THE EFFECTS OF DIETARY SODIUM INTAKE ON HEAT ACCLIMATION AND THERMOREGULATION DURING HEAT EXPOSURE

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ABSTRACT

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THE EFFECTS OF DIETARY SODIUM INTAKE ON HEAT ACCLIMATION AND THERMOREGULATION DURING HEAT EXPOSURE

by Adrian John Allsopp

The effects of dietary sodium intake upon the physiological heat acclimation responses were investigated over 8 days. In a 'temperate' study (25°C), two dietary sodium groups: MNa - 170 mmol/d (n=4); and MLNa - 170 mmol/d reduced to 70 mmol/d from day 4 (n=5), were examined. In two 'hot' investigations subjects were exposed to 25°C for 3 days, followed by 40°C (between 0800h and 1800h) for 5 days whilst dietary sodium intake was controlled as follows: HNa - 340 mmol/d (n=7); MNa - as above (n=9); LNa - 70 mmol/d (n=9); and MLNa - as above, n=8.

In the 'temperate' environment, sodium balance was restored by a reduction ($P<0.05$) in renal and sweat excretion. The MLNa group experienced a greater ($P<0.05$) loss of body weight and plasma volume than the MNa group. In the 'hot' environment sodium balance was maintained for the HNa and MNa groups, but LNa and MLNa experienced a deficit upon heat exposure, which tended to be smaller and restored more rapidly ($P<0.1$) in the LNa group. Plasma aldosterone increased upon heat exposure and this was potentiated ($P<0.05$) by a reduced sodium intake (LNa). The increase of aural temperature during exercise diminished as heat acclimation progressed, the magnitude of this change being smaller ($P<0.05$) for MLNa compared to all the other subjects (HNa, MNa and LNa).

These results support the hypothesis that aldosterone secretion in the heat is potentiated by prior reduction of sodium intake. Furthermore, thermoregulation is attenuated by a negative sodium balance. Thus dietary sodium manipulation has implications for heat acclimatisation and the avoidance of heat illness.

CONTENTS

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[1] - MNa: 170 mmol/d; MLNa: 170 mmol/d reduced to 70 mmol/d on day 4; 25°C: temperature throughout.

[2] - HNa: 340 mmol/d; MNa: 170 mmol/d; 40°C: temperature between 0800h and 1800h on days 4 to 8.

[3] - LNa: 70 mmol/d; MLNa: 170 mmol/d reduced to 70 mmol/d on day 4; 40°C: as above.

Figure *Page*

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[4] - MLNa: 170 mmol/d reduced to 70 mmol/d from day 4; MNa: 170 mmol/d; 25°C: environmental temperature throughout.

[5] - HNa: 340 mmol/d; MNa: 170 mmol/d; 40°C: environmental temperature between 0800h and 1800h from day 4.

[6] - LNa: 70 mmol/d; MLNa: 170 mmol/d reduced to 70 mmol/d from day 4; 40°C: environmental temperature between 0800h and 1800h from day 4.

[7] - HNa: 340 mmol/d; MNa: 170 mmol/d; LNa: 70 mmol/d; MLNa: 170 mmol/d reduced to 70 mmol/d from day 4; 40°C: environmental temperature between 0800h and 1800h from day 4.

"La fixité du milieu interne est la condition de la vie libré."

- Claud Bernard

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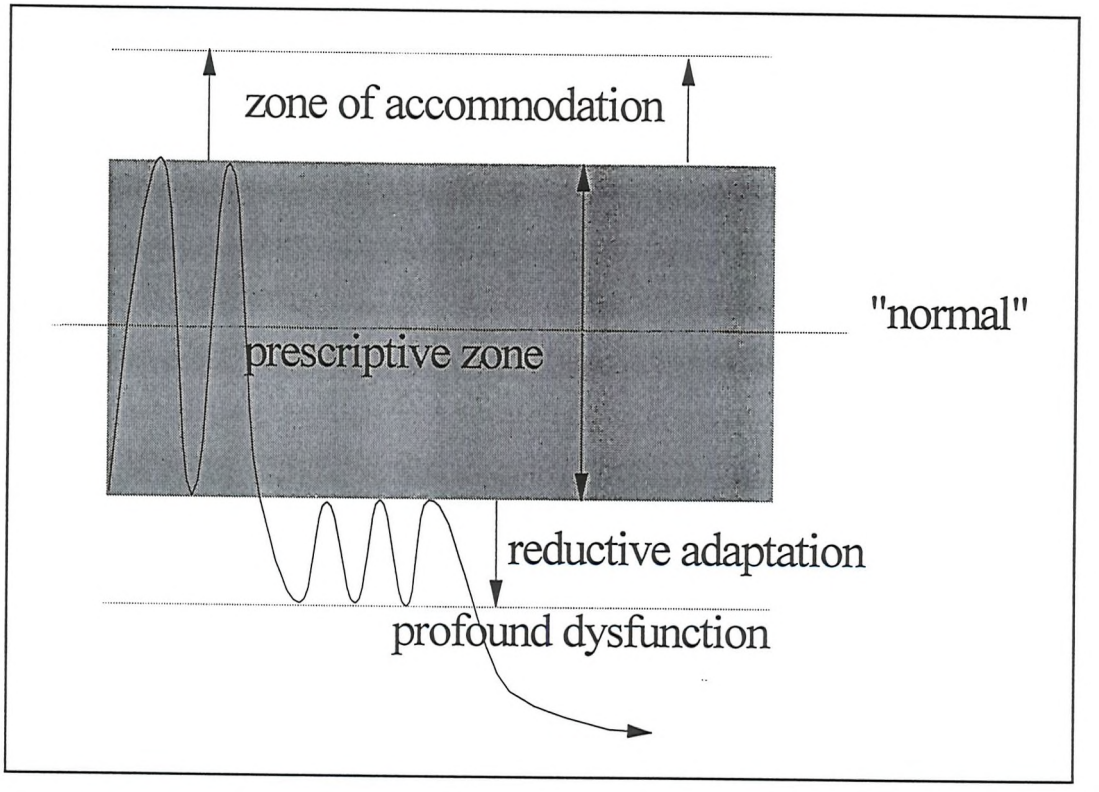
CHAPTER 1.

INTRODUCTION.

For the human body to remain in thermal balance, heat loss must equal the heat gain from metabolism and the environment. Upon heat exposure, compensatory responses are initiated which facilitate the greater heat dissipation required to preserve this balance. These responses, such as an increased peripheral blood flow, help to maintain the body temperature within a narrow prescriptive zone. It should be noted however, that such responses are a "reductive" adaptation in the sense that a "cost" is incurred; in the example above, the greater demand for cardiac output to maintain skin blood flow. Repeated stimulation results in physiological adaptation, as occurs with heat acclimatisation following successive days of exposure. This adaptation is clearly of benefit since following acclimatisation the body can thermoregulate more efficiently at higher levels of thermal stress. If however, in stimulating these compensatory responses body temperature is driven beyond the prescriptive zone, then the "cost" associated with this becomes more severe, - as in cardiovascular insufficiency consequent to heat exhaustion. This theoretical model is presented in Figure 1.1. From the above generalised example of a balance model, it can be seen that whilst it may be desirable to stress the system to initiate adaptation, over stimulation may have profound non-desirable consequences. This thesis investigates whether one such system, sodium balance, can be manipulated to achieve a desirable effect, that of improved heat acclimatisation, without any impairment.

A high rate of sweat production during heat exposure will increase the loss of body sodium, and may lead to sodium depletion, particularly if sweating is prolonged and the individual is unacclimatised. Following heat acclimatisation however, although sweat production is higher, the sweat is more dilute and the sodium loss via this route is reduced. Sweat sodium loss is regulated physiologically by aldosterone, but its action upon the sweat gland to increase sodium reabsorption requires two or three days of successive heat exposure. As aldosterone action appears to be the only mechanism whereby sweat sodium can be regulated (unlike renal loss of this ion) its control is critical to sodium balance in these circumstances. In addition, it has been demonstrated previously that alterations in dietary sodium intake affect aldosterone secretion.

Figure 1.1. General model of adaptation.



According to the general model above, any homeostatic mechanism (e.g. body temperature) is regulated within a prescribed zone. A stimulus (e.g. metabolic heat production) may force the system to operate beyond this prescriptive range, but within a zone of accommodation, with only relatively minor reductions in regulatory control; for example a reduced cardiac reserve in response to elevated body temperature through the shunting of blood flow for peripheral cooling. A major perturbation of the system however, (e.g. an excessive heat load), may push the control system beyond this boundary with profound consequences, as occurs in heat stroke.

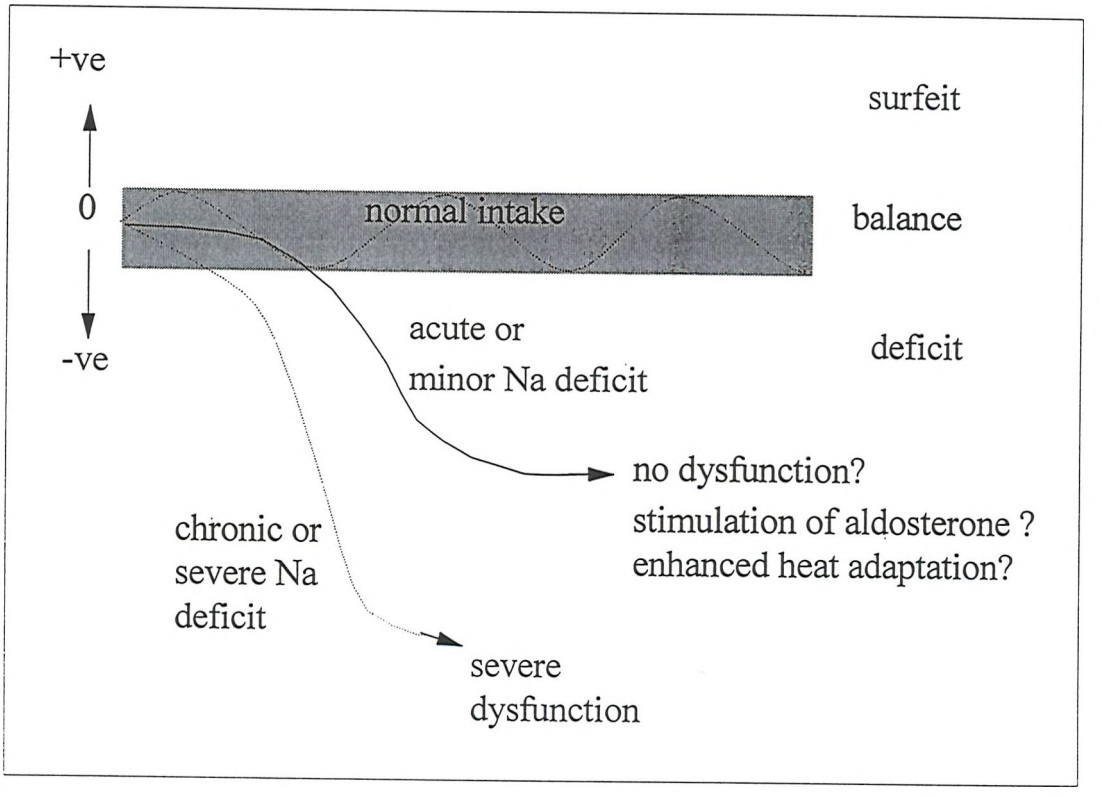
Increased sodium intake in the heat is commonly advocated, particularly if unacclimatised. This advice, however, gives little consideration to the general balance model which has been described above, indeed it is directly counter to it. Supplementation is based upon earlier work which was inadequately controlled with respect to either the dietary sodium intake or the time course for renal compensation to ingestion of sodium. Furthermore, it is apparent that the human body can adapt to hot environments and low sodium intakes without harm and there is a general trend supported by Governmental advisory bodies for a policy of reducing dietary sodium intake. Assuming aldosterone secretion to be the key compensatory mechanism in this model of sodium balance in the heat, then it is feasible that an adaptive response could be stimulated by manipulation of dietary sodium (Figure 1.2). Hence this thesis investigates the following hypothesis:

> *Sodium intake can be manipulated sufficiently to stimulate aldosterone secretion, reducing the loss of sodium in the sweat and so maintaining extracellular fluid volume. As a consequence thermoregulation in the heat will be improved.*

To be practical, potentiation of the aldosterone response needs to be effected without incurring any severe dysfunction associated with the reduced sodium intake. This thesis examines the inter-relationships between dietary sodium, aldosterone secretion, heat acclimation, and subsequent effects upon thermoregulation. In so doing, it attempts to draw conclusions regarding advice for sodium requirements during heat acclimatisation.

In view of the possible implications of this work for human performance in the heat, this work has been sponsored by the Department of the Surgeon General, within the Ministry of Defence and conducted at the Institute of Naval Medicine. The military scenario in mind was the rapid deployment of specialised forces to a hot location, with limited logistical support and inadequate time to acclimatise.

Figure 1.2. Putative mechanism of sodium balance upon aldosterone secretion.



The previous general model (Figure 1.1) has been applied to sodium balance to give the working model above. In this model, a sodium deficit which is beyond the prescribed zone but within the zone of accommodation, causes an adaptive reduction, (a reduction of effective circulating blood volume) which stimulates aldosterone secretion. This is hypothesised to potentiate heat acclimation through improved sodium retention. A greater sodium deficit, however, is not accommodated and may result in a severe dysfunctional response, such as heat syncope for example.

CHAPTER 2.

REVIEW OF THE LITERATURE.

Introduction.

This review will firstly examine the responses to heat exposure and the mechanisms for heat acclimatisation with particular reference to the control of sweating. Present advice regarding sodium requirements in the heat, with reference to current guidelines for military personnel, and how this requirement is altered by acclimatisation status will then be considered. The principles of sodium and volume homeostasis are included together with the actions of relevant regulatory hormones including arginine vasopressin (AVP), the renin-angiotensin system, atrial natriuretic peptide (ANP) and aldosterone. The final section of this review will give an account of the control of aldosterone secretion before considering the role of this hormone in heat acclimatisation. The intention of this review is to illustrate the links between heat acclimatisation and sodium balance, and to highlight those areas in which current knowledge is incomplete with regard to these two factors.

Heat Exposure.

On initial exposure to heat or increased metabolic heat production, the primary response is a rise in body temperature. This stimulates the mechanisms for heat dissipation: peripheral vasodilatation and sweating. These responses are neurally controlled with the hypothalamus being the central point of integration. Re-establishment of body temperature then depends upon the balance of heat flux between the increased thermal conductance and evaporative sweat loss compared to the degree of heat load from the thermal burden. Hence heat exposure causes peripheral vasodilation, increased peripheral blood flow and, with elevation of body temperature, sweating. These physiological responses facilitate the loss of heat through convection and evaporation of sweat. However, they constitute a threat to the cardiovascular system by the concomitant reduction in circulating blood volume, and gradual salt and fluid loss. The immediate threat to circulating blood volume is diminished by a reduced blood flow to the splanchnic and renal vasculature (Rowell, 1983) and hence a dramatic fall in urinary output and urinary sodium excretion

(Poortmans, 1984). The reduced renal blood flow and subsequent reduced glomerular filtration rate conserve body sodium.

Heat Acclimatisation.

Heat acclimatisation results in compensatory responses which lessen the effects of heat exposure and assist thermoregulation. These include an increased blood volume, improved peripheral blood flow and increased sweating.

Cardiovascular Changes.

Secondary to the increased dissipation of heat, there is a redistribution of blood, primarily through a reduced renal and splanchnic blood volume. This change in circulation is necessary in order to maintain cardiac output and blood pressure in the face of extensive peripheral vasodilatation (Rowell et al, 1967). With the onset of acclimatisation over a period of days, there is a marked improvement in circulatory efficiency such that blood and extracellular fluid volumes increase by up to 15-20 % and cardiac output is maintained by an increased stroke volume with a parallel reduction in heart rate (Rowell et al, 1967; Rowell, 1983).

Haemodynamic Changes.

Changes in stroke volume and cardiac output have been reported in the literature but it is difficult to differentiate between heat acclimation *per se* rather than exercise training since the endocrine responses during and following exercise (increased renin activity, aldosterone, AVP, and ANP) are similarly stimulated by heat exposure (Wade & Freund, 1990; Follenius et al, 1979). It is generally agreed that exercise induces haemodilution (Harrison, 1985) and this response is enhanced in the heat (Senay, 1975). Expansion of the plasma volume can be explained by either: sodium and water retention (i.e. osmolar and baroceptor control together to achieve the "isotonic" expansion of plasma volume); protein movement from the interstitial

compartment to the vasculature, such that whilst total protein content increases the concomitant expansion of plasma volume ensures that protein concentration remains relatively constant; or a combination of both of these mechanisms.

Sweating.

Sweat production. Eccrine glands are distributed over all the skin, their density being higher on the palms of the hands and soles of the feet. Each consists of a single duct which spirals from the skin surface to the dermal tissue where it is coiled in the body of the gland. This body has clear and dark cells involved in the production of the primary secretion, with a composition similar to plasma, with the exception of the plasma proteins (McCance, 1936), containing sodium (140 mmol/L) and chloride (105 mmol/L) as well as urea, lactic acid and potassium ions. The gland is innervated by cholinergic sympathetic nerves, and can also respond to circulating catecholamines. It is suggested that a layer of myoepitheliocytes between the basement membrane and the basal aspect of the secretory cells, are responsible for expressing the primary secretion (Williams & Warwick, 1980). The duct is composed of two layers of cells; luminal cells which have filaments at the luminal membrane together with sodium and chloride transport channels, and basal cells which contain Na-K dependent pumps to power the absorption of sodium (Sato, 1993). Typically, for an unacclimatised person, the sodium concentration of the sweat is 50-60 mmol/L when sweating maximally, and lower than this if sweat rate is lower (Robinson & Robinson, 1954).

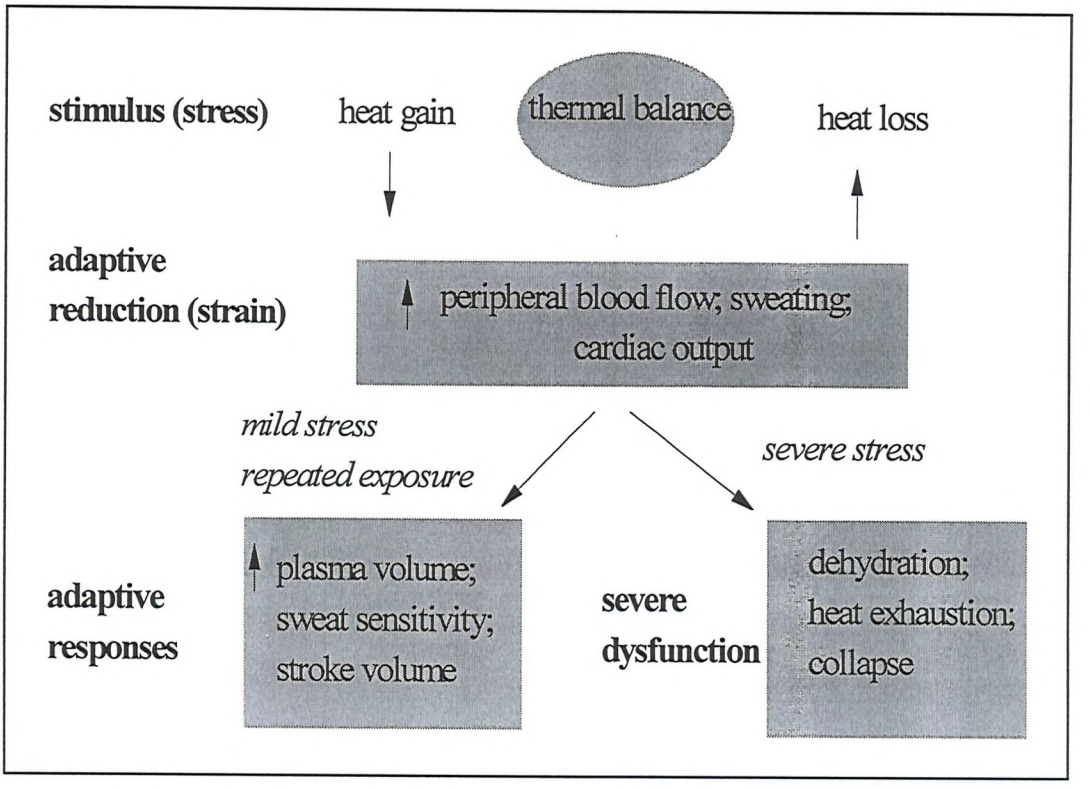
Sweat sensitivity. Sweat loss following a period of heat acclimatisation may increase twofold (Wyndham et al, 1965). The rate of sweat production appears to increase most rapidly in the first few days of acclimatisation and thereafter becomes less marked with maximum capacity being reached after two or three weeks. This occurs together with an increase in sweat sensitivity, such that a greater sweat production is elicited by a similar increase in core temperature, with an earlier onset of sweating (Wyndham, 1967). The lower threshold therefore extends the range over which

sweating exerts thermoregulatory control. Exercise training appears to increase the sensitivity but not the onset of this response (Nadel et al, 1974), whereas repeated heat exposure lowers the threshold but not the sensitivity (Henane & Valatx, 1973). Most acclimatisation procedures involve a combination of exercise in the heat so this distinction is of little consequence. The loss of water by sweating is partly compensated for by a persistent oliguria and renal sodium conservation (Conn 1949). Whilst these renal responses are seen within a few hours of heat exposure, reduced salt loss by the sweat glands takes several days.

Summary of heat acclimatisation responses.

Initial exposure to heat incurs an additional physiological strain to the body as is apparent from the increased demands for cardiac output through a higher skin blood flow and sweat losses. After repeated days of heat exposure this additional thermal load is reduced, and the compensatory adaptive mechanisms by which this occurs are collectively termed heat acclimatisation (or heat acclimation if induced artificially). Heat acclimatisation results in: increased sweat rate and sensitivity; a reduced cardiovascular strain through increased stroke and plasma volume; and a reduced thermal strain as measured by a lower increase core temperature following a given level of thermoregulatory stress or exercise. These responses are summarised in Figure 2.1.

Figure 2.1. Summary of responses in heat acclimatisation.



In the above model a stimulus which stresses the thermoregulatory system within the zone of accommodation produces an adaptive reduction. If this stimulus is repeated it results in the beneficial adaptive responses of heat acclimatisation. A stimulus which cannot be accommodated, however, may lead to heat illness.

Heat acclimatisation and the control of sweating.

Effect of core versus skin temperature. Sweat is produced in response to an increase in body temperature, the effect of increased core temperature (Tc) being much greater than a similar increase in mean skin temperature (Ts), (Wyndham, 1965; Nadel et al, 1974). The magnitude of this effect of core compared to skin temperature is reduced in hotter conditions when Ts is elevated; thus sweating elicited by a degree rise in Tc may be four to five times greater than the response elicited by a similar rise in Ts at a skin temperature of 36°C, whereas in cooler conditions (a Ts of 26°C) this response may be 10 times greater (Wyndham, 1965). Sweat production slows as heat exposure progresses (Ladell et al, 1944; Fox et al, 1963) and it has been postulated to reflect swelling of the stratum corneum to occlude the sweat duct orifices (Peiss et al, 1956), a condition termed hydromeiosis. As this secretion flows through the sweat duct, sodium chloride is reabsorbed by an amount dependent upon the rate of sweat production, thus more dilute sweat is produced when the sweat glands are stimulated slightly compared to a higher sweat rate. (Weiner & van Heyningen, 1952).

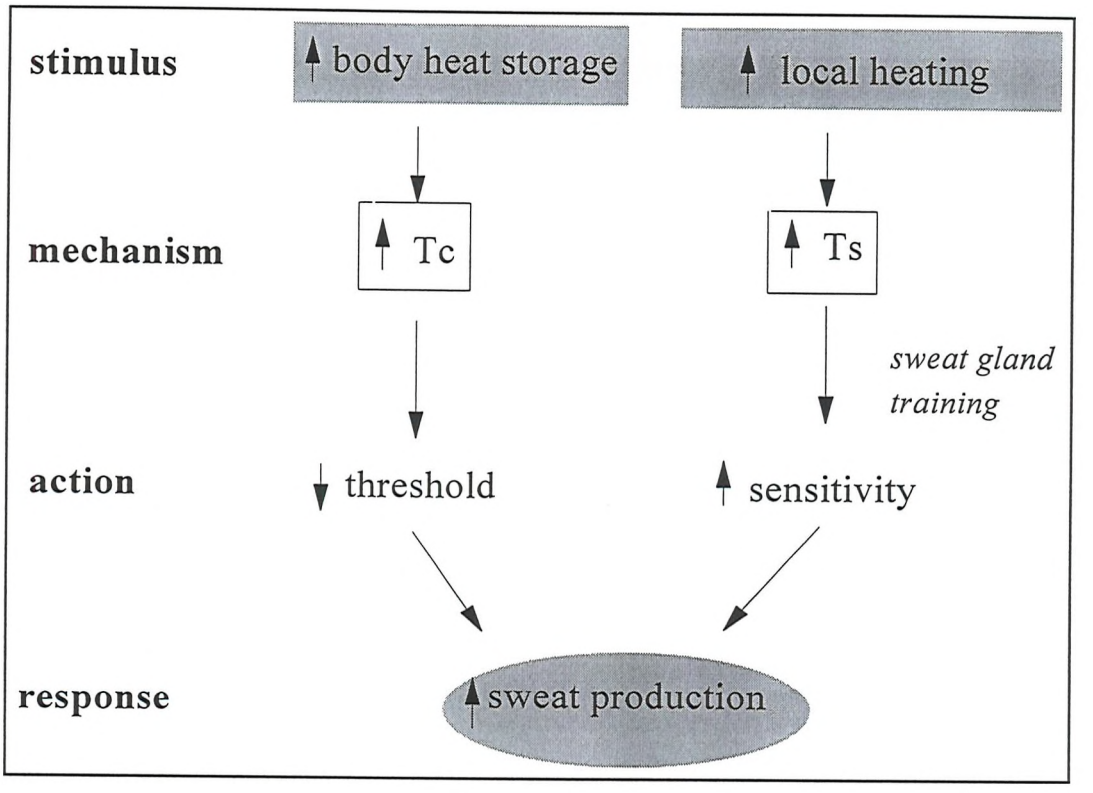
Peripheral versus central adaptation of the sweat glands. The mechanism whereby the capacity of the sweat response is enhanced in acclimatisation in terms of peripheral (skin temperature) as opposed to central stimulation (core temperature) has been one of debate. The increase in sweat rate seen with acclimation is due in part to an increased sweat gland sensitivity to sympathetic stimulation (Wenger, 1988; Nadel et al, 1974). However, other authors have stated that there is also an increase in each sweat gland's capacity which can be increased by "training" of the sweat glands, (Brebner & Kerslake, 1963; Collins et al, 1963; Fox et al, 1964; Collins et al, 1965; Collins et al, 1966). This effect is attributable to a local glandular effect rather than an increased stimulation via the central nervous system. This was shown after water immersion of one arm in warm water, resulting in an increased sweat production on heat exposure in the limb which had been previously immersed but an absence of the phenomenon in the control arm (Fox et al, 1964).

Sweat sodium concentration and peripheral stimulation. Sodium concentration in sweat increases with sweat rate, although heat acclimation appears to increase the sweat glands capacity for sodium retention resulting in a reduced concentration of sweat at any given sweat rate and a reduction in the slope of this relationship between sweat concentration and sweat rate (Allan & Wilson, 1971). Thus it appears that whereas the sweating threshold is centrally controlled, the rate of sweating depends upon the activity of the sweat gland which is altered by training (Yoshimura, 1964), and this impacts upon the concentration of the sweat produced. Thus higher rates of sweating through peripheral stimulation will yield more concentrated sweat. The control of sweat production is depicted in Figure 2.2.

Sweat loss in other populations. Indigenous populations living in tropical climates sweat less and stay relatively dry during exercise compared to non-indigenous individuals who are acclimatised to the heat. In reviewing the literature, Wenger (1988) suggested that long-term residence induces changes in the pattern to sweating to make it more efficient, over and above heat adaptations seen in short-term exposure, allowing a lower sweat production and less circulatory strain.

Summary of control of sweating and sodium concentration. Sweat sodium concentration is dependent upon the acclimatisation status of the individual and changes with sweat production such that the sweat becomes less dilute as sweat rate increases. A further factor in the concentration of sweat is the usual dietary sodium intake as it affects sodium balance. This is considered in the following sections.

Figure 2.2. Mechanism for increased sweat production: Tc indicates core, and Ts skin temperature.



Sweat production is initiated by an increase in body temperature. The effect of a degree rise in core temperature (Tc) is much greater than the equivalent rise of skin temperature (Ts). Increased sweating following heat acclimatisation is attributable to: a reduced sweat threshold on increasing Tc, mediated via a greater sympathetic stimulation; and increased sweat sensitivity, thought to be a local glandular phenomenon.

Heat acclimatisation and sodium requirements.

Sweat sodium losses. Sweat evaporation is the predominant route of heat loss in hot environments and is essential for human thermoregulation. Sweat production can therefore be a significant route of sodium depletion when the production rate of sweat is high (greater than approximately 3 L/day). An excessive salt deficit has been shown to constitute a threat to plasma osmolality and the volume of the extracellular fluid. It is this hyponatraemia, leading to secondary dehydration of the intracellular fluid, that was characterised by the classical study of McCance (1936) which is reviewed later. These clinical observations, together with the studies of Ladell et al (1944), provided the basis for existing advice regarding additional salt requirements in the heat (Leithead, 1963).

Present guidelines for sodium requirements in the heat. The Reference Nutrient Intake or RNI (COMA, 1991) for sodium is 70 $mmol.d^{-1}$. This is based upon the likely maximum sweat secretion of unacclimatised and acclimatised individuals, living in a temperate climate, who are moderately active. This amount may be inappropriate for those unacclimatised individuals who travel to, or inhabit a hotter climate. Thus additional dietary intake may be advised when excretory losses of sodium are in excess of normal intake, depending upon the environmental temperature and work rate. Therefore it has been recommended previously that acclimatised men in a relatively steady state of salt balance require no sodium supplement beyond their dietary intake, but unacclimatised men should have their diet supplemented by 10 g (425 mmol) of sodium per day unless water is in short supply (Leithead, 1963)

Advice for military personnel. The advice to supplement sodium intake on initial exposure to heat is still adhered to today although the duration and amount of this additional intake are not stipulated (COMA, 1991). No reference is made to how existing salt status may affect this requirement. Current UK military guidelines suggest that sodium requirements are met by rations in all but exceptional circumstances, when personnel may require supplements until acclimatised,

particularly if regular meals are not taken or if sweating profusely (unpublished guidelines from the Book of Regulations (BR) 2170(2)).

Sodium supplementation during heat acclimatisation. As already discussed in the previous section, the initial renal responses of reduced blood flow and subsequent fall in glomerular filtration act to conserve sodium. In contrast, reduced sodium loss via the sweat glands takes several days, as acclimatisation proceeds. Thus it is apparent that salt supplementation may only be necessary prior to these adaptations occurring, and only if the level of salt depletion (dependent upon the duration and intensity of heat stress) exceeds usual sodium intake in food.

Summary of sodium requirements during heat acclimatisation. The requirement for supplementary sodium during heat exposure is therefore dependent upon the magnitude of sodium loss via the sweat glands, which in turn will be a function of acclimatisation status. Unacclimatised individuals are advised to take additional amounts of salt without regard for how this may subsequently affect their sweat gland sodium reabsorption.

The maintenance of body sodium homeostasis, plasma osmolality and effective circulating volume.

Introduction.

As the major cation in the extracellular fluid, the flux of sodium is a precursor to fluid distribution and hence the control of body sodium is a critical element to extracellular fluid volume homeostasis. The cellular concentration of sodium is also essential for the maintenance of electrochemical gradients for nervous conduction, cellular transport and consequent energy processes. Thus the maintenance of extracellular sodium concentration is also of primary importance. Both of these processes are inextricably linked, and also impinge upon thermoregulatory mechanisms. This section will review these topics commencing with normal body sodium content and a review of the theoretical basis of sodium balance.

Distribution of body sodium.

An adult man weighing 70 kg has a total body sodium of approximately 4,300 mmol; of this approximately 280 mmol is in the intracellular fluid at a concentration of 10 mmol/L, and 1500 mmol is sequestered in bone. The remaining "exchangeable" sodium of approximately 2500 mmol, is found in the extracellular fluid including the plasma (450 mmol), interstitial fluid (1900 mmol), and transcellular fluid (150 mmol) (Geigy, 1987; COMA, 1991). Although the extracellular spaces are exchangeable, it is the plasma which, being the effective circulating volume, is of primary importance in volume homeostasis.

Plasma sodium.

The maintenance of a constant sodium concentration within the extracellular fluid space is critical to fluid volume regulation and synaptic transmission. Whereas the transport of these solutes is an active process, water can pass freely across cell

membranes driven by hydrostatic and osmotic pressure gradients. Note that it is the relative amount of sodium which is controlled, the sodium concentration, not the absolute amount of sodium and that a reduction in plasma sodium concentration can therefore be achieved by: a reduction in total body sodium or potassium, or an increase in total body water. Hence it is apparent that the maintenance of body sodium is of critical importance to volume homeostasis and plasma sodium (concentration) is the fluid space by which total body sodium is maintained.

Sodium intake.

Estimates of sodium intake between population groups. Salt intake varies widely according to cultural differences between populations (Intersalt, 1988), and also between individuals within the same population (Baldwin, 1960; Simpson, 1988). A recent UK survey estimated the daily mean sodium intake of 842 adult males (aged 16-64) as 173 mmol/d (sem 2.6 mmol/d) (Gregory et al, 1990). This estimate (from 24-hour urinary excretion rates) agrees with that reported earlier for three centres in the UK with between 149.9 and 151.8 mmol/d (Intersalt, 1988). However, the same paper reports an extreme range of sodium excretion ranging from 0.2 mmol/d in the Yanomamo Indians of the Andes, Brazil, to 242 mmol/d in north China (Intersalt, 1988).

Advised daily sodium intake. These actual intakes compare to the advised UK Reference Nutrient Intake (RNI) value of 70 mmol/d (COMA, 1991) i.e. roughly half of what is presently eaten. The lower reference nutrient intake (LRNI, defined as the amount for a few people who have a low requirement) is 25 mmol/d. This is similar to the estimated minimum sodium intake estimated by Truswell (1985) of 20 mmol/d. Note that urinary loss of sodium is depressed during periods of low sodium intake and can be reduced to virtually zero when sodium is being avidly conserved, for example after a period of fasting (Drenick, 1972). This is clearly relevant to the present thesis which will investigate sodium balance in the heat when ingesting a low sodium diet.

Estimation of the dietary sodium intake of military personnel. Prior to these experiments the normal sodium intake of the Royal Navy and Marine personnel, both at sea and ashore, needed to be verified. This was examined by the technique of 24h urinary sodium excretion using two markers of completeness, *p*-amino benzoic acid (PABA) and creatinine, together with a questionnaire. From this survey a sample of 185 collections thought to be complete by criteria of creatinine recovery yielded a mean sodium excretion of 180 (sem 4.3) mmol/d (Allsopp & Whetton, unpublished data), which was similar to the UK population in general (Gregory et al, 1990).

Sodium deficiency.

Clinical salt deficiency has been characterised, and moderately severe deficiency is represented by a deficit of 500 mmol in a 70 kg man. Experimental sodium deficiency was investigated in the classical observations of McCance (1936). In this study a radiant heat bath was used as a sweating chamber for four subjects who ingested a very low sodium diet (3 mmol/d) for up to eleven days. This procedure resulted in the loss of an estimated one-third (1,014 mmol) of sodium in one subject. Results from this work showed the initial loss in sodium to be matched by an equivalent (for sodium in plasma) weight loss, and hence attributable to an extracellular fluid loss. After the first four days, however, this relationship no longer held and the bodyweight ceased to fall. McCance concluded from this that the initial loss was an iso-osmotic hypovolaemic response, whereas the latter represented a sacrifice of osmolality to maintain plasma and extracellular fluid volume. Certainly, during this period the plasma concentration was reduced and there were abnormalities in water regulation. This secondary phase suggests that fluid is transferred from the intracellular to the extracellular fluid compartments, causing the reduction of plasma osmolality. These experiments concur with the clinical diagnosis of secondary dehydration (termed Type I heat exhaustion by Ladell et al) arising through sodium depletion as opposed to simple fluid deficiency (Laddell et al, 1944; Leithead & Lind, 1964).

Sodium balance.

The concept of sodium balance. In the absence of considerable physical exercise or heat stress, the amount lost via the skin is small, (approximately 5 mmol/d; Cogan, 1991) and similarly, faecal losses are also negligible (0.1 - 8.0 mmol/d) even when sodium intake is high (149-299 mmol/d; Baldwin et al, 1960). Thus the kidneys are the primary route for sodium excretion. Homeostatic mechanisms of control help to minimise the effect of altered sodium intake on extracellular fluid volume and total body sodium. When daily sodium intake is constant, then the urinary sodium excretion matches the level of intake (allowing for a small amount of non-renal loss), and the individual is said to be in sodium balance (Simpson, 1988). If, however, sodium intake is suddenly reduced to a very low level, then urinary sodium excretion falls in an exponential fashion over 3-5 days to virtually zero.

The Strauss model of sodium balance. The above model of sodium balance was proposed by Strauss et al (1958). They proposed that excretion was altered in an exponential fashion to match intake and developed the concept of a baseline state between surfeit and deficit to which body sodium falls when sodium intake is reduced to a very low level (Strauss et al, 1958). The rate of this exponential change is given by the predictive equation:

$$Na_U(t) = Na_U(t_0)\, e^{-kt}$$

$Na_U(t)$ is the urinary excretion of sodium at time t
$Na_U(t_0)$ is the initial sodium excretion
t is the time in days
e is the base for natural logarithms
k is the time constant

This translates to a more general predictive equation for a step change in sodium intake (reduction or increase) given by:

$$Na_U(t) = Na_U(t_0) + \Delta Na_{in}(1\text{-}e^{-kt})$$

ΔNa_{in} is the step change in intake, The half life, h (h=24 x 0.693/k), is approximately 24 hours but longer with increased age (Epstein & Hollenberg, 1976).

