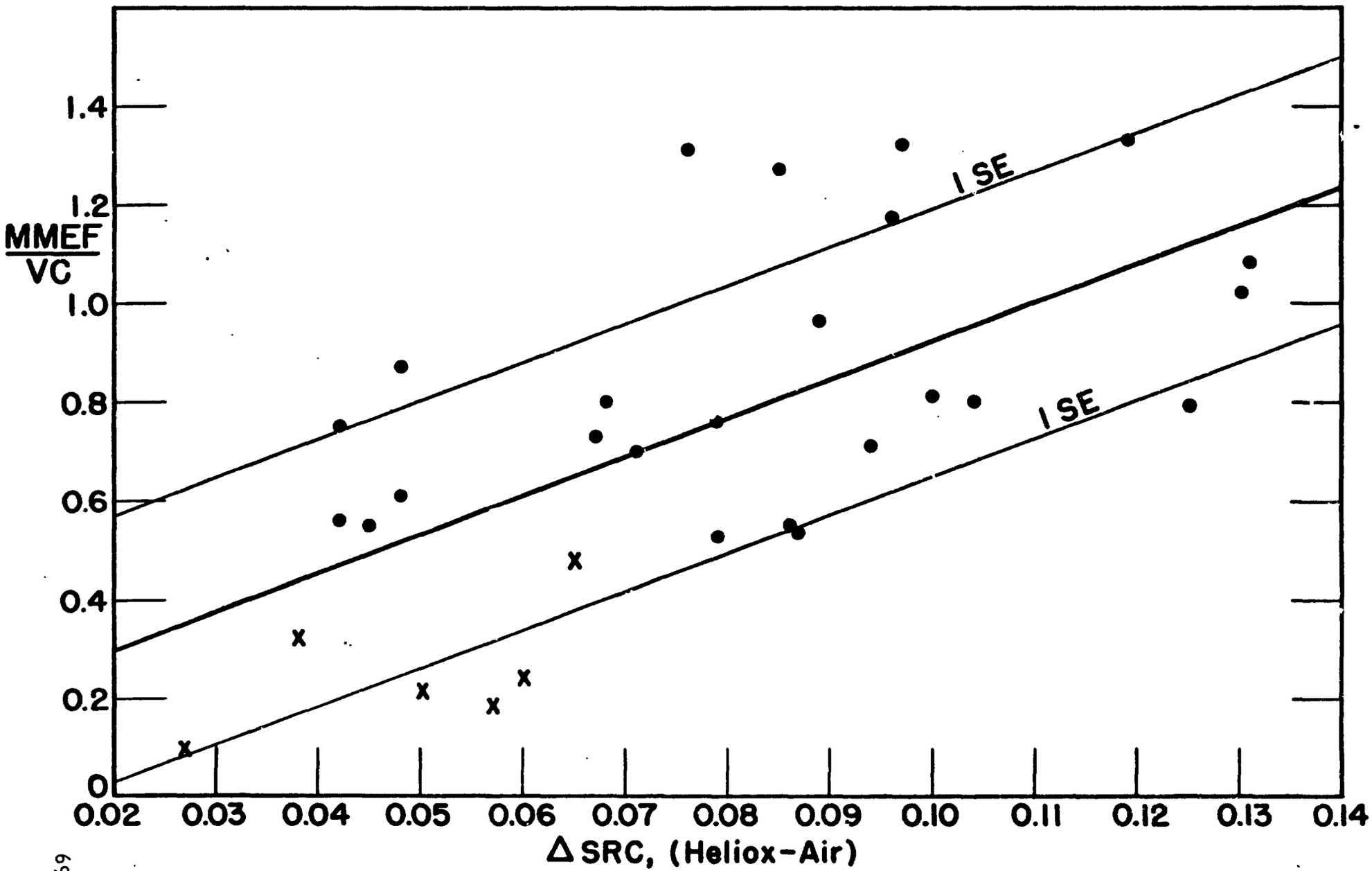

Figure 1. Correlation between MMEF/VC and the difference between specific respiratory conductance breathing heliox and air.

