Sodium balance, fluid homeostasis and the renin–aldosterone system during the prolonged exercise of hill walking

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Summary

1. The effect of 5 consecutive days of hill walking on electrolyte balance, fluid homeostasis, plasma renin activity and plasma aldosterone concentration was studied in five male subjects.

2. The 5-day exercise period was preceded by a 4-day control period and followed by a 4-day recovery period. Throughout the 13-day study subjects ate a fixed diet.

3. After 5 days of exercise subjects had retained a mean of 264 mmol (SD 85) of sodium. Plasma sodium concentration remained constant at 142.0 mmol/l (SD 5.4). This indicates an expansion of the extracellular space by 1.84 litres.

4. By the end of the exercise period there was a positive water balance of about 0.9 litre. Thus there was a net movement of 0.94 litre of fluid from the intracellular to the extracellular space.

5. Packed cell volume decreased from a mean of 43.5% to 37.9% after 5 days of exercise, indicating that about 0.9 litre of the extracellular fluid entered the vascular compartment. The remaining fluid may be responsible for the significant increase in lower leg volume.

6. During the exercise period plasma aldosterone concentration and plasma renin activity rose, and there was a highly significant correlation between these values and the sodium retention. There was also a significant correlation between sodium retention and the increase in leg volume, which suggests that oedema may be the result of prolonged exercise of this type.

Key words: aldosterone, exercise, extracellular fluid, oedema, plasma volume, renin, sodium balance.

Introduction

Williams et al. [1] found that exercise in the form of hill walking caused a retention of sodium and water, and a reduction in packed cell volume. These findings were interpreted as indicating a movement of water from the intracellular to the extracellular space and an expansion of the plasma volume. The responses seemed to be related to changes in the handling of electrolytes rather than of water, because the plasma concentration of arginine vasopressin did not change throughout the study, but the urinary sodium/potassium excretion ratio increased on stopping exercise, suggesting a change in the secretion of aldosterone.

Costill et al. [2] found a similar sodium-conserving response lasting for 36 h after a single period of exercise of 1 h duration. Smiles & Robinson [3] and Costill et al. [4] also found sodium retention when inducing heat acclimatization by performing successive days of exercise in the heat. In all of these studies the
renin–angiotensin–aldosterone system was implicated in the sodium-conserving response, but the hormonal basis of the response to successive days of exercise alone was not examined. The purpose of this study was to investigate possible mechanisms by which the exercise of a period of consecutive days of hill walking resulted in sodium retention and consequent alterations in fluid homeostasis, and to examine if these changes could be involved in the development of ankle oedema described by Williams et al. [1] as a response to hill walking.

Methods

Outline

The 13-day study was divided into three phases. During the first 4 days (the control period) subjects were semi-sedentary. For each of the next 5 days (the exercise period) they exercised by hill walking. During the final 4 days (the recovery period) they were again semi-sedentary.

Subjects

Five male subjects whose normal mode of life was semi-sedentary took part in this study. Their ages, heights and weights are given in Table 1.

Exercise

The exercise consisted of hill walking for 7–8 h daily, covering distances of 26–31 km at altitudes up to 1070 m. This form of exercise was similar to that described by Williams et al. [1] and increases the energy expenditure of the control days by about 70%. The weather was generally bad with high winds, rain and snow, and temperatures of about 0°C. Subjects were adequately clothed for the conditions and did not report feeling either chilled or over-heated.

Diet

Throughout the study subjects ate a basic diet consisting of 91 g of fat, 75 g of protein and 384 g of carbohydrate (calculated total energy content 11.2 MJ). It contained 140 mmol of sodium and 86 mmol of potassium (measured by chemical analysis). During the exercise period subjects ate an extra 7.3 MJ/day in the form of 437 g of electrolyte-free carbohydrate and fat. Measured volumes of electrolyte-free water were available ad libitum.