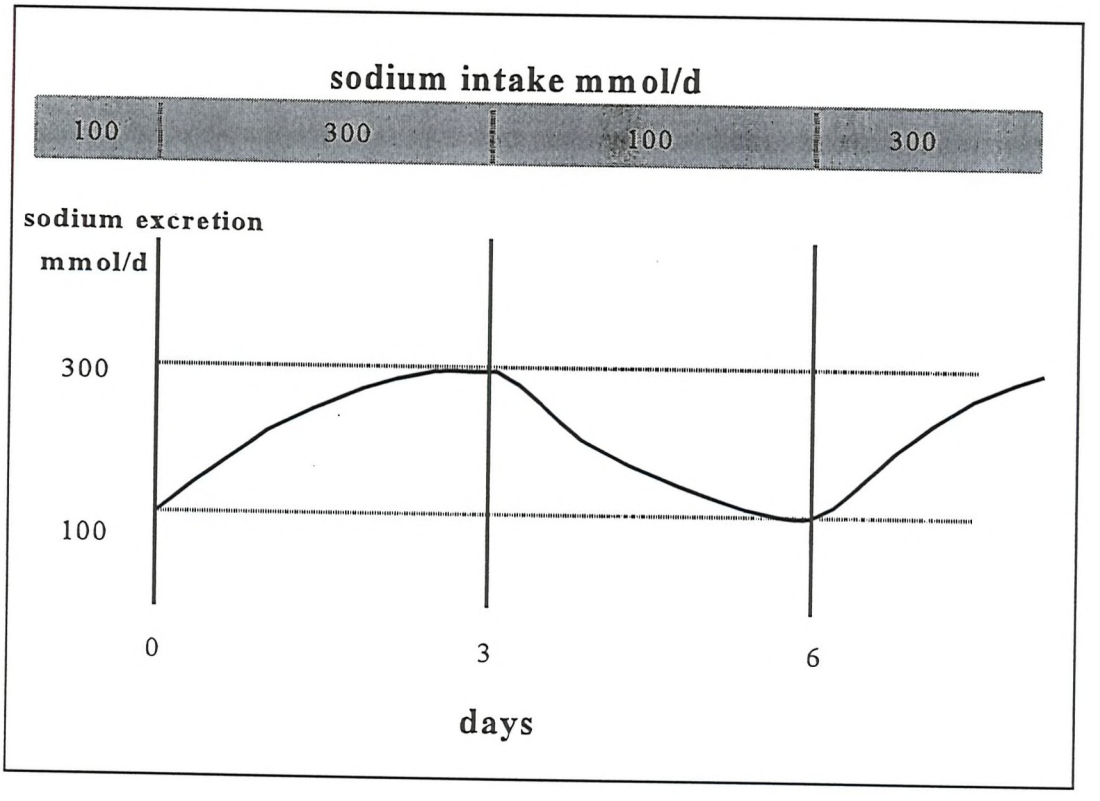
Implications of sodium balance for the present study. The relevance of this equation to the present thesis is that the period for a constant state of sodium balance (i.e. when the rate of excretion is equivalent to the rate of ingestion) following a step change in intake can be predicted as approximately three days. Thus in the present work a control period of this duration was needed in order to establish a steady-state prior to further intervention.

Hollenberg's set-point or a basal state. The above "balance" state has been called the "set-point" by Hollenberg (1980) although it might better be termed the basal level since it is not a fixed absolute but dependent upon the regulation of sodium excretion as reviewed below. Basal sodium, the state between surfeit and deficit, is thought to be determined predominantly by the action of aldosterone (Simpson, 1988). The above principles are illustrated in Figure 2.3.

Summary of normal body sodium and its maintenance.

Sodium balance is achieved by matching of sodium excretion to sodium ingestion in order to minimise the effect of a sodium load on total body sodium and hence alterations of body fluid volume. Sodium excretion is therefore altered in an exponential manner in response to a step change in sodium intake. Thus, for any study of the effects of dietary sodium intervention upon human physiological responses, it is essential that sufficient time is permitted for subjects to attain a new steady-state, and that this state is maintained (in the absence of other factors) by adequate control of dietary sodium ingestion.

Figure 2.3. Schematic diagram of the sodium set-point or basal state principle.



This Figure (revised from Simpson, 1988) illustrates the exponential rise and fall of sodium excretion which theoretically occurs in response to a stepped change of sodium intake, to maintain sodium balance in accordance with the Strauss model (Strauss et al, 1958).

Maintenance of plasma osmolality by arginine vasopressin (AVP) or antidiuretic hormone (ADH).

The responses to a sodium load. For a large increase in dietary sodium intake the time course of excretion, as described by the "set point" predictive equation above, will be several days whereas the time for distribution of that sodium load within the extracellular fluid (ECF) will be a less than an hour (Cogan, 1991). In the interim period, the maintenance of plasma osmolality requires that any acute change in total sodium (in the ECF) be accompanied by an increase or decrease of that volume of fluid to maintain plasma osmolality. Water can pass freely by osmosis and will move from the intracellular to the extracellular fluid to maintain the osmotic equilibrium (within hours of ingestion of the sodium load). Hence there is an initial shift from the intracellular fluid space (ICF) to the ECF, thereby expanding the extracellular volume but, at this point, total body water remains unchanged (Cogan, 1991).

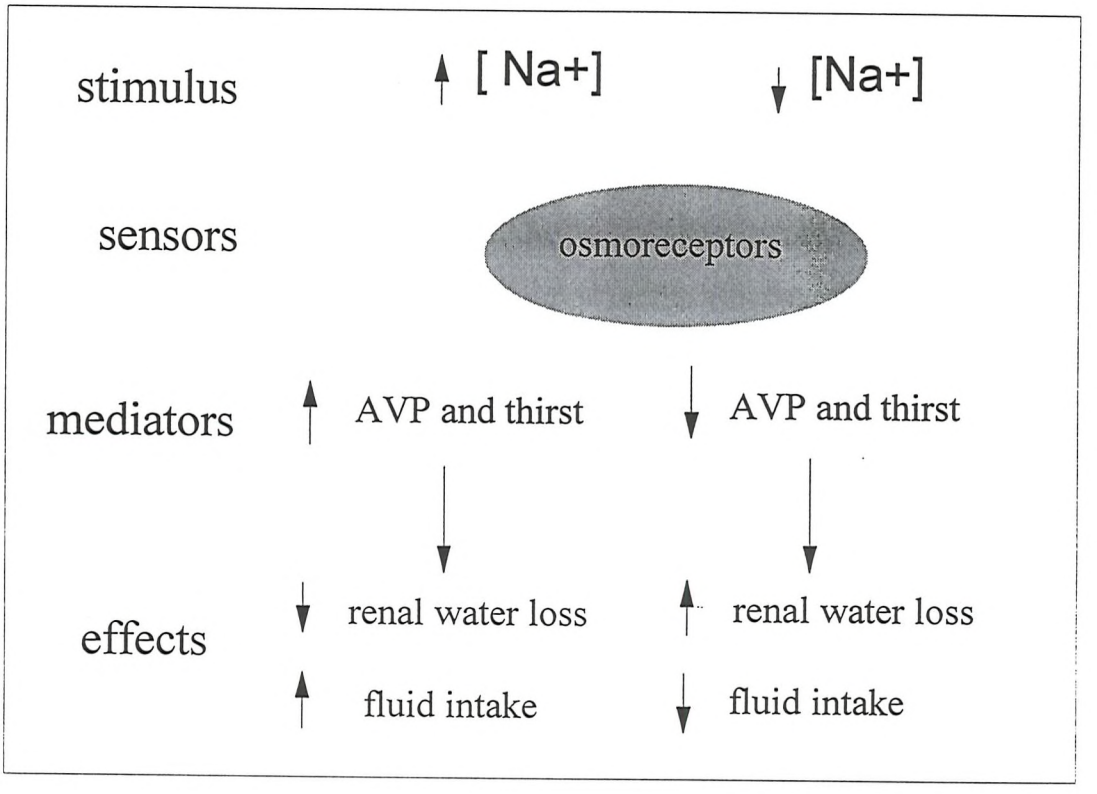
The osmotic regulation of arginine vasopressin (AVP). The renal response to this increase in sodium load is a greater water reabsorption to further expand the ECF volume. This occurs since the increased plasma osmolality is sensed by the osmoreceptor cells of the hypothalamus. The exact location of these cells remains debatable but a recent review by Share (1996) favoured the organum vasculosum of the lamina terminalis, rather than the paraventricular and supra-optic nuclei of the anterior hypothalamus. These latter cells are implicated as they have vasopressin containing axons which project to the posterior pituitary (Zerbe & Robertson, 1987). These cells respond to a change in plasma osmolality by releasing a prohormone of neurophysin, a glycopeptide and arginine vasopressin (AVP), also termed antidiuretic hormone (ADH) because of its action upon urinary output. This concept of osmotic control of vasopressin release was first demonstrated by Verney (1947). Below a threshold stimulus of approximately 280 mosm/kg (an equivalent plasma sodium concentration of 137.5 mmol/L) these cells are quiescent and secretion of AVP is minimal, above this threshold however, AVP secretion increases linearly with plasma osmolality (Zerbe & Robertson, 1987; Share, 1996). The prohormone is transported

to the posterior pituitary and cleaved to release AVP in response to osmoreceptor (or baroreceptor) stimuli. AVP has only a short half-life of 10-20 minutes (Norman & Litwack, 1987).

Overall effect of AVP release. The anti-diuretic action of AVP is to increase the permeability of the distal tubule by binding with specific receptors in the cortical collecting duct and the intramedullary collecting duct, thereby increasing the reabsorption of water, and inhibiting diuresis. The speed of the response suggests effects on existing membrane structures rather than a protein induced mediated process. One theory suggests that AVP causes the insertion of water channels called aggrephores into the cell membrane (Harris et al, 1991). AVP acts to increase the ECF volume in order to dilute the plasma, reducing its osmolality. Hence the overall effect of this response is an increase in total body water and so an increased body weight. If on the other hand a reduction in sodium intake occurred, then plasma osmolality would fall, AVP secretion be depressed, and the volume of the ECF reduced. This feedback mechanism is depicted in Figure 2.4.

The contribution of thirst receptors. The thirst receptors of the hypothalamus, located in the anteroventral region of the third ventricle and the subfornical organ, are less sensitive to small reductions in plasma osmolality compared with the osmoreceptor cells, although they appear to operate by a similar process. Hence when only a small change in plasma osmolality occurs the primary response is an alteration in renal water reabsorption and urinary excretion, rather than a change in fluid intake. The dipsogenic threshold is thought to be approximately 295 mosm/kg (a plasma sodium concentration of 145 mmol/L) (Cogan, 1991). Note that hypovolaemia will also trigger an increase in AVP secretion (even if the plasma is iso- or hypotonic) but this is thought to occur only when the hypovolaemia is severe; an approximate reduction in ECF volume in excess of 10%. The threshold for AVP release however, is dependent upon the relative level of either hypo- or hypervolaemia.

Figure 2.4. The maintenance of plasma osmolality.



An increased plasma sodium concentration (above approximately 137.5 mmol/L) stimulates acts via osmoreceptors to secrete AVP, which acts to increase the permeability of the cortical collecting duct, resulting in a greater reabsorption of water. Thirst receptors are less sensitive to increased osmolality, but will trigger a thirst response at higher plasma sodium concentrations (approximately 145 mmol/L).

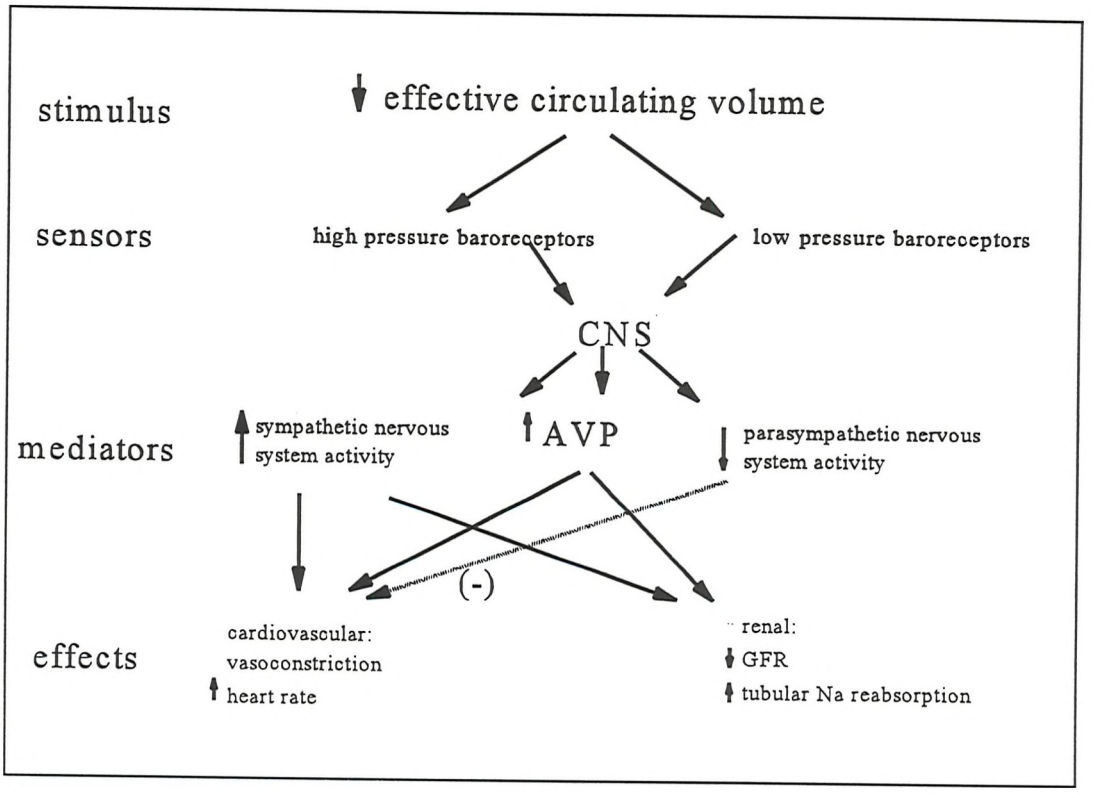
Summary of the maintenance of plasma osmolality. The control of plasma sodium concentration in normal circumstances is via the osmoreceptors which, once plasma osmolality exceeds the normal threshold (280 mOsm/kg) stimulate the production of AVP, reducing the urinary output of water. This response is poorly assisted by the stimulation of thirst to ingest more water which occurs at a higher threshold (295 mOsm/kg). The consequences of plasma sodium homeostasis upon fluid balance and thermoregulation are discussed in the next section.

The maintenance of effective circulating fluid volume.

Starling forces. The "effective" circulating volume is a conceptual device used to describe the integrated effects of circulating plasma volume and arterial pressure. Effective circulating volume is maintained by changes in total peripheral resistance through altered hydrostatic pressure, or by buffering of the plasma volume by the interstitial fluid volume elicited by movement of electrolytes and water. The steady-state plasma volume to interstitial fluid volume is maintained by the balance of Starling forces, i.e. the difference between the net hydraulic pressure (capillary-interstitial difference) minus the net oncotic pressure (capillary - interstitial difference).

Stimuli and responses in the control of effective circulating volume. The afferent limb of the effective blood volume homeostatic mechanism are the low and high pressure baroreceptors located in the atria (low or volume receptors), aortic arch and carotid sinus (high) (Reineck & Stein, 1987). Stretch of these receptors in response to increased venous return and blood volume (low pressure) or elevated arterial blood pressure (high pressure) activates vagal unmyelinated fibres to the medulla. Stimulation of these receptors inhibits the tonic discharge to sympathetic nerves which regulate peripheral vasoconstriction and augments vagal discharge. A decrease in effective circulating blood volume has the opposite effect. The efferent activity of the renal nerves is also reduced, which has a direct effect upon renin release (reviewed in a later section). These responses are summarised in Figure 2.5.

Figure 2.5. The maintenance of effective circulating fluid volume.



A reduction of effective circulating fluid volume is sensed by the pressure and volume receptors. In response, there is an increase in vasomotor tone and heart rate mediated by an increase in sympathetic nervous and reduced vagal (parasympathetic) activity. Vasopressin (AVP) has both cardiovascular and renal effects to increase vasomotor tone and reduce glomerular filtration rate (GFR) whilst increasing tubular sodium reabsorption. (Figure adapted from: Lote, 1994; and Cogan, 1991).

Interaction with the secretion of AVP. The consequence of an altered AVP release is an isotonic volume expansion or depletion as described above. This volume change is detected by low and high pressure volume and baroreceptors in the atria, aortic arch and carotid sinuses. When osmolality is maintained or even if the plasma becomes hypo-osmotic which would suppress AVP secretion, then gross reduction in effective circulating blood volume (>10%) will over-ride osmoreceptor control and stimulate AVP secretion.

Summary of maintenance of plasma osmolality and ECF volume.

Volume and sodium homeostatic mechanisms are critical to the maintenance of plasma osmolality and ECF volume which act in concert to minimise the effects of altered sodium ingestion and excretion. In the absence of sweating, sodium homeostasis is dependent upon renal sodium excretion as determined by glomerular filtration and reabsorption. These processes are regulated by the renin-angiotensin system, ANP and aldosterone which are reviewed in a later section. In heat exposure, however, sodium balance is also dependent upon the process of sweating, and the loss of fluid via this route impacts upon the maintenance of effective circulating fluid volume and thermoregulation.

Fluid Balance in the heat and thermoregulation.

Involuntary dehydration. Thermoregulation during heat stress is known to be reliant upon adequate fluid intake to maintain water balance (Sawka et al, 1985). Whilst at rest in normal temperate conditions, thirst is an adequate stimulus to replace lost fluid, such that daily regulation of body weight is possible to within 120-280 g (Adolph & Dill, 1938). This equates to approximately 0.3 % of body weight, or 0.5 % of body water for an 70 kg man. In the heat, however, humans are commonly unable to rehydrate fully after thermal or exercise-induced dehydration. Thus although a person will drink to satisfy their thirst a water debt remains. This was first called 'voluntary

dehydration' but is more appropriately termed 'involuntary dehydration' precisely because the dehydrated person has no volition to drink.

Euhydration and thermoregulation. The need to maintain fluid balance in order to reduce the risk of heat illness has been repeatedly reported since being highlighted by the pioneering work of Adolph (1943). This showed that the rate of rise of core temperature, and therefore reduced work tolerance in the heat, was proportional to the level of dehydration. Thermoregulation, and thus sustained physiological performance in the heat, is dependent upon hydration status, or the volume of total body water available to maintain thermal conductance and evaporative heat loss (Sawka et al 1985; Sawka et al, 1992). The maintenance of water balance (euhydration) is normally dependent upon the movement of body fluids and electrolytes (primarily body sodium), via receptors of osmolality, volume and pressure (as discussed above), these control feedback mechanisms being linked.

Osmolality and thermoregulation. Hydration status, in terms of the available ECF volume is not the only factor which influences thermoregulation in the heat. Harrison et al (1978) demonstrated that increases in plasma osmolality alone, without accompanying changes in blood volume, were sufficient to cause significant elevation of body temperature during exercise. Similarly, hyperosmolality (294 mosmol/kg), induced by replacing lost plasma volume with 3% saline following dehydration, has been shown to modify thermoregulation by elevating thresholds for vasodilation and sweating even without decreases in plasma volume (Fortney et al 1984). This is not to recognise that hypovolaemia *per se* will potentiate these hyperosmotic effects. Fortney et al hypothesise that the shift in sweating threshold which they observed may have been due to either direct action of ions on hypothalamic neurons controlling body heat loss, or indirectly through an increased cerebral concentration of AVP. During short term exercise (30 minutes), however, the influence of hyperosmolality (achieved by infusion of saline) appeared to have a negligible effect (Fortney et al, 1988), possibly because of the short duration of the exercise. The excessive consumption of salt may cause a reduced heat tolerance if this is not accompanied by

an increased fluid intake (Dassler et al, 1973), possibly through the effects of plasma hyperosmolality on sweating as suggested above. In addition to reportedly impairing heat acclimation (Wyndham, 1973) salt "loading" has also been implicated in hypokalaemia and rhabdomyolysis in heat injury (Knochel & Vertel, 1967).

The effect of dehydration on the maintenance of sweating capacity. Dehydration has been associated with either a reduced or unchanged sweat rate during exercise in the heat (Sawka et al, 1985). Investigators who reported reduction in sweating still observed an elevated core temperature indicating an impaired heat dissipation, probably associated with the increased plasma hyperosmolality and a reduced sweat response as indicated above (Sawka & Pandolf, 1990). An iso-osmotic hypovolaemia may also lead to a reduced sweat rate by increasing the core temperature threshold at which sweating is initiated (Sawka, 1992). According to Sawka et al (1985) hyperosmolality is more strongly associated with the reduced sweat rate than is hypovolaemia. Several authors have also proposed that AVP acts of the sweat gland to reduce water loss during dehydration. This hypothesis, however, is unsupported and would appear unlikely given the lack of any demonstrable effect of AVP on sweat rate (Pearcy et al, 1956).

Summary of fluid balance in the heat. The maintenance of the effective circulating blood volume, to maintain an adequate peripheral blood flow for heat dissipation, appears to be critical to thermoregulation in the heat. Whilst salt supplementation has been used in the past during the heat acclimatisation phase, there is some evidence to suggest that an increased plasma osmolality results in impaired heat tolerance, and that over-supplementation may lead to potassium depletion.

Control of renal sodium excretion.

Introduction.

In temperate conditions, sodium homeostasis is primarily dependent upon renal sodium excretion, a processes which is regulated by the renin-angiotensin system, ANP and aldosterone. The integration of these control mechanisms is reviewed here.

Filtration and reabsorption.

The filtered sodium load. Renal sodium is excreted by means of differential rates of filtration and reabsorption. In the average male the glomerular filtration rate (GFR) is approximately 125 ml/min so 180 L of filtrate per day. If the sodium concentration of this filtrate is 140 mmol/L then the amount of filtered sodium will be 25,200 millimoles of sodium compared to a daily excretion of approximately 170 mmol/d, thus over 99% of the filtered load is reabsorbed.

Reabsorption of sodium. Approximately 65% of this filtered load is reabsorbed from the proximal tubule, by active transport of sodium (the Na/K ATPase basolateral membrane pump) coupled with passive diffusion of sodium chloride. The active mechanism is regulated by sympathetic renal nerve activity, modulated by angiotensin II (Cogan, 1991) whereas the passive transport is affected by Starling's forces controlling the formation of filtrate and the plasma oncotic pressure in the efferent arteriole. The remainder of the filtered load is absorbed primarily in the late diluting segment of the distal nephron, the cortical collecting tubule, and the cortical and medullary collecting duct, under the control of aldosterone.

The renin-angiotensin system.

Renin and juxtaglomerular feedback. When renal arterial pressure or perfusion is decreased, renin, a glycoprotein hormone, is released from the cells of the

response is also related to the delivery of sodium to the cells of the macula densa, which is situated adjacent to the juxtaglomerular apparatus, by the so-called tubuloglomerular feedback mechanism. This involves the "sensing" of sodium delivery to these cells. Renin is also released neurogenically by the renal sympathetic nerves, a response which is mediated by adrenergic β2 receptors. The production of renin is the rate limiting factor in the conversion of angiotensinogen to angiotensin I, which is then further cleaved to form angiotensin II (Ang II) by angiotensin converting enzyme.

Actions of angiotensin II (Ang II). Ang II has the capability of influencing urinary sodium excretion in a number of ways: it increases the synthesis and release of aldosterone (see below), stimulates AVP and thirst and regulates GFR directly by its vasoactive effect upon renal blood flow (Hall, 1986). Ang II also has a direct effect upon sodium reabsorption by modulating the activity of renal nerves as indicated above. A paracrine system also exists whereby locally formed angiotensin II acts locally (Johnston et al, 1993). Thus the renin-angiotensin system acts in concert with renal nerves, and changes in renal perfusion and plasma oncotic pressure, to increase the proximal reabsorption of sodium and to stimulate aldosterone production.

Atrial natriuretic peptide (ANP).

Overview of atrial natriuetic peptide (ANP). ANP is synthesised in the muscle cells of the heart where it is stored in granules (Jamieson & Palade, 1964) and released in response to atrial stretch as a result of an elevation of central venous pressure (Schutten et al, 1987) or increased venous return. ANP secretion elicits natriuresis, diuresis, relaxation of vascular smooth muscle and therefore a reduction in total peripheral resistance. It has been regarded as a potent defence mechanism against volume overload, countering the vasoconstrictor and anti-natriuretic activities of angiotensin and aldosterone (Winaver et al, 1995). It is a 28-amino acid peptide which is rapidly degraded by an endopeptidase and removed by binding to a clearance

receptor (Cogan, 1990a). Its action is mediated via a secondary messenger, cyclic guanosine monophosphate (cGMP) (Cogan, 1990b).

Renal actions. ANP increases glomerular filtration rate without increasing renal blood flow (Norman & Litwack, 1987), by afferent arteriole dilation and efferent arteriole constriction. ANP inhibits sodium transport in the intramedullary collecting duct either: directly via cyclic guanidine monophosphate (cyclic GMP) inhibition of sodium channels in the cell membrane (Cogan, 1990b); or by stimulating PGE2 synthesis, which inhibits the ATPase of the Na/K pump (Zeidel, 1993). In addition, ANP causes dilation of smooth vascular muscle and antagonises the antinatriuretic and antidiuretic action of aldosterone and AVP. This latter action is effected by the inhibition of: renin release by the juxtaglomerular apparatus; aldosterone secretion by the zona glomerulosa cells; and AVP secretion of the hypothalamus-posterior pituitary (Maack et al, 1985; Norman & Litwack, 1987; Cogan, 1991). Dilator actions of ANP are mediated by receptors acting with guanylate cyclase to produce cyclic GMP (Ganguly, 1992).

Aldosterone.

Release of aldosterone. Aldosterone is a mineralocorticoid hormone secreted from the cells of the zona glomerulosa of the adrenal cortex in response to four dominant stimuli: angiotensin II; potassium; adrenocorticotrophic hormone (ACTH); and, lastly, sodium (Funder, 1993; Verrey & Beron, 1996). Atrial natriuretic peptide (ANP) also has an indirect effect upon aldosterone secretion by inhibiting the action of potassium, angiotensin II and ACTH. Potassium potentiates the action of Ang II on aldosterone release by increasing the availability of Ang II receptor sites or by increasing the permeability of the cells to potassium (Funder, 1993; Verrey & Beron, 1996).

Action and specificity of aldosterone. Aldosterone increases potassium excretion and sodium reabsorption in the principal epithelial cells located in the late diluting segment of the distal nephron, the cortical collecting tubule, the cortical collecting

aldosterone are not unique but share a common homology with glucocorticoids, with which they have a similarly high affinity. These receptors are inhibited by spironolactone which binds to the receptor site but does not induce gene transcription. Activity of these type I receptors to aldosterone in preference to cortisol or corticosterone is conferred by the presence of 11-β-hydroxysteroid dehydrogenase which when present, converts these glucocorticoids to cortisone and 11-dehydroxycorticosterone, which have no affinity for the site (Horisberger & Rossier, 1992). Aldosterone is resistant to the action of 11-β-hydroxysteroid dehydrogenase by virtue of its aldehyde group on the C18 carbon. Note that the action of 11-β-hydroxysteroid dehydrogenase can be inhibited by glycyrrhetinic acid found in licorice and that the symptoms of acute mineralocorticoid excess, hypertension, sodium retention, hypokalaemia, are due to the absence of this enzyme.

The gene-regulated theory of aldosterone action. The time course for aldosterone action has been previously reported as between two to eight hours. Hence this is linked to an "upregulation" of Na/K ATPase activity via chromosomal transcription and the production of aldosterone induced proteins. There are several theories as to how this protein transcription then mediates an increase in the unidirectional flux of sodium ions across the luminal membrane by: opening of previously closed amiloride sensitive sodium channels; the potentiation of sodium entry by the Na/K ATPase pump; or by increasing the availability of ATP, Na/K ATPase or mitochondrial enzymes (Bastl & Sebastian, 1987).

A new mechanism of aldosterone action. More recently a more rapid response of aldosterone has been demonstrated which cannot be explained by the classical gene-transcription mediation model (Horisberger & Rossier, 1992). This second component of aldosterone action is mediated through a plasma membrane receptor which is specific for aldosterone and DOCA (Wehling et al, 1992). This membrane receptor increases the production of inositol triphosphate, which increases the activity of Na/H antiporter activity to increase intracellular sodium concentration. As the

availability of sodium ions is rate limiting, this accelerates the Na/K ATPase pump.

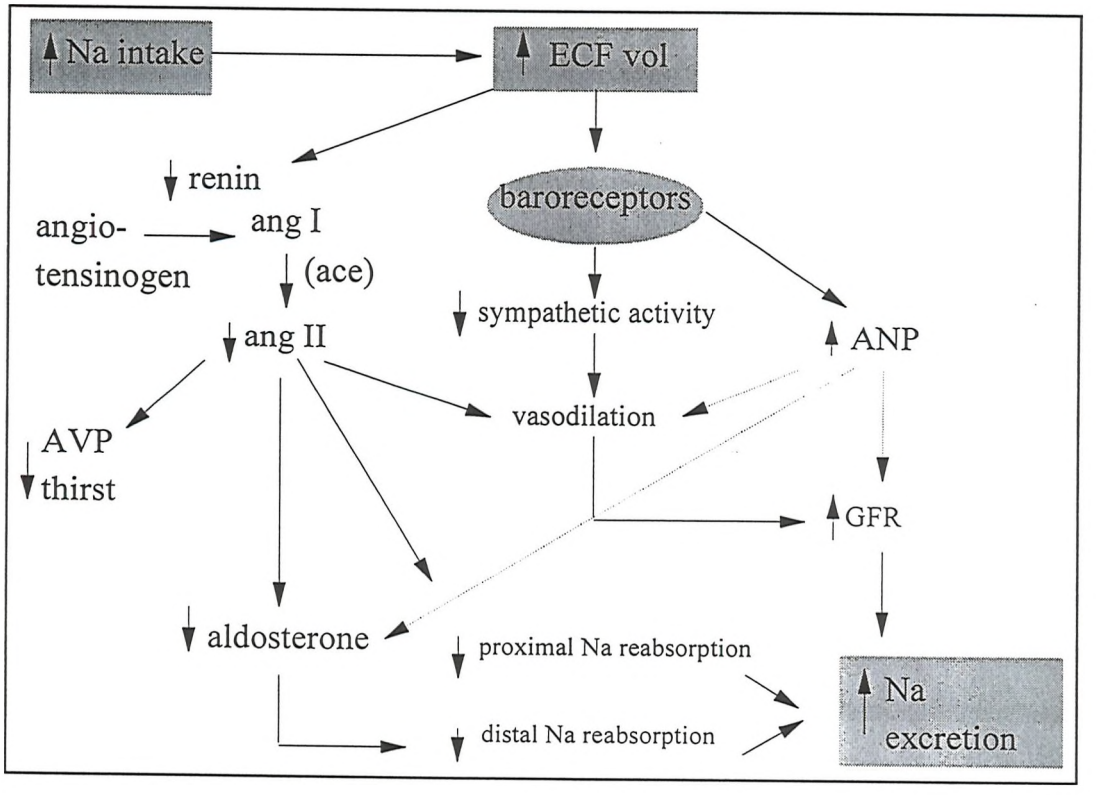
Hepatorenal receptors.

A recent review has highlighted the existence of hepatorenal receptors which may play an important role in the control of sodium excretion (Hosomi & Morita, 1996). These authors postulate that a receptor for sodium exists in the hepatoportal region which augments urinary sodium excretion via renal verve activity, in a negative feed-forward control system. This conclusion is based upon the decrease in renal nerve activity and increased urinary sodium excretion which occurred in conscious (vagotomised and sinoaortic denervated) rabbits following sectioning of the hepatic nerve (Hosomi & Morita, 1996). The significance of this and other studies of the hepatorenal and hepatointestinal reflexes to sodium homeostasis is unclear, although these authors suggest that classical negative feedback control systems are secondary to this feed-forward process.

Summary of the control of sodium excretion: renal and non-renal losses.

Urinary sodium excretion sodium in response to an expansion of ECF volume is mediated by: renal nerve activity; renal perfusion and plasma oncotic pressure; and angiotensin II which act to alter proximal reabsorption. Distal reabsorption of sodium is regulated primarily by aldosterone, the actions of which are modulated by ANP. ANP also acts directly inhibits sodium reabsorption, regulates GFR and inhibits the secretion of renin, aldosterone and AVP. These control mechanisms are summarised in Figure 2.6. With respect to the influence of aldosterone on sodium homeostasis in temperate conditions, it would appear that aldosterone has a subsidiary role to Ang II and ANP. In contrast, in conditions of heat stress when sweat production is profuse, losses via the sweat glands may be substantial. As the reabsorption of sodium by the sweat glands is regulated by aldosterone (Conn, 1949b), this hormone plays a primary role in sodium homeostasis in these conditions. This pivotal role of aldosterone to sodium homeostasis will be reviewed in the next section.

Figure 2.6. The author's summary view of regulatory mechanisms with respect to renal sodium excretion.



Atrial natriuretic peptide (ANP) has antagonistic actions to angiotensin II (Ang II) and aldosterone (dotted lines); ace - indicates angiotensin converting enzyme.

Non- renal sodium loss and the physiological control of aldosterone in the heat.

Introduction.

As stated previously, in temperate conditions the primary route of sodium loss is via the kidneys, and it would appear from the above review of the literature that aldosterone plays a facilitative, rather than a primary role in renal regulation of sodium excretion in these circumstances. In a hot environment however, sweat sodium losses will also determine sodium balance and loss of sodium by this route will increase as sweating continues. Hence in the heat, reabsorption of sweat by the sweat glands, controlled by aldosterone, elevates the importance of this hormone in the regulation of total body sodium homeostasis.

Experimental factors to be considered when investigating aldosterone secretion.

As noted above, aldosterone is secreted in response to: an increased plasma potassium concentration; angiotensin II activity; ACTH; and a reduction in plasma sodium concentration. Angiotensin II is released in response to renin release when the effective circulating blood volume, arterial pressure, or renal perfusion pressure are reduced. Thus redistribution of blood volume as occurs during heat exposure, exercise or alterations of posture will also influence the secretion of aldosterone (Davies et al, 1981; Kirby & Convertino, 1986). The magnitude of the response may also be affected by the type, intensity and duration of the exercise performed, the level and type of heat stress (hot-humid versus hot-dry) and individual differences in fitness and acclimatisation status. A further consideration is the appropriate manner of blood sampling, which is discussed in the methods section of this thesis. These stressors need to be taken into account when attempting to examine the effects of sodium balance upon aldosterone secretion. Hence in the present studies the environmental and dietary conditions were carefully controlled and physical activity kept to a minimum.

The role of aldosterone on sodium balance in the heat.

Aldosterone and sweat sodium sparing. Excessive salt loss from the body in the first days of heat exposure is controlled by markedly reduced urine production and renal conservation of sodium. This renal conservation of sodium occurs within the first few hours of heat exposure but not earlier than six hours for the sweat glands (Robinson et al, 1955). The rate of re-establishment of positive salt balance is primarily dependent upon heat acclimatisation status and also sodium intake. Individuals who are well acclimatised to heat secrete very dilute sweat (5 mmol/L or less) compared with unacclimatised people (40-60 mmol/L), who are therefore more likely to incur a negative sodium balance (Costill, 1977). Mineralocorticoids were implicated in this sodium sparing response by Conn (1949a) who demonstrated reductions in sweat sodium secretion following injections of adrenocorticotrophin (ACTH) and deoxycorticosterone (DOCA). Helman et al (1956) observed a similar reduction of sweat sodium during heat acclimatisation which was accompanied by an increased urinary excretion of aldosterone (Helman et al, 1956). Furthermore, Collins has shown that sweat sodium secretion can be influenced by injection of either aldosterone or spironolactone (an aldosterone antagonist) in subjects not exposed to the heat (Collins, 1966).

Is aldosterone stimulated by a negative sodium balance ? It has been suggested that the primary stimulus to this secretion of aldosterone, and hence subsequent enhanced conservation of sodium during heat acclimatisation, is a negative sodium balance incurred during the first few days of exposure (Collins & Weiner, 1968). This hypothesis is based upon earlier work by Taylor et al (1943) who observed a reduced sweat sodium concentration occurs following experimental salt depletion. Robinson et al (1950) demonstrated a reduction in sweat sodium concentration in two subjects without heat acclimatisation when sodium ingestion was reduced to approximately 15 mmol/d; sweat chloride concentration fell from 60.7 to 39.5 mmol/L in one subject and from 39.6 to 25.5 mmol/L in the other. Sodium sparing by the sweat glands in response to dietary sodium intake has been ascribed to aldosterone activity since salt

deprivation in temperate conditions increases excretion of urinary aldosterone and salt loading decreases it (Collins & Weiner, 1968).

Sodium losses and dietary intake of sodium. Hence there appear to be two separate processes to reduce sweat sodium secretion: heat acclimatisation by sweat gland training causing an increased sweat production rate, as reviewed earlier; and a reduced sodium intake to achieve a net body sodium deficit. Both would appear to be mediated by aldosterone secretion. Enhanced sweat sodium reabsorption as a result of reduced dietary intake raises the possibility that salt supplementation may be non-facilitative of the heat adaptive processes. Indeed, previous authors have suggested that a negative sodium balance status is required to initiate these responses (Smiles & Robinson, 1971). Such a hypothesis would support the earlier finding that when heat exposure was repeated in conditions of generous salt intake, there was no reduction in sodium concentration of sweat, compared to that seen from similar heat exposure but when sodium depleted (McCance, 1938). This is despite the "training" stimulus to the sweat glands which is presumed to have taken place. This finding was confirmed by Taylor et al (1943) who also reported an absence of sodium conservation in heat acclimatised subjects given a high salt diet. This would suggest that reduced sweat sodium loss, although important to achieving sodium balance on a restricted salt diet, is not a necessary part of heat acclimatisation.

Summary of investigations to elucidate the effects of sodium status upon aldosterone secretion and subsequent sodium balance in the heat.

Experiments whereby sodium deficit has been prevented by the administration of saline have concluded that: the reduced sweat sodium concentration following acclimatisation was unaffected by this procedure (Allan & Wilson, 1971); and that increased secretion of plasma aldosterone and increased plasma renin activity were attenuated but not prevented (Davies et al, 1981). The latter authors concluded that aldosterone secretion is determined primarily by the acute stress of exercise in the heat rather than in response to a sodium deficit *per se* (Davies et al, 1981). In these

experiments, however, dietary sodium intake was uncontrolled and hence a positive sodium balance could only be assumed and not demonstrated. Whilst the above research has concentrated upon the effects of a negative sodium balance on mineralocorticoid production, there is a similar uncertainty whether a positive sodium balance reduces the stimulus for acclimatisation (Finberg & Berlyne, 1977; Smiles et al, 1971). Furthermore, in experiments where no significant rise in plasma aldosterone concentration has been reported following heat acclimation, it is unclear whether this is as a result of no sodium deficit being incurred (Davies et al, 1981). Attempts to study the mechanism of aldosterone on sodium reabsorption and heat acclimatisation using deoxycorticosterone acetate (DOCA), an aldosterone agonist, have also been inconclusive; although DOCA mimics the fluid and electrolyte changes of acclimation it does not alter the time course of acclimation or produce pre-acclimation.

Summary of this review of the literature.

It is apparent from the above review of the literature that there has been a wealth of previous research into heat acclimatisation. Much of this has concentrated upon the thermal and cardiovascular responses to reduce heat strain; fewer studies have investigated endocrinological aspects of heat acclimation, with particular attention to total body sodium balance. Some studies in which sodium balance was of concern have had inadequate control of dietary sodium intake (e.g. Ladell et al, 1944), or have not permitted enough time to ensure sodium balance prior to heat exposure. Other studies have used several hours of exercise in the heat as a means of achieving heat acclimatisation (for example Armstrong et al, 1993) and the results have therefore been confounded by the this factor. Similarly, since the work of Smiles and Robinson (1971), the effects of pre-existing sodium intake on the rate of restoration of sodium balance have been ignored.

Hence the stimulus for increased sodium reabsorption by the sweat glands remains unclear; is it a consequence of sodium deficit, as suggested by McCance (1938) or purely an adaptive response to repeat stimulation by the heat? Can the sweat glands be "trained" by manipulation of dietary sodium intake to create a net sodium deficit prior to heat exposure, or is the conservation of sodium by this route an indirect effect of increased sweating in response to repeated peripheral stimulation? What are the consequences of reduced and supplemented sodium upon thermoregulation during heat exposure, and can dietary supplementation be manipulated to assist the process of heat acclimation?