TABLE 1

Subject No.	Sex	Age (yr)	Ht (cm)	Wt (kg)	FRC (l)	VC (l)	MMEF (l/sec)	MMEF/ VC	SRC-Air TRC/FRC	SRC-He TRC/FRC	Δ (He-Air)	Δ %
						NORMAL						
1	M	31	174	71.9	2.29	5.00	5.38	1.08	.149	.280	.131	88
2	F	50	164	63.5	2.57	3.35	4.46	1.33	.173	.292	.119	69
3	F	29	170	72.5	3.13	3.92	5.13	1.31	.114	.190	.076	67
4	M	37	185	63.0	3.95	5.72	3.17	0.55	.091	.136	.045	49
5	M	33	183	82.5	2.87	4.68	3.56	0.76	.070	.149	.079	113
6	M	35	184	88.0	2.27	5.02	6.62	1.32	.179	.276	.097	54
7	M	30	188	81.0	3.73	6.69	5.85	0.87	.119	.167	.048	40
8	M	45	180	63.8	3.73	5.58	3.41	0.61	.160	.208	.048	30
9	M	28	183	63.9	4.47	5.75	3.23	0.56	.095	.137	.042	44
10	M	45	176	68.1	2.89	5.16	4.93	0.96	.122	.211	.089	73
11	M	28	173	59.9	3.00	5.39	3.92	0.73	.113	.180	.067	59
12	M	29	185	89.3	3.38	6.26	4.70	0.75	.043	.085	.042	98
13	M	29	173	76.7	3.26	5.78	3.19	0.55	.117	.203	.086	74
14	M	26	184	82.5	3.34	5.13	2.74	0.53	.073	.152	.079	108
15	M	37	174	63.9	2.67	4.69	4.77	1.02	.115	.245	.130	113
16	M	34	195	116.4	2.44	5.51	3.85	0.70	.121	.192	.071	59
17	M	27	168	59.6	2.65	5.21	4.15	0.80	.112	.180	.068	61
18	M	28	175	63.9	2.48	4.48	5.23	1.17	.107	.203	.096	90
19	M	32	174	73.3	1.88	4.33	3.41	0.79	.184	.309	.125	68
20	F	53	165	62.5	2.78	4.15	5.25	1.27	.084	.169	.085	101
21	F	21	184	54.0	2.80	4.35	2.37	0.54	.128	.215	.087	68
22	M	33	178	87.6	2.28	5.44	4.37	0.80	.119	.223	.104	87
23	M	28	175	75.5	2.32	5.31	4.31	0.81	.153	.253	.100	65
24	M	23	183	84.0	2.62	5.69	4.02	0.71	.101	.195	.094	93
						ABNORMAL						
1	M	31	179	69.0	3.75	6.22	3.00	0.48	.112	.177	.065	58
2	M	65	180	81.5	3.99	4.73	1.52	0.32	.107	.145	.038	36
3	M	47	173	65.0	4.42	4.79	1.07	0.22	.075	.125	.050	67
4	F	56	156	39.5	2.62	1.71	0.33	0.19	.078	.135	.057	73
5	M	81	165	58.0	2.21	2.11	0.50	0.24	.126	.186	.060	48
6	M	71	177	63.5	5.97	1.74	0.17	0.10	.033	.060	.027	82

TABLE 2

TOTAL RESPIRATORY CONDUCTANCE

NORMAL

Subj.		AIR Record	AIR Slope	HELIOX Record	HELIOX Slope	Mean Δ Heliox-Air absol.	Mean Δ Heliox-Air %
1.	a.	.316	.320	.635	.693		
	b.	.318	.346	.682	.616		
	c.	.375	.373	.580	.640		
	M	.336	.346	.632	.650	.300	88
2.	a.	.362	.320	.770	.697		
	b.	.415	.320	.737	.746		
	c.	.664	.581	.745	.802		
	M	.480	.407	.751	.748	.306	70
3.	a.	.354	.376	.604	.581		
	b.	.367	.361	.581	.581		
	c.	.332	.346	.639	.581		
	M	.351	.361	.608	.581	.239	67
4.	a.	.334	.333	.564	.550		
	b.	.388	.361	.553	.495		
	c.	.369	.376	.558	.495		
	M	.364	.357	.558	.513	.175	49
5.	a.	.200	.222	.495	.376		
	b.	.181	.187	.449	.410		
	c.	.223	.187	.404	.429		
	M	.201	.199	.449	.405	.227	114
6.	a.	.379	.410	.618	.616		
	b.	.428	.393	.604	.616		
	c.	.420	.410	.654	.654		
	M	.409	.404	.625	.629	.[illegible]	55
7.	a.	.447	.449	.634	.581		
	b.	.435	.449	.682	.616		
	c.	.429	.449	.638	.581		
	M	.437	.449	.651	.593	.179	41
8.	a.	.663	.616	.781	.802		
	b.	.623	.550	.767	.746		
	c.	.584	.550	.753	.802		
	M	.623	.572	.767	.783	.178	30
9.	a.	.425	.449	.636	.616		
	b.	.440	.376	.653	.550		
	c.	.430	.429	.609	.616		
	M	.432	.418	.633	.594	.189	45