Electrolyte balance

To calculate electrolyte balance it is necessary to know electrolyte input and output. In this study input consisted only of the electrolytes in the diet; output comprised the electrolyte losses in the urine, faeces and sweat (electrolyte loss in the blood samples was small, and there was no loss by vomiting). Although it is possible in principle to measure faecal and sweat electrolyte loss, in practice it is extremely difficult to do so in field experiments. In this study we overcame this difficulty in the following way. If electrolyte input is constant it follows that when urinary electrolyte excretion is constant the electrolyte loss in the faeces and sweat is constant. So the equilibrium value of urinary sodium or potassium excretion can be taken as ‘input’ and no further correction is needed for faecal or sweat electrolyte loss. The urinary sodium and potassium excretions at the end of the control period (day 4) were therefore taken as input and 24-h electrolyte balances for the period 07.30–07.30 hours were obtained by subtracting from this figure the subsequent daily urinary excretion of sodium and potassium. From these data the cumulative electrolyte balance for each subject was calculated.

Water balance

Daily 24-h water balance for the period 07.30–07.30 hours was calculated by the method

| Table I. *Age, height, weight and body fat content of individual subjects* |
|---|---|---|---|---|
| Subject | Age (years) | Height (cm) | Body weight (kg) | Body fat (%) |
| | Before exercise | After exercise | Before exercise | After exercise |
| 1 | 23 | 185 | 64.1 | 64.1 | 14.3 | 13.2 |
| 2 | 48 | 175 | 74.8 | 75.2 | 17.2 | 16.4 |
| 3 | 43 | 183 | 80.0 | 80.2 | 15.5 | 14.4 |
| 4 | 53 | 178 | 68.8 | 68.7 | 12.1 | 11.3 |
| 5 | 34 | 178 | 75.0 | 75.0 | 17.1 | 16.9 |
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of Consolazio et al. [5]. Water balance is the difference between water intake and water output. Water intake consists of the volume of any water drunk, water in the diet and metabolic water. Water is lost in the urine and faeces, and as insensible water of perspiration and respiration. The methods used to measure or estimate each of these components have been described in detail by Williams et al. [11]. From the daily water balances the cumulative water balance was calculated for each subject.

Blood samples

For those measurements requiring blood or plasma samples, blood was withdrawn from an antecubital vein into ice-cold heparinized tubes which were stored on ice until centrifuged (at a temperature below 6°C). Plasma was separated and stored at -20°C for subsequent analysis. Samples were taken at 16.00 hours daily during the control and recovery periods, and within 2 min of stopping exercise during the exercise period.

Packed cell volume

The packed cell volume (PCV) of venous blood was determined daily using the Hawksley microtechnique. Duplicate tubes were set up on each occasion. Measurements were made immediately after sampling and are reported with no correction for trapped plasma, for differences between venous and whole body PCV, for possible changes in red cell volume due to changes in plasma osmolality or for the loss of red cells in the blood samples. This is justified because the important feature of these results is the change in packed cell volume induced by exercise, rather than the absolute value of this variable.

Plasma volume

Plasma volume was measured at the end of the control period (day 4), at the end of the exercise period (day 9) and at the end of the recovery period (day 13). Plasma volume was measured at about 17.00 hours. A blood sample was taken for the estimation of background radioactivity. Then 3-4 μCi of 125I-labelled human serum albumin was injected into an antecubital vein. Exactly 10 min later a blood sample was taken from the other arm. The plasma was counted for radioactivity with standards from each injected dose and plasma volume was calculated. The standard error of this method is approx. 2%.

Total body water

Total body water was determined in the early mornings of the same days as the estimates of plasma volume. To allow at least 5 h after the last food or drink, at 04.30 hours the subject emptied his bladder, a blood sample was taken and about 100 μCi of tritiated water was drunk. The exact dose was determined gravimetrically. At 07-30 hours the subject again emptied his bladder and an aliquot of the measured volume of urine was taken. A further blood sample was then taken. The 3H content of vacuum distilled water samples from plasma and urine was determined by liquid-scintillation counting by the method described by Haxhe [6], and the total body water was calculated. The standard error on an estimate of total body water by this method is about 3%, of which 1% is due to laboratory methods.