Almost thirty years ago, Collins and Weiner (1968) first postulated that a negative sodium balance prior to heat exposure might potentiate the secretion of aldosterone and assist subsequent sodium retention. This hypothesis still needs to be addressed in a series of systematic experiments which are well controlled with respect to dietary sodium intake, thermal environment and physical activity.

CHAPTER 3.

AIMS AND HYPOTHESES.

From the above review of the literature it is clear that there is a need for systematic studies of the effects of dietary sodium intake on body sodium, fluid balance, thermoregulation and heat acclimation during chronic heat exposure. These studies should ensure significant sodium depletion and sweat losses without the confounding effects of exercise or immersion as have occurred in other heat acclimation studies.

The central hypothesis was that:

> *Sodium intake can be manipulated sufficiently to stimulate aldosterone secretion, reducing the loss of sodium in the sweat and so maintaining extracellular fluid volume. As a consequence thermoregulation in the heat will be improved.*

The outcome of this sodium sparing effect upon measures of heat acclimation (plasma volume, heart rate and body temperature responses) will be such that adaptation to the heat is not compromised, and possibly enhanced, compared to other sodium states. This model is depicted in Figure 3.1.

Three studies are described in the following chapters which investigate:

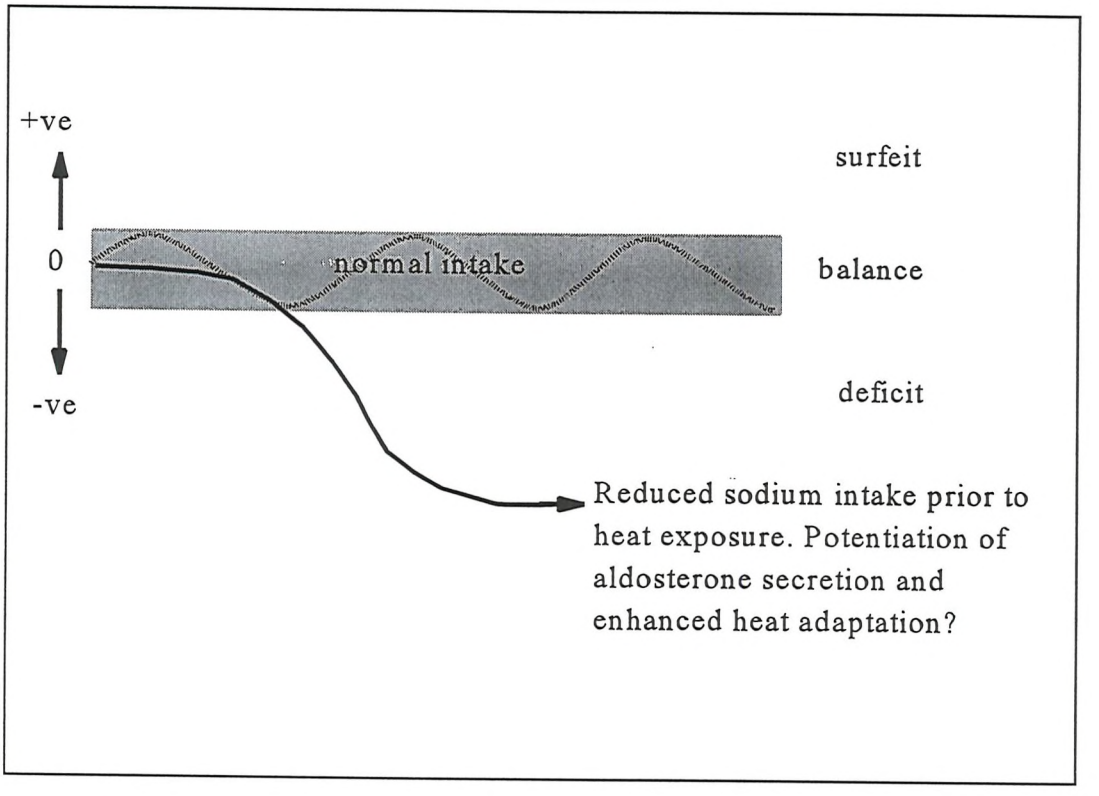
a. the effect of a low sodium diet in normal temperate conditions;

b. the effects of heat exposure when ingesting a normal or a high (supplemented) sodium diet;

c. the effect of a low sodium intake prior to heat exposure compared with a restriction of intake upon heat exposure.

A common protocol was used for b and c above which allowed further comparisons between the various dietary groups.

Figure 3.1. Putative mechanism for a net sodium deficit prior to heat exposure potentiating the secretion of aldosterone and facilitating heat adaptive responses.



The author's schematic representation of the central hypothesis, after Collins & Weiner, 1968.

Aims.

In order to test the above central hypothesis in the present thesis, four major aims were addressed:

1. The aim of experiment one (Chapter 5) was to examine the effects of a restricted dietary sodium intake in a temperate climate in order to test the hypotheses that:

   a. such a restriction of dietary sodium produces a net negative sodium balance until balance is restored within approximately three days;

   b. a sodium deficit reduces the extracellular fluid volume and stimulates the secretion of aldosterone.

These hypotheses were tested by the comparison of a restricted dietary sodium intake group with a control (non-restricted) group. Sodium balance was assessed by measured intake and excretion. Changes in extracellular fluid volume were estimated from altered plasma volume and body weight. Plasma aldosterone concentration was measured daily.

2. The aim of experiment two (Chapter 6) was to examine the effects of sodium supplementation, before and during heat exposure, in order to test the hypotheses that:

a. in a hot climate, dietary supplementation with sodium prior to heat exposure has no beneficial effect upon parameters of thermoregulation and heat acclimation;

b. sodium supplementation reduces aldosterone secretion, attenuating those physiological responses initiated by heat exposure to conserve sodium.

These hypotheses were tested by the comparison of two dietary groups, a supplemented sodium group versus a control, prior to and during exposure to the heat. In addition to the measures above, thermoregulation and heat acclimation were assessed by measuring body temperature and heart rate during an exercise tolerance test, performed daily. Sweating and sweat sodium excretion were also estimated.

3. The aim of experiment three (Chapter 7) was to compare the effects of a low sodium diet administered *prior* to and during heat exposure with a normal dietary sodium intake which was suddenly restricted to a low level on exposure to the heat. This experiment tested the hypotheses that:

a. in a hot climate, *prior* reduction of sodium intake increases aldosterone secretion;

b. this dietary manipulation potentiates the increased aldosterone secretion normally seen in response to heat exposure;

c. the enhanced aldosterone secretion caused by the combination of above effects attenuates sodium losses via sweat glands and so assists the maintenance of sodium balance.

These hypotheses were tested by the comparison between two groups, one adhering to a low sodium prior to and during heat exposure, the second having sodium intake reduced during heat exposure. Measures were performed as for experiment two.

In a further chapter (Chapter 8), the results from experiments two and three above were combined to compare the thermoregulatory, circulatory and endocrine responses of the four dietary sodium groups. Specifically, the hypothesis that, adherence to a low sodium diet prior to and during heat exposure does not result in a reduced heat tolerance, was tested by comparison of the data from all the dietary groups investigated.

CHAPTER 4.

GENERAL METHODS.

This Chapter will describe the general methods used, details of methodology specific to a particular experiment will be given separately within the Chapters which follow.

Subjects.

All subjects were Royal Navy and Royal Marine male volunteers and gave their written informed consent prior to participation. These experiment were conducted in the Winter and Spring months and so subjects were assumed to be unacclimatised to heat. None of the subjects reported to participate in regular strenuous physical training. Subjects wore shorts and sandals during the exercise period but wore long cotton underwear during the sweat washdown days as described below for estimation of sweat sodium.

Dietary conditions.

Throughout the experiments the level of sodium intake of each subject was carefully controlled and monitored, each subject being assigned to one of the four dietary groups as follows:

a. A restricted or moderate to low sodium intake (MLNa): a target intake of 170 mmol/d for the first three days of the experiment which was reduced to 70 mmol/d from day 4 onwards.

b. Moderate sodium intake (MNa): a target intake of 170 mmol/d for the entire experiment.

c. High sodium intake (HNa): a target intake of 340 mmol/d for the entire experiment.

d. Low sodium intake (LNa): a target intake of 70 mmol/d for the entire duration of the experiment.

Note that the low category coincided with the RNI value (COMA, 1991), whereas the moderate intake was similar to that reported as normal for adult males in the UK (Gregory et al, 1990; Allsopp & Whetton, unpublished data). Water was permitted ad-libitum. Other drinks, fruit juice, milk and tea, were permitted as part of a daily food allowance. No coffee was allowed due to its high potassium content.

Subjects, (aged 19 to 40 years; body weight 64 to 102 kg; BMI 20 to 34 $kg.m^2$) were allocated to one of the above dietary sodium intake levels on the basis of their energy expenditure estimated from age and body weight (Schofield, 1985), calculated as 1.4 times the predicted BMR. Their activity was categorised as light (COMA, 1991).

Climates.

All experiments were conducted within the Environmental Medicine Unit Chamber at the Institute of Naval Medicine. This chamber has full temperature (10-50°C) and humidity (30-85% RH) control. It comprises a main living area of approximately $50m^2$ with sleeping (12 bunks), toilet and kitchen facilities attached to this central space. Environmental conditions were maintained constant using one of the following procedures:

a. Experiment 1: a constant dry bulb of 25°C dry-bulb, 17°C wet-bulb, (40% relative humidity), with a constant air velocity of 0.5 $m.sec^{-1}$ throughout the entire trial.

b. Experiments 2 and 3: a constant dry bulb of 25°C dry-bulb, 17°C wet-bulb, (40% relative humidity, ambient vapour pressure 1.3 kPa), with a constant air velocity of 0.5 $m.sec^{-1}$ for the first three days of the trial

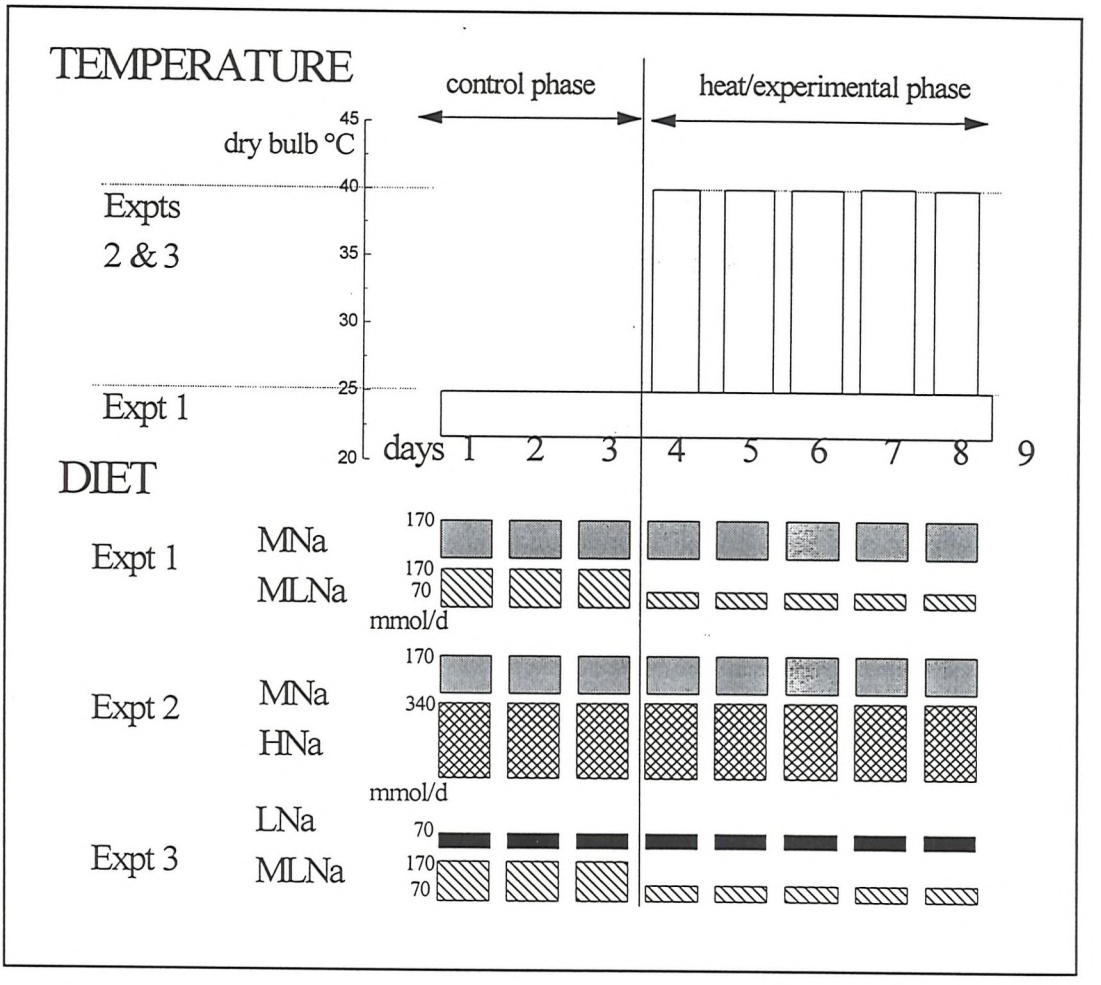
(control period). This was followed by five days of heat exposure during which environmental temperature was cycled during each 24-hours: 40°C dry bulb/27-28°C wet bulb, 40% RH/2.8 kPa, between 0800 -1800h; and 23-25°C dry bulb/16-17°C wet bulb, 40% RH/1.4 kPa between 2000 - 0600h).

The hot-dry environment was chosen to reduce the risk of sweat dripping from the skin and the effects of hidromeiosis on sweat production. The sweat sodium secretion for the daily 10 hour heat exposure under these conditions was estimated at 155 mmol of sodium (i.e. close to the level of MNa above), depending upon the method of estimation (Kerslake, 1972) and the estimated salt content of the sweat (40 mmol/L of sodium assumed here (Geigy, 1987)).

Procedure.

Eight to ten subjects, depending upon which trial, entered the chamber on the evening prior to day 1 of the experiment and commenced their dietary regime that evening with their first meal. The subjects arose between 0700-0730h each day, after having their blood sampled (see below), when they were weighed nude. Their pattern of activity was similar each day and was sedentary in nature with the exception of one hour of light exercise each day, a standardised exercise step-test. They retired to their bunks at approximately 2300-2400h each evening. All subjects continued with this regime until the morning of day 9 when the experiment ended (at approximately 0800h). The procedure for the protocol is depicted in Figure (4.1).

Figure 4.1. Schematic diagram of the experimental procedure.



Experiment 1 was conducted at an environmental temperature of 25°C (dry bulb) throughout, whereas in Experiments 2 and 3, this was increased to 40°C between the hours of 0800h to 1800h daily from the morning of day 4 until the evening of day 8.

Experimental Measures.

Food and beverage intake. All meals were controlled with respect to sodium content by use of standard pre-prepared menus of identified (weight and content) food items. These items have been previously identified as having a high accuracy with respect to nutrient content and hence sodium intake was estimated from the manufacturer's nutrient analysis data. The quantity of tea drunk was recorded by the number of standardised cups recorded that day. All other fluid intake (water, milk and fruit juice) was recorded by weight.

Analysis of urinary sodium and potassium excretion.

Urine was collected in 2-litre plastic containers. The amount of urine was determined either by weight measurement, the specific gravity was also determined (see below) and the weight corrected to volume as necessary. An aliquot of the urine (10 ml) was then taken and analysed within the next 24-hours for concentration of electrolytes. Specific gravity (of the complete 24h sample) was measured daily by a colorimetric reagent strip (N-Multistix SG (Ames), Bayer Diagnostics, Berks) previously shown to correlate well (r=0.93) with the results of hydrometry in healthy individuals (Frew et al, 1982).

Urinary sodium was analysed by Inductive Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES; Perkin-Elmer Plasma 400) following sample preparation; the urine sample (5 ml) being mixed on a roller mixer for 5 minutes prior to 1:10 dilution in doubly distilled water and mixing for a further 5 minutes. Samples were then analysed in triplicate against a calibration standard (National Institute of Standards and Technology, Gaithersburg, Maryland: NIST 2670). A 500 mg/L solution of this standard was serially diluted, analysed and the results recorded as a calibration file for reference against all batches of urine subsequently analysed. In each batch, triplicates of each sample were analysed and the mean result recorded provided that the coefficient of variation was <2%. Samples falling outside this range

were re-analysed. The calibration standard (NIST 2670) was analysed prior to the analysis of sample batch and after every fifth sample as a Quality Control (QC). All QC values were required to fall within accepted operational limits (within 3 standard deviations from the mean). If any QC value fell beyond these limits then the calibration file was repeated as above and the analysis repeated.

Urinary potassium was analysed similarly by ICP-AES but using a 1000 mg/L solution of the NIST 2670 calibration standard to create a calibration file.

Estimation of sweat sodium excretion.

Whole body sweat sodium losses were estimated using the whole-body washdown technique (See Figure 4.2) described by Collins et al (1971) in six 12h washdown periods as follows:

a. the evening of day 3 (1930-2030 h) to the morning of day 4 (0730- 0830 h);
b. the morning of day 4 (0730-0830 h) to the evening of day 4 (1930-2030 h);
c. the evening of day 7 (1930-2030 h) to the morning of day 8 (0730-0830 h);
d. the morning of day 8 (0730-0830 h) to the evening of day 8 (1930-2030 h).

The same washdown periods were used for all groups with the exception of the MNa subjects in Experiment 1 which were as follows:

e. the evening of day 5 (1930-2030 h) to the morning of day 6 (0730- 0830 h);
f. the morning of day 6 (0730-0830 h) to the evening of day 6 (1930-2030 h).

The washdown procedure was as follows:

1930-2030 previous night: The subject showered and then was sprayed with distilled water from a garden-type sprayer (at skin temperature), dried and weighed before dressing in standard cotton long underwear.

0730-0830 next day: The subject undressed and was weighed, before stepping into a large polythene (survival) bag, sprayed with warm distilled water from a pressure spray of known quantity (approximately 4 litres), before stepping out of the bag, towelling dry on the underwear and being reweighed as before. The clothing was returned to the bag. The subject then dressed in second set of clean underwear.

1930-2030: The subject undressed and was weighed before stepping into a second polythene bag and sprayed, dried and reweighed as previously.

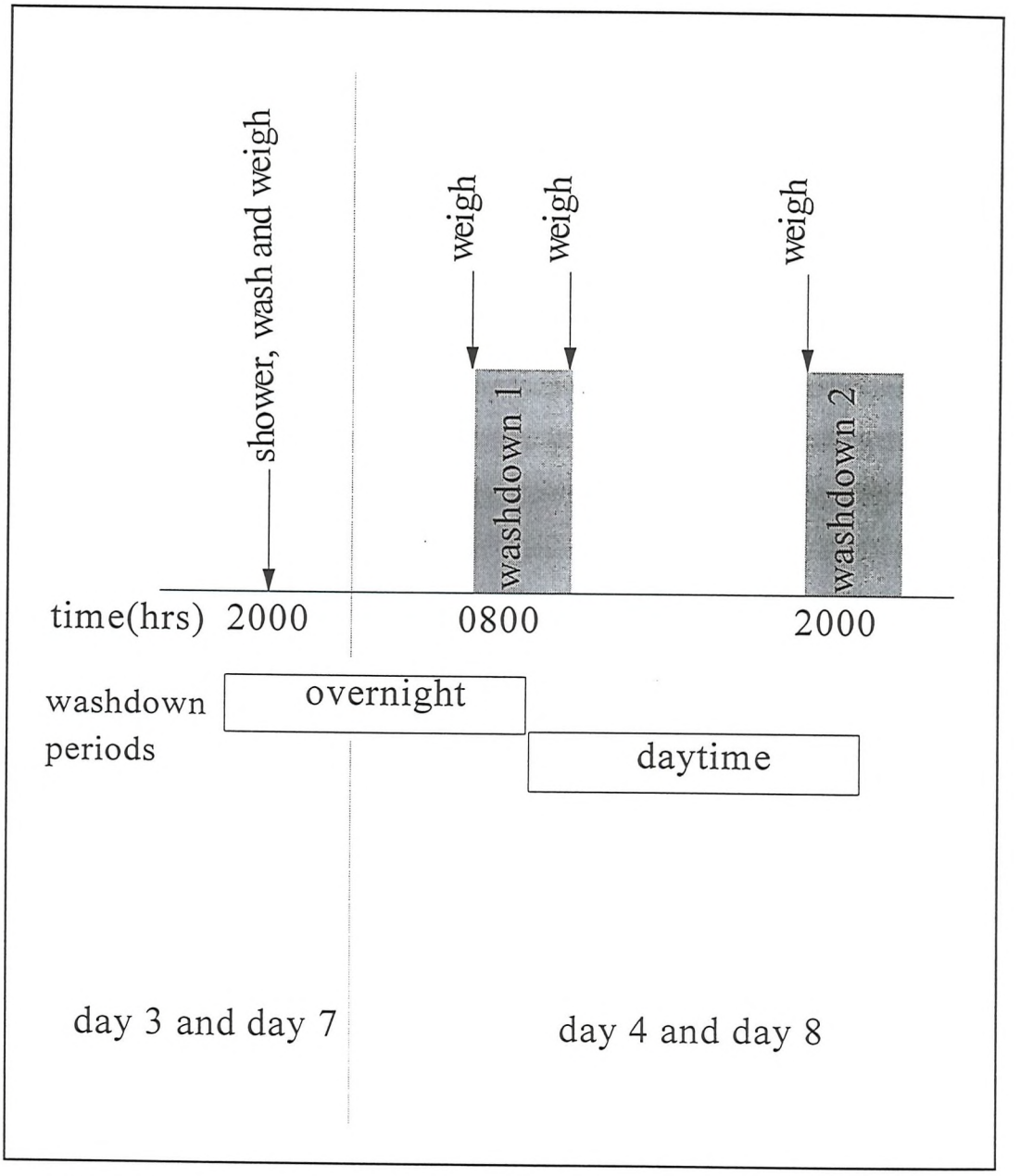
The clothing was well mixed in the washdown before removing an aliquot (approximately 100 ml) for later analysis of sodium content. To clean the clothes, they were washed in a machine four times, the last three times being without soap powder prior to use to minimise contamination. The polythene bags were also rinsed thoroughly with tap water and dried before re-use.

The washdown procedure was evaluated by recovery of a known amount of sodium which had been allowed to dry on the surface of the skin after application of 500 ml of the standard solution by a pressure sprayer. A recovery of approximately 85% (80-90% was achieved by this procedure.

Sweat sodium was analysed (within 48 hours) following the same procedure as described for urinary sodium above, without prior dilution of the sample.

The proportion of recovery of sodium from the skin using this technique is clearly a limiting factor in the subsequent analysis. A more accurate assessment of completeness of the washdown could have been made if a known quantity of a inert marker had been sprayed onto the skin and the recovery of this analysed together with the washings. Thus the results for sweat sodium and net sodium balance need to be interpreted with this limitation in mind.

Figure 4.2. Schematic diagram of the sweat washdown procedure.



The subjects were dressed in clean long underwear during each of the washdown periods. This clothing was removed and then used to towel the body dry before being added to the washing water.

Faecal weight loss and faecal sodium excretion. The weight of faeces was estimated from differences in body weight (pre- and post-defaecation) as necessary. In addition, stools were collected on days 3 and 8 and frozen for subsequent analysis. The weight and sodium concentration of the wet stool was determined to estimate faecal sodium excretion. Wet stools were homogenised with a known volume of distilled water and centrifuged (4,000 rpm for 25 minutes). One millilitre of supernatant was removed into a boiling tube with 2 ml of concentrated nitric acid and left to denature any remaining solids for 30 minutes before adding a further 1 ml of 2M nitric acid and heating to 100°C for 45 minutes. This was washed into a volumetric flask and made up to 20 ml with distilled water.

Body temperatures. Aural and skin temperature at 4 sites (Grants thermistors) were monitored and recorded (Grants 1200 data logger, Grants, Cambridge, UK) during the exercise step test. In addition, sub-lingual and urine temperatures were monitored at approximately 4 hourly intervals during the hours 0700 - 2000. Sublingual temperature was taken by a clinical thermometer positioned beneath the tongue for a minimum of 2 minutes. Urine temperature was measured by asking the subject to urinate into a container through the neck of a funnel into which a standard clinical thermometer (previously shaken) had been inserted. This funnel was punctured with holes such that the flow of urine into the container was slowed but not impeded. This method has been shown to be effective unless the volume of urine is less than approximately 200 millilitres. The urine was transferred to the 24-hour collection container following temperature measurement. All thermometers and thermistors were tested for accuracy (to within 0.1 °C) prior to experimentation using a water bath and calibration thermometer (National Physics Laboratory).

Sublingual temperature. In the heat trials (experiments 2 and 3) sublingual temperature was measured with a standard clinical thermometer at the following approximate times throughout the day: 0700h; 1000h; 1400h; 1800h; and 2200h.

Heart rate. Heart rate was monitored during the exercise period via a 3-lead electrocardiogram from disposable electrodes and display monitor (S&W Diascope, Sweden) and recorded manually every five minutes.

Haematological measures.

Blood sampling. Posture and site are critical to the haematocrit ratio (Eisenberg 1963) and to alterations in blood volume (Senay et al 1985). Circulating blood volume is greater in the supine (11 %) than in the upright posture, and these fluid shifts mean that haematocrit and plasma volume do not become reasonably stable for at least 20 minutes after subjects have moved from standing to supine or vice-versa. Failure to control posture introduces a serious errors due to local fluid transudation. Similarly haemoconcentration incident to venostasis will lead to false serum protein concentrations.

All blood was sampled by trained personnel whilst the subjects reclining in their beds prior to rising, at 0700 hours daily. In all but two cases, subjects had been recumbent for a minimum of six hours prior to blood collection, and in most cases this was 6-7 hours. Blood was sampled from the antecubital vein or another convenient site in either arm, without stasis, using a needle and vacutainers. Five millilitres of blood was collected in EDTA for analysis of haematocrit ratio and haemoglobin concentration. A further 6-8 ml of blood collected in lithium/heparin was centrifuged and the plasma concentration of sodium and potassium determined. The remaining plasma was stored at -35°C until analysed for aldosterone concentration.

Preparation of plasma from blood samples. The lithium/heparin sample was mixed on a roller mixer for 5 minutes before centrifugation at 3000 rpm for 15 minutes. The plasma was aspirated and 1 ml diluted 1:10 (using a Hamilton automated dilution syringe) in doubly distilled water. This dilute plasma sample was re-mixed prior to analysis for sodium (see below). To remove all plasma proteins, 400 μL of undiluted plasma was diluted 1:25 in 10% nitric acid. This was well mixed by inversion before

centrifugation at 2500 rpm for 10 minutes. The supernatant was then eluted and re-mixed prior to analysis for potassium.

Analysis of plasma sodium and potassium. Plasma sodium was measured by ICP-AES as described above against a suitable calibration and quality control standard (NYCOMED 010017; Nycomed Pharma AS Diagnostica, Oslo) diluted as for the sample. Plasma potassium was measured similarly using the same standard for quality control, every 5 samples as for urine analysis. Calibration files for plasma sodium and potassium analysis were constructed prior to batch analysis of samples, from serially diluted calibration standards (NYCOMED 010017) of 500 mg/L sodium and 10 mg/L potassium respectively. All results were corrected for dilution.

Accuracy of measurement. To illustrate the method of quality assurance used throughout analysis of sodium and potassium, data from one of the calibration files created prior to the sample batch are reproduced here as follows: 27 plasma sodium QC samples were processed giving a mean of 126.5 mmol/L, and standard deviation of 2.4 mmol/L. This gave 95% confidence limits of 121.8 to 131.2 mmol/L. If any QC fell outside this range then the QC sample was repeated; if outside the computed 99% confidence limits then the calibration file was reconstructed and the batch analysis repeated. Individual samples were analysed in triplicate and repeated if the coefficient of variation was greater than 2%. Approximately 10% of the urine and 25% of the plasma sodium samples exceeded this limit.

Measurement of plasma aldosterone concentration. Aldosterone concentration was measured by radio immunoassay (Maia procedure, Serono Diagnostics, Rome) against standards of 0 - 6.925 nmol/L aldosterone. Aldosterone tracer (0.1 ml) and antiserum (0.1 ml) were incubated with 0.05 ml of each sample and 0.1 ml of distilled water at a temperature of 37°C for 60 minutes following thorough mixing of reagents. 1.0 ml of magnetic separation reagent was then added to each sample tube, including standards, and re-mixed before a further 10 minutes incubation at room temperature. The sample was then separated by exposure to a magnetic field for 10 minutes to assist

sedimentation of the residue. The supernatant was then decanted before radiation counting of 125-iodine commenced. Concentration of aldosterone was estimated by referral to a standard calibration curve. The coefficient of variation of three quality controls of 227, 440 and 936 pg/ml were < 12.8%, < 10%, and < 8.4% respectively for 9 repeated measures.

Measurement of plasma volume, haemoglobin and haematocrit. The EDTA tube was analysed for haemoglobin and haematocrit ratio using the principle of light scatter (Technicon H1E, Bayer, Newbury). This method of analysis measures the cell volume by light scatter following isovolumetric sphering and fixation (to eliminate differences due to cell shape and orientation). This method also counts the red corpuscles for RBC, and detects the wide-angle scatter as a measure of the density or haemoglobin concentration of each cell. Changes in plasma volume were computed from these measures by the method of Dill and Costill (1974) as follows:

$$\%\Delta PV = [(hbg_1 \times (1\text{-}hct_2)/hbg_2 \times (1\text{-}hct_1))\text{-}1] \times 100$$

where:

ΔPV is the change in plasma volume;

hbg is the haemoglobin concentration ;

hct is the haematocrit ratio or packed cell volume;

and subscripts 1 and 2 indicate initial and final values.

Estimation of sweat losses. Estimates of sweat loss whilst at rest were made in the heat by difference in body weight adjusted for any fluid intake and respiratory water loss (see below). Only one period of approximately one hour was allowed for this estimate. Sweat losses during the physical performance (step) test was assessed by change in body weight (adjusted for fluid intake and respiratory water loss), measured just prior to, and immediately after the exercise period.

Respiratory water vapour loss and energy expenditure. Respiratory water vapour losses were estimated from measures of temperature and ventilatory volume of exhaled air conducted by collection of several timed expired gas samples, during rest and exercise. Gas volumes were measured by a wet gas meter (PK Morgan, Rainham, UK) and temperature by a thermistor (Grant, Cambridge UK) and calibrated temperature meter (Grant meter, Grants Cambridge, UK). Energy expenditure during the physical performance test was calculated from measured oxygen consumption calculated from similar expired gas samples analysed for oxygen and carbon dioxide (gas analysers, ADC UK). Resting energy expenditure was also measured from expiratory gas collected over approximately 15-30 minutes, following a period of rest of at least 30 minutes. These latter measurements were conducted in the evening during the control and heat exposure periods.

Measurement of total body water. In experiments 2 and 3 total body water (TBW) volume was assessed by the dilution of deuterium oxide (D_2O). Following the collection of a baseline urinary sample (a minimum of 3 ml) for background measurement of D_2O, precisely 12 g of this stable isotope was administered orally to the subject prior to retiring on the first night before heat exposure and again on the last night. At least 6 hours equilibration was permitted overnight (with no food nor water permitted during this period) before collection of duplicate 5 ml samples of the first void urine. These samples were frozen at -35°C and subsequently their deuterium concentration analysed (isotope-ratio mass spectrometry, Europa Scientific, UK).

Anthropometric measures. Body weight was measured on rising each morning, following the first micturition.

Ethical and safety considerations.

These studies were approved by the Institute's local Ethical committee which operates according to guidelines defined by the joint Medical Research Council / Royal Navy

Personnel Research Committee. All subjects were volunteers who were fully informed regarding the nature of the trial and gave their written consent to participate, in accordance with the Helsinki Declaration. All were permitted to withdraw from the trial at any time, and an Independent Medical Officer was appointed for the trial to safeguard their interests. All subjects, who were deemed fully fit (no previous predisposing history of heat intolerance or renal complications) underwent a thorough medical examination including a 12-lead ECG. As a final precaution, access to emergency resuscitation, cooling facilities and i.v. fluids in EMU sickbay was maintained at all times. During the one hour exercise step tests a Royal Navy Medical Branch Rating (a trained paramedic) was in attendance. The Institute's safety limits regarding physical exercise in the heat were adhered to.

Statistical analysis.

All analyses were conducted using a software programme (Statistical Package for the Social Sciences, SPSS version 6.1).

For experiment one, which dealt with small numbers of subjects (MNa - 4 subjects; MLNa - 5 subjects) non-parametric statistics were used following examination of the distributions. Data are presented as median values with a range rather than mean and standard deviations. The Mann-Whitney U rank sum test and the Wilcoxon matched pairs signed-rank test were used to assess differences between the two groups and within each group respectively. These tests were preferred to *t*-tests as the samples were small and not normally distributed. The assumptions for these tests are that the samples are random and the values can be ordered.

For the latter experiments which used a minimum of seven subjects, parametric statistics were preferred, since this permitted the use of more powerful techniques, and the data were normalised where necessary. Statistical significance was assessed by a repeated measures analysis of variance. The assumptions underlying this technique

are that the variances of the populations are equal and that any covariances are zero. Observation of the dispersion of each of the variables revealed that not all data sets were normally distributed, necessitating square root transformation of the total sodium excretion and logarithmic transformation of the sweat sodium excretion and plasma aldosterone concentration data prior to statistical testing. Data presented are means and one standard error of the mean (with the exception of the environmental temperature data which are mean and one standard deviation).

When testing between groups, an additional assumption is that of symmetry, which means that the variance-covariance matrices must be equal for all levels of the between subjects factors. These assumptions were tested by Mauchly's test of sphericity. F ratio values were adjusted by the Greenhouse-Geisser Epsilon correction in repeated measures ANOVA where the assumption of sphericity appeared to be violated. Use of the Greenhouse-Geisser gives a more conservative estimate of significance.

CHAPTER 5.

THE EFFECTS OF DIETARY SODIUM RESTRICTION ON SODIUM AND FLUID BALANCE IN A TEMPERATE (25°C) ENVIRONMENT.

Introduction.

This thesis is primarily an investigation of altered dietary sodium intake upon physiological responses to heat exposure over several days. Before this, the effects of manipulating dietary sodium intake *per se* needed to be examined in the absence of any thermal stress. This would enable the results from the heat exposure experiments which followed to be attributed to either altered dietary intake, or heat exposure, or a combination of these stressors. Hence this first chapter examined the effects of dietary sodium control over a period of days and the subsequent effects of a restricted sodium intake.

Aims and main hypotheses.

The aim of this experiment was to examine the effects of a restricted dietary sodium intake in a temperate climate in order to test the hypotheses that:

a. such a restriction of dietary sodium produces a net negative sodium balance until balance is restored within approximately three days;

b. a sodium deficit reduces the extracellular fluid volume and stimulates the secretion of aldosterone.

This experiment was to establish whether sodium balance could be achieved at two different levels of sodium intake within a three day "control" period. It also presented an opportunity to examine the physiological effects of a reduced dietary sodium intake on sodium and volume homeostasis, and plasma aldosterone concentration in temperate (25°C) conditions before proceeding with further manipulations of dietary sodium intake in the heat.

Secondary hypotheses.

Secondary hypotheses examined by this experiment were that:

a. daily ingestion of a constant amount of sodium for three days would result in sodium balance such that the total amount of sodium excreted was similar to that ingested;

b. daily ingestion of two distinct different levels of sodium (i.e. a 'moderate' and a 'low' intake) by two separate groups of subjects would result in those groups having significantly different total sodium excretions within three days;

c. a restriction of dietary sodium intake would reduce: body weight, fluid intake, and circulating plasma volume;

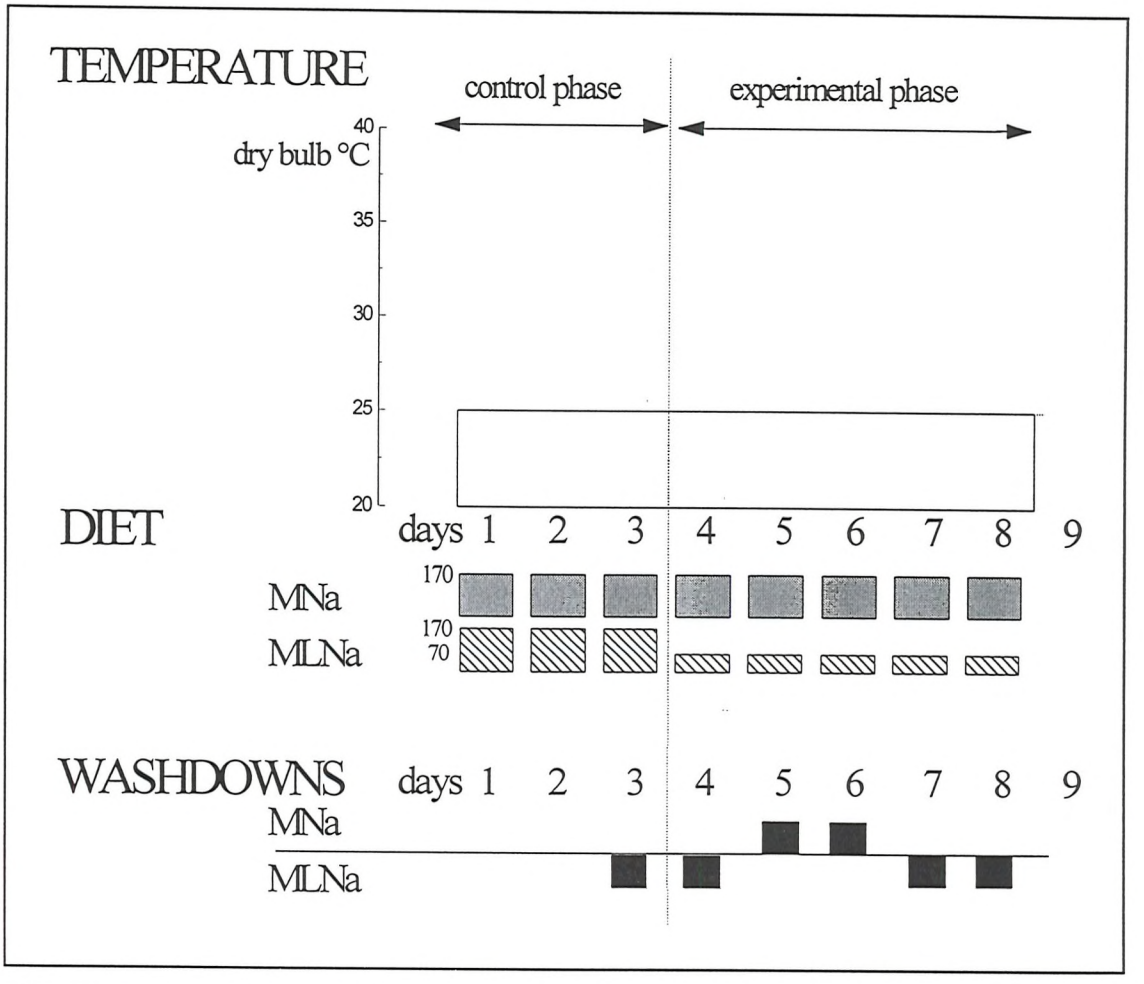
d. a restriction of dietary sodium intake would have no effect upon: the body temperature of individuals during exercise; heart rate during exercise;

e. a restriction of dietary sodium intake would increase the plasma concentration of aldosterone.