TABLE 2a

Subj.		AIR Record	AIR Slope	HELIOX Record	HELIOX Slope	Mean Δ Heliox-Air absol.	Mean Δ Heliox-Air %
10.	a.	.361	.320	.576	.616		
	b.	.399	.320	.583	.616		
	c.	.338	.376	.644	.616		
	M	.366	.339	.601	.616	.256	73
11.	a.	.319	.320	.541	.495		
	b.	.367	.333	.529	.521		
	c.	.366	.333	.575	.581		
	M	.351	.329	.548	.532	.200	59
12.	a.	.134	.140	.271	.297		
	b.	.169	.130	.295	.297		
	c.	.141	.151	.276	.286		
	M	.148	.140	.281	.293	.143	100
13.	a.	.374	.376	.710	.654		
	b.	.423	.376	.669	.654		
	c.	.353	.393	.667	.616		
	M	.383	.382	.682	.641	.279	73
14.	a.	.136	.193	.538	.471		
	b.	.356	.230	.590	.471		
	c.	.308	.230	.527	.449		
	M	.267	.218	.552	.464	.266	110
15.	a.	.297	.286	.565	.616		
	b.	.340	.297	.586	.746		
	c.	.326	.297	.662	.746		
	M	.321	.293	.604	.703	.347	114
16.	a.	.286	.297	.446	.471		
	b.	.266	.308	.467	.495		
	c.	.295	.320	.456	.471		
	M	.282	.308	.456	.479	.173	59
17.	a.	.282	.320	.469	.495		
	b.	.287	.308	.470	.495		
	c.	.267	.308	.438	.495		
	M	.279	.312	.459	.495	.182	62
18.	a.	.259	.308	.446	.550		
	b.	.234	.297	.482	.550		
	c.	.214	.275	.442	.550		
	M	.236	.293	.457	.550	.239	91
19.	a.	.379	.308	.558	.616		
	b.	.316	.308	.567	.616		
	c.	.472	.297	.546	.581		
	M	.389	.304	.557	.604	.234	71

TABLE 2b

Subj.		AIR Record	AIR Slope	HELIOX Record	HELIOX Slope	Mean Δ Heliox-Air absol.	Mean Δ Heliox-Air %
20.	a.	.234	.275	.489	.521		
	b.	.225	.238	.451	.471		
	c.	.209	.222	.431	.449		
	M	.223	.245	.457	.480	.235	101
21.	a.	.348	.308	.661	.495		
	b.	.347	.320	.614	.581		
	c.	.462	.361	.680	.581		
	M	.386	.330	.651	.552	.244	68
22.	a.	.262	.265	.512	.471		
	b.	.272	.297	.531	.495		
	c.	.250	.286	.524	.521		
	M	.261	.283	.522	.496	.237	88
23.	a.	.329	.320	.528	.581		
	b.	.398	.333	.535	.616		
	c.	.386	.361	.603	.654		
	M	.371	.338	.555	.617	.232	67
24.	a.	.303	.238	.540	.495		
	b.	.242	.222	.534	.495		
	c.	.320	.256	.482	.521		
	M	.288	.239	.519	.504	.248	96
1-24	M	.335		.565		.230	69
	SD	.094		.105		.048	
	Coeff. var.	28%		19%		21%	