Aldosterone and renin

Plasma aldosterone concentration was measured by using a commercial radioimmunoassay kit [ALDOCTK-125, Sorin CIS(U.K.) Ltd.]. All plasma and calibration samples were run in duplicate. Plasma renin activity was determined by a radioimmunoassay of the rate of generation of angiotensin I at pH 5.5 by the method of Menard & Catt [7] modified only in that Dimercaptol was omitted from the mixture of angiotensinase inhibitors.

Body fat

The percentage of body fat in each subject was estimated from measurements made at the end of the control period (day 4) and after exercise on the final day of the exercise period (day 9) of skinfold thickness at four sites. Triplicate measurements were made on the right side of standing subjects using the method of Durnin & Rahaman [8]. The sum of the skinfold thickness was used to calculate body density from the equation:

\[ \text{Body density} = 1.721 - 0.0706 \times \log(\text{sum of four skinfolds}) \]

Body density was then converted to percentage body fat by using the equation of Siri [9]. The same site of measurement was used on both occasions.

Leg volume

The anthropometric method of Jones & Pearson [10] was used to quantify changes in the
volume of the leg below the knee. The subject stood erect with an inter-malleolar distance of 20 cm and the circumference of the leg at several locations was measured: (1) minimum circumference below the knee; (2) maximum circumference of the calf; (3) circumference at a mid-point between (2) and (4); (4) minimum ankle circumference; (5) circumference of the foot at the point mid-way between the middle of the medial malleolus and the first metatarsophalangeal joint.

The sites were marked with a dermographic pen, the measurements were made in triplicate by using a flexible steel tape and [except for site (5)] their heights above the floor were measured with a modified anthropometer. Each subject was measured daily from day 2 at a time between 20:30 and 22:30 hours. All measurements were made by the same person.

Statistical evaluation of results

Unless otherwise stated, all results were analysed by the two-factor analysis of variance technique. The factors used in the evaluation were subjects and days. Where days showed a significant difference, selected contrasts were carried out to ascertain the significance levels between days.

Results

Electrolyte homeostasis

Throughout the study there was no statistically significant change in the plasma sodium concentration. The mean value was 142.0 mmol/l (SD 5.3). Plasma potassium concentration also remained constant at 4.1 mmol/l (SD 0.4).

Mean 24-h urinary sodium excretion on days 1 to 4 was 167 mmol (SD 15), 110 mmol (SD 14), 132 mmol (SD 17) and 117 mmol (SD 14) respectively. The large fall in excretion from day 1 to day 2 suggests that the fixed diet eaten during the study contained less sodium than did the subjects' usual diets. The difference between sodium intake (140 mmol) and excretion on days 2, 3 and 4 is accounted for by the sodium lost in the faeces and sweat. On day 5 (the first day of the exercise period) sodium excretion fell to 77 mmol (SD 18), and for the remainder of the exercise period it showed no statistically significant change. The mean value for the whole of the exercise period was 66 mmol (SD 19). During the recovery period sodium excretion rose to 111 mmol (SD 32), 184 mmol (SD 24), 179 mmol (SD 32) and 160 mmol (SD 24) on days 10, 11, 12 and 13 respectively. As explained in the Methods section, sodium excretion on day 4 was used as the datum from which cumulative sodium balance was calculated.

All subjects retained sodium during the exercise period and unloaded it during the recovery period (Fig. 1). The group mean sodium retention after 5 days of exercise was 264 mmol (SD 85), but there were large differences between subjects. For example, by day 9 (after 5 days of exercise) subject 5 had accumulated 356 mmol of sodium compared with a retention of only 159 mmol by subject 4. The two subjects who retained the most sodium during the exercise period continued to retain sodium on the first day of the recovery period (day 10), whereas on this day the other subjects had already begun to unload sodium.

During the exercise period there was no significant change in potassium balance (Fig. 1). However, during the recovery period all subjects tended to retain potassium, with the greatest retention being in those subjects who had previously retained the greatest amount of sodium and who were therefore having the greatest sodium diuresis.