Methods.

Nine male volunteer subjects were allocated to two dietary groups, MLNa (restricted intake from 170/d mmol to 70 mmol/d) and MNa (170 mmol/d) as described previously. The environmental conditions were maintained constant at 25°C dry-bulb, 17°C wet-bulb, (40% relative humidity), with a constant air velocity of $0.5 m.sec^{-1}$. All procedures were as described in Chapter 4. The protocol used is schematised in Figure 5.1.

Figure 5.1. Schematic diagram of the experimental protocol.



There were two dietary conditions in this experiment: MLNa (n=5) - 170 mmol/d falling to 70 mmol/d from day 4; MNa (n=4) - 170 mmol/d throughout. The environmental temperature was regulated at 25°C throughout the entire 8 days. Washdowns were performed on days 3,4 and 7,8 for the MLNa group but on days 5,6 for the MNa group. All other measures were as described in the General Methods (Chapter 4).

Results

Sodium intake. The sodium intake of all nine subjects averaged over the first three days was 175.5 mmol/d. During days four to eight this was reduced to an average daily sodium of 66.9 mmol/d for the MLNa subjects, but maintained at 174.4 mmol/d for the MNa group.

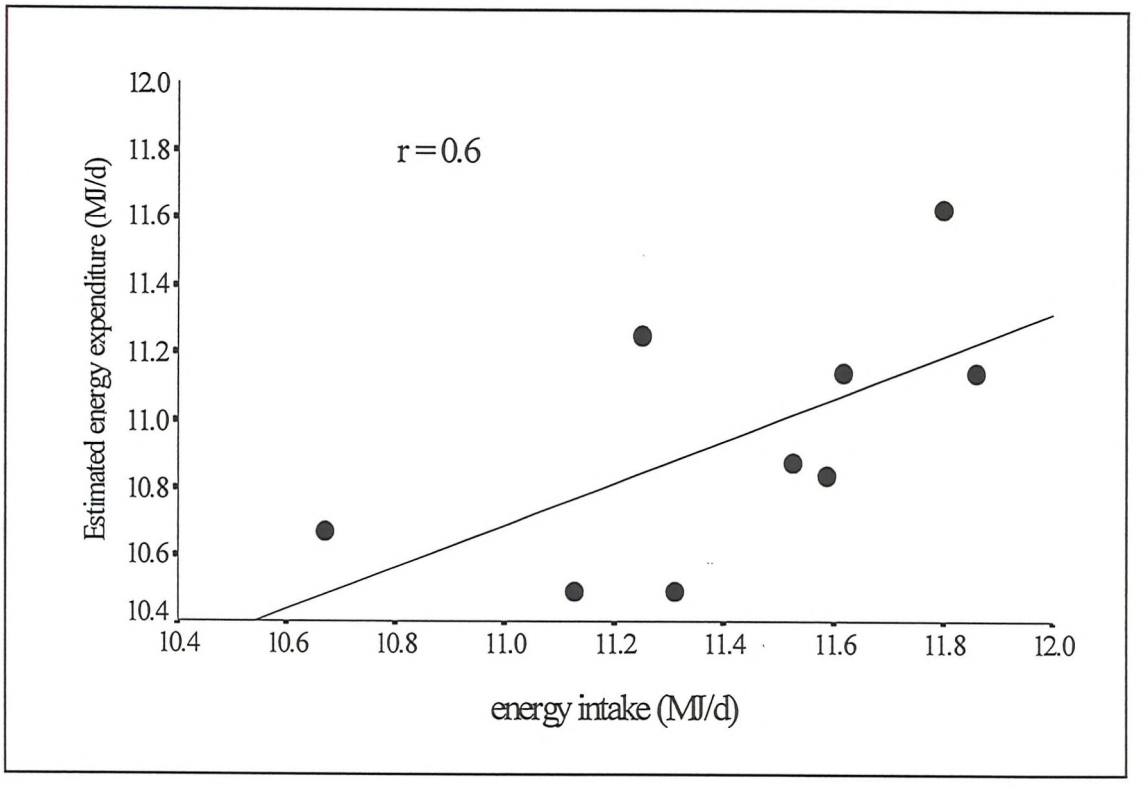
Energy intake and estimated energy expenditure. Mean (11.4 MJ) and range (10.7-11.9 MJ) of energy intake was similar to estimated energy expenditure (mean 11.0 MJ; range 10.5-11.6 MJ). The Pearson coefficient (r) for this correlation was 0.6 ($P=0.07$). The association between these two measures is shown in Figure 5.2.

Urinary sodium excretion. Twenty-four hour urinary excretion of sodium (UNa) is presented in Figure 5.3. Sodium excretions were similar on days 1 and 2 but on day 3 UNa excretion of the MLNa group (188 mmol) was significantly higher than for MNa (144 mmol). During the experimental phase this difference was reversed and the median UNa excretion of the MLNa group was consistently lower ($P<0.05$) than for MNa thereafter by approximately 15% on day 4, falling to approximately 60% lower by day 8.

Urinary potassium excretion. Values of 24h urinary potassium excretion (UK) on day three (final day of the "control" phase) ranged between 58-69 mmol/d and 39-80 mmol/d for MLNa and MNa respectively, and 71-117 mmol/d and 64-109 mmol/d respectively three days later (day six). There was no statistical difference between the two groups.

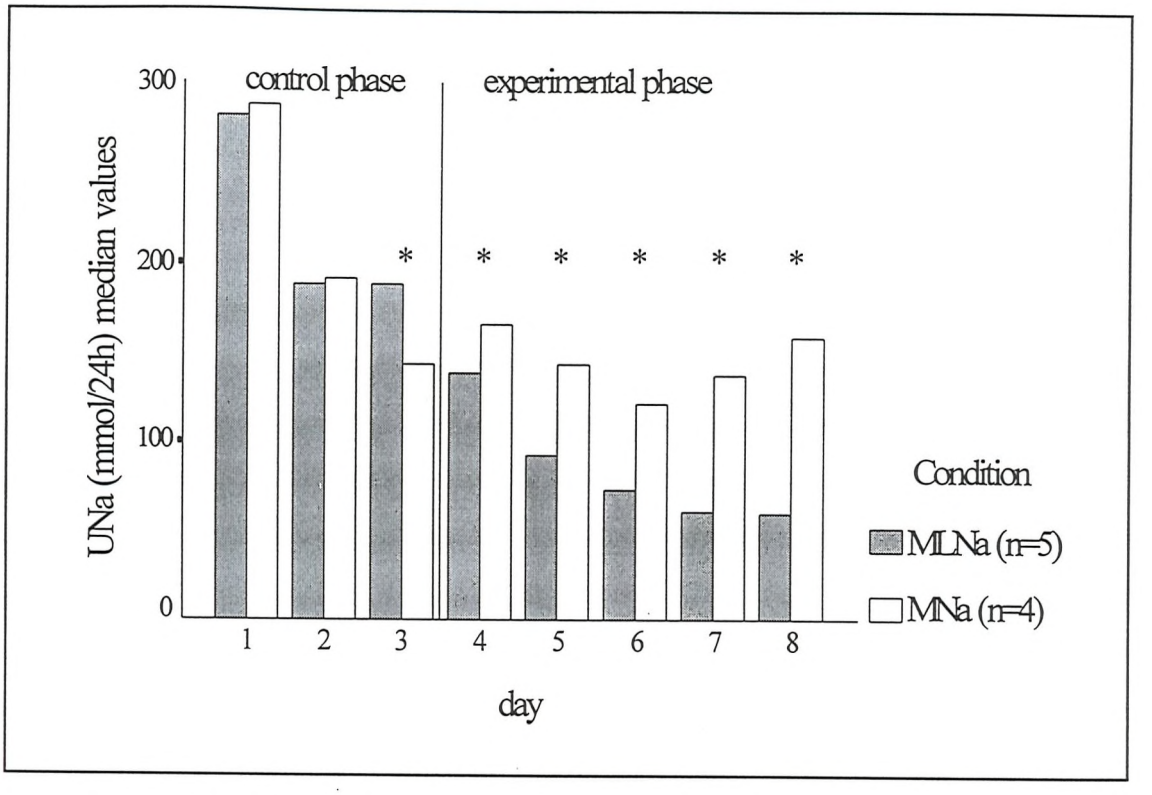
Ratio of urinary sodium to potassium excretion. As shown in Figure 5.4, the urinary sodium to potassium (UNa/UK) ratios of the two groups were statistically similar during the control phase. In the experimental phase, this ratio was consistently lower ($P<0.05$) for the restricted condition by approximately 20-60%.

Figure 5.2. Estimated energy expenditure versus energy intake of the nine subjects.



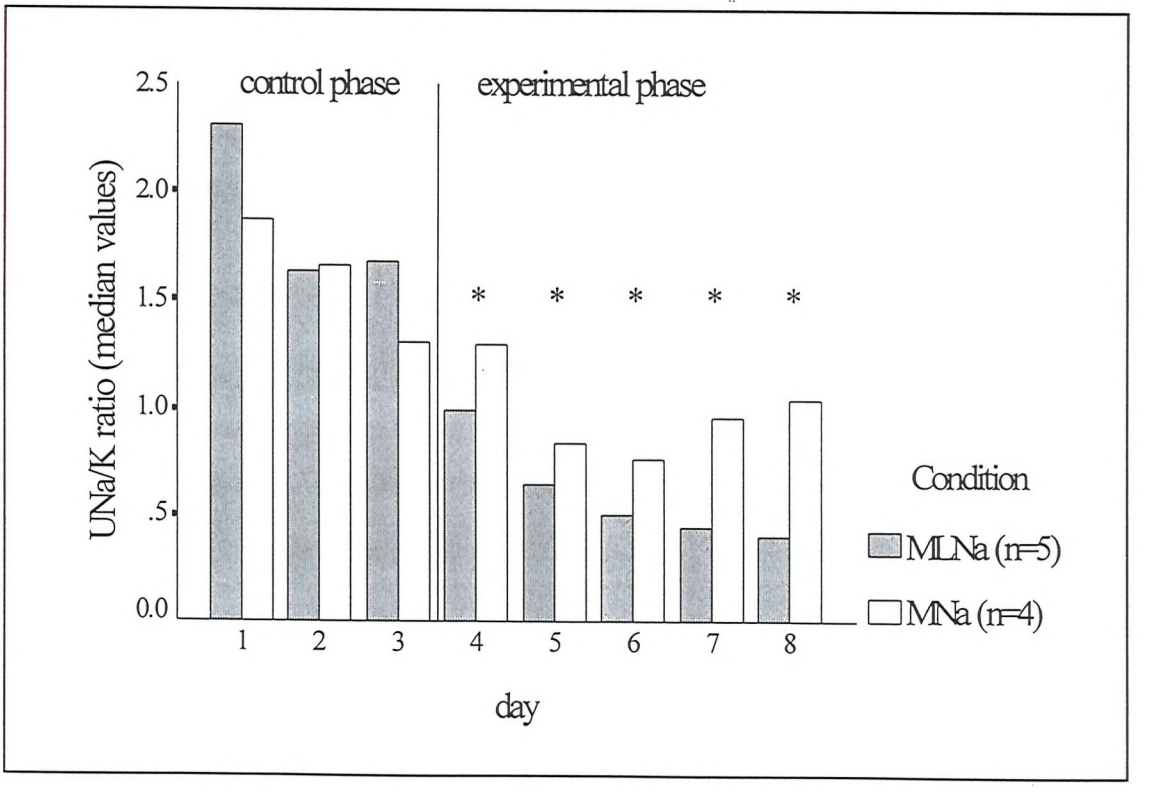
Estimated energy expenditure was calculated from indirect calorimetry, and intake was calculated from the supplier's food tables. The Pearson coefficient (r) for this correlation was 0.6 (P=0.07).

Figure 5.3. Urinary sodium excretion of the two groups.



** - indicates a significant (P<0.05) difference between the two groups.*

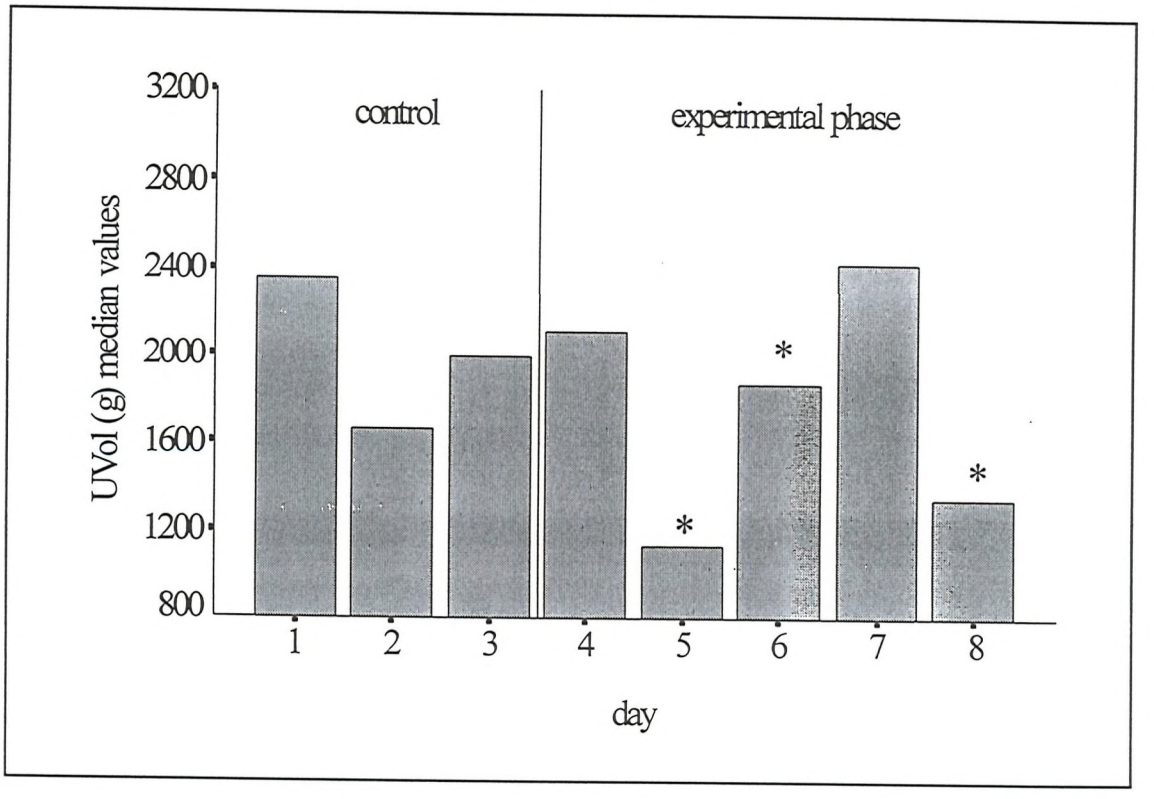
Figure 5.4. Urinary sodium to potassium ratio.



Urinary output. Daily urinary output for the two groups during the experimental period ranged from 628 g to 3957 g for the MLNa group, and 1132 g to 4193 g for the MNa condition. Within the MLNa group, urinary output was lower ($P<0.05$) on days 5,6 and 8 compared to day 3, whereas there were no significant changes in the MNa group. Figure 5.5. shows the urinary output of the MLNa group.

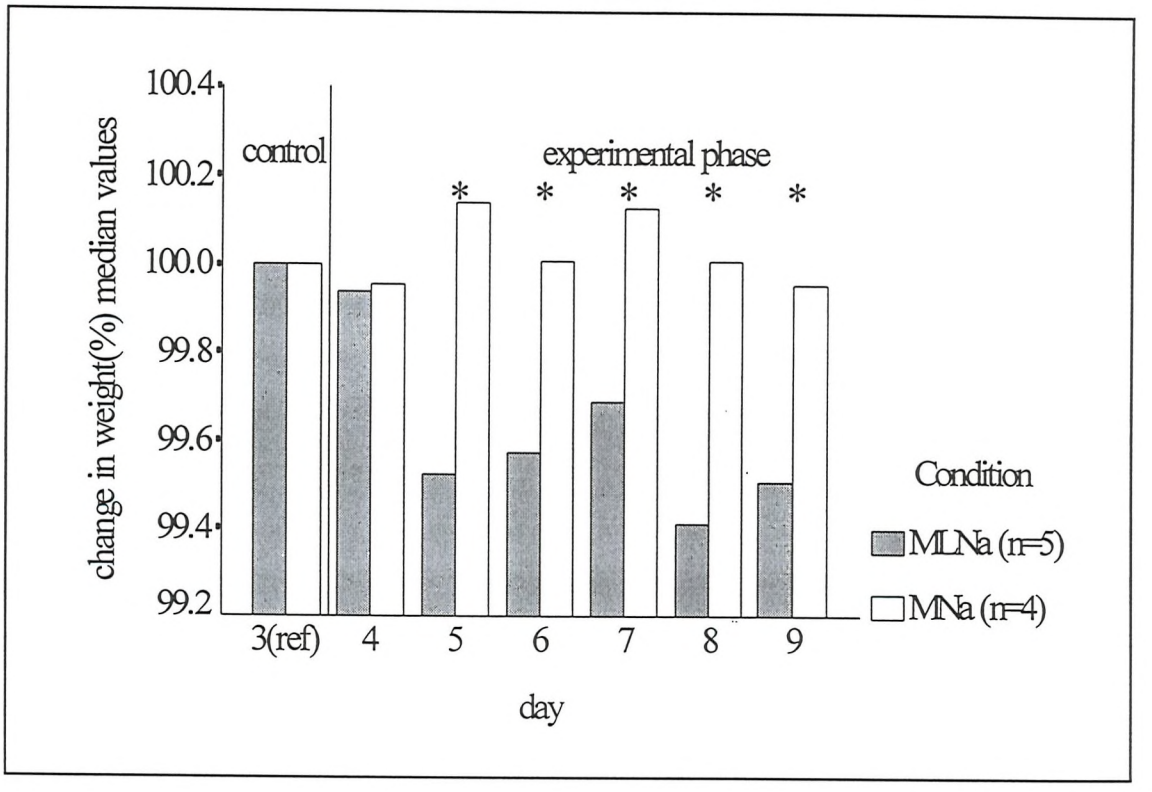
Bodyweight. The median bodyweight of the two groups on day 3 was 81.56 kg and 83.40 kg for MLNa and MNa respectively. To normalise this disparity of bodyweight between the two groups, the change in body weight during the experimental phase was expressed as a percentage change from that on the morning of day 3 (baseline). In the MNa there was little variation in %body weight about this 100% baseline value (ranging from 99.2% to 100.9%). In the MLNa condition, percent changes were greater (ranging from 98.5% to 100.4%) and after day 4, all values were below 100% indicating a fall in body weight (unlike the MNa condition). The median values for both groups are shown in Figure 5.6. A comparison between the groups revealed that from day 5 onwards there was a fall in % body weight in the MLNa group, which was not apparent in the MNa subjects. This difference between the groups was indicated ($P<0.1$) on day 5 and more apparent on day 6 ($P<0.05$). Within group analysis showed that, compared to day 3, this reduction of % body weight for the MLNa group was significant ($P<0.05$) from day 5 onwards. Weight loss, in absolute terms, was also significantly ($P<0.05$) lower than for day three in the MLNa group but not the MNa subjects.

Figure 5.5. Urinary output of the MLNa subjects (n=5).



The urinary output of the MLNa subjects was lower ($P<0.05$) on days 5,6 and 8 compared to the baseline (day 3) values.

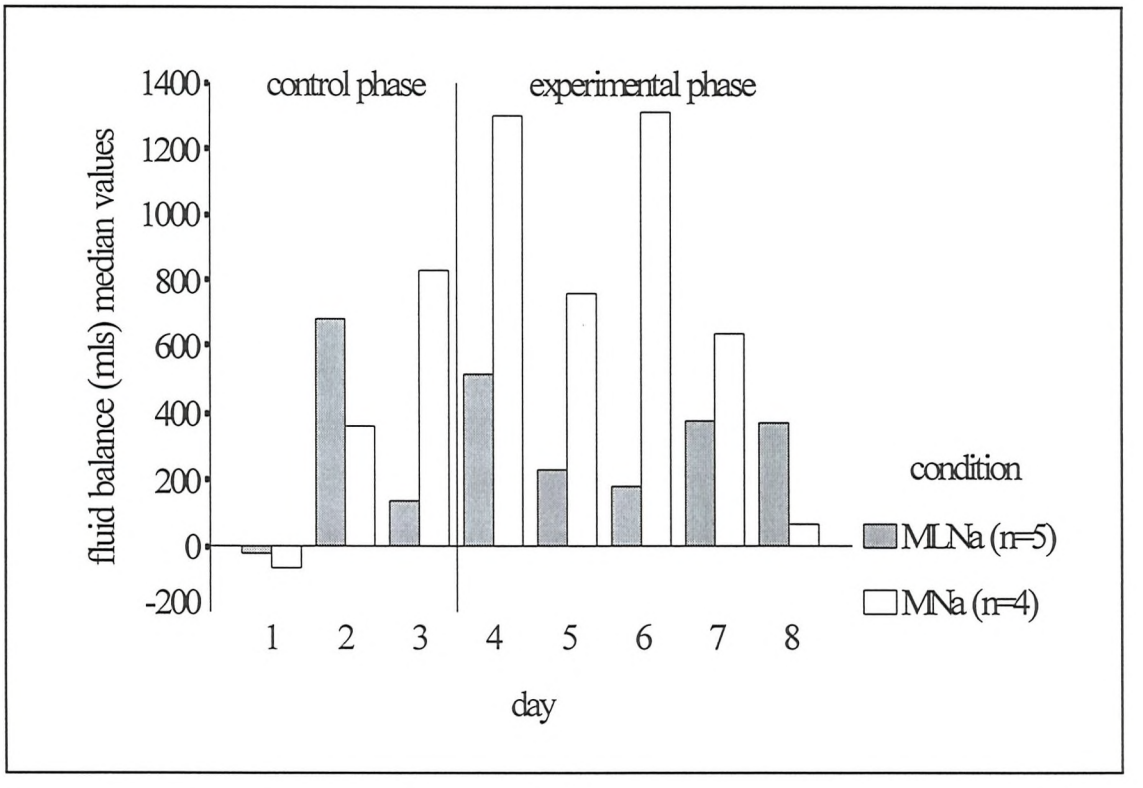
Figure 5.6. Percentage change in body weight for the two groups.



Within subject analysis indicated that, compared to day 3, there was a significant ($P<0.05$) reduction in % body weight from day 5 onwards in the MLNa group, but this was not apparent in the MNa subjects. The difference between these groups on day 6 (of approximately 0.5%) was also significant ($P<0.05$).

Fluid intake and fluid balance. Fluid intakes ranged from 1020-5885 g for MLNa and 1640-4320 g for the MNa group (not including water ingested with food). These absolute values were expressed as g per kg of body weight prior to comparison between groups. Median values across all days of the experimental phase were 23.5 g/kg and 39.3 g/kg for the MLNa and MNa groups respectively, but no significant differences were observed. A within group comparison of intake on day three compared to each of the following days revealed a higher ($P<0.05$) median intake on day seven (28.4 g/kg) compared with day three (20.8 g/kg) for the MLNa group, but this amount on day seven was not significantly higher than the quantity of fluid drunk on either of the other two control days. Fluid balance (fluid intake less urinary output, i.e. excluding insensible and evaporative sweat loss) did not change significantly within each group of subjects with respect to time, nor was there any significant difference between the two groups, although a tendency to a more positive balance was suggested in the MNa subjects compared to MLNa (Figure 5.7).

Figure 5.7. Fluid balance of the two groups.



Plasma sodium concentration. Median and range values are presented in Table 5.1; these ranged from 131.4 mmol/L to 166.1 mmol/L for the MLNa group and 131.4 mmol/L to 146.7 mmol/L for the MNa subjects. Thus there was more variability in this measure for the MLNa subjects. On day 7 the value for the MLNa group (135.4 mmol/L) was lower ($P<0.05$) than the value for the MNa group (139.1 mmol/L).

Table 5.1. Plasma sodium concentration (mmol/L): median and range values.

day	MLNa (n=5)			MNa(n=4)		
	median	min	max	median	min	max
1	135.8	131.3	138.2	135.0	134.2	136.9
2	135.8	132.2	149.7	136.3	134.2	137.2
3	136.3	133.0	166.1	136.9	135.6	137.8
4	136.7	131.6	147.5	135.2	133.8	139.4
5	136.1	133.4	138.2	134.7	133.7	139.5
6	136.8	135.0	137.8	135.7	131.4	138.4
7	135.4*	134.7	138.3	139.1*	135.5	146.7
8	134.8	132.1	160.7	137.4	134.8	138.5
9	135.7	135.2	151.6	138.3	136.9	143.0

** indicates significant ($P<0.05$) difference between the two groups (Mann-Whitney U test).*

Plasma potassium. Median and range values are presented in Table 5.2; these ranged from 2.94 mmol/L to 5.05 mmol/L for the MLNa group and 2.74 mmol/L to 4.08 mmol/L for the MNa subjects. On day 5, the median value for the MLNa group (4.63 mmol/L) was higher ($P<0.05$) than the baseline value on day 3 (3.61 mmol/L), and also higher ($P<0.05$) than the MNa value on day 5 (3.82 mmol/L).

Table 5.2. Plasma potassium concentration (mmol/L).

day	MLNa(n=5)			MNa(n=4)		
	median	min	max	median	min	max
1	3.41	2.94	3.98	3.49	3.43	3.51
2	3.67	3.39	4.14	3.05	2.74	3.58
3	3.61	3.13	3.69	3.88	3.41	4.04
4	3.84	3.17	4.46	3.78	3.13	4.08
5	4.63*†	4.48	5.05	3.82	3.72	3.97
6	3.30	3.08	3.96	3.38	2.99	3.56
7	3.58	3.14	3.78	3.38	2.83	4.00
8	3.23	3.05	3.69	3.38	2.93	3.43
9	3.67	3.45	4.57	3.42	2.92	3.46

** indicates significant ($P<0.05$) from day 3 (Wilcoxon matched pairs test); † indicates significant ($P<0.05$) from MNa (Mann-Whitney U test).*

Change in plasma volume. Change in plasma volume relative to day three varied widely between subjects. Median and range values for days 4, 6 and 9 (when all subjects were sampled without difficulty) are presented in Table 5.3. There was a fall in plasma volume on day 6 for both MLNa (a 1.8% reduction) and MNa (a 0.9% reduction). When these values were compared within each group to the initial increase in plasma volume seen on day 4 (0.4% for MLNa and 2.1% for MNa), only the change seen in the MLNa subjects was found to be significant ($P<0.05$).

Table 5.3. Change in plasma volume (relative to day 3): Median and range values.

	MLNa (n=5)			MNa (n=4)		
day	median	min	max	median	min	max
4	0.5	-2.0	7.0	2.1	0.0	6.5
6	-1.8*	-8.0	3.9	-0.9	-3.2	6.2
9	3.1	0.0	6.4	4.3	3.8	10.7

*Footnote: * indicates significantly lower (P<0.05) than day 4 (Wilcoxon matched pairs test).*

Faecal sodium. One of the subjects in the MLNa did not produce a faecal sample for the experimental phase. No difference was observed between the two groups when faecal sodium concentration was expressed as mmol/g of wet stool weight or if expressed as absolute sodium excretion (mmols). There was a reduction within the MLNa group from the control sample to the experimental phase sample, but this was not significant. The MNa group exhibited a faecal excretion of sodium similar to the experimental phase of the MLNa (Table 5.4).

Table 5.4. Faecal sodium excretion of the two groups (mmol/d).

	MLNa (n=4)			MNa (n=4)		
	median	min	max	median	min	max
control phase	15.6	6.4	27.4	8.4	4.7	13.3
experimental phase	7.9	3.2	12.7	7.0	4.5	24.5

Sweat sodium secretion. Sweat sodium secretion (SNa) was measured over two twelve-hour periods, in whole-body washdowns performed on day 4 (am and pm) and again on day 8 (am and pm) for the MLNa group. In the MNa group, washdowns were only performed on day 6 (am and pm). Overnight collections ranged from 1.20 mmol/12h to 3.26 mmol/12h, whereas daytime collections were higher being 2.27 mmol/12h to 7.39 mmol/12h. A significant ($P<0.05$) reduction in SNa was found in the overnight samples for the MLNa group, from a median sodium secretion of 2.44 mmol/12h to 1.62 mmol/12h. This latter value was also significantly ($P<0.05$) lower than that of the MNa group in the am sample on day 6 (2.51 mmol/12h).

Table 5.5. Sweat sodium secretion of the two groups (mmol/washdown period).

	MLNa (n=5)			MNa (n=4)		
	median	min	max	median	min	max
control overnight	2.44*	2.06	3.26			
experimental phase overnight	1.62*†	1.20	2.36	2.51†	1.97	2.63
control daytime	5.16	2.38	7.39			
experimental phase daytime	2.44	2.27	6.02	4.15	3.77	6.56

** and † indicate values are significantly ($P<0.05$) different within the group (control overnight) and between the groups.*

Aldosterone. Plasma aldosterone concentration is shown in Table 5.6 for the two groups on all days with the exception of 5 and 7 due to missing samples. The range of values during the experimental phase was between 116 pmol/L and 981 pmol/L for the MLNa subjects and 313 pmol/L to 549 pmol/L for the MNa subjects. No significant differences between the groups were apparent from day to day, nor was there any significant change in concentration (relative to day 3) within each group.

Table 5.6. Plasma aldosterone concentration of the two groups (pmol/L).

	MLNa (n=5)			MNa (n=4)		
day	median	min	max	median	min	max
1	289	180	348	265	235	354
2	324	167	467	243	184	408
3	331	204	758	343	237	657
4	288	151	595	328	313	494
6	475	257	981	400	335	445
8	475	228	932	478	359	549
9	379	116	847	369	334	421

Heart rate during work. Heart rate increase in the first ten to fifteen mins of work and then was maintained at approximately the same level of 95-100 beats per minute for the remainder of the hour. Within each group, heart rates at 40 and 50 minutes of exercise were compared on day 3 and day 6 and no significant differences were found.

Aural temperature. Aural temperatures increased from an average of 36.6°C at the commencement of exercise to a value of 36.9-37.1°C after 30 minutes after which no further change occurred. Within each group, temperatures at the 40 and 50 minute time points were compared on day 3 and day 6. This analysis indicated no significant change in temperature in either condition.

Mean skin temperature and evaporative weight loss during exercise. Mean skin temperature ($\bar{T}_{sk}$) on day 3 was compared with that on day 6. In the MLNa group $\bar{T}_{sk}$ increased from a mean value of 30°C by a degree to 31°C during exercise on day 3, whereas on day 6, a similar increase in $\bar{T}_{sk}$ was seen but at a higher absolute temperature (31.8°C to 32.6°C). This difference between day 3 and day 6 was significant ($P<0.05$). a similar, if slightly higher change in absolute $\bar{T}_{sk}$ was noted for

the MNa subjects, although this was not significant (P=0.07). Taking the baseline $\bar{T}_{sk}$ of all nine subjects together, analysis of variance revealed a significant (P<0.01) difference between day of exposure which was unaffected by diet. The mean (sd) evaporative weight loss on day 8 was 298 g (42 g).

Environmental temperature. Environmental temperature was recorded at 15 minute intervals throughout the trial. Mean (sd) results for day 3 were: dry bulb 25.0±0.1°C; wet bulb 17.6±0.3°C, and on day 6: dry bulb 24.8±1.3°C; wet bulb 17.6±0.3°C.

Conclusions.

These conclusions will be confined to discussing only those points pertinent to this chapter. The relevance of these findings to the issue of sodium balance and heat acclimation will be addressed in the general discussion (Chapter 9). The purpose of this experiment was to examine sodium balance; firstly to assess whether this could be achieved after three days of a fixed daily sodium intake, and secondly, if this could be restored following an acute restriction of dietary sodium. Of particular interest was the speed of this re-establishment of sodium balance and how it was achieved physiologically.

That sodium intake can be successfully manipulated by the methods used here was evident from the sodium excretory data. This indicated that balance could be achieved after three days of dietary control, and that a clear separation of dietary groups was possible following a dietary restriction. Urinary sodium excretion was reduced within 24h of the restriction of dietary sodium intake. The UNa/K ratio also discriminated between the two groups successfully from the day of restriction. The diet was designed to meet predicted energy expenditure and this too appeared to be achieved as judged by the association between intake and expenditure, which approached significance, and the stable bodyweight of the moderate intake (MNa) group. This would support the inference that loss of bodyweight is attributable to

fluid loss, i.e. dehydration. Note however that the discrepancies between weight loss and positive fluid balance (compare the MLNa condition in Figures 5.6 and 5.7) indicate error in the calculation of this latter measure. This error indicates the difficulty of estimating fluid balance from weight differences, errors in the estimation of faecal, insensible water and metabolic weight losses, and highlights the assumption of a steady-state upon which this estimation rests.

Dehydration, as determined by bodyweight loss, appears to have occurred during the first 48 hours of sodium restriction in the MLNa group, and this was achieved despite a reduction in urinary output during the restriction period. This fall in weight must therefore have been achieved by a negative fluid balance, and as sweat and insensible losses were presumably similar for both groups, then this must be attributable to differences in fluid intake and urinary output. This is supported by the reduction in urinary output for the MLNa group on days 5, 6 and 8 compared to day 3, and the generally lower (although not significant) fluid intake over the experimental phase. The fluid balance of the MLNa group did appear to be less positive on days 4 and 6 of the experimental phase (Figure 5.7), also supporting this hypothesis. The median sodium deficit in the interim period (days 4-6) was 103 mmol, roughly equivalent to 0.7 litres of plasma, almost 1% of body weight. This would suggest that a proportion of the weight loss could be accounted for by this negative sodium balance, assuming a reduction of the extracellular fluid volume.

Median plasma sodium appeared to be higher in the case of the MNa diet compared to the restricted diet on day 7. The reason for this is unclear since the value for plasma sodium concentration on this day (135.4 mmol/L), although the lowest of all the days, was not substantially reduced compared to the control period. The increase in plasma potassium on day 5 is of interest and may be indicative of a sodium deficit during the previous day, (the first day of sodium restriction) leading to a reduced cell membrane sodium/potassium exchange. The previous day differences in food intake were slight: the MLNa group ate 240 g meal of roast beef and Yorkshire pudding and a 100 g chocolate mouse; the MNa group ate 227 g of beef stew and dumplings with a 128 g

caramel dessert. Roast beef has a higher potassium content (370 mg/100g) than stewed beef (230 mg/100g) (Holland et al, 1991). Assuming the meat weight to be 10% of the total, the estimated difference in potassium intake from dietary beef would have been only 5 mg.

The reduction in sweat sodium secretion (overnight samples) and also faecal sodium (not significant) during the period of dietary restriction may be indicative of compensatory increases in sodium reabsorption; Greater sodium retention by the sweat glands and colon may have been mediated by an increased secretion of aldosterone, although this response was slight. The extreme values of faecal sodium were higher than have been reported elsewhere (Geigy, 1987; Collins et al, 1971).

The exercise test was intentionally limited to a light work rate for just one-hour, although this appeared to be sufficient to increase body temperature by almost 0.5 C. No adverse effect of reduced sodium intake was shown on thermoregulation or exercise heart rate in these conditions. The amount of evaporative loss on day 8 (mean of 298 g) was typical of each day. Assuming the sodium loss during the day to be sweat loss, this would give a sweat sodium concentration estimates for the two groups of: between 8 and 17 mmol/L. This lower value of 8 mmol/L is similar to that found for acclimatised subjects in three studies cited by Robinson & Robinson (1954), whereas 17 mmol is lower than the values cited for unacclimatised subjects (ranging from 25 to 60 mmol/L). The explanation for this could be the low rate of sweat production, since less concentrated sweat would normally result in these circumstances (Allan & Wilson, 1971). Rather it may be a consequence of incomplete sodium collection, the uncollected portion probably being from the uncovered areas of the body, the hands and feet, areas which sweat more profusely having a higher distribution of sweat glands.

The increase in mean skin temperature on day 4 was unexpected but was small, and was almost certainly attributable to a rise in the ambient vapour pressure as the environmental conditions were well controlled in the control phase (coefficient of

variation <2%) to within one degree of the target (dry and wet) temperatures, but a less well controlled dry bulb (for technical reasons) after day 3 (CV <5%). This serves to underline the importance of climatic control during experiments of this nature.

In summary these conclusions support the central hypothesis that reduced dietary sodium will cause a reduction in ECF volume in order to maintain plasma osmolality and that these changes will be well tolerated in this environment. Additionally, they indicate that reduced dietary sodium intake may potentiate the secretion of aldosterone and that sodium balance can be restored in 3-4 days. The new working hypothesis is that:

> *restriction of dietary sodium intake initiates increased urinary loss in the short-term (within hours) to maintain plasma osmolality, but increased renal reabsorption of sodium in the longer term (3-4 days) to restore sodium balance. Restriction also appears to reduce sweat sodium secretion and faecal sodium loss. These changes may be in response to an increase in plasma aldosterone.*

As a consequence, further questions needed to be addressed:

a. What would be the effect of a greater sodium depletion through increased sweating?
b. How would these changes in ECF volume be tolerated in the heat?
c. Can the sodium deficit be prevented by dietary supplementation, and if so, is this advisable?
d. Can the aldosterone response to a low sodium intake be manipulated to assist in maintaining sodium balance?

CHAPTER 6.

THE EFFECTS OF HEAT EXPOSURE AT MODERATE AND HIGH LEVELS OF SODIUM INTAKE.

Introduction.

The model for the investigation of sodium manipulation in the heat adjusts two variables: sodium intake and environmental temperature. Hence the first investigation needed to establish that sodium balance could be achieved and maintained at two altered levels of sodium intake within a period of 2-3 days of commencing a strictly controlled sodium diet. This experiment was intended to investigate the effects of heat exposure, at both the normal level of sodium intake, which was estimated to maintain approximate balance (i.e. sodium excretion would be equal but opposite to sodium intake). As a comparison a higher sodium intake was also included, as this condition was predicted to be in positive sodium balance throughout the heat exposure and also represented the likely situation with regard to sodium supplementation upon exposure to heat.

Aim and main hypotheses.

The aim of this experiment was to examine the effects of sodium supplementation, before and during heat exposure, in order to test the hypotheses that:

a. in a hot climate, dietary supplementation with sodium prior to heat exposure has no beneficial effect upon parameters of thermoregulation and heat acclimation;

b. sodium supplementation reduces aldosterone secretion, attenuating those physiological response initiated by heat exposure to conserve sodium.

These hypotheses were tested by the comparison of two dietary groups, a supplemented sodium group versus a control, prior to and during exposure to the heat. Thermoregulation and heat acclimation were assessed by measuring body temperature and heart rate during an exercise tolerance test, performed daily. Sweating and sweat sodium excretion were also estimated.

Secondary hypotheses.