TABLE 3

TOTAL RESPIRATORY CONDUCTANCE

ABNORMAL

Subj.		AIR Record	AIR Slope	HELIOX Record	HELIOX Slope	Mean Δ Heliox-Air absol.	Mean Δ Heliox-Air %
1.	a.	.510	.410	.645	.697		
	b.	.397	.361	.653	.616		
	c.	.426	.410	.681	.697		
	M	.444	.394	.660	.670	.246	60
2.	a.	.447	.429	.590	.495		
	b.	.413	.410	.618	.550		
	c.	.435	.429	.630	.581		
	M	.432	.423	.613	.542	.150	35
3.	a.	.320	.308	.595	.550		
	b.	.325	.308	.572	.521		
	c.	.354	.361	.573	.521		
	M	.333	.326	.580	.531	.226	69
4.	a.	.192	.200	.361	.333		
	b.	.204	.214	.375	.346		
	c.	.207	.214	.371	.333		
	M	.201	.209	.369	.337	.148	73
5.	a.	.246	.297	.406	.449		
	b.	.209	.320	.377	.376		
	c.	.312	.286	.443	.410		
	M	.256	.301	.409	.412	.132	49
6.	a.	.197	.193	.364	.361		
	b.	.201	.200	.362	.361		
	c.	.203	.200	.357	.361		
	M	.200	.198	.361	.361	.162	82
1-6	M	.310		.487		.177	57
	SD	.101		.130		.047	
	Coeff. var.	33%		27%		27%	

PART III

VALIDATION OF THE ALCOHOL DILUTION METHOD FOR TOTAL BODY WATER AND FAT FREE MASS

ABSTRACT

In order to validate and possibly improve on a method for estimating total body water (TBW) by alcohol dilution measured by periodic breath analyses, a series of experiments were performed on 35 subjects to compare the results obtained by the alcohol method with the tritium (HTO) dilution method. Each subject ingested 25 μCi Tritiated water and 0.35 g/kg ethanol. Breath analyses were started 60 min thereafter, using an infra-red alcohol analyzer and repeated every 15 minutes until the blood level had dropped below 0.01g%. The blood alcohol concentration at zero time, as if absorption and distribution had been instantaneous was retropolated by least squares regression of the decay curve to calculate TBW. It was also calculated from the HTO activity in a urine sample taken 3-4 hours after ingestion of HTO. The results showed no systematic difference between the two procedures, the mean values being only 1.5% of body weight apart. The alcohol method has the advantage of being simple to perform, non-invasive, non-radioactive and can be repeated in less than 12 hours.

INTRODUCTION

Stimulated by some publications in the European literature about 20 years ago reporting the successful use of an alcohol dilution method for the estimation of total body water (1) this laboratory embarked upon a pilot study to explore the usefulness and reliability of this method and its applicability to the manned space program. At the same time we were aware that a simple non-invasive, non-radioactive method for total body water would be useful in the diagnosis and management of many different clinical conditions as well as a valuable research tool. The results of our preliminary study on only 10 subjects indicated that the alcohol dilution method using breath analyses compared quite favorably with the commonly used tritium method. However it appeared desirable to confirm these findings with a larger number of subjects and to optimize the experimental protocol particularly as to the minimum dose of alcohol necessary to obtain reliable results.

METHODS

Several changes were made in the analytical methods as compared to the previous pilot study. Blood alcohol levels were estimated from exhaled air using an Intoxilyzer (Omicron Corp. Model 4011) which is based on infra-red absorption. This instrument has both greater sensitivity and stability than the gas chromatograph (Alco-Analyzer, Luckey Laboratories) used before and is easier to calibrate with the breath simulator using freshly prepared alcohol solutions. The simultaneous HTO determinations were performed following the procedure used at the Johnson Space Center on the SKY-LAB Astronauts. The dose ingested was 25 μCi instead of 250 μCi used previously according to Logsdon et al. (2) and the measurements were made on distilled urine samples instead of on serum with a Beckman Liquid Scintillation System (LS-100C).

In view of the superior resolution of the Intoxilyzer the alcohol test dose could also be reduced from 0.5g/kg body weight to 0.35g/kg. Fat free mass (FFM) was determined on the subjects from body density by hydrostatic weighing as described previously (3).