During the control period the Na/K urinary excretion ratio remained constant, the mean of all subjects throughout this period being 2.48 (SD 0.8). On the first day of exercise the ratio fell to 1.32. Throughout the exercise period the ratio remained at a mean value of 1.36 (SD 0.4). On
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FIG. 2. Effect of exercise on plasma aldosterone concentration and plasma renin activity in individual subjects.

the first day of the recovery period (day 10) the value rose to 4.05 (SD 1.0), and on days 11, 12 and 13 it was 4.24 (SD 0.6), 3.54 (SD 0.5) and 2.80 (SD 0.4) respectively.

Plasma renin activity and aldosterone were constant during the control period and rose sharply on the first day of the exercise period (day 5) (Fig. 2). They reached plateau values early in the exercise period and began to decline before the exercise period was over. By the first day of the recovery period (day 10), the levels were close to those found during the control period. There was great variation in plasma hormone levels between subjects, but the ranking of subjects was the same for both hormones, and was exactly that seen for sodium retention, which suggests a close relationship between these variables.

**Fluid homeostasis**

During the exercise period there was a small retention of water (Fig. 3), and there was a net loss of water accompanying the sodium diuresis during the recovery period.

Total body water increased significantly from a mean of 44.04 litres in the control period to 45.96 litres during the exercise period ($P < 0.01$ by paired t-test). Individual changes did not correlate with the level of sodium retention ($r = 0.3$). Total body water fell to a mean of 44.84 litres during the recovery period, but there was a considerable scatter between individual results;

FIG. 3. Effect of exercise on cumulative water balance (continuous lines) in individual subjects, and on the mean change of total body water (broken line), using the same scale. The bars represent 1 SD.
the broken line in Fig. 3 shows the change in total body water. The discrepancy between the calculated water balance (+0.12 litres) and the measured increase in total body water (+1.92 litres) on day 9 may be related to the metabolism of some body fat during the exercise period (Table 1). This has the effect of giving an erroneously low estimate of water balance, and is considered further in the Discussion section.

During the control period PCV remained constant at a mean value of 43.5% (SD 1.9). During each day of the exercise period there was a statistically significant \( (P < 0.01) \) fall in packed cell volume (Fig. 4) from a mean of 43.2% on the first day (day 5) to a mean of 37.9% on the final day (day 9). If red cell mass is assumed to remain constant, this represents an increase in plasma volume of 26% over control values.

The measured plasma volume (Fig. 4) rose by 25% during the exercise period, from a mean value of 3.6 litres on day 4 to 4.5 litres on day 9. However, because the scatter in the results was considerable, this change was not statistically significant (paired t-test). The plasma volume fell towards, but did not match, control values during the recovery period. At this point packed cell volume had not attained control values.

**Oedema**

Mean leg volume increased during exercise (Fig. 5), with the increases being statistically significant \( (P < 0.05) \) on the second day, and \( (P < 0.001) \) on the fourth and fifth days of exercise. During the first day of the recovery period the leg volume remained elevated \( (P < 0.001) \). Thereafter, it began to decrease until by the final day of the recovery period (day 13) the volume was not significantly different from control values.

The relationship between the changes in leg volume \( (LV) \) and the cumulative sodium balance \( \Sigma Na \) was examined by calculating for each subject the linear regression and correlation coefficients for the two variables to satisfy the general equation \( LV = a\Sigma Na + b \). The results are shown in Fig. 6. In each subject there was a high degree of correlation between the amount of sodium retained and the increase in the leg volume.

**Discussion**

**Electrolyte homeostasis**

The results of this study confirm the findings of Williams et al. [1] that the prolonged exercise of hill walking causes a cumulative retention of sodium. The mean sodium retention after 5 days of exercise was 264 mmol. It is first appropriate to consider the accuracy of this figure.

Because the amount of sodium lost in the faeces and sweat was not measured directly (see the Methods section), there is a possibility that
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Fig. 5. Change in the volume of the lower leg in individual subjects during exercise and recovery.