Secondary hypotheses examined by this experiment were that:

a. daily ingestion of a high or moderate level of sodium (HNa and MNa) would result in sodium balance being achieved after three days at normal temperature and within five days of heat exposure;

b. the lower level of sodium intake would increase secretion of plasma aldosterone in the heat due to a state of negative sodium balance.

c. the higher level of sodium intake (HNa) would have no effect on thermoregulation nor heat acclimation compared to a moderate intake (MNa) at this level of heat stress;

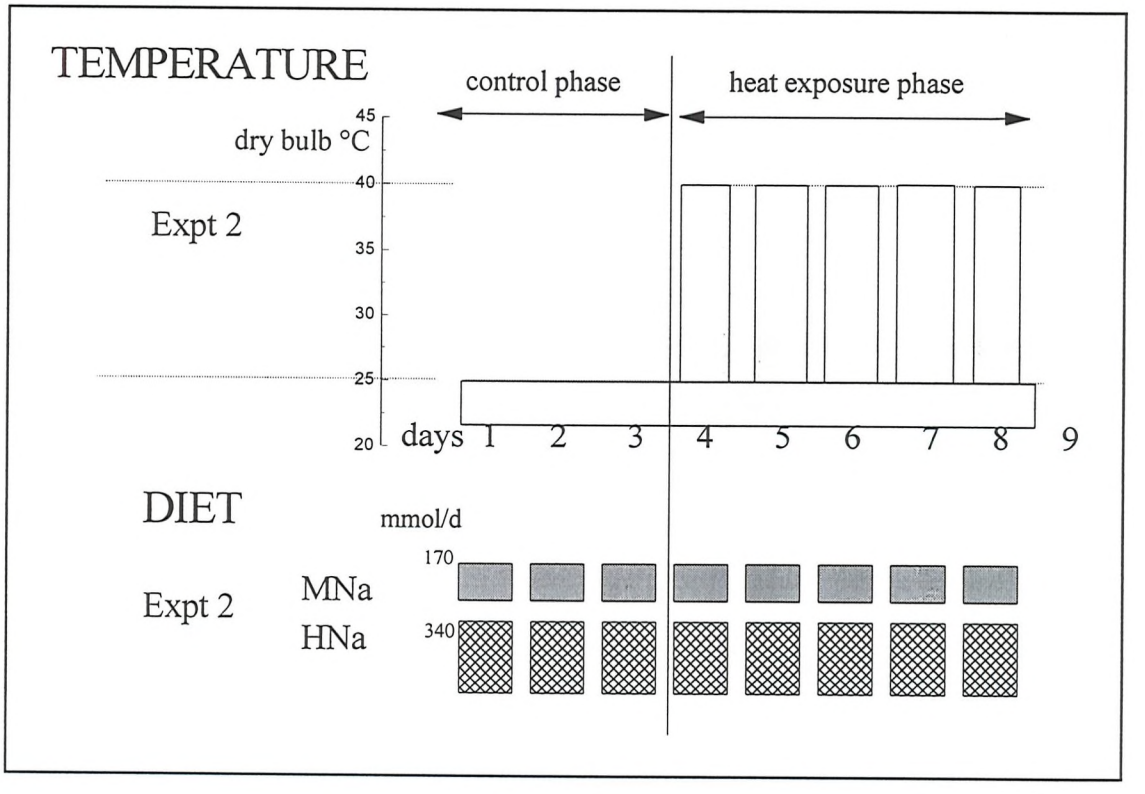
Methods.

The model of investigation was as described in the methods section with dietary control commencing on the evening prior to day one and heat exposure (40°C) between the hours of 0800h to 1800h on days four to eight inclusive. All measures were as previously described. Sixteen male volunteer subjects were recruited and each subject assigned to either:

a. Moderate sodium intake (MNa): a target intake of 170 mmol/d; or
b. High sodium intake (HNa): a target intake of 340 mmol/d.

The experimental protocol is summarised in Figure 6.1.

Figure 6.1. Schematic diagram of the experimental protocol.



Dietary control commenced from the evening prior to day one on arrival at the chamber. During the heat exposure phase, ambient temperature was increased from 25°C to 40 °C between the hours 0800h to 1800h, this change taking approximately one hour.

Results. Summary statistics are given as mean±sem unless otherwise indicated.

Anthropometric measures. Age, height and weight were: MNa group - 27 (2) years; 178.8 (1.5) cm; 81.8 (3.9) kg; HNa - 26 (3) years; 175.0 (1.6) cm; 90.19 (3.0) kg. There were no differences between the dietary groups with respect to these measures.

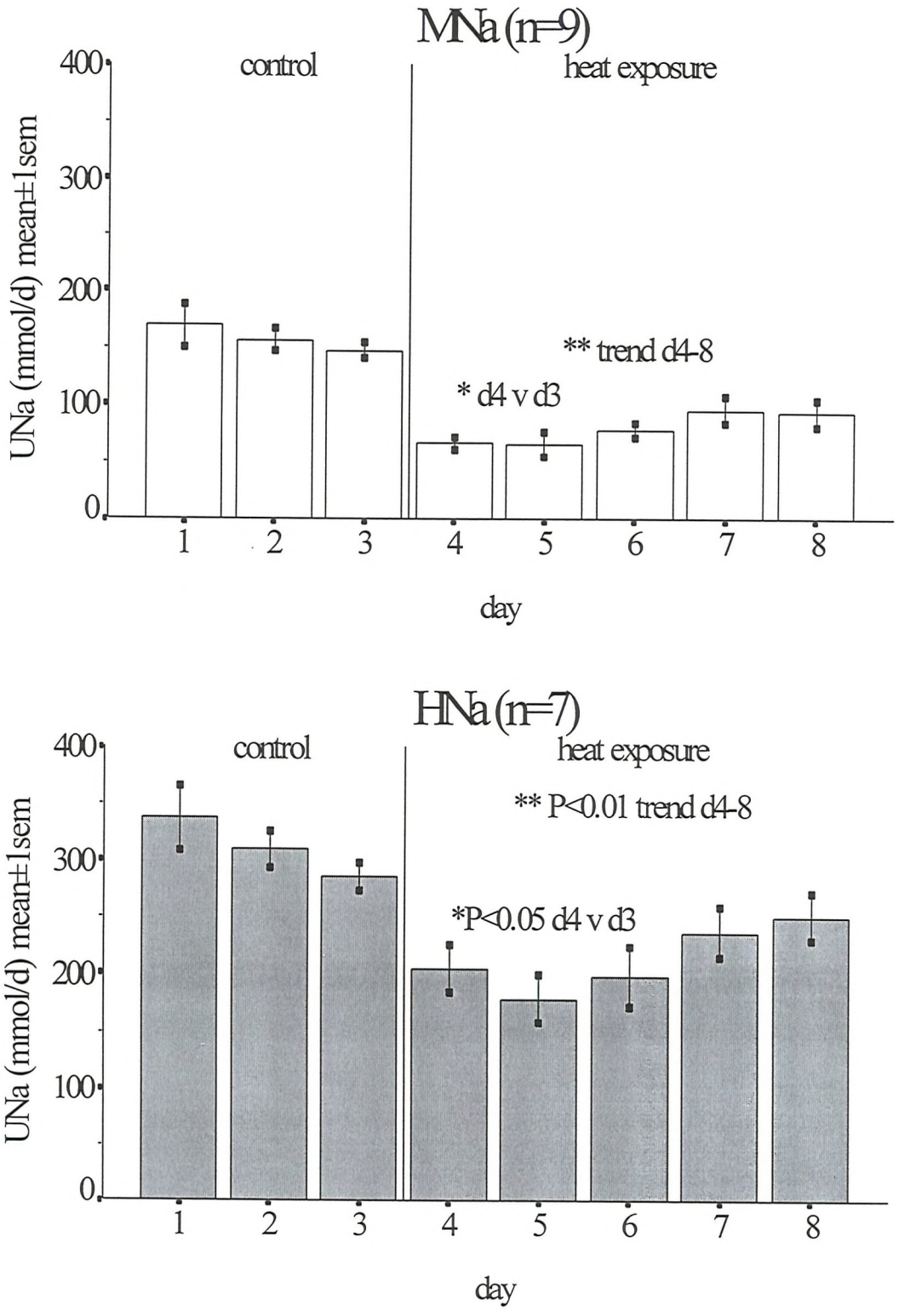
Sodium intake. The mean estimated intakes of sodium for each of the groups were as follows: MNa 174.1±0.6 mmol/d; and HNa 348.4±0.8 mmol/d.

Urinary output. Mean urinary output during the control phase was 2490±513 ml for the MNa condition and 2625±788 ml for the HNa group. On heat exposure there was a pronounced oliguria with mean urinary output falling significantly ($P<0.001$) to mean values of 1522±400 ml and 1387±391 ml for MNa and HNa respectively. There were no significant differences in urinary volume between the two groups either during the control period or the days of heat exposure.

Urinary sodium excretion. The difference in excretion of urinary sodium between the two dietary groups was apparent from day 1 of the experiment, being approximately two-fold higher for the HNa group, reflecting the higher sodium intake. This divergence between the groups remained throughout the experiment (Figure 6.2). There was a 25-50% reduction ($P<0.05$) in sodium excretion by this route in both dietary groups on day 4 compared to day 3. This initial fall continued until days 5 and 6, before there was a small but significant ($P<0.01$) rise.

Urinary sodium potassium ratio. The sodium/potassium (Na^+/K^+) ratio of the MNa group fell from a mean value of 5.0±1.1 on day 3 to 1.4±0.3 on day 4, and fell further to 1.3±0.3 the following day before rising to a mean of 2.4±0.7. Corresponding values for the HNa condition were 7.7±1.3 on day 3, falling to 5.6±3.2 on day 4, and further to 3.4±1.1 on day 6, before rising on the final day to 4.4±1.1. The (square-root transformed) ratio of the HNa group was higher ($P<0.05$) than for the MNa group, this difference being entirely attributable to differences in Na^+ and not K^+ excretion.

Figure 6.2 Urinary sodium excretion of the two groups.

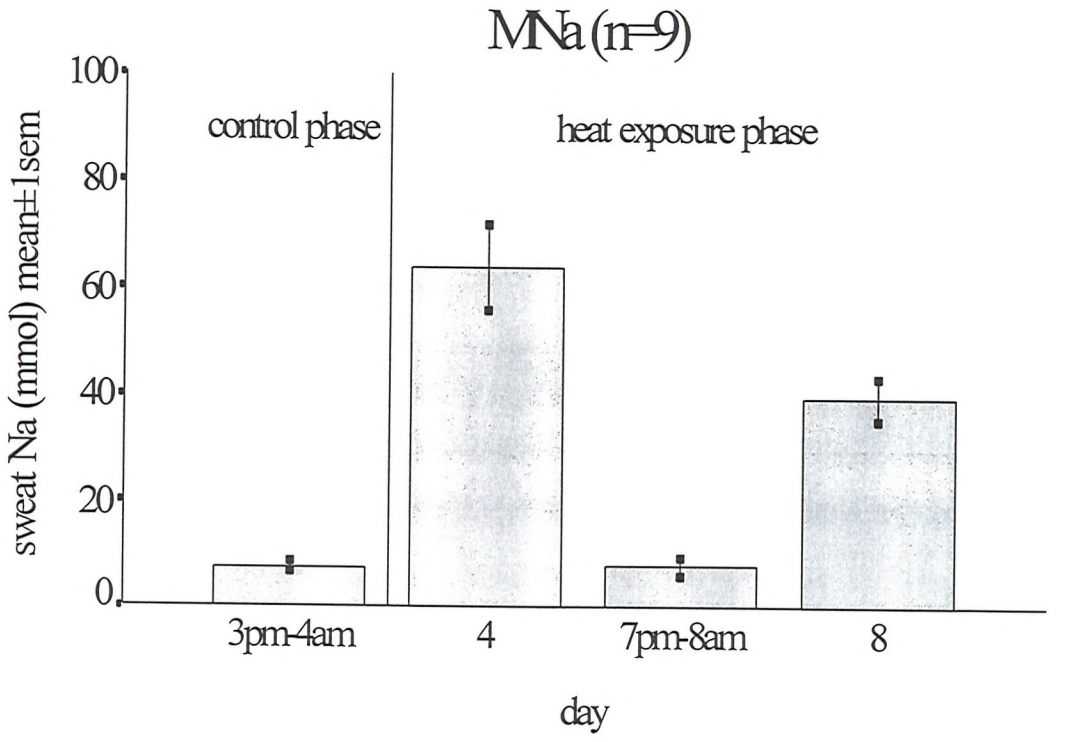


Sweat sodium secretion. Sweat sodium secretion (for the 12-hour periods) was significantly ($P<0.01$) higher for the daytime washdowns compared to the overnight washdowns. The four 12h washdowns (overnight and daytime on days 3,4 and 7,8) washdowns gave mean±sem values of 7.7±0.9 mmol; 63.8±8.0 mmol; 7.8±1.7 mmol; and 39.1±4.0 mmol for the MNa condition. The same washdowns for the HNa group yielded values of 14.6±2.9 mmol; 78.3±10.3 mmol; 13.7±1.3 mmol; and 52.6±6.2 mmol (Figure 6.3). All values were transformed (natural logarithm) prior to ANOVA analysis. The overnight samples of the two groups were similar whereas the daytime washdown of the HNa group was greater than the MNa group, although this difference was not significant. There was a reduction ($P<0.01$) in sodium secretion across both groups from day 4 to day 8.

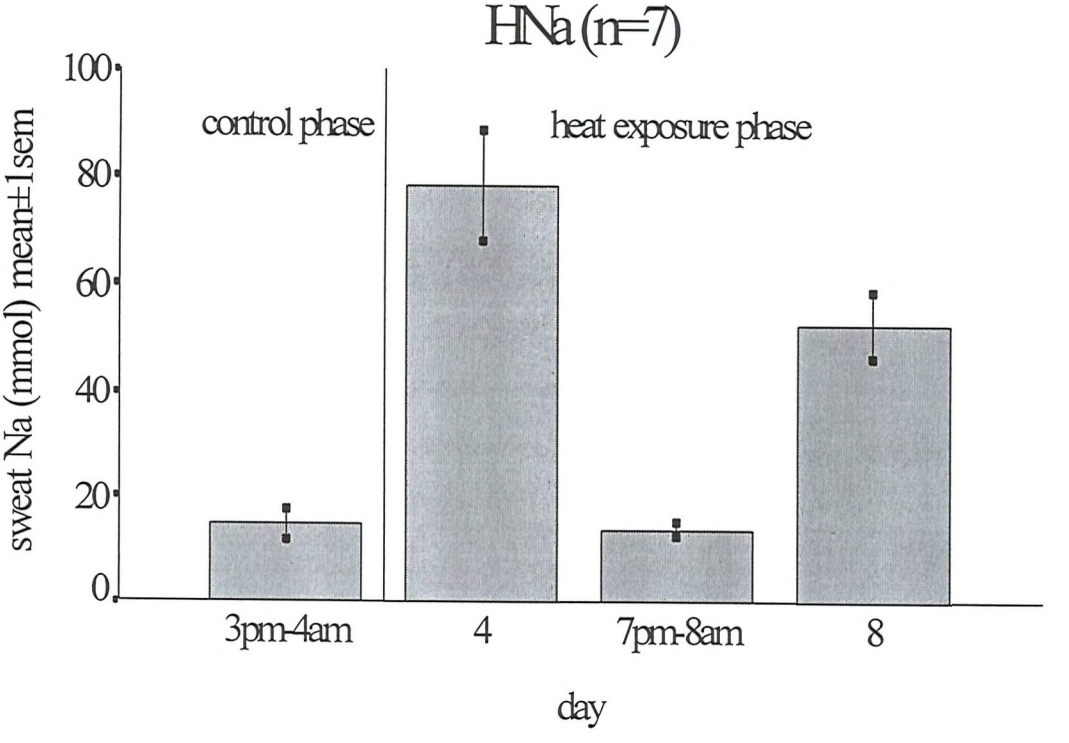
Faecal sodium excretion. Two subjects, one from either group were unable to produce faecal samples on both of the two occasions (days 3 and 8) when samples were collected and used to estimate faecal sodium loss. In view of the reduced numbers of subjects and the distribution of the data median and range data are presented here. Day 3 and day 8 median (range) values for MNa (n=8) were 1.1 (0.7 to 19.6) mmol/d and 2.1 (0.9 to 23.5) mmol/d respectively; similarly values for the HNa group (n=6) were 15.8 (1.3 to 19.5) mmol/d and 13.8 (0 to 38.6) mmol/d respectively.

Net sodium balance. Sodium balance (intake minus total losses including faecal) on days 3, 4 and 8 was positive in both groups, mean±sem values for each of these respective days being: 12.1±6.8 mmol, 36.3±6.7 mmol, and 34.5±9.3 mmol for MNa; and 33.2±11.1 mmol, 50.5±15.5 mmol, and 29.0±19.6 mmol for the HNa. There was no difference between the two dietary conditions nor within each condition for the effect of day of exposure.

Figure 6.3. Sweat sodium secretion.

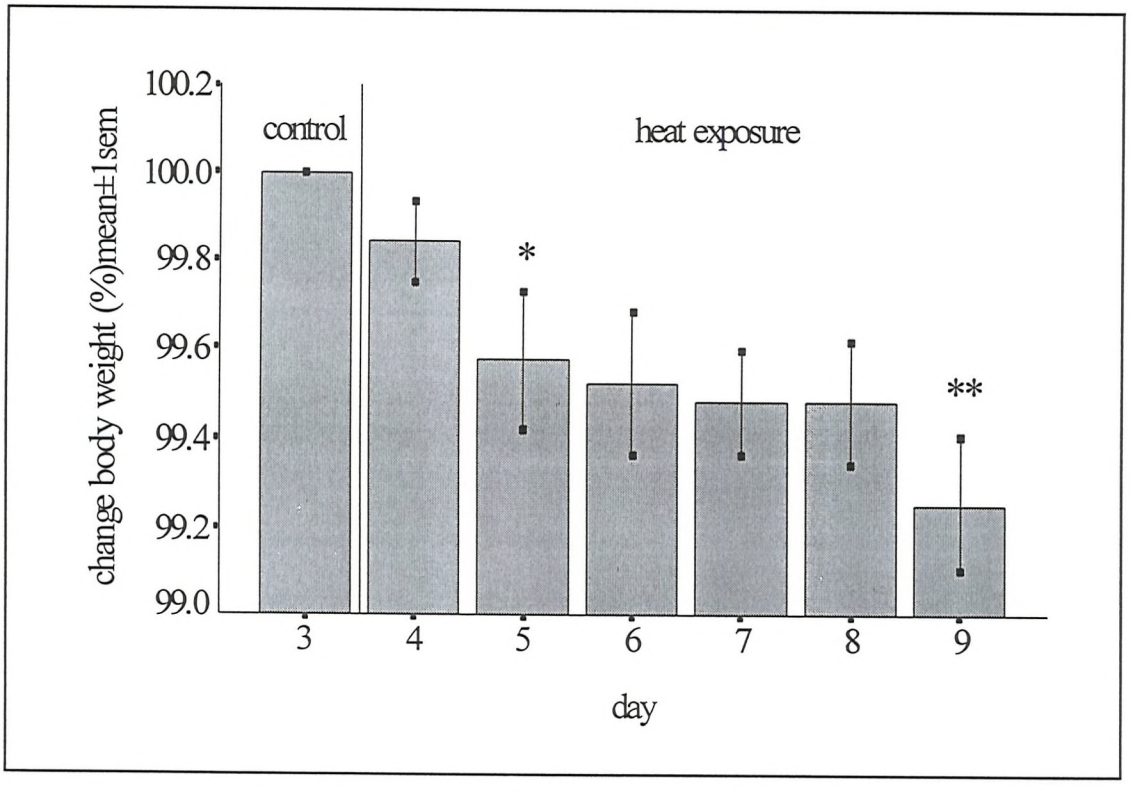


There was a reduction (P<0.01) in sodium from day 4 to day 8 for both the MNa and HNa groups.



Bodyweight. On commencing the trial, the mean±sem bodyweight for MNa was 81.75±3.09 kg compared with 90.09±2.96 kg for the HNa group, although this difference between dietary groups was not significant (P=0.08) when examined using repeated measures ANOVA for all days. Body weights for day 3 (MNa 81.68±3.1 kg; HNa 89.94±3.0 kg) were reduced (P<0.05) on day 4 (MNa 81.56±3.10 kg; HNa 89.78±3.00), and further reduced throughout the heat exposure period to values on day 9 of 81.15±3.03 kg (MNa) and 89.09±2.83 kg (HNa). To investigate this further the percentage change in bodyweight (relative to day 3) was examined for days 4 to 9 (Figure 6.4). The percentage bodyweight on day 5 was significantly (P<0.05) lower than for days 3 and 4, but there was no further significant fall in bodyweight until day 9, the mean value of which was significantly (P<0.05) lower than for all preceding days of heat exposure.

Figure 6.4. Percentage change in body weight during the heat exposure (n=16)



** indicates day 4 was lower (P<0.05) than day 3; ** indicates day 9 was lower than all preceding days in the heat.*

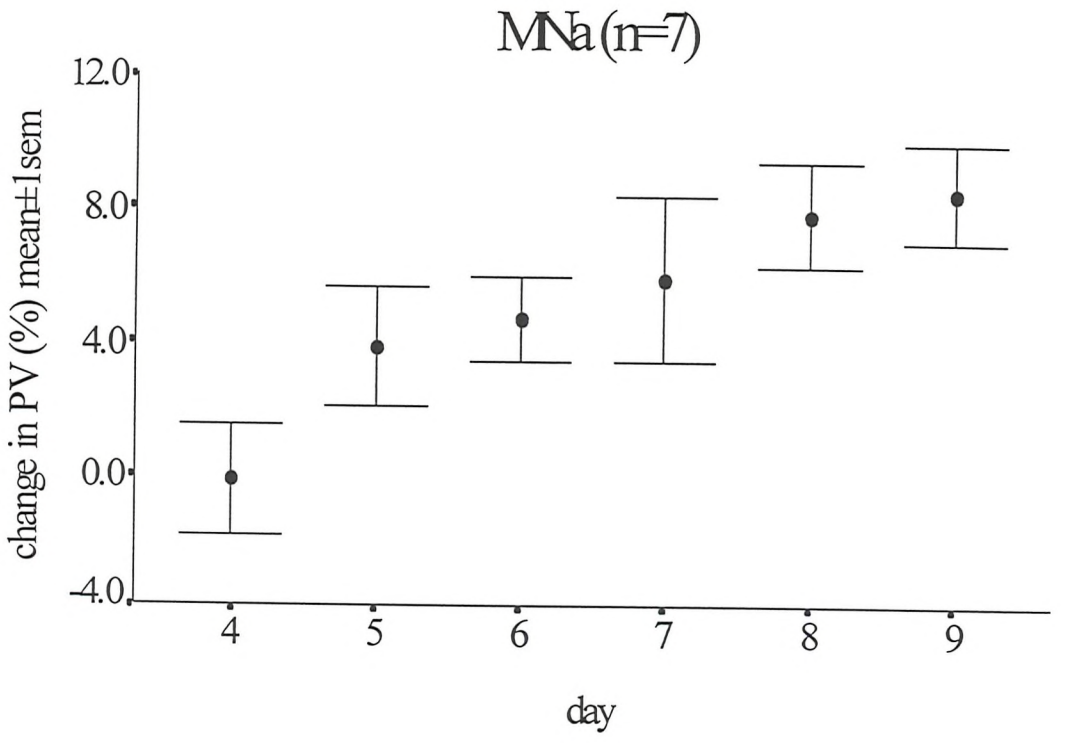
Fluid intake and sweat loss in the heat. Subjects' fluid intakes are shown (mean and sem) in Table 6.1. The highest intakes (approximately 7 to 8 litres) each day in each group were of the same two individuals (one in each group) who drank water at very frequent intervals. Fluid intakes of both groups increased by approximately two litres per day on exposure to heat. Fluid balance could not be assessed without accurate estimates for sweat loss, which were not measured. Estimates of sweat losses (from fluid intake and urine loss adjusted for weight loss) in the heat, assuming daily fluid balance, were an average of 3361 (sem 184) g/d.

Table 6.1. Daily fluid intakes (g/d) of the two groups

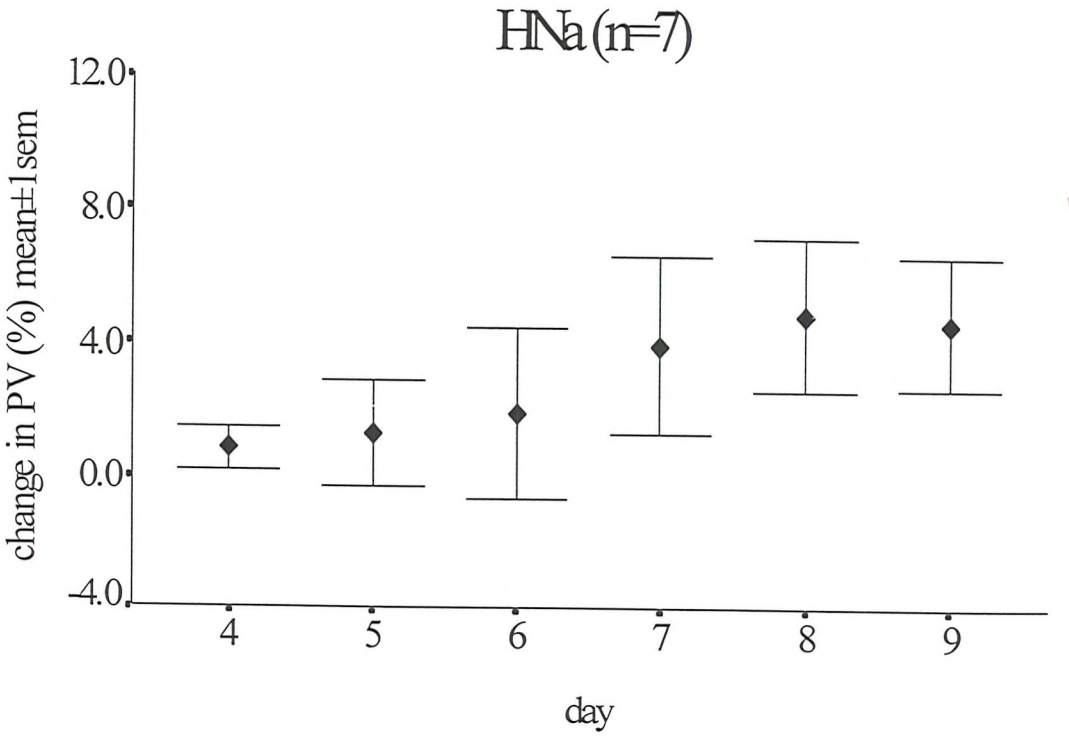
day	MNa (n=9)		HNa (n=7)	
	mean	sem	mean	sem
1	3732	867	3281	794
2	3320	591	4156	1062
3	3126	496	2770	479
4	4863	456	5424	979
5	5300	381	5335	328
6	5500	345	5041	412
7	4976	493	5549	409
8	5244	425	4765	231

Plasma volume. The percentage change in plasma volume relative to the third day, was calculated for the fourteen subjects from whom blood samples were successfully taken on all five days of heat exposure. For the MNa, there was a small fall on day four (-0.6±1.4%), which increased to 4.2±1.4% on day five and 8.35±1.2% by day nine. ANOVA gave an indication (P=0.6) of this rise during heat exposure (Figure 6.5). For the HNa group, the corresponding values were: day four 0.7±0.6%; day five 2.8±2.0%; day nine 4.6±1.7%. This trend was not significant. Taken together, this rise was confirmed (P<0.01) but there was no difference between the 2 groups.

Figure 6.5 Change in plasma volume (PV) during heat exposure.



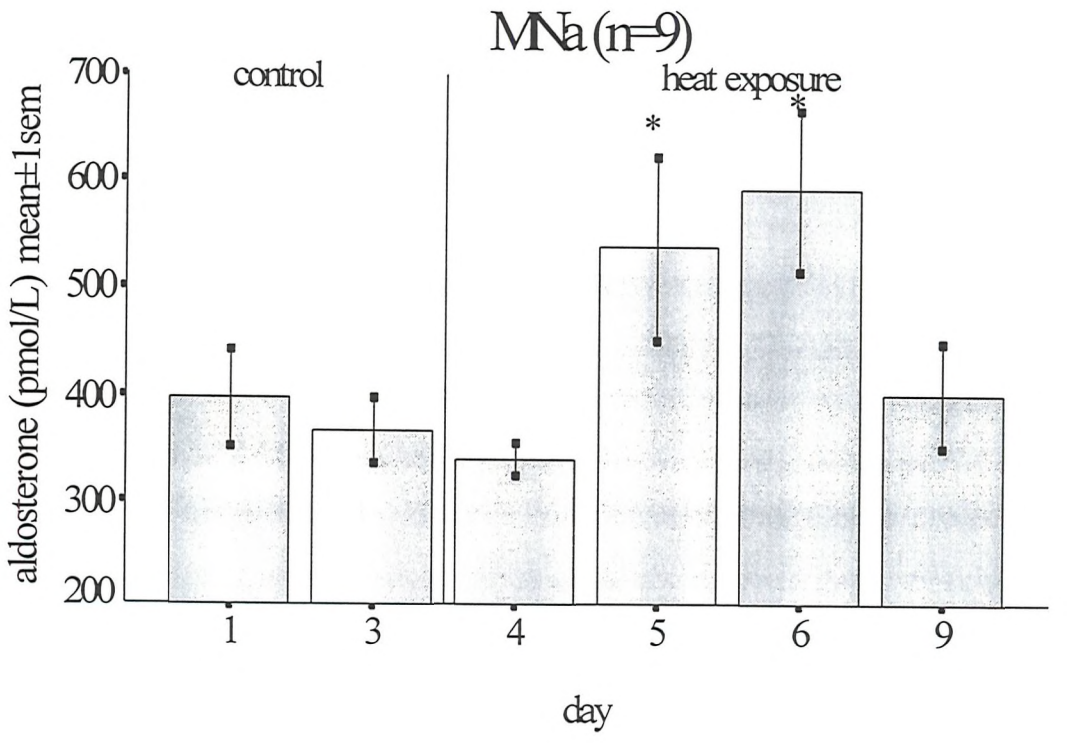
The day-to-day trend for MNa was indicative (P=0.6) of an overall increase. No similar trend was detected for HNa.



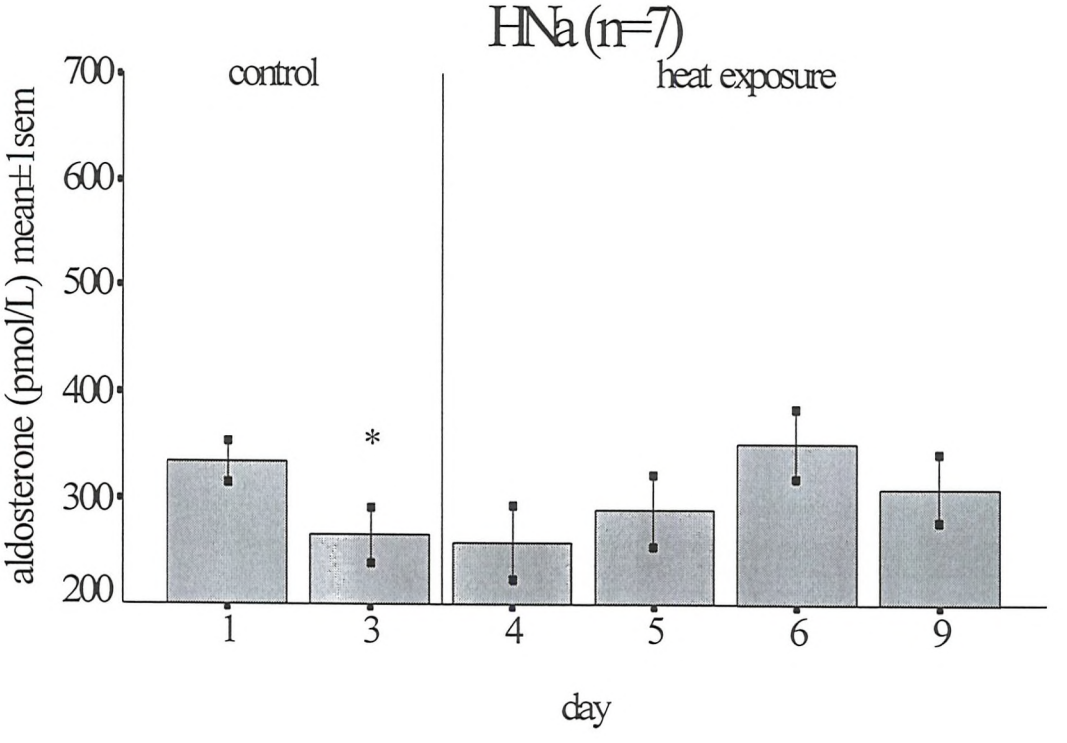
Plasma sodium and potassium concentration. Daily mean plasma sodium for the MNa group ranged from 136.5±2.2 mmol/L to 148.8±4.5 mmol/L whereas mean values for HNa were generally higher ranging between 141.3±1.8 mmol/L to 155.8±4.2 mmol/L. This difference between the group means was not significant. Daily mean plasma potassium was similar for both groups with mean values ranging from 3.9±0.2 mmol/L to 4.3±0.3 mmol/L for MNa and 3.9±0.1 mmol/L to 4.4±0.1 mmol/L for HNa.

Plasma aldosterone concentration. All data were transformed (natural logarithm) prior to analysis. On day one, aldosterone concentration was higher ($P<0.05$) for the MNa (396±45 pmol/L) compared to HNa (334±19 pmol/L). The day three values for the two groups were similar (366±31 pmol/L and 266±26 pmol/L for the MNa and HNa conditions respectively). This reduction from day one to day three was significant ($P<0.05$) for the HNa group. During the heat exposure, aldosterone concentrations increased ($P<0.01$) for both groups; for the MNa this rise reached significance ($P<0.05$) on day five (537±85 pmol/L) and day six (591±76 pmol/L), whereas values for HNa on these days (289±33 pmol/L and 351±33 pmol/L respectively) were not significantly higher than for the control. ANOVA on the two groups combined across all days examined, revealed that plasma aldosterone concentration was higher ($P<0.01$) for the MNa compared to the HNa group (Figure 6.6). The plasma concentrations of the two groups on day 9 (400±50 pmol/L and 309±32 pmol/L for MNa and HNa) were similar.

Figure 6.6. Plasma aldosterone concentration of the two groups.



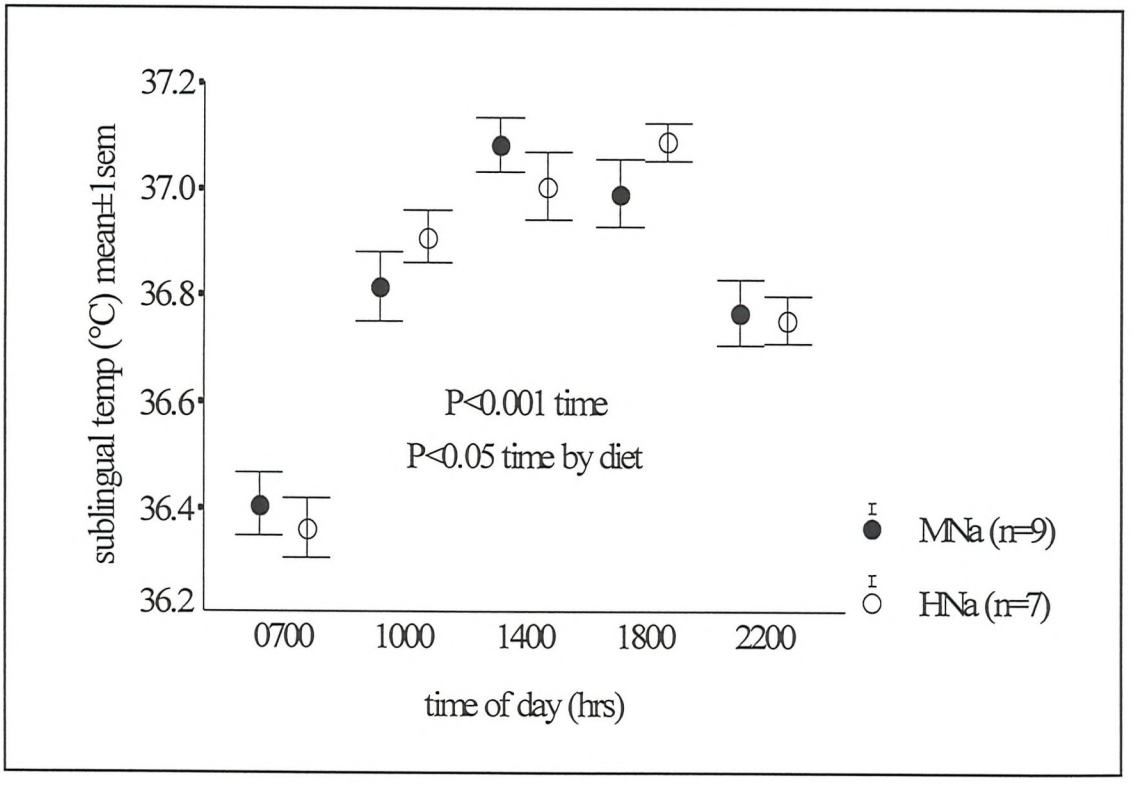
** indicates higher (P<0.05) than day 3.*



** indicates lower (P<0.05) than day 1.*

Sublingual temperature. During the control period, daily sublingual temperatures (across all five time points) for the MNa group were: 36.5±0.1 °C on day 1; 36.6±0.04 °C on day 2; 36.6±0.04 °C on day 3. For the HNa group these were 36.5±0.1 °C; 36.5±0.1 °C; and 36.5±0.1 °C respectively. On day 4 sublingual temperatures of the two groups increased ($P<0.01$) to mean values of 36.9±0.1 °C and 36.8±0.1 °C for the MNa and HNa conditions respectively. Sublingual temperature was consistently lowest at 0700 h (approximately 36.4°C), rose steadily during the morning (to approximately 36.8°C at 1000h) to peak at around 37.0°C- 37.1°C during the afternoon, before falling again in the evening (to approximately 36.8°C at 2200h). This diurnal variation is shown in Figure 6.7. ANOVA of this diurnal data for days 4,6 and 8 revealed no differences with respect to sodium intake and no day-to-day trend during the heat exposure, whereas the diurnal pattern was highly significant ($P<0.001$), and differed ($P<0.05$) between the two dietary groups, with the MNa temperature peak occurring earlier than for the HNa.