PROCEDURE

The subjects reported in the early morning in the fasting state, but had been requested to drink about 8 oz of a non-alcoholic beverage on retiring the night before to ensure adequate hydration. The hydrostatic weighing was performed first whereupon they gave a baseline urine sample for the HTO measurement. Then they drank 10ml of water containing 25 μCi of HTO and flushed the container with another 20ml of pure distilled water. The alcohol solution was prepared to contain 0.35g/kg pure ethanol and diluted with water to make a 25% solution. A small teaspoon of dry cocktail mix was added to taste. The potion was consumed within a 15 min period. Measurements on the Intoxilyzer started 60 min after beginning the drink and were repeated at 15 min intervals until the readings on the Intoxilyzer showed 0.010g% or less. Each measurement consisted of three breaths from which a mean was taken and with the mean time gave a point on the alcohol elimination curve. Duration of the test varied from individual to individual ranging from 2 hr 17 min to 4 hr 33 min (average 3 hr 5 min). All subjects voided urine 90 min after receiving the HTO solution to ensure that the final test sample for HTO taken at least 3 hours after the drink was at complete equilibrium with body fluids. When alcohol in g% was plotted against time, all curves showed a linear decay after 90 min and the alcohol concentration at zero time could be retropolated from a least squares regression using all points from 90 min to the end of sampling. In this manner a minimum of 4 up to 13 points were obtained. Total body water by this method (TBW-Alco) was calculated from the equation given by Grüner in percent of body weight.

$$\text{TBW-Alco} = \frac{\text{Alco} \times 0.8}{\text{Co} \times 10}$$

where Alco = dose of alcohol in g/kg weight; Co = blood alcohol concentration at zero time (retropolated) and 0.8 the ratio: blood alcohol to total body alcohol (1) .

RESULTS

The results of the TBW determination with alcohol (TBW-Alco) and tritiated water (TBW-HTO) are presented in Table 1 with body weight and fat free mass (FFM) as well as the water fraction of FFM (TBW/FFM) in

the last columns. The data on the first 25 subjects were obtained by the methods described in this report, while the last ten subjects were studied two years before as reported previously (NAS 9-12572, 1974). It seems justified to incorporate their results with the others, since the experimental protocol was the same and the results were very similar.

The mean values from the two methods for TBW, both in percent of weight and in terms of liters, were in good agreement. The average for TBW-HTO was 1.0 liter or 1.5% higher than for TBW-Alco and the difference was statistically not significant. Of the 35 pairs of values 17 differed by less than 5%, 9 were between 5 and 10%, 8 between 10-15% and one between 15-20%. A graphic comparison of the two methods is plotted in Figure 1. The regression line is very close to the identity line and the correlation coefficient was $r = .896$ and statistically highly significant ($p < .0001$).

Because the fat fraction of total body mass contains a minimum amount of water the water content of FFM should presumably be much less variable than that of the body as a whole. Therefore the consistency of the ratio TBW/FFM should be good criterion of the reliability of any method for estimating TBW. In the last two columns of Table 1 it can be seen that both methods gave remarkably consistent results with the mean for TBW-Alco = 0.717 and for TBW-HTO = 0.735. The coefficients of variation are extremely small being 7.1% for the former and 4.9% for the latter method. In Figure 2 the concurrent values for TBW/FFM are plotted with the two regression lines.

TBW-Alco = -6.76 + 0.825 FFM, r = .906, SE 3.29 and

TBW-HTO = 1.64 + 0.708 FFM, r = .938, SE = 2.28

Both correlations are highly significant statistically but the HTO method appears to be slightly superior with a smaller standard error of estimate.

In view of the tight relationship between TBW and FFM the estimation of FFM from TBW with either method appears well justified, if direct measurements of body composition are not available.

Of the 35 subjects shown in Table 1, 14 were regularly engaged in a program for running or other endurance exercises. The following table summarizes a statistical comparison between the 14 runners and the 21 less active subjects using the results for TBW with the alcohol method.