Fig. 6. Relationship between the change in volume of the leg and cumulative sodium retention in individual subjects. The correlation coefficient ($r$) was as follows: subject 1, 0.82; 2, 0.64; 3, 0.59; 4, 0.71; 5, 0.76.

The positive sodium balance merely represents the amount of sodium lost in the faeces and sweat during the exercise period. This was considered in some detail by Williams et al. [1] who concluded that the net effect of ignoring the faecal and sweat loss of sodium in excess of that on the control days would result in a calculated sodium retention about 60 mmol too high after 5 days of exercise. In addition, there is no doubt that at the end of the exercise period large amounts of sodium were lost in the urine (this was measured directly). This sodium had to come either from sodium stored during the exercise period, or from the body's normal supply of intracellular and extracellular sodium. Since the plasma sodium concentration remained constant throughout the
study, it seems highly unlikely that the amount of sodium lost in the urine on the recovery days could have come from depletion of previously normal reserves of sodium, thereby inducing a considerable sodium deficit. It seems more probable that the sodium lost during the recovery period was that stored during the exercise period, and that homeostatic forces were responding to some stimulus to restore the sodium content to normal.

During the control period plasma renin activity and plasma aldosterone concentration remained constant (Fig. 2). However, on the first day of exercise the hormone levels rose sharply and remained elevated throughout the exercise period. At the end of the exercise period the levels returned to control values. It seems probable therefore that the retention of sodium was a direct consequence of the elevated plasma aldosterone concentration, which in turn is a consequence of the elevated plasma renin activity. The loss of sodium in the recovery period would therefore be caused by the removal of the sodium conserving stimulus.

Some evidence to support this contention comes from the consistency of rankings between the three variables; subjects with the highest plasma renin activity also had the highest plasma aldosterone concentration and the highest sodium retention. This relationship was repeated for each subject (see Figs. 1 and 2), and the correlation on an individual basis both between the two hormones ($r = 0.84-0.97$) and between aldosterone concentration and sodium retention during exercise ($r = 0.75-0.86$), was very close.

The effect of exercise on renin activity has been studied by a number of authors [11-14]. These workers showed that exercise of short duration (15-30 min) resulted in increased plasma renin activity lasting from 20-60 min, depending on the severity of exercise. Bozovic et al. [15] showed that longer term exercise (an 85 km ski race) also resulted in increased plasma renin activity. Costill et al. [2] showed that a single bout of exercise lasting 1 h caused reduced urine output, sodium retention and increased plasma aldosterone concentration. The effect was apparent throughout the day of exercise and sodium was retained on the following recovery day. For the full effects of this mechanism on sodium and fluid homeostasis to be seen, the exercise would need to last for several days (as it did in the present study).

The stimulus giving rise to increased plasma renin activity during exercise is uncertain. It seems probable that the two most important factors are increased plasma concentrations of catecholamines and increased sympathetic nerve stimulation [16]. Kotchen et al. [12] suggested that the latter was the most important mechanism, and showed that renin secretion was correlated with the severity of the exercise. This may explain why in our experiment the ranking of the sodium retention matched the subjective feeling of fatigue as expressed by the subjects during the exercise period. Kotchen et al. [12] also suggested that a reduction in renal blood flow was a crucial factor in the events leading to renin release during exercise. Because the hypoxia of altitude reduces renal blood flow, this mechanism may explain the intimate relationship between exercise, hypoxia, and acute mountain sickness [17].

**Fluid homeostasis**

In the present study the calculated cumulative water balance was +0.12 litres by the end of the exercise period. However, as has been pointed out, these calculations assume a constant body composition. If fat is metabolized, the loss of body weight is calculated as insensible water loss, and this gives an erroneously low water balance. A further error is introduced because each gram of fat metabolized produces 1.07 ml of water. However, to offset this the ‘respiratory factor’ (the correction for the fact that the weight of carbon dioxide eliminated is not equal to the weight of oxygen taken in) is altered in the opposite direction when more fat is metabolized. Thus the net effect is that for each 100 g of fat metabolized the calculated water balance will be 137 ml too low.