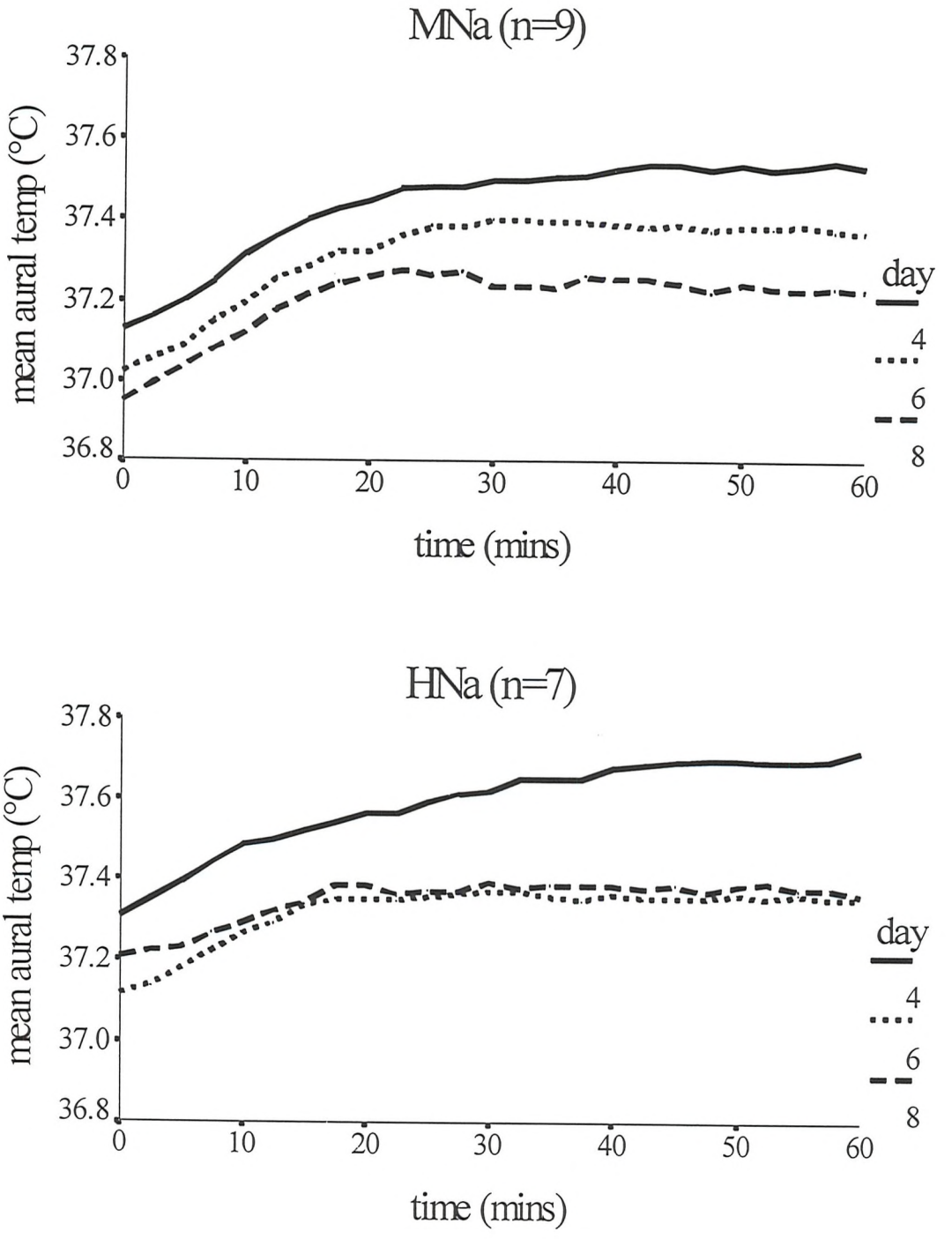
Figure 6.7. Sublingual temperature of the two groups throughout the day.



Aural temperature during the exercise step task: within group analysis. The change in aural temperature as exercise progressed on days 4, 6 and 8 is shown in Figure 6.8. The mean aural temperatures at the commencement of exercise in the MNa condition (37.1°C, 37.0°C and 36.9°C for days 4, 6 and 8 respectively), were approximately 0.2°C lower than for the HNa group (37.3°C, 37.1°C and 39.2°C for days 4, 6 and 8 respectively). The rate of rise of temperature during the first 15-20 minutes on each of these days was similar, but the temperatures attained at the cessation of exercise differed. In the MNa condition this plateau occurred at: 37.6°C on day 4; 37.4°C on day 6 and 37.3°C on day 8. For the HNa group a plateau was not attained on day 4 (aural temperature was rising slowly throughout), but this was achieved on days 6 and 8 at approximately the same temperature (37.4°C). For both dietary groups, analysis indicated that the plateau was significantly ($P<0.01$) lower on days 6 and 8 compared to day 4, whereas the reduction between day 6 and 8 was not significant.

Aural temperature during the exercise step task: between group analysis. To examine differences between the two groups the temperature differential (δ°C)was calculated for each of the 13 five-minute periods during exercise for day4-day6, day4-day8 and day6-day8. This calculation was necessary to avoid any changes due to the dissimilar diurnal pattern noted in the body (sublingual) temperature above; since not all the subjects performed the step test at the same time of day, these diurnal changes may have otherwise confounded the comparison between diets. These differential analyses indicated that δ°C altered significantly with time as exercise progressed for the comparisons of day 4 minus day 6 ($P<0.05$) and day 4 minus day 8 ($P<0.001$), but the profiles for day 6 minus day 8 were similar. There was no significant effect of dietary sodium on these measures.

Figure 6.8. Aural temperature on days 4, 6 and 8 for both groups.

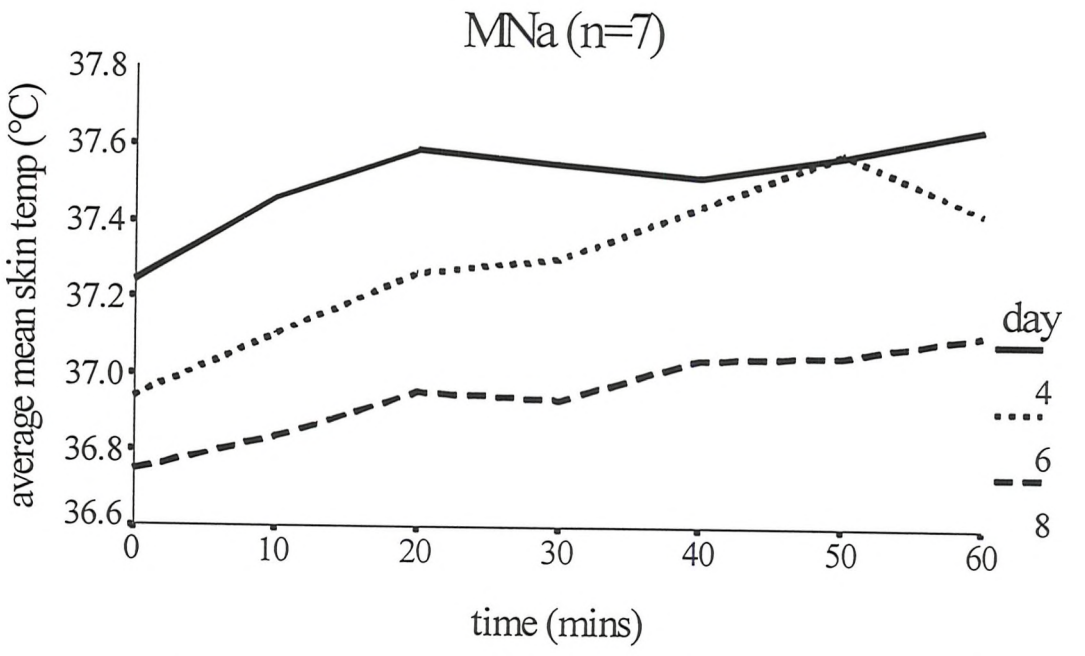


Aural temperature on day 4 was significantly higher ($P<0.01$) than days 6 and 8 which did not differ significantly, there was no difference between the two diets.

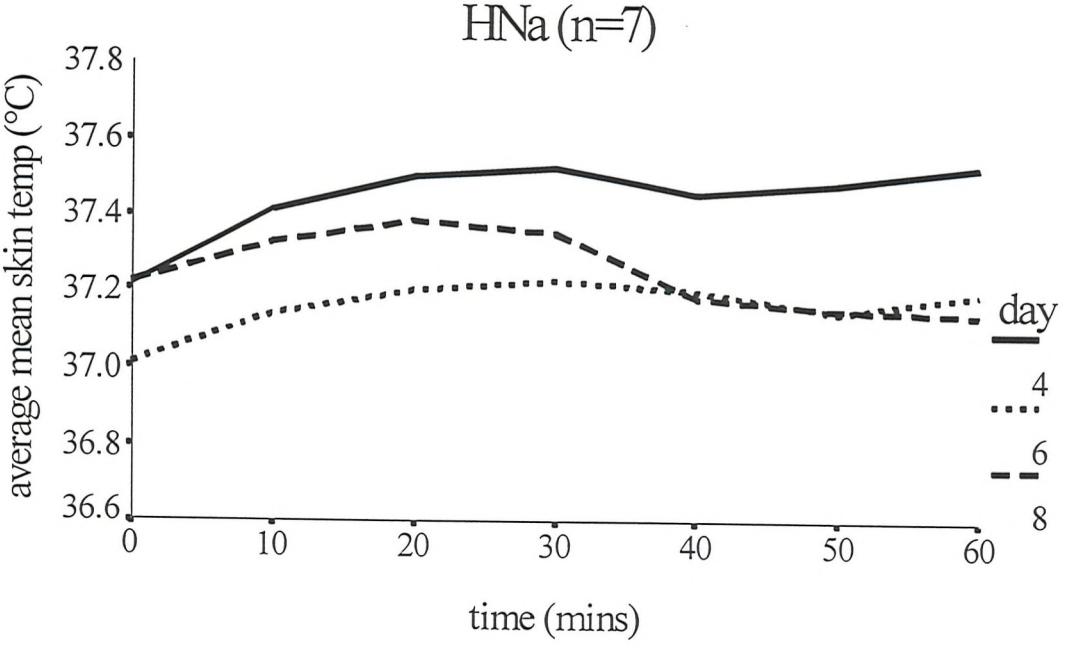
Skin temperature during the exercise step task: within group analysis. Mean skin temperature ($\bar{T}_{sk}$) was calculated from skin temperature recorded at 2.5 minute intervals, and these values averaged over each successive ten minute period to 'smooth' small fluctuations. There were two subjects with missing data (one or more skin thermistors had become unattached) in the MNa group and these have been omitted from Figure 6.9 and all analyses. For the MNa group, $\bar{T}_{sk}$ rose slightly during the step exercise procedure (from an average of 37.31 °C to 37.69 °C on day 4 for the MNa group), and tended to fall as heat acclimation progressed from day-to-day: thus the final average $\bar{T}_{sk}$ for the MNa on days 4, 6 and 8 was 37.69 °C, 37.45 °C and 37.05 °C on days 4, 6 and 8 respectively (Figure 6.9). For the HNa subjects, the temperature profile was similar on day 4 (rising from 37.21°C to 37.53°C) but this increase was less marked on day 6 and absent on day 8 (there was a fall in $\bar{T}_{sk}$ after 30 minutes). Day-to-day changes were less consistent for the HNa subjects; the temperature values for day 8 being slightly higher than day 6 during the initial 40 minutes of the test. Values for each twenty minute time point (0, 20, 40 and 60 mins) were analysed on days 4, 6 and 8 for each group of subjects. For the MNa subjects the overall average $\bar{T}_{sk}$ on day 4 (37.57°C) was reduced ($P<0.01$) on day 6 (37.32°C) and fell further ($P<0.01$) on day 8 (36.89°C). For the HNa group the corresponding overall average $\bar{T}_{sk}$ was 37.45°C on day 4, falling ($P<0.01$) to 37.16°C on day 6, but rising to 37.25°C on day 8. The greatest reduction in overall $\bar{T}_{sk}$ during the acclimation period was therefore approximately 0.7 °C for the MNa and 0.3 °C for the HNa subjects. For the HNa group there was no significant change in $\bar{T}_{sk}$ across time points or over successive days.

Skin temperature during the exercise step task: between group analysis. To examine these dissimilarities between the two groups, repeated measures ANOVA was conducted on the differences in mean skin temperature ($\delta\bar{T}_{sk}$) at the same twenty-minute intervals throughout exercise. This $\delta\bar{T}_{sk}$ was significantly ($P<0.05$) greater for the MNa group compared to HNa, indicating a greater fall in $\bar{T}_{sk}$ over the period of acclimation. This analysis also indicated ($P=0.09$) a difference between diets in this acclimation process from day-to-day, seen in the $\bar{T}_{sk}$ profiles of the two groups.

Figure 6.9. Average mean skin temperature for the two dietary groups.



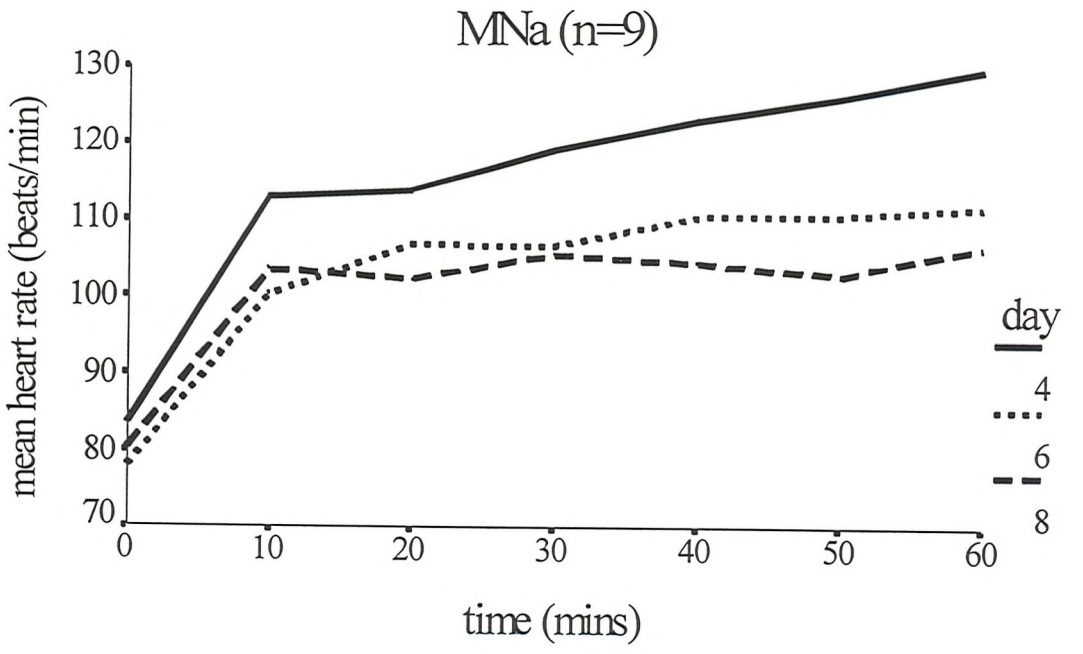
Overall average mean skin temperature on day 4 was higher (P<0.01) than for day 6 which was higher (P<0.01) than for day 8.



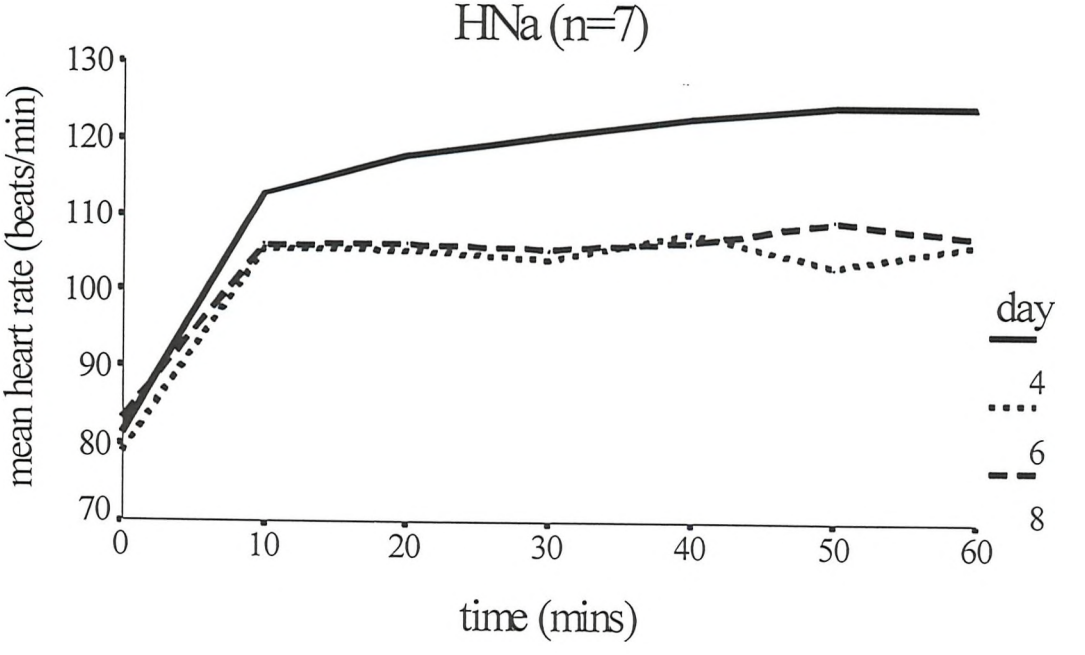
Overall average mean skin temperature on day 4 was higher (P<0.01) than for day 6 which was similar to day 8.

Heart rate. Heart rate was monitored at 2.5 minute intervals throughout the exercise bout. These data were time averaged every 10 minutes to reduce any minor fluctuations. Figure 6.10 shows the mean values for these time-averaged rates every ten minutes during the step test, the initial steep rise corresponding to the change from rest to exercise. For the MNa subjects, heart rate increased after this first ten minutes, as exercise progressed (the so-called "cardiac drift"). This rise was less marked as the heat exposure continued - average values for the MNa subjects were: 119 to 137 bpm on day 4; 104 to 116 bpm on day 6 and 106 to 112 bpm on day 8. For the HNa subjects, only the day 4 data increased over this period (from 113 to 126 bpm); the data for days 6 and 8 were almost identical and mean values were unchanged appreciably from 105 - 108 bpm. ANOVA was performed on data for five time points (10 - 60 minutes) and three days (4, 6 and 8). This indicated that, for the MNa group, there was a significant ($P<0.001$) reduction in average heart rate; from 123 bpm on day 4 to 106 bpm on day 6, which was further decreased ($P<0.05$) on day 8. The HNa group demonstrated a significant ($P<0.01$) reduction in heart rate from day 4 (116 bpm) to day 6 (103 bpm) but this was unchanged on day 8 (103 bpm). There was no difference between the two diets as analysed by either the absolute heart rate on each of the days 4, 6 and 8 or the differential (δ) in heart rate between these pairs of days.

Figure 6.10. Mean heart rate during the exercise step test.



Heart rate was reduced (P<0.001) from day 4 to day 6, and further reduced (P<0.05) on day 8.



Heart rate was reduced (P<0.01) from day 4 to day 6, but there was no further reduction thereafter.

Sweat evaporation. Sweat evaporation following the step test was estimated by weight difference corrected for fluid intake and estimated body surface area (Du Bois & Du Bois, 1916). Average values for the two groups are presented in Table 6.2 with the exception of one subject for whom the data was thought to be incorrect on one of the days due to a technical error. These values show a numerical increase in sweat evaporation after day 4 but there was no apparent increase in sweat evaporation with successive days of heat exposure. Note that the individual variation in this measure was almost 200%, ranging from 371g/m^2 to 681g/m^2. Analysis (MANOVA) of sweat evaporation indicated no significant effect of sodium diet nor day of heat exposure.

Table 6.2. Estimated sweat evaporation (g/m^2) during the step test.

	MNa (n=8)				HNa (n=7)			
	mean	sem	min	max	mean	sem	min	max
day 4	433	28	371	569	495	46	365	652
day 5	452	21	383	526	520	40	387	681
day 6	485	34	411	665	523	47	371	676
day 7	443	12	413	498	509	33	424	649
day 8	447	18	386	546	489	39	377	678

Conclusions.

Only those conclusions which are pertinent to this chapter will be discussed here. Results which have a bearing upon the central hypothesis of dietary sodium manipulation and heat acclimation will be addressed in the general discussion. The purpose of this experiment was to examine the effects of dietary sodium supplementation, taken both prior to and during heat exposure, upon parameters of thermoregulation and sodium conservation.

Effect of heat exposure and heat acclimation on sodium balance. Adherence to the dietary sodium regime was indicated by the divergence between the mean values for urinary sodium excretion of the two groups throughout the trial. There were no clinical signs of salt depletion or heat exhaustion in any subject and this suggests that net sodium balance was maintained in environmental conditions which were designed to incur a slight deficit if sodium intake was at or below 170 mmol/day (i.e. MNa). The thermal responses of reduced aural temperature, reduced skin temperature and heart rate, together with an expansion of plasma volume, indicate that the intensity and duration of the heat sojourn were sufficient to stimulate heat acclimation.

This maintenance of sodium balance was achieved by marked reductions in urinary and sweat sodium excretion. The data for sodium balance (estimated from urinary, sweat and faecal losses on days 3, 4 and 8), indicating a net positive balance for both groups, may be attributable to methodological errors in the estimation of sodium loss by the various routes.

The time course of the increased renal reabsorption was within 24 hours, as judged by the pronounced oliguria (in the face of increased fluid intake) and the significant reduction in sodium excretion on day 4, which was at its lowest level on day 5. It is not possible to assess the time course of the sweat gland adaptation because washdown procedures were performed only at the start and end of the heat exposure. However, it was of interest to note the significant reduction in sweat sodium secretion

over the period of heat exposure. Sweat production was not measured, although it had been the original intention to measure this by weight difference over the period of the washdowns. It became apparent, however, that this method presented difficulties in either controlling, (which was not desirable) or measuring, the quantity of all food and fluid intake and all faecal and urinary output. Whilst measures of volume and quantity were recorded, the time of ingestion of fluid and food (before or after the washdown period) was less easily controlled and was a potential source of error. This method also assumes that the subjects are in a state of fluid balance (Robinson & Robinson, 1954), which clearly is not the case. Previous attempts to measure sweat production by this method (for example Collins et al, 1971) have used a shorter time period of four hours and excluding any meals.

The downward trend in plasma aldosterone concentration on days 1, 3 and 4 suggests that the prescribed diets of these subjects were higher in sodium than their dietary intake prior to the trial. This reduction and the higher aldosterone concentration of the MNa group compared to HNa indicates that aldosterone secretion is sensitive to manipulations of sodium intake as reported previously (Follenius et al, 1979). The results here support the hypothesis that plasma aldosterone secretion is dependent upon sodium status. The data also support the argument that a sodium deficit will potentiate the response, for although the mean sodium balance of the MNa group was positive, two of the subjects were in negative balance on day 3, one subject on day 4, and two on day 8. The difference in values between the two groups on day one may indicate that aldosterone secretion is potentiated by a relative deficit rather than an absolute one, i.e. the response is initiated by a relative fall in plasma or extracellular sodium even in the absence of a net negative sodium balance.

Faecal losses seen in this experiment were median values of 1.1 to 2.1 mmol for the MNa group, similar to the range of 2-4 mmol/d reported by Bates et al (1991). Higher values were reported by Collins et al (1971) but the median and maximum values for the HNa are much higher than reported elsewhere. This could be a reflection of the high level of sodium ingestion and suggests that if sodium is over-supplemented, then

much of the sodium will be excreted.

The similarity in the reductions of sweat sodium secretion (Figure 6.3) for the MNa and HNa diet is discussed in a later chapter. The results here would indicate that the adaptive process of sodium reabsorption was similar in both conditions, which may reflect either a similarity of sodium balance or suggest that sweat sodium reabsorption is not influenced by the level of dietary sodium intake.

The effect of acute and repeated heat exposure on body temperature. There was a clear increase in average sublingual temperature (taken throughout the day) on exposure to the heat. The diurnal pattern in this variable is shown in Figure 6.7 and it is apparent that the HNa had a higher value at 1800h compared to the MNa group. This difference between the two dietary groups with respect to this variation is explained by the methodology whereby some of the MNa and HNa groups performed the exercise step test at different times of day, that of the majority of HNa subjects being later in the afternoon when the diurnal phase of body temperature would have been highest. Examination of any effect of dietary sodium on heat acclimation therefore had to be a within-subject comparison of temperature change over the period of heat exposure, to avoid this potentially confounding factor. This form of analysis also had the advantage of being unaffected by other differences between subjects such as heat acclimatisation status, fitness and metabolic rate.

Thermoregulatory and cardiovascular responses to exercise in heat acclimation.

Aural temperature and mean skin temperature. The additional strain which results when exercising in a hot environment compared to cool conditions was apparent from the step increase in aural temperature on day 4. Heat acclimation occurred during the trial to reduce this heat strain. This was shown by the progressive decline (more so in the MNa subjects) in aural temperature, mean skin temperature, and heart rate from day to day. Further confirmation of heat acclimation was evidenced by the rate of change of aural and skin temperature during the exercise bout; with successive days

the aural temperature profiles reached a constant level (plateau) at an earlier time point. Thus it can be seen that the heat exposure elicited thermal responses consistent with heat acclimation in both the MNa and HNa subjects. The aural temperature profiles (Figure 6.8) suggest that heat acclimation was more fully achieved in the MNa condition, since a further reduction in temperature occurs from day 6 to the final day.

Sweat evaporation. The reduced core (aural) temperature with heat acclimation must result from increased heat loss from the periphery, hence the reduction in mean skin temperature. Increased heat loss in these conditions could only be achieved by increased sweat evaporation. In this experiment, although sweat evaporation was higher after day 4, there was no consistent pattern, other than a tendency for the highest sweat evaporation to be achieved on day 6. Thus the observed reductions in aural and mean skin temperature cannot be directly attributed to an increased sweat evaporation. Whilst a more prolonged or intense period of exercise might have allowed a greater discrimination of results with respect to this measure, this would have also increased the potential for introducing further confounding factors in response to the increased physical activity rather than heat stress *per se*.

The effect of sodium intake on thermoregulation and cardiovascular responses in the heat.

Significant differences between the subject groups have only been found with respect to mean skin temperature. This analysis indicated that the size of the reduction ($\delta\bar{T}_{sk}$) in mean skin temperature was similar for day 6 compared to day 4, but there were differences between the two groups in these measures for day 4 and day 6 compared to day 8. Whether these apparent differences are attributable to dietary sodium intake is debatable or some other factor is uncertain and these results are therefore compared to those subjects on a low sodium diet in a later chapter.

Summary.

In summary, the above results of sodium excretion indicate that increased renal and sweat sodium reabsorption occurred on exposure to the heat. This increased reabsorption was noted from the reduction in urinary sodium excretion at the onset of heat exposure. A reduced sweat sodium loss was also noted from the comparison of whole-body washdown collections at the start and end of the exposure. Both conditions appeared to remain in sodium balance, despite the heat stress calculated to produce a theoretical sweat sodium loss of 170 mmol in an unacclimatised man. It is suggested that sodium reabsorption was augmented in the MNa condition by an increased secretion of plasma aldosterone and that this increase was initiated by a sodium deficit in this group. Hence it seems likely that sodium balance can be maintained provided that the secretion of aldosterone is potentiated. Further experimentation to explore whether reducing dietary sodium intake prior to heat exposure further increases this potentiation of aldosterone secretion is the subject of the following chapter.

The repeated days of exposure appeared to acclimatise the subjects with respect to thermoregulatory and cardiovascular responses. The extent to which these responses were affected by the dietary sodium intake was difficult to assess but there were indications that the time course of these adaptations differed significantly between the two groups in the case of mean skin temperature, possibly as a consequence of differences in dietary sodium intake. This requires further investigation by comparison with a lower dietary sodium intake group and will be explored in the following chapter. Two of the three secondary hypotheses in the introduction were answered: sodium balance was attained within three days of control diet and five days after heat exposure - on both moderate and high sodium intakes; and aldosterone secretion was enhanced in the MNa condition prior to and during heat exposure. These outcomes indicate that aldosterone mediates sodium balance in these conditions. The third question regarding the effect of dietary sodium on heat acclimation requires investigation of subjects in negative sodium balance.

CHAPTER 7.

THE EFFECTS OF HEAT EXPOSURE WHEN SODIUM INTAKE IS RESTRICTED PRIOR TO, OR DURING HEAT EXPOSURE.

Introduction.

In the previous chapter it was apparent that heat exposure did not result in a sodium deficit for all subjects when ingesting a diet containing 170 mmol/d. It is of practical importance to assess whether a lower sodium intake would suffice in similar conditions. In the context of sudden travel to hot climates it is likely that normal food intake will be reduced because of the disorientation caused by travel through time zones. In military operations a reduced daily sodium intake may arise when insufficient supplies are provided, or there is a breakdown of the logistical support chain such that food provisions are inadequate. This is more likely to occur when troops are deployed rapidly and the supply chain for provisions is lengthy, i.e. it is likely to occur when personnel are unacclimatised to the heat. The adequacy or otherwise of a dietary intake of sodium lower than 170 mmol/d is also important in view of the policy to reduce dietary sodium intake to the target Reference Nutrient Intake value of 70 mmol/d (COMA, 1991).

In the last chapter it was suggested that sodium balance was dependent upon the secretion of aldosterone, the level of which is influenced by pre-existing dietary sodium intake. Thus it is plausible that reabsorption of sodium during heat acclimation could be augmented by higher levels of circulating plasma aldosterone initiated by prior dietary sodium restriction, as suggested by Collins (1963). Such a response, if sufficiently rapid, would obviate the need for supplementation with dietary sodium, indeed additional salt would be counterproductive to the aldosterone production mechanism.

Aims and main hypotheses.

The aims of this experiment were to:

a. examine sodium balance in the heat when sodium intake was maintained at a low level (70 mmol/d);

b. assess the thermoregulatory and cardiovascular responses to a period of heat acclimation in subjects who were maintained on a 'low' dietary sodium intake heat (70 mmol/d);

c. assess any effects of prior sodium restriction on the above responses to chronic heat exposure.

The hypothesis that a *prior* low sodium intake, as opposed to a sudden restriction of dietary sodium, would enable an improved tolerance to heat exposure, and a more rapid restoration of sodium balance, was tested by measures of thermoregulation, sodium losses and fluid balance.

Secondary hypotheses.

The secondary hypotheses were that a low sodium diet prior to heat exposure would:

a. potentiate aldosterone secretion;
b. diminish and possibly prevent a sodium deficit occurring on exposure to heat;
c. reduce the extent of dehydration in the heat;
d. have no adverse effect on cardiovascular stability and thermoregulation in the heat compared to a condition in which sodium intake was restricted upon heat exposure.

Methods.

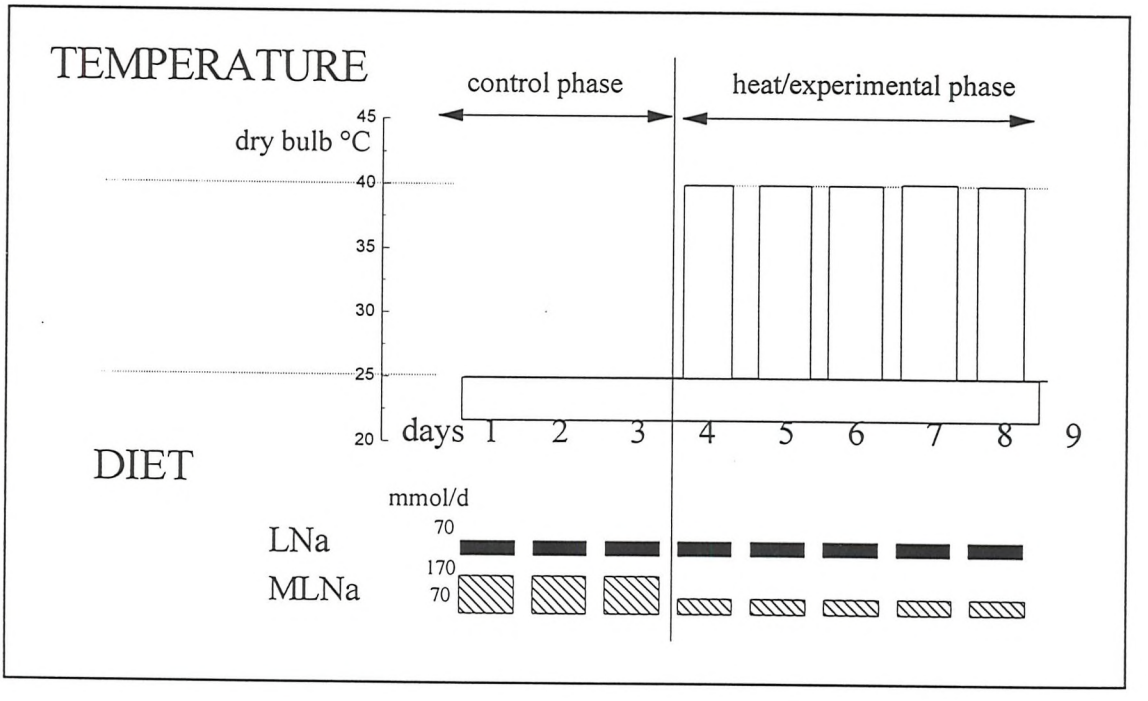
Subjects. Seventeen male volunteer subjects were recruited and assigned to one of the two following dietary conditions:

a. Moderate to low sodium intake (MLNa) n=8: an intake of 170 mmol/d of sodium for the first three (control) days, reduced to an intake of 70 mmol/d during the five day heat exposure.

b. Low sodium (LNa) n=9: An average daily intake of 70 mmol/d of sodium prior to, and during the heat exposure.

A "control" period of 3 days at 25°C was followed by five days in the heat (40°C) during the hours 0800 to 1800h (Figure 7.1). All other methods were as described in Chapter 4.

Figure 7.1. Schematic diagram of the experimental protocol.



Dietary control commenced from the evening prior to day one on arrival at the chamber. During the heat exposure phase, ambient temperature was increased from 25°C to 40 °C between the hours 0800h to 1800h, this change taking approximately one hour.

Results. All summary values given are mean and sem unless otherwise stated.

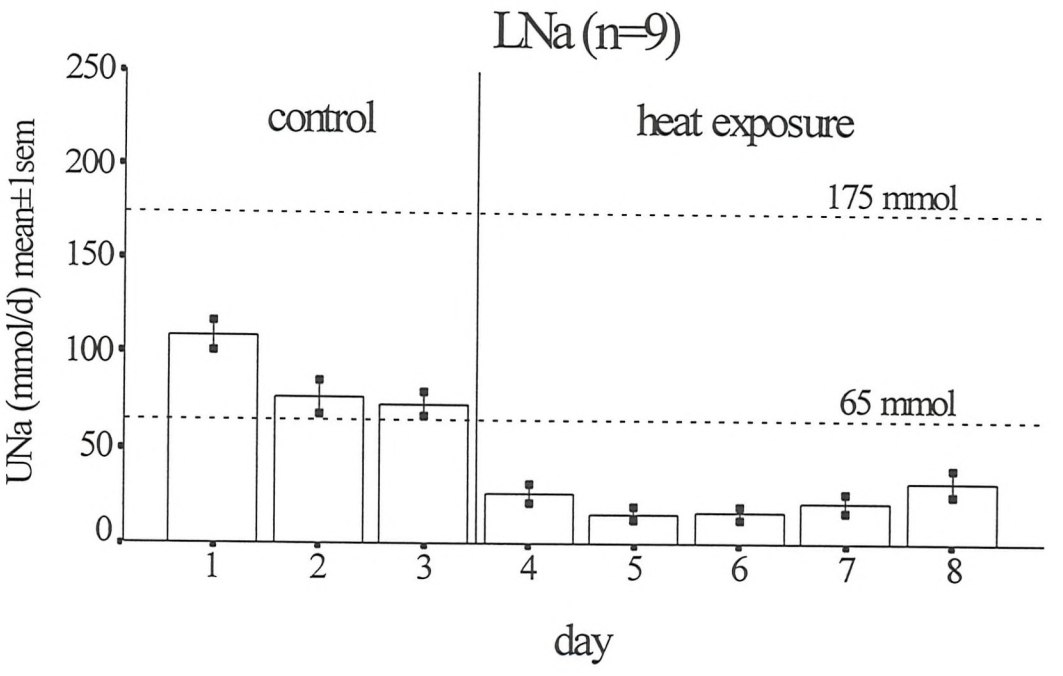
Anthropometric measures. Age, height and weight were as follows: LNa group - 28 (2) years; 172.0 (2.0) cm; 71.0 (1.6) kg; MLNa - 27 (1) years; 178.5 (2.3) cm; 75.94 (2.6) kg. No statistical differences were found between the two groups.

Sodium intake. Actual sodium intakes for the two groups were: 66.3 mmol/d for the LNa group and 175.3 mmol/d reduced to 65.1 mmol/d on day four and thereafter for the MLNa group.

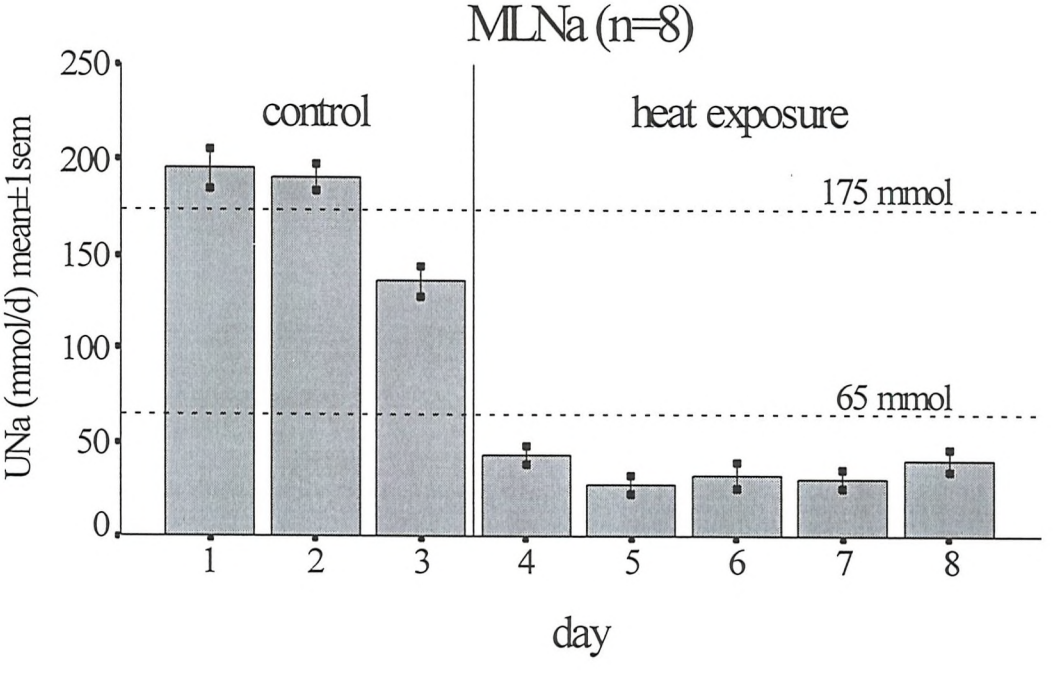
Urinary output. Urinary output fell ($P<0.01$) on heat exposure in both groups. For the LNa subjects this was from 2335±353 ml on day 3, to 1184±334 ml on day 4. In the MLNa group these values were 2140±184 ml and 983±164 ml respectively. The volume of urine remained low for the duration of the heat exposure, day 8 values being 962±170 ml and 804±199 ml for LNa and MLNa respectively. There were no differences between the two groups.

Urinary sodium excretion (UNa). There was a marked reduction in UNa excretion on heat exposure. For the LNa group, UNa was reduced ($P<0.01$) from 73±6 mmol on day 3 to 27±5 mmol on day 4, this fell further to a low of 17±3 mmol on day 5 before rising slightly to 33±7 mmol on day 8. In the MLNa subjects there was a fall ($P<0.01$) from 136±8 mmol on day 3 to 43±5 mmol on day 4, falling further to 27±5 mmol on day 5 before rising to 40±6 mmol on day 8. Over all five days of heat exposure, the lower UNa of the LNa group (114±19 mmol) compared with MLNa (174±22 mmol) approached significance ($P=0.06$). The day 4 value for LNa (27±5 mmol) was lower ($P<0.05$) than that of the MLNa group (43±5 mmol). Analysis also revealed that the pattern of reduction in urinary sodium excretion on days 4 and 5, followed by a gradual increase over the remainder of the heat exposure significant ($P<0.01$). This pattern of change was similar in both dietary groups (Figure 7.2).