	Wt kg	FFM	FFM/Wt	TBW%	$\frac{TBW(L)}{FFM}$
Runners (14) M:	74.4	66.1	.891	63.9	.718
Non-runners (21) M:	76.9	61.6	.809	57.9	.716
t	0.53	1.53	4.80	3.29	0.08
Probability	$p < .20$	$.10 < p < .20$	$p < .0001$	$p < .01$	$p < .20$

The non-runners weighed 2.5kg more than the runners, but had 4.5kg less FFM, neither difference being significant. However the two groups were clearly separated on the basis of FFM/Wt and TBW% of Wt, while TBW/FFM was practically identical in both groups. This again substantiates the fact pointed out earlier that the water content of FFM is highly consistent and is apparently the same regardless of the level of physical activity.

CONCLUSION

The results of this study in essence confirms and consolidates the conclusions reached in the preliminary experiments reported previously supported by a larger number of subjects and certain improvements in methodology. Although the HTO method may be slightly more precise, there is no strong statistical evidence for its superiority. On the other hand the alcohol method has several important advantages both for possible in-flight application and for general use in clinical medicine and research. The analytical procedures are much simpler and less time consuming and expensive. The procedure does not involve ingestion of radioactive material and could be repeated every 5-6 hours if necessary. With the reduced dose of 0.35g/kg, which has proved to be adequate to obtain a good decay curve, there are no adverse intoxicant effects.

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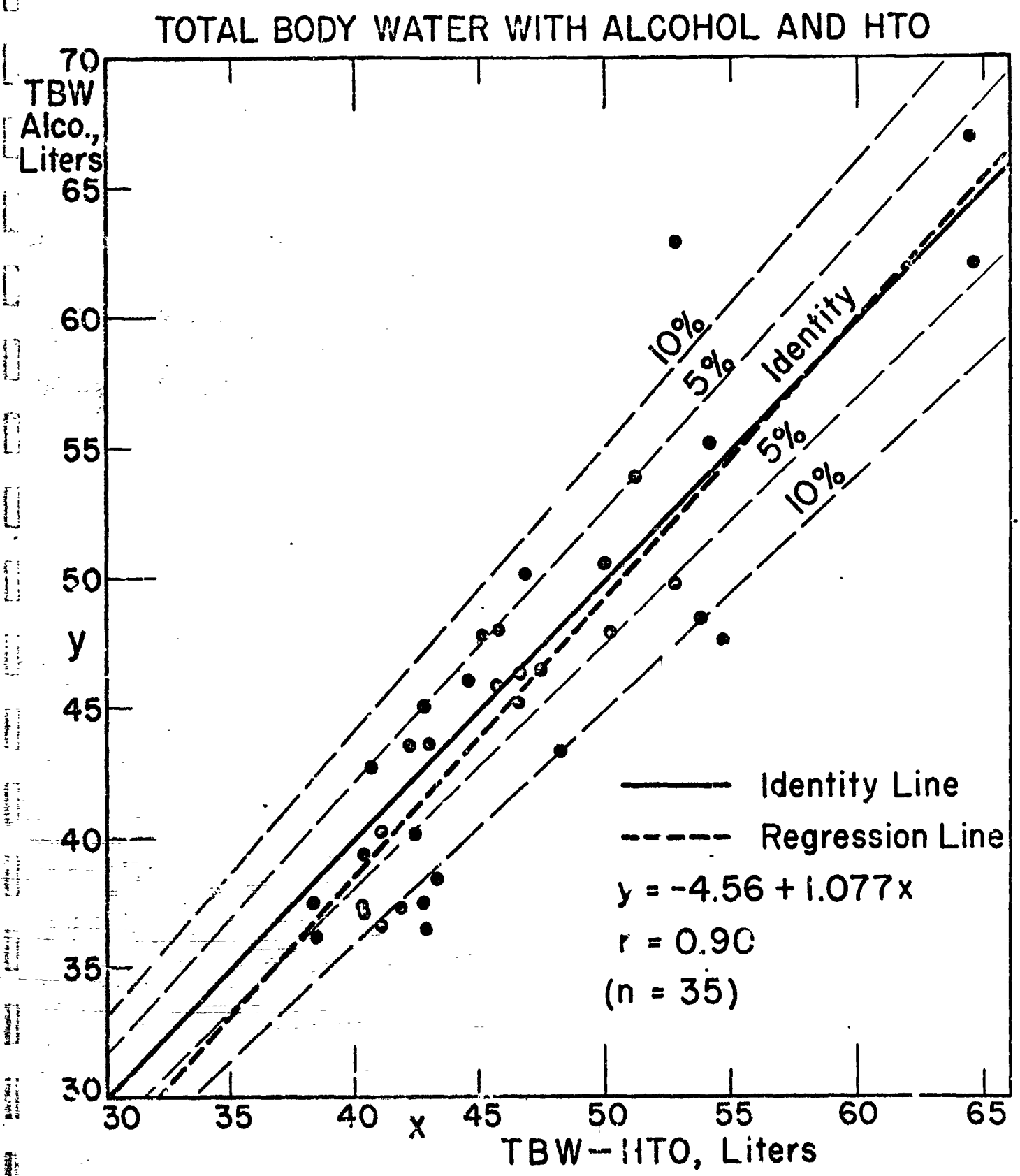