Estimates of body fat content were made with this problem in mind. All subjects showed a reduction in the fat mass after exercise, with a mean loss of 0.8% of body weight (about 580 g of fat). It is probably not justifiable to use this figure to calculate the actual error in fluid balance, but only to point out the general direction and magnitude of any correction. In this experiment the calculated water balance would be about 800 ml too low by the end of the exercise period. This would result in a net water balance of about +920 ml at the end of the exercise period and of about 0 at the end of the study (uncorrelated value −900 ml). The calculated mean water balance of +920 ml is within the limits of error of the mean increase in total body water of +1.92 litres (3% of about 45 litres).

During the exercise period, the mean sodium retention was 264 mmol and the mean plasma sodium concentration was 142 mmol/l. Assuming that the intracellular sodium concentration is unchanged and knowing that there was no significant change in extracellular sodium con-
centration we can predict that for every 142 mmol of sodium retained the extracellular space is expanded by 1 litre. Thus for 264 mmol of retained sodium, the extracellular space is expanded by 1.84 litres. There was a retention of about 0.9 litres of water, so that part of the expansion of extracellular fluid was caused by water retention. The remainder (0.94 litres) must have been caused by a movement of water from the intracellular to the extracellular compartments. The concept that in some cases oedema can be caused by a shift of fluid from intracellular to extracellular compartment rather than by an increase in total body water is supported by the finding that most patients with cor pulmonale who become oedematous do so without an increase in body weight [18]. It is of interest to consider the fate of this extracellular water.

PCV was reduced on starting exercise and returned towards control values at the end of the recovery period. This is interpreted as an expansion of the plasma volume with exercise and its return during recovery. Arguments for this interpretation have been given in full by Williams et al. [1]. They are now supported by measurements of plasma volume which show an increase of 0.9 litres during the exercise period. Pugh [19] showed a similar reduction in PCV and increase in blood volume after one day of hill walking. The increase in plasma volume can be viewed as a part of the increase in extracellular fluid. However, whereas the change in PCV was the same for all subjects, there was a wide range of sodium retention (and hence of increase in extracellular fluid). This would suggest that the mechanism for plasma volume expansion is not necessarily the same as that for sodium retention. Just what that mechanism may be is not clear and deserves further study. The fate of that extracellular fluid which did not enter the vascular compartment is not certain, but it may be the cause of the increase in leg volume measured in the present experiment.

Leg volume and oedema

There was a significant increase in leg circumference in all subjects, providing objective evidence for the previously anecdotal contention that oedema is present during hill-walking. The frank pitting oedema illustrated by Williams et al. [1] was not seen even in their subject, but the increase in lower leg volume is interpreted as sub-clinical oedema. It is suggested that fluid in the interstitial space is free to gravitate to the dependent parts of the body resulting in an increase in leg circumference and in more extreme cases in frank oedema.

Other explanations for the increase in leg volume should be considered. For example, it may be due to hypertrophy of the leg tissue. This explanation has been rejected for two reasons: firstly, it seems improbable that the time scale of the changes in tissue build up and loss would match exactly the changes in accumulation of sodium; and secondly, if tissue hypertrophy is the cause of the increased leg volumes, the close correlations between leg volume and sodium retention would be spurious, and this seems improbable.

The findings of the present study document the responses to the prolonged exercise of consecutive days of hill walking. A stimulus (or series of stimuli) gives rise to increased production of renin. In turn this elevates plasma aldosterone concentration which results in sodium retention (as in secondary hyperaldosteronism). Sodium retention is accompanied by a positive water balance. The fluid retained expands the extracellular space, some causing an expansion of plasma volume with concomitant reduction in packed cell volume, and some entering the interstitial space where it is free to gravitate to the dependent parts of the body. There is also some redistribution of fluid from the intracellular to the extracellular fluid compartment. These general disturbances may be factors which contribute to the formation of high-altitude pulmonary and cerebral oedema.

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