Figure 7.2. Urinary sodium excretion of the two groups.



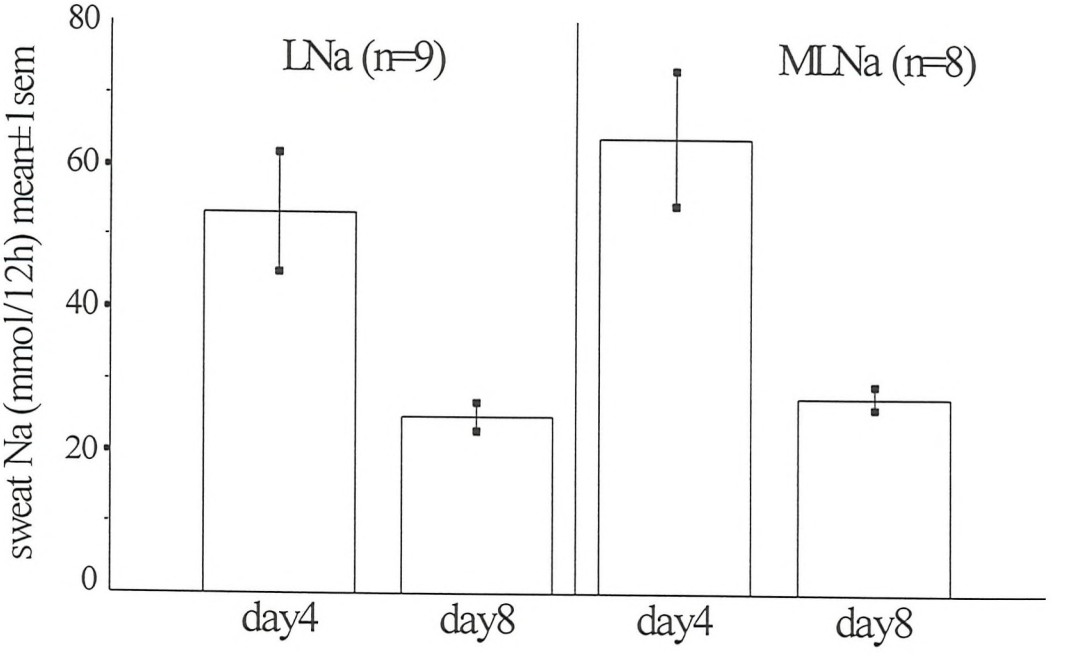
There was a reduction (P<0.01) in UNa upon heat exposure in both dietary groups. During heat exposure, there was an indication (P=0.6) that UNa was higher in the MLNa condition. The pattern fall and rise of UNa of both groups during heat exposure was significant (P<0.01).



Urinary sodium to potassium ratio Urinary sodium potassium ratio fell dramatically from mean values on day 3 of 1.52±0.33 and 1.76±0.09 for LNa and MLNa respectively, to 0.45±0.21 and 0.57±0.03 on day 4. There was no significant difference between the two groups during the heat exposure.

Sweat sodium excretion. For the LNa group, sweat sodium secretion from each 12h washdown was: (4.9±0.8 mmol for overnight day 3 pm to day 4 am; 53.5±8.5 mmol for day 4; 7.2±1.5 mmol for overnight day 7 pm to day 8 am; and 24.8±2.1 mmol for day 8. For the MLNa group these values were: 5.3±0.6 mmol; 63.9±9.5 mmol; 4.3±0.8 mmol; and 27.8±1.7 mmol (Figure 7.3). Analysis (ANOVA) of the transformed (logarithmic) data within each group, indicated no differences between the two overnight collections but a reduced ($P<0.01$) sodium secretion during the day on day 8 compared to day 4. Between group analysis indicated no differences with respect to sodium secretion in any of the four washdown periods.

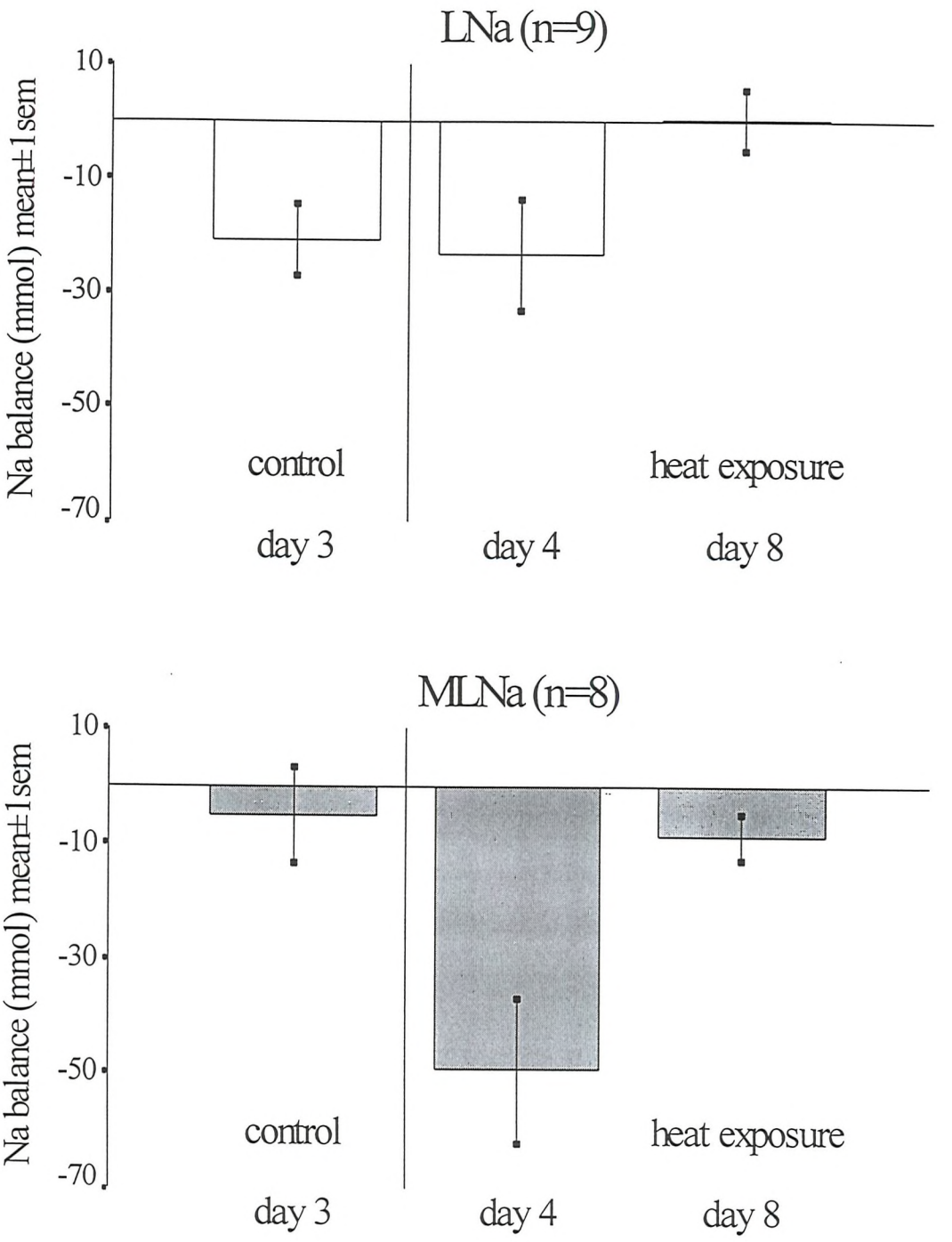
Figure 7.3. Sweat sodium secretion for the two groups on day 4 (first day of heat exposure) and day 8 (last day).



There was a significant ($P<0.01$) reduction in sweat sodium secretion from day 4 to day 8, but differences between the two dietary groups.

Net sodium balance. Sodium balance (intake minus total losses) on days 3,4 and 8 is presented in Figure 7.4. In the LNa group, net sodium balance was slightly negative (-20.8±6.4 mmol) on day 3 and this deficit remained on the first day of heat exposure (-23.3±9.8 mmol), but was corrected by day 8 (0.2±5.1 mmol). In comparison, the MLNa were in slight negative balance on day 3 (-5.1±8.2 mmol) but incurred a greater deficit on day 4 (-49.3±12.5 mmol), which approached correction on day 8 (-8.8±4.1 mmol). There was no difference between groups in the average sodium balance across these time points. Sodium balance was significantly ($P<0.01$) more negative on day 4 compared to the other two days. There was an indication ($P=0.06$) of an interaction between diet and day, which became significant ($P<0.01$) if day 3 was compared to the other two days (day 4 and 8), indicating that the change in sodium balance during the period of heat exposure (i.e. a more negative balance on day 4 in the MLNa condition compared to LNa), was affected by diet.

Figure 7.4. Sodium balance of the two groups on days 3, 4 and 8.

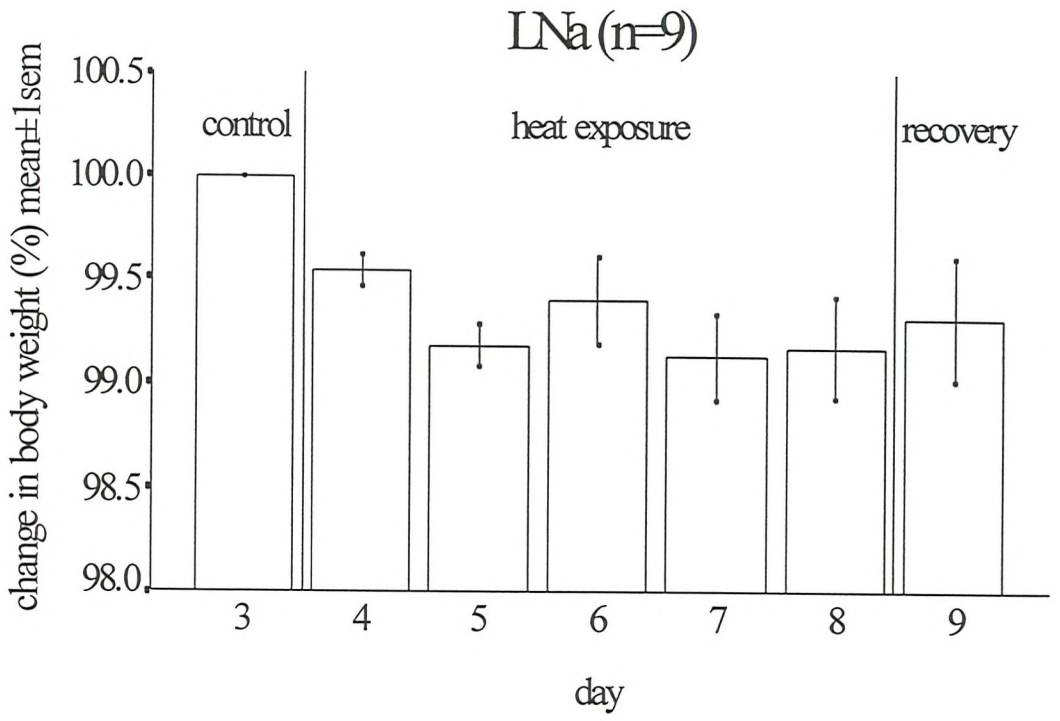


Sodium balance was more negative (P<0.01) on day 4 compared to the other two days (4 and 8). There was no difference between the two groups across all three days. There was an indication (P=0.06) that the trend of sodium balance was affected by diet.

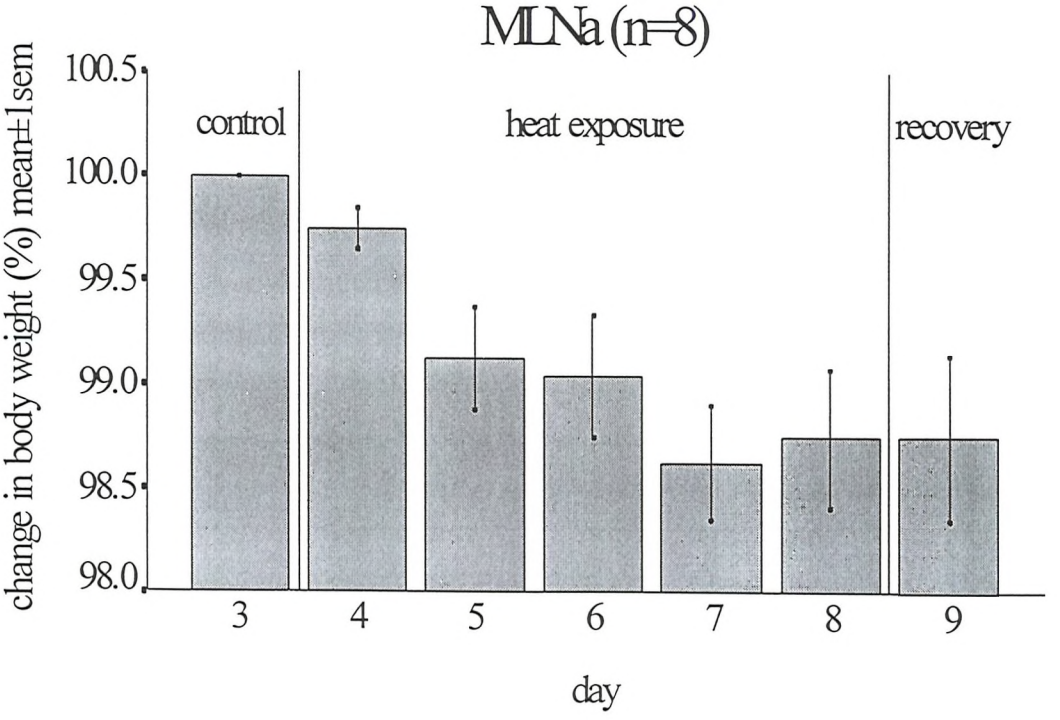
Body weight. The mean body weights of the LNa and MLNa subjects on day 3 were 70.94±1.73 kg and 75.53±2.73 kg respectively. This difference was not significant, but percentage change in body weight compared to day 3 (100%) was used to explore weight changes during the heat exposure phase. In the LNa condition this fell to 99.5±0.1% on day 4, and was further reduced to 99.1±0.2% on day 7 before recovering slightly to 99.3±0.3% on day 9. In the MLNa condition the mean on day 4 was 99.8±0.1%, falling further to 98.6±0.3% on day 7 before a slight recovery to 98.7±0.4% on day 9. These changes are shown in Figure 7.5. The fall in body weight during the exposure period was significant ($P<0.01$), irrespective of dietary condition on overall weight loss (days 4 to 9). ANOVA revealed, however, an interaction effect ($P<0.05$) between dietary group and day of heat exposure such that weight loss continued until day 7 in the MLNa group, whereas change in bodyweight stabilised by day 5 in the LNa group.

Fluid intake and estimated sweat loss. Fluid intake on day 3 was 2651±343 g and 2334±245 g for LNa and MLNa respectively. This increased ($P<0.01$) on day 4 to 4242±356 g for LNa and 3861±149 g for MLNa. Fluid intake was maintained at this level (LNa range 2753 g to 7035 g; MLNa range 3145 g to 6198 g) during heat exposure. There was no difference between the two dietary groups. Estimated sweat loss (calculated from fluid intake minus urine output allowing for reductions in bodyweight) was similar for both groups during the heat exposure being 2671±190 g/d for LNa and 2684±184 g/d for the MLNa.

Figure 7.5. Percentage change in body weight (relative to day 3) during heat exposure.



There was a reduction in body weight (P<0.01) during heat exposure which stabilised earlier (by day 5) in the LNa group but progressed until day 7 in the MLNa condition.



Plasma volume. Blood sample collection was incomplete for three of the LNa subjects, hence the analysis presented here is based upon the six "common" subjects. In the LNa condition there was a slight reduction in mean plasma volume (-0.4±1.7%), which recovered on day 5 (3.5±2.5%) and steadily increased up to 10.3±2.0% on day 9. Note that the values for day 4 and day 5 were slightly higher (0.1±1.5% and 4.2±1.9% respectively) when all LNa subjects (nine for day 4 and eight for day 5) were included in the analysis. The overall increase in plasma volume was therefore approximately 10% for the LNa condition. In the MLNa group, there was a fall in plasma volume on initial heat exposure (-2.1±1.0%) which recovered on day 5 (4.3±2.4%), and then rose in an irregular manner to 8.9±1.4% on day 8 (Figure 7.6). There were no significant differences between the two dietary groups with respect to these changes.

Plasma sodium concentration. In the LNa group, plasma sodium concentration increased from a mean value of 139.5 mmol/L on day 4 to a value of 144.5 mmol/L on day 7, whereas for the MLNa condition, the mean value on day 4 was lower (131.3 mmol/L) and this rose to a higher level (151.9 mmol/L) on day 7. The differences between these mean values were significant ($P<0.05$). ANOVA over the five day heat exposure period gave an indication ($P<0.1$) that the pattern of change in the MLNa (a more dramatic change in concentration) was less stable than for the LNa group.

Plasma potassium concentration. Average plasma potassium concentration during the control period (across all three days) was higher ($P<0.01$) for the LNa group (4.5±0.1 mmol/L) compared to the MLNa subjects (3.9±0.1 mmol/L). During the heat exposure period the overall mean values were similar (4.2±0.2 mmol/L and 4.1±0.1 mmol/L for LNa and MLNa respectively).

Figure 7.6 Plasma volume change during the heat exposure phase.

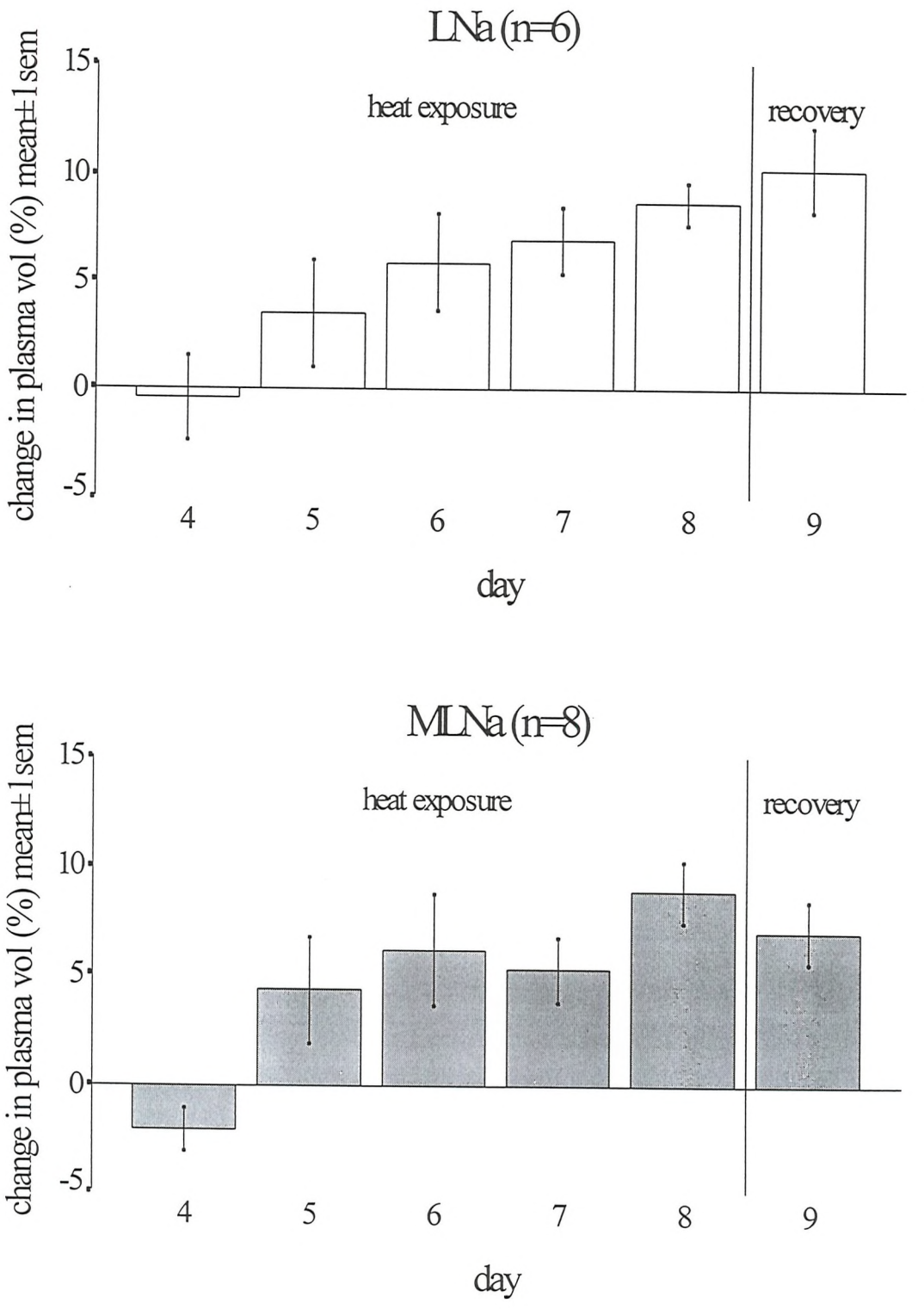


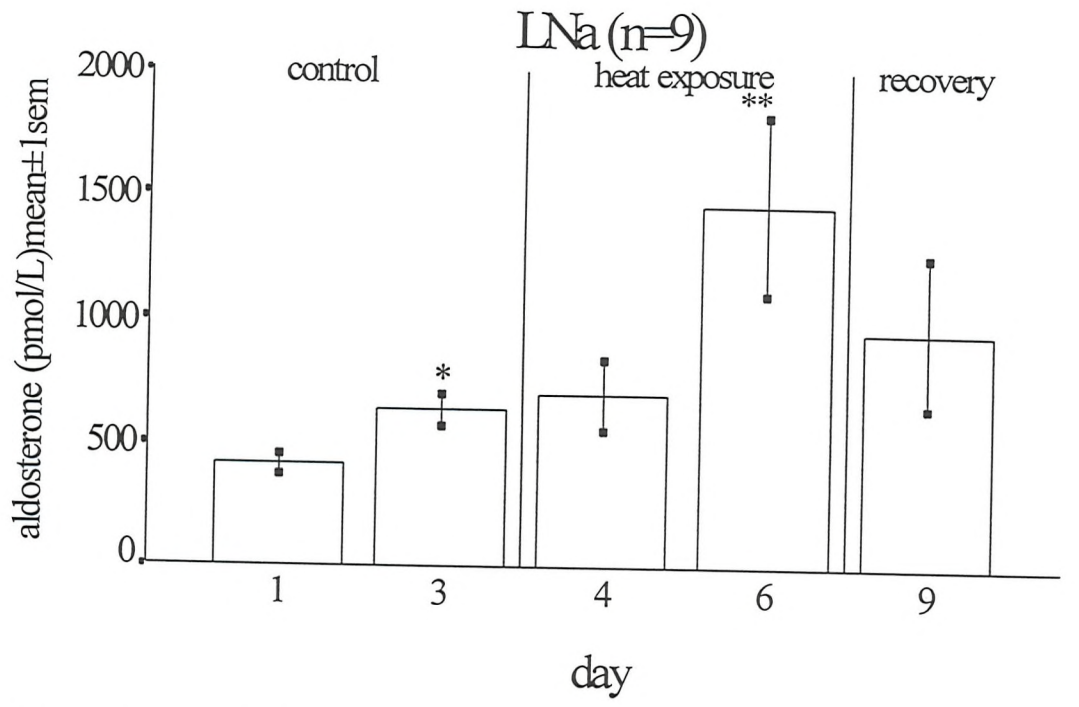
Table 7.1. Plasma sodium and potassium concentration (mmol/L).

Day	Sodium (mean±sem values)		Potassium (mean±sem values)	
	LNa (n=9)	MLNa (n=8)	LNa (n=9)	MLNa (n=8)
1	136.4±3.0*	138.6±1.1*	4.4 ±0.1	3.9±0.1
2	137.2±2.6*	128.3±1.9*	4.7±0.2	4.1±0.1
3	139.5±2.0*	132.0±1.6*	4.4±0.1	3.6±0.1
4	139.5±3.0	131.3±0.7	4.1±0.1	3.9±0.1
5	139.2±3.3	134.7±1.8	4.4±0.3	4.3±0.1
6	142.0±5.1	135.1±5.5	4.8±0.3	4.1±0.1
7	144.5±4.0	151.9±8.0	3.9±0.1	4.3±0.2
8	134.9±2.0	146.1±5.0	3.7±0.1	4.0±0.1
9	137.6±3.1	148.5±4.3	4.2±0.1	4.0±0.1

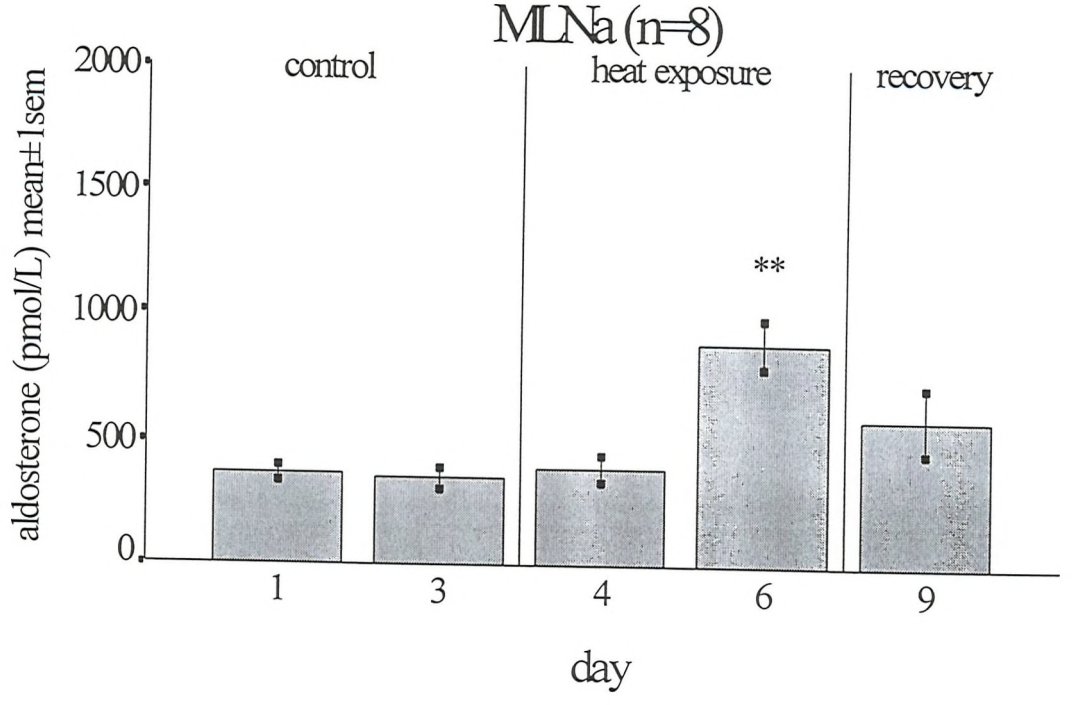
** - indicates a significant ($P<0.05$) difference between the two groups during the control period; there was also an indication ($P<0.1$) of an interaction between dietary sodium intake and day of heat exposure suggesting that day-to-day fluctuations in the MLNa group were more dramatic or less stable than for the LNa.*

Plasma aldosterone concentration. ANOVA were performed on logarithmic transformed data. In the LNa group, mean aldosterone concentration on day 3 (638±70 pmol/L) was higher ($P<0.05$) than for day 1(412±41 pmol/L). Plasma concentration for this group increased further ($P<0.01$) to a mean of 1461±355 pmol/L on day 6. In the MLNa condition, there was no difference between the day 1 and day 3 values (371±34 pmol/L and 351±44 pmol/L respectively). An increase occurred upon heat exposure to a higher ($P<0.01$) mean value on day 6 of 891±94 pmol/L. There was a difference ($P<0.001$) between the aldosterone concentration of these two groups overall.

Figure 7.7. Plasma aldosterone concentration of the two groups.



** day 3 was higher (P<0.05) than day 1 and ** day 6 was higher (P<0.01) than day 3.*

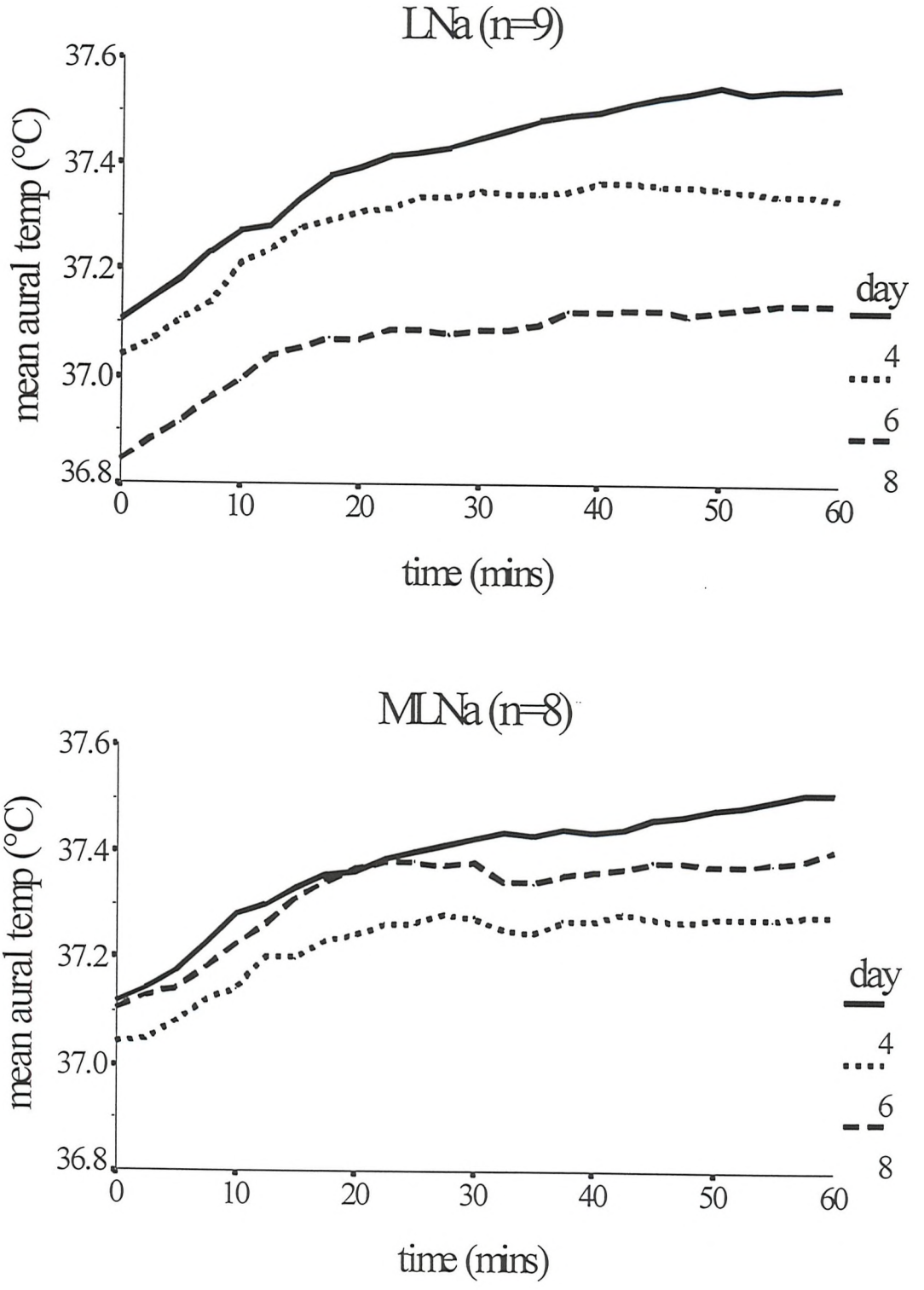


*** - indicates higher (P<0.01) than day 3.*

Aural temperature during the exercise step task: within groups analysis. The changes in aural temperature as exercise progressed are shown in Figure 7.8. The rates of rise of temperature during the first 15-20 minutes on each of these days were similar. The temperatures attained after 45 minutes (at which point there was a tendency for the temperature to plateau) for the LNa group were: 37.5±0.1 °C on day 4; 37.4±0.1 °C on day 6; and 37.1±0.1 °C on day 8. For the MLNa group these mean values at the same 45 minute period were: 37.5±0.1 °C on day 4; 37.3±0.1 °C on day 6; and 37.4±0.1 °C on day 8. For the LNa this plateau was significantly ($P<0.05$) lower on days 6 and 8 compared to day 4, but there was no further reduction from day 6 to day 8; for MLNa the three temperature profiles did not differ significantly at these times points. Furthermore, for the MLNa condition, the day 8 profile was intermediate between days 6 and 4.

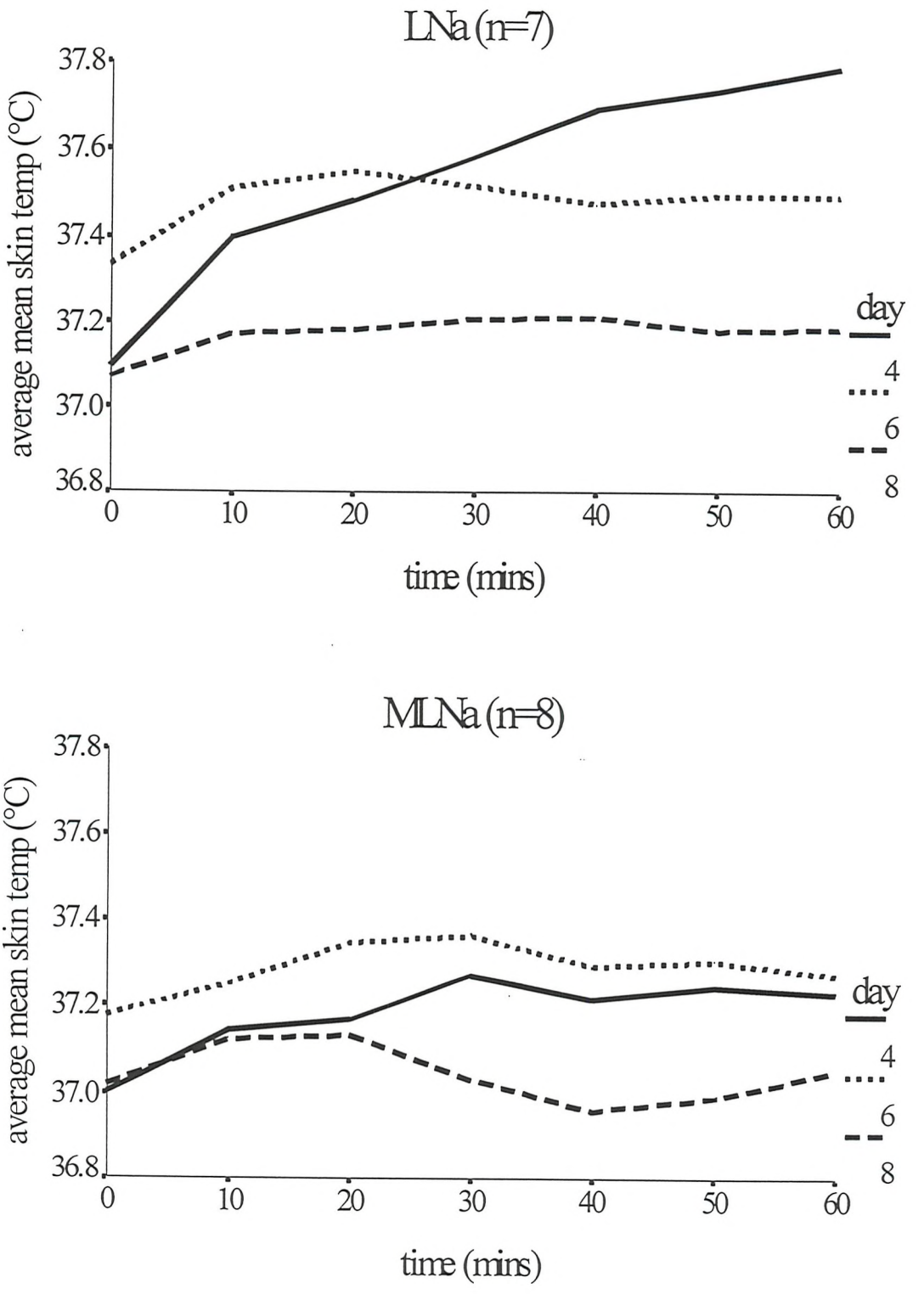
Aural temperature during the exercise step task: between groups analysis. To analyse differences in aural temperature as heat acclimation proceeded, the temperature differential (δ°C) has been calculated for: day 4 - day 6; day 4 - day 8; and day 6 - day 8 (as previously). ANOVA were conducted on these thirteen differential 5-minute time points during the 60 minute exercise period for each of these comparisons. The average differentials between day 4 and 8 for the two groups were not significantly different ($P=0.13$) nor were other between day comparisons.

Figure 7.8. Aural temperature during the exercise step test.



Mean skin temperature during the exercise step task: within and between group analysis. Two of the subjects in the LNa had incomplete data with respect to this variable on one of the days and hence these subjects have been excluded from the analyses and the graphs of average mean skin temperature ($\bar{T}_{sk}$) at Figure 7.9. For the LNa group, the day 4 $\bar{T}_{sk}$ profile increased with time from an initial value of 37.09±0.11 °C to 37.79±0.06 °C at the end of the exercise, whereas the profile for day 6 was relatively flat (overall $\bar{T}_{sk}$ of 37.54±0.04 °C), as was that for day 8 (overall average $\bar{T}_{sk}$ of 37.17±0.05 °C). All three temperature profiles for the MLNa group changed very little over the course of the exercise with overall average $\bar{T}_{sk}$ values of 37.18±0.04 °C on day 4; 37.29±0.06 °C on day 6; and 37.05±0.04 °C on day 8. Within group analyses (ANOVA) of the temperature differentials ($\delta \bar{T}_{sk}$ °C) for each of three days (4, 6 and 8) indicated that the rise in temperature for the LNa group on day 4 was significant ($P<0.01$). The reduction in temperature from day-to-day (across the two dietary groups) was significant ($P<0.05$). Between group comparison showed no interaction between day and diet, indicating that this reduction was independent of diet. In contrast, whilst the increase in temperature over time was significant ($P<0.01$) for the two groups combined, there was an indication ($P=0.09$) that the profiles of skin temperature over time were different for the two dietary conditions.

Figure 7.9. Average mean skin temperature of the two groups on days 4, 6 and 8.