Figure 1

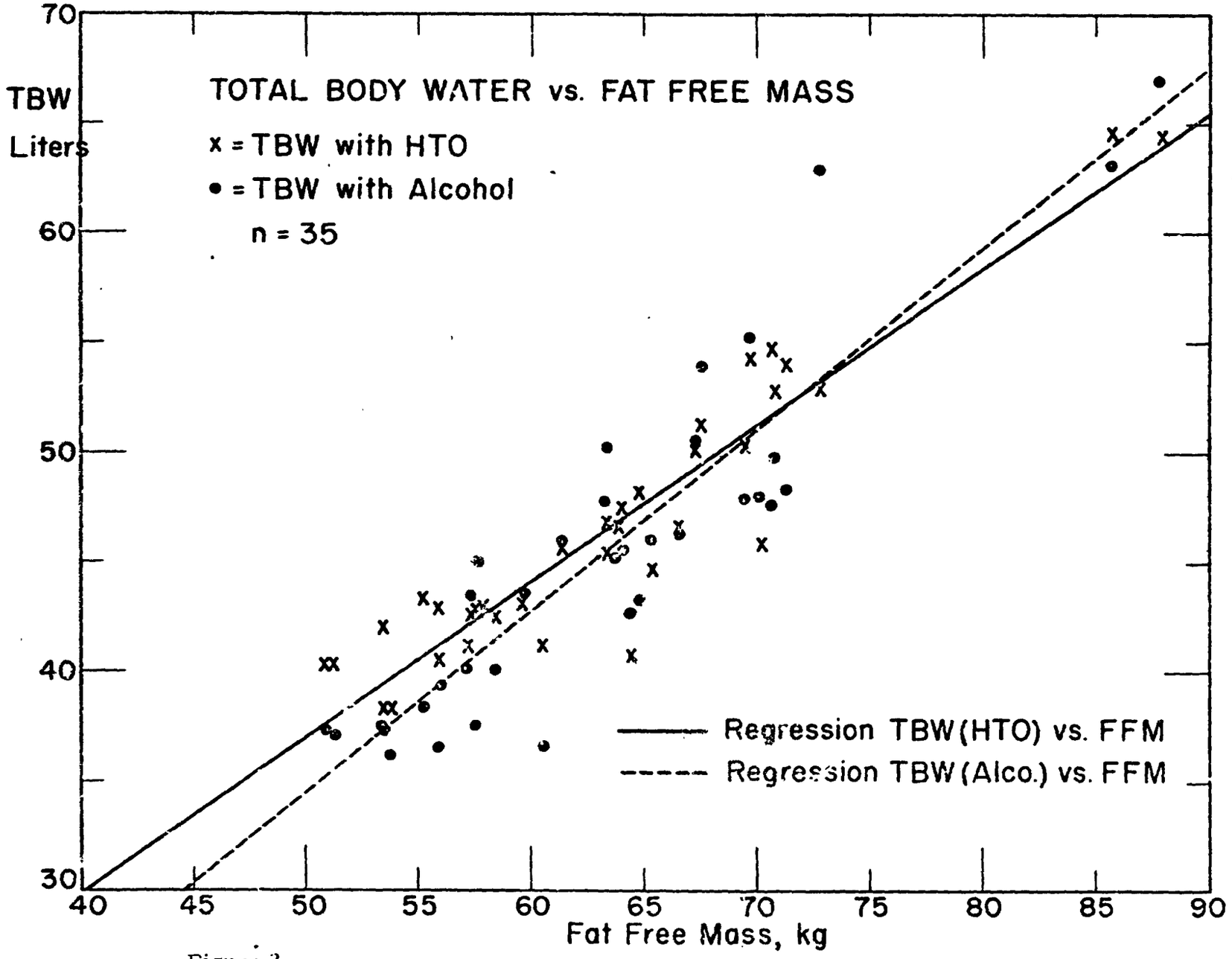
TOTAL BODY WATER vs. FAT FREE MASS
x = TBW with HTO
• = TBW with Alcohol
n = 35
TBW
Liters
70
60
50
40
30
40
45
50
55
60
65
70
75
80
85
90
Fat Free Mass, kg
Regression TBW(HTO) vs. FFM
Regression TBW(Alco.) vs. FFM

Figure 2

TABLE 1

No.	Wt kg	FFM kg	TBW% Alco.	TBW% HTO	TBW, Liters Alco.	TBW, Liters HTO	TBW/FFM Alco.	TBW/FFM HTO
1.	71.9	53.4	51.9	58.3	37.3	41.9	.699	.785
2.	73.7	64.8	58.8	65.4	43.3	48.2	.668	.744
3.	67.7	60.5	54.1	60.7	36.6	41.1	.605	.679
4.	59.9	55.1	64.1	72.3	38.4	43.3	.697	.786
5.	75.5	57.2	53.2	54.4	40.2	41.1	.703	.719
6.	81.0	69.7	68.2	66.9	55.2	54.2	.792	.778
7.	76.7	71.3	63.1	70.3	48.4	53.9	.679	.756
8.	63.9	57.6	58.6	67.0	37.4	42.8	.649	.743
9.	59.9	50.9	62.4	67.3	37.4	40.3	.735	.792
10.	81.4	67.6	66.2	62.9	53.9	51.2	.797	.757
11.	82.5	70.7	57.7	66.3	47.6	54.7	.673	.774
12.	70.6	63.4	71.1	66.3	50.2	46.8	.792	.738
13.	63.9	57.6	70.4	67.0	45.0	42.8	.781	.743
14.	82.5	67.3	61.3	60.6	50.6	50.0	.752	.743
15.	78.6	64.1	59.1	60.3	46.5	47.4	.725	.739
16.	88.0	72.8	71.5	60.1	62.9	52.9	.864	.727
17.	73.3	58.4	54.7	57.8	40.1	42.4	.687	.726
18.	71.9	59.7	60.6	59.8	43.6	43.0	.730	.720
19.	116.4	87.8	57.7	55.3	67.2	64.4	.765	.733
20.	59.6	51.1	62.2	67.8	37.1	40.4	.726	.791
21.	74.3	56.0	52.9	54.4	39.3	40.4	.702	.721
22.	77.2	66.6	60.0	60.4	46.3	46.6	.695	.700
23.	126.0	85.7	49.3	51.3	62.1	64.6	.725	.754
24.	77.7	69.5	61.6	64.7	47.9	50.3	.689	.724
25.	81.6	65.4	56.4	54.7	46.0	44.6	.703	.682
26.	60.4	57.3	72.0	69.9	43.5	42.2	.759	.736
27.	71.7	63.3	66.7	63.0	47.8	45.2	.755	.714
28.	68.4	64.4	62.4	59.5	42.7	40.7	.663	.632
29.	80.8	70.8	61.6	65.3	49.8	52.8	.703	.746
30.	79.1	70.2	60.7	57.9	48.0	45.8	.684	.652
31.	77.1	63.7	58.6	60.4	45.2	46.6	.710	.732
32.	64.2	53.4	58.4	59.7	37.5	38.3	.702	.717
33.	78.7	61.4	58.3	58.1	45.9	45.7	.748	.744
34.	67.9	55.9	53.8	63.0	36.5	42.8	.653	.766
35.	71.6	53.7	50.6	53.6	36.2	38.4	.674	.715
Mean:	75.9	63.4	60.3	61.8	45.5	46.5	.717	.735
SD:	±13.6	±8.6	±6.0	±5.2	±7.8	±6.5	±.051	±.036
Coeff Var:	17.9%	13.6%	10.0%	8.4%	17.1%	14.0%	7.1%	4.9%