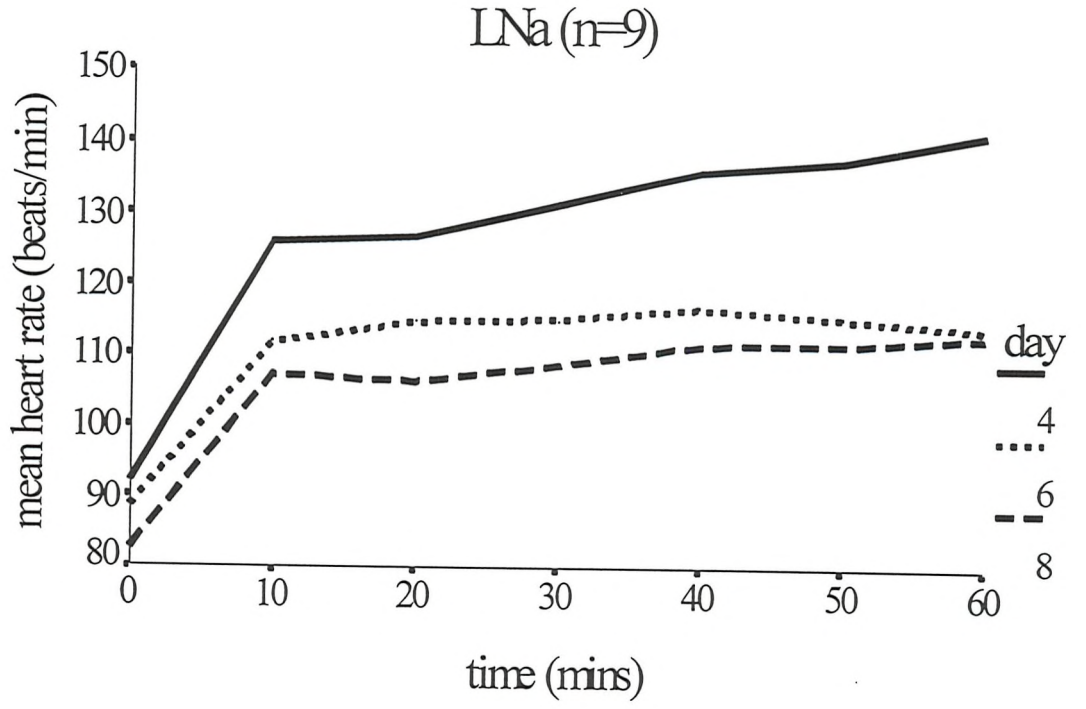
Heart rate during exercise. Heart rates during exercise are presented in Figure 7.10. For the LNa group, heart rate after ten minutes of exercise was 126±6 bpm rising to 141±8 bpm at the cessation, on day 4. These values were reduced to 111±5 bpm rising to 114±6 bpm on day 6, which were similar to day 8 (107±5 bpm rising to 113±5 bpm). A similar pattern was observed in the MLNa group; average heart rates on each of these respective days after 10 and 60 minutes were: 113±6 rising to 125±6 bpm; 104±6 to 109±3 bpm; and 102±4 to 105±3 bpm. ANOVA of this data (two groups, days 4, 6 and 8 with six time points) indicated that the fall ($P<0.01$) in heart rate from day 4 to day 6 occurred independently of sodium intake.

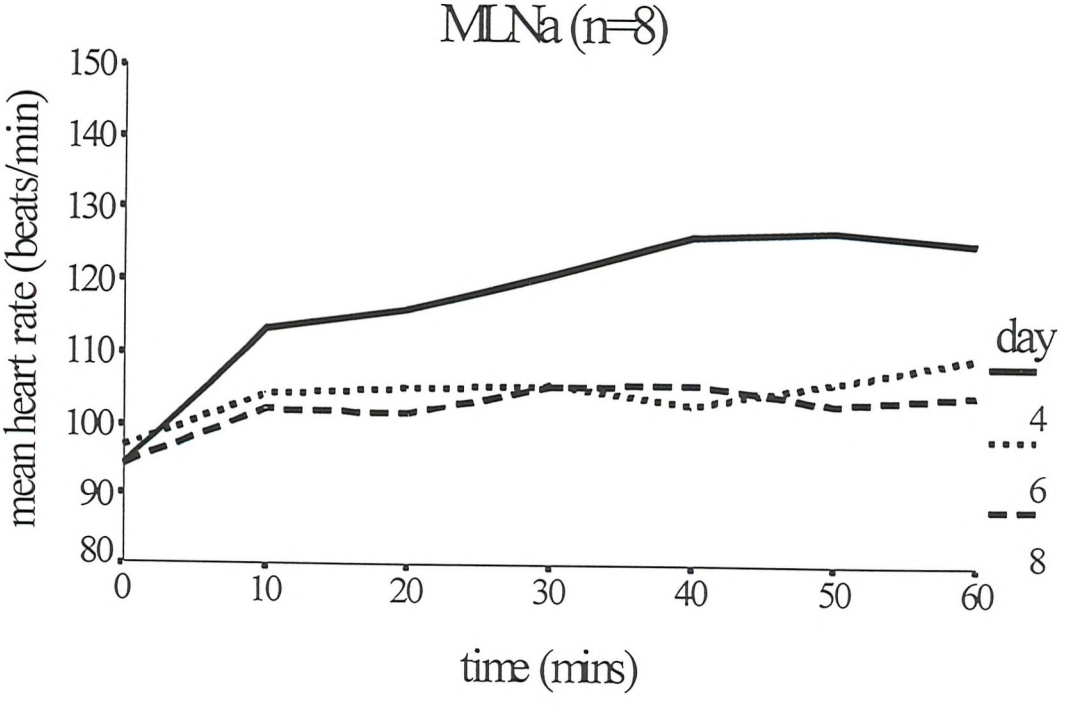
Sweat evaporation. Absolute sweat evaporation (g/m2) and percentage sweat evaporation over the exercise period were estimated from the difference in clothed body weight, pre- and post exercise, corrected for fluid intake. The average values are presented in Table 7.2. There was no apparent increase in evaporative sweat loss as determined by this methodology, nor differences between the two groups.

Table 7.2. Average sweat evaporation estimated over the exercise period.

	LNa (n=9)				MLNa (n=8)			
	g/m2		%		g/m2		%	
	mean	sem	mean	sem	mean	sem	mean	sem
day 4	436	37	1.10	0.10	384	10	0.98	0.03
day 5	429	34	1.09	0.09	462	18	1.19	0.05
day 6	418	23	1.06	0.06	430	18	1.10	0.04
day 7	419	18	1.06	0.05	444	9	1.15	0.03
day 8	424	29	1.07	0.07	409	19	1.05	0.05

Figure 7.10. Mean heart rate during the exercise step test.





Conclusions.

Only those conclusions which are pertinent to this chapter will be discussed here. Results which have a bearing upon the central hypothesis of dietary sodium manipulation and heat acclimation will be addressed in the general discussion.

The aim of this experiment was to examine the effects of dietary intake of sodium (with respect to thermoregulation, sodium and fluid balance), when this was reduced either *prior* to heat exposure, or upon the first day of heat exposure.

Sodium balance.

The effect of restricting dietary sodium intake prior to heat exposure on subsequent sodium balance in the heat was that a smaller sodium deficit appeared to be incurred compared to that experienced by the sodium restricted group. This was indicated by the interaction of diet with day of heat exposure for the sodium balance data. The amount of this deficit for the MLNa group, approximately 50 mmol Na, was over twice that experienced in the LNa group. Assuming that these deficits were recovered in an exponential manner over the next four days, the total sodium depletion would be of the order of 100 mmol and 50 mmol for MLNa and LNa respectively, or assuming a normal plasma concentration of 145 mmol/L, approximately 1% and 1.5% of bodyweight. This estimate is similar to the actual reduction in bodyweight measured and therefore, it is likely that this loss is due to dehydration consequent to a sodium depletion.

Aldosterone.

The reduction in dietary sodium caused an elevation of plasma aldosterone concentration even prior to the heat exposure (Figure 7.7). The stimulus for this is likely to be the sodium deficit as indicated in Figure 7.4. There was evidently an interaction between this sodium deficit and the heat exposure as evidenced by the

further potentiation of aldosterone secretion during heat exposure in the LNa condition. Thus aldosterone concentration was higher in the LNa condition on day 6 despite the greater deficit presumably being incurred in the MLNa group. This point reiterates the desirability of avoiding other potential stimuli of aldosterone (e.g. exercise) in this experimental design.

Sweat sodium retention.

The similarity between the dietary groups with regard to sweat sodium reabsorption would suggest that despite the earlier stimulation of aldosterone secretion in the LNa condition, this was insufficient or too late to effect any response of sodium reabsorption at the sweat glands. The time course of this response is thought to be up to three days (Collins and Weiner, 1963). An alternative explanation could be that the local activity of the sweat gland is the primary factor in retaining sodium, the so-called sweat gland training, and that aldosterone secretion has a secondary lesser role. This issue is addressed in the general discussion.

Thermoregulation and heat acclimation.

Reduction in body temperature. A number of thermoregulatory changes were observed which served to reinforce the conclusion that this experimental procedure produced partial or complete heat acclimation as before; the decline in heart rate, lower aural temperatures and increased plasma volume. The greater reduction in plasma volume on day 4 for the restricted group could be critical if working sufficiently hard in the heat as to be at risk from cardiovascular collapse. The reduction in body temperature over the heat acclimation period was numerically greater for the LNa subjects (0.45°C) than for MLNa (0.20°C) and the apparent divergence between the two groups was reinforced by a similar changes to the aural temperature in the mean skin temperature profiles.

Sweat production. There was no detectable change in sweat production to cause this reduction in mean skin temperature, hence it may be postulated that reduced peripheral bloodflow consequent either to dehydration (Coyle & Hamilton, 1990) or altered plasma osmolality (Fortney et al, 1984) influenced heat dissipation. The observation of further dehydration in the MLNa subjects from day 6 to day 8 may therefore explain the reversal of the two aural temperature profiles for these days (Figure 7.7). This hypothesis is discussed further in Chapter 9.

The observed differences in temperature above were relatively small (approximately 0.3 °C), and unlikely to constitute a significant burden to thermoregulation within the constraints of the methodology used here. What is of interest however, is whether these thermoregulatory/heat adaptation differences are real, whether they are attributable to dietary sodium intake, and if so, whether they may constitute a significant thermal penalty in more severe circumstances. One interpretation of this difference between the LNa and MLNa groups with respect to the adaptation of thermoregulatory responses, is that the LNa group acclimated to the heat stress more completely or to a greater extent. Alternatively it might be considered that the thermoregulatory responses of the LNa were impaired on the initial day of heat exposure and so any subsequent reduction in temperature appeared to be greater than for the other groups. Note however, that the initial temperature profiles for LNa and MLNa were similar, (mean aural temperature rose to approximately 37.5 °C in both groups on day 4) and so this latter explanation is less likely.

Summary.

In summary, a low sodium intake prior to heat exposure stimulated aldosterone secretion prior to heat exposure. During the exposure the aldosterone response was further potentiated to increase sodium reabsorption, to diminish the total sodium deficit which was incurred. Whilst a statistically significant difference was not demonstrated in this experiment, a physiologically significant reduction was achieved in respect of the subsequent effects upon loss of body weight and heat acclimation.

Since the statistical tests applied to the above data have fallen short of statistical discrimination between dietary conditions, it was considered desirable to further explore the link between heat acclimation and sodium balance by direct comparison of all the dietary groups exposed to heat. This is presented in the chapter which follows.

CHAPTER 8.

COMPARISON OF SODIUM BALANCE AND HEAT ACCLIMATION RESPONSES: LOW, MODERATE AND HIGH SODIUM INTAKES COMPARED WITH DIETARY SODIUM RESTRICTION.

Introduction.

Experiments two and three were conducted using the same generic protocol hence any common outcomes of interest may be investigated across all dietary groups. Of particular concern to the central hypothesis of this thesis was whether plasma aldosterone concentration was related to dietary sodium intake, and if so, how these two factors subsequently effected sodium and fluid balance and thermoregulation in the heat. It was considered therefore, that comparison of the earlier results from all groups in the same environment (HNa, n=7; MNa, n=9; LNa, n=9; and MLNa, n=8) would enable firmer conclusions to be drawn from the data concerning these adaptive responses in the heat. Attention was focused upon the MLNa condition compared to the other dietary groups, as this condition represents a restriction of dietary sodium intake; restriction simulates a likely outcome of heat exposure if appetite or food availability is reduced, and is also the most physiologically stressful condition in which the stressors of heat exposure and sodium depletion were applied simultaneously.

Aim and hypothesis.

The aim of this comparison was to re-examine the results of experiments two and three to assess whether sodium balance and heat acclimation responses varied according to dietary sodium intake. The hypothesis was that altered sodium intake had no effect upon: heat acclimation, sodium and fluid balance, as measured by: the rate of adaptation of body temperature and heart rate, and changes in sweat sodium secretion, bodyweight, plasma volume and aldosterone concentration.

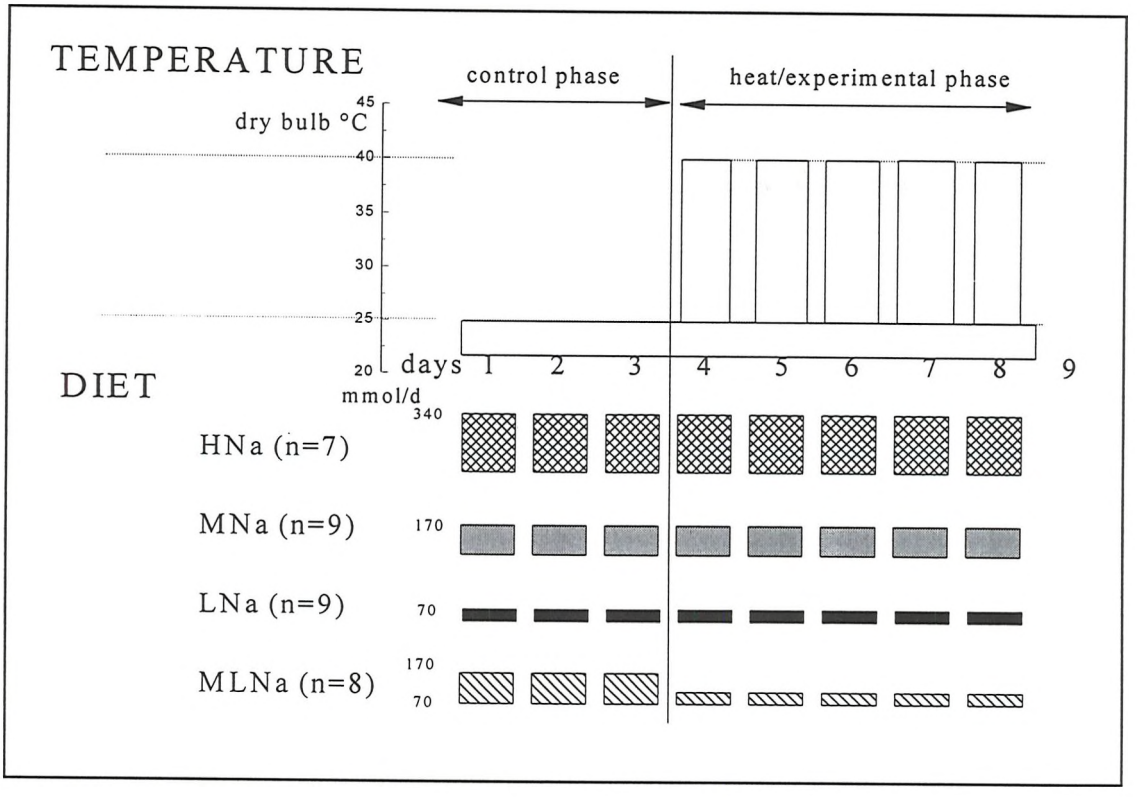
Methods.

The subjects were the same 33 male volunteers reported in the previous experiments (2 and 3) who were confined to an environmental chamber for a period of eight days, the first three at 25°C and the following five days at 40°C during the hours 0800h until 1800h each day. The dietary groups compared were therefore:

a. High sodium intake (HNa) 340 mmol/d (n=7);
b. Moderate sodium intake (MNa): 170 mmol/d (n=9);
c. Low sodium intake (LNa): 70 mmol/d (n=9);
d. Moderate to low intake (MLNa) 170 mmol/d reduced to 70 mmol/d (n=8).

These groups are shown in the schematic diagram of the experimental protocol below. All methods were as previously reported in Chapter 4.

Figure 8.1 Schematic diagram of the experimental protocol.



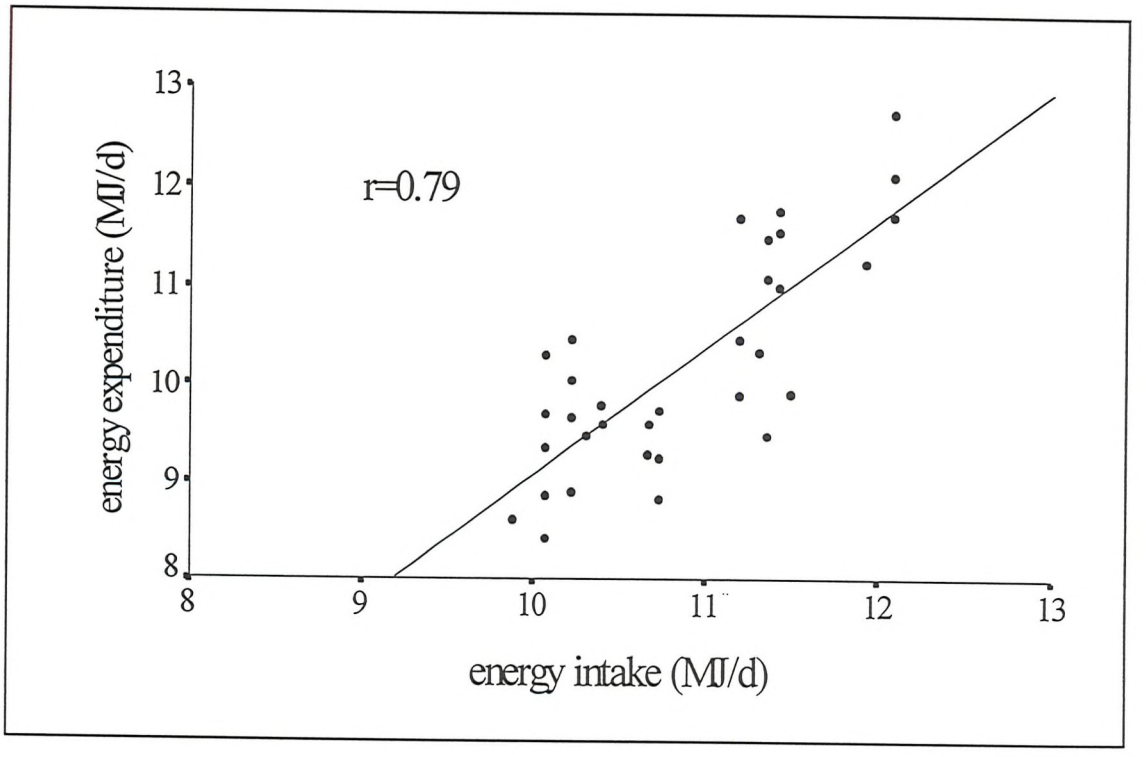
Ambient temperature was increased to 40°C between the hours of 0800h to 1800h daily from the morning of day 4 until the evening of day 8.

Results.

Symptoms of heat illness. All subjects completed the heat exposure successfully with only minor symptoms of heat stress, these being two subjects who reported feeling faint on the first and second day of exposure (one in the LNa group and the other in the MNa group) and one subject who developed a mild heat rash (a HNa subject). One subject (in the LNa group) did not complete the 60 minutes step test as he began to hyperventilate and experienced paraesthesia of the mouth, fingers and toes after 45 minutes of stepping. This subject requested to stop at which point he had a core temperature of 38.2°C. After resting and re-breathing into a paper bag for 10-15 minutes, he was fully recovered. He completed the exercise test on the days which followed without experiencing any difficulties.

Energy balance. The main determinant of fluid balance in this experiment was change in bodyweight. For this to be so, then it must be demonstrated that the subjects were in energy balance. Figure 8.2 shows the correlation between energy intake and expenditure, as estimated from oxygen consumption at rest (lying and seated) and during the step test. There was a significant ($P<0.001$) correlation between these two variables ($r=0.79$).

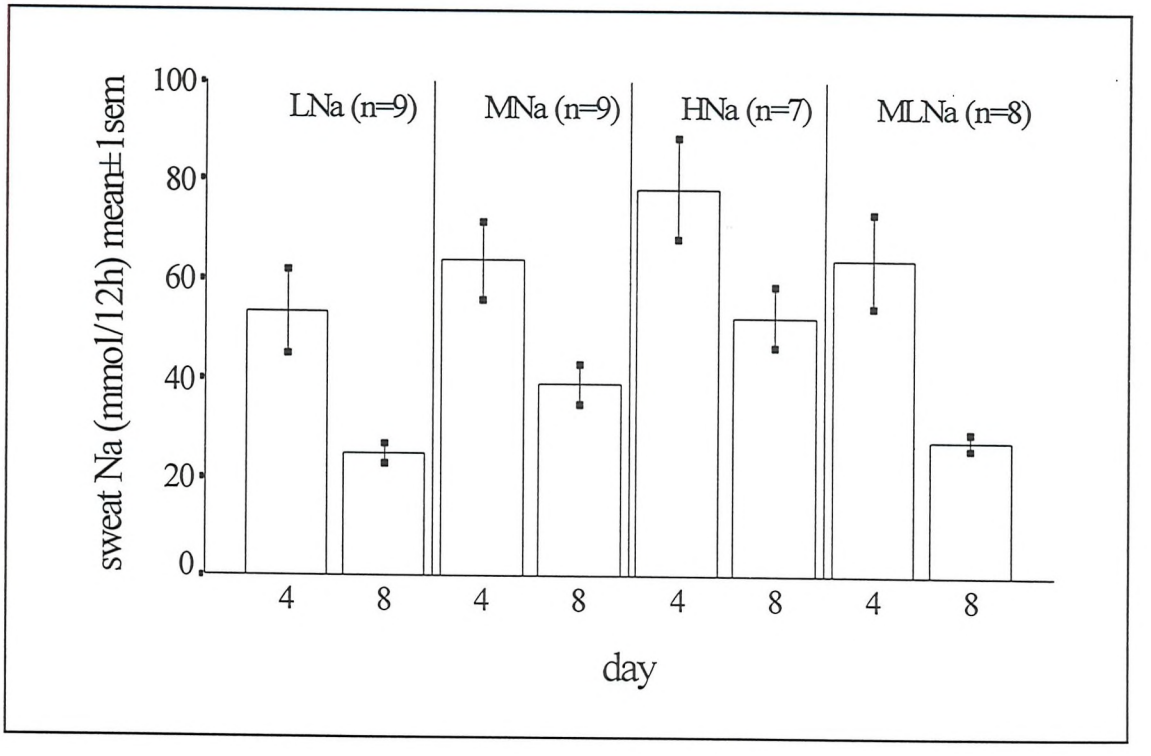
Figure 8.2. Estimated energy intake versus expenditure (n=33).



Energy intake was estimated from the food composition data supplied by the manufacturer, calculated for each subject. Energy expenditure was measured by indirect calorimetry during the control phase whilst at rest (lying), seated, and during the exercise step test. Measured values were used to calculate energy expenditure for each of these three activities, and summated over the course of 24 hours to estimate daily energy expenditure.

Sweat sodium secretion and sweat production. Figure 8.3 presents the mean sweat sodium loss for the washdown period on day 4 and day 8. The reduction in sweat sodium secretion (i.e. the day 4 - day 8 difference) during the heat acclimation period was dissimilar as indicated by a significant ($P<0.05$) interaction between day of exposure and diet in the ANOVA of the transformed data. The mean reductions in sweat sodium secretion were: HNa 24.0 mmol; MNa 27.4 mmol; LNa 37.5 mmol; MLNa 50.4 mmol. ANOVA of transformed data with post-hoc comparison between pairs of means (Scheffé) indicated that were no differences between groups on day 4 whereas on day 8 MLNa and LNa were significantly ($P<0.05$) lower than the HNa and MNa groups. There were no differences between the groups with respect to daily sweat production during the heat exposure (as estimated from fluid balance).

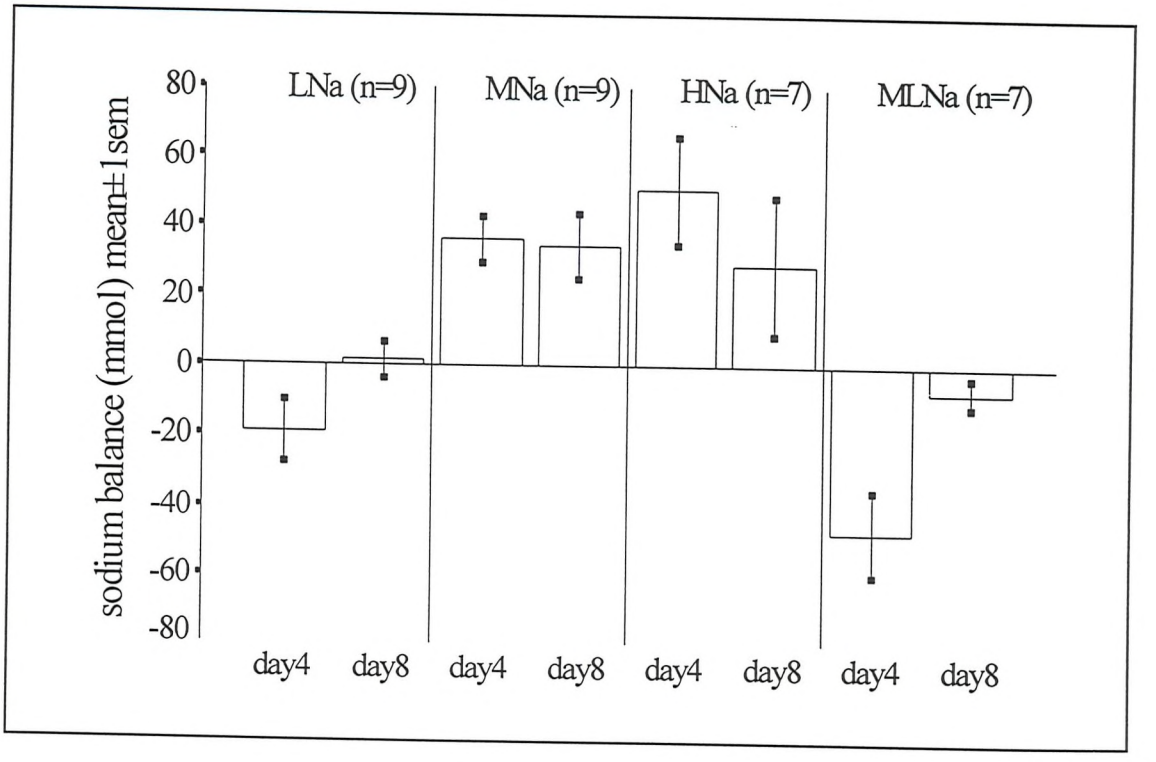
Figure 8.3. Sweat sodium secretion for all four heat exposure groups.



Sweat sodium secretion derived from washdowns performed on the first (day 4) and last (day 8) day of heat exposure. There was an effect ($P<0.05$) of diet upon the difference in sweat secretion on these days, this being a greater reduction for the LNa and MLNa groups ($P<0.05$).

Sodium balance. Figure 8.4 presents the mean sodium balances of the four groups on the first and last days of the heat exposure. The MNa and HNa groups were in a positive balance on both days of measurement, (MNa values were: 36.3±6.7 mmol and 34.5±9.3 mmol; and for HNa 50.5±15.5 mmol and 29.0±19.6 mmol for days 4 and 8 respectively). LNa and MLNa were in negative balance on day 4 (-18.9±8.7 mmol and -47.1±12.4 mmol), although this was restored by day 8 in the LNa group (1.6±5.1 mmol), but not for the MLNa group (-6.9±4.4 mmol). ANOVA revealed a difference ($P<0.01$) between the overall sodium balance (across both days) with respect to diet and an effect of diet ($P<0.05$) upon sodium balance from day 4 to day 8. Post-hoc Scheffé testing indicated that on day 4 the mean value of HNa was higher ($P<0.05$) than for the LNa or MLNa groups. On day 8 both the MNa and HNa means were higher ($P<0.05$) than the LNa and MLNa, which were similar.

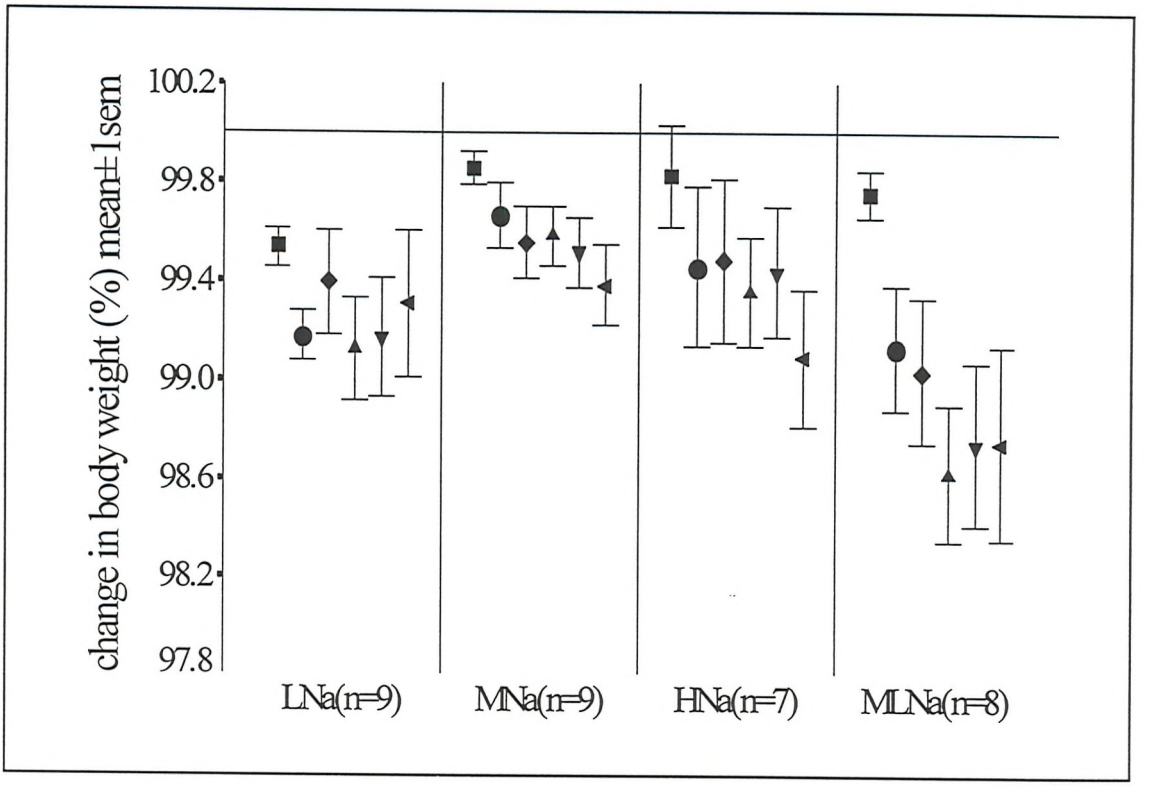
Figure 8.4. Sodium balance of each of the four groups on day 4 and day 8.



(See text above for results regarding differences between these groups)

Percentage change in bodyweight. On the first day of heat exposure (day 4), the greatest percentage reduction in body weight (compared to day 3) occurred in the LNa condition (0.47±0.08 %) although this was not significantly greater than for any other group (HNa, 0.17±0.20%; MNa, 0.14±0.07%; MLNa, 0.25±0.10%). After this first day in the heat, body weight continued to fall in the LNa group until day 7, a reduction of 0.88±0.21%. In the MNa group, this loss of body weight was less, the greatest percentage loss occurred on day 9 (0.62±0.16%). In the HNa group this reduction (by 0.91±0.28%) was also greatest on day 9. In the MLNa subjects the greatest reduction in bodyweight occurred on day 7 (1.38±0.28%). The fall in body weight (across all dietary groups) was significant ($P<0.001$). ANOVA of all four dietary groups revealed that this trend was not significant when all groups were compared with each other. As reported in Chapter 7, however, there was an indication ($P<0.1$) of a difference in this pattern of weight loss when the MLNa group (n=8) were compared with the LNa (n=9) subjects. Furthermore, if the MLNa subjects were compared to all the other subjects (n=25), then this trend was more apparent ($P<0.05$). Thus on day 7, the mean percentage body weight of the MLNa subjects (98.62±0.28 %) was lower ($P<0.01$) than the mean of all the other subjects (99.35±0.11%) as it was ($P<0.05$) on day 8 (MLNa, 98.74±0.33 %; rest, 99.37±0.12 %).

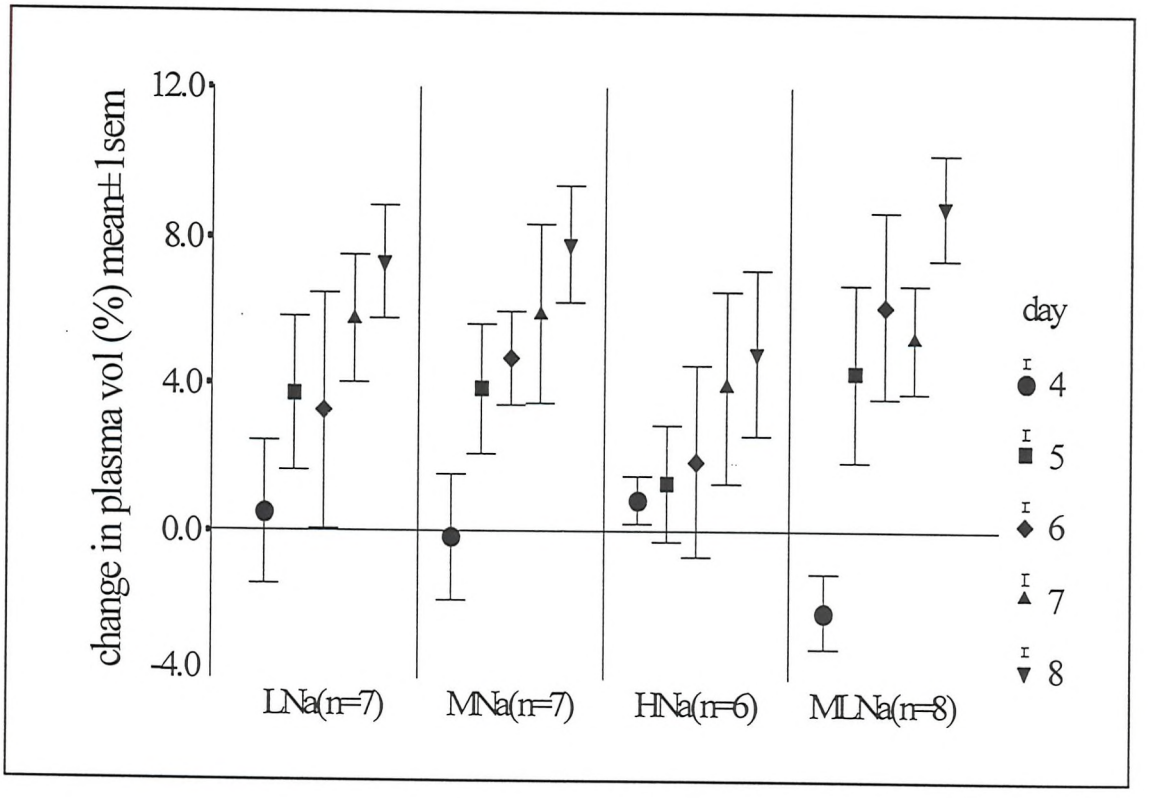
Figure 8.5. Percentage change in body weight of the four groups during the heat exposure.



Percentage change in weight over days 4, 5, 6, 7 and 8 compared to day 3 (at 0700h daily) Each bar represents a new day. There was a fall ($P<0.001$) of body weight from day-to-day. This reduction was not affected by diet if all four groups were compared within the ANOVA. If however, the restricted (MLNa) group were compared to the LNa group, then there was an indication ($P<0.1$) that the rates of fall in body weight were different. If the restricted group were compared to all other ($n=25$) subjects, then the faster ($P<0.05$) rate of loss was confirmed.

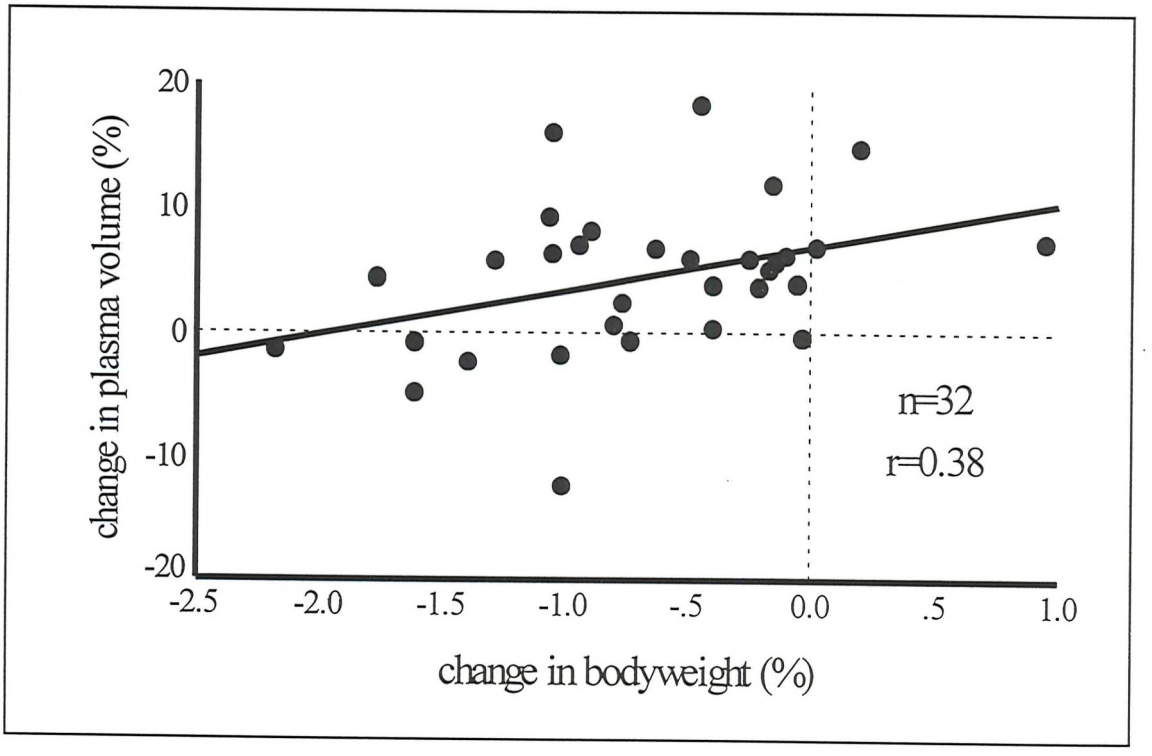
Percentage change in plasma volume. Plasma volume expansion is shown in Figure 8.6. Note how the restricted (MLNa) group incurred an initial reduction in plasma volume on day 4. Percentage change in bodyweight was significantly ($P<0.05$) correlated with change in plasma volume on day 6 ($r=0.38$; Figure 8.7) and day 7 ($r=0.38$), but not the remaining days.

Figure 8.6. Percentage change in plasma volume of the four groups during the heat exposure.



Percentage change in plasma volume over days 4, 5, 6, 7 and 8 compared to day 3 (at 0700h daily) Each bar represents a new day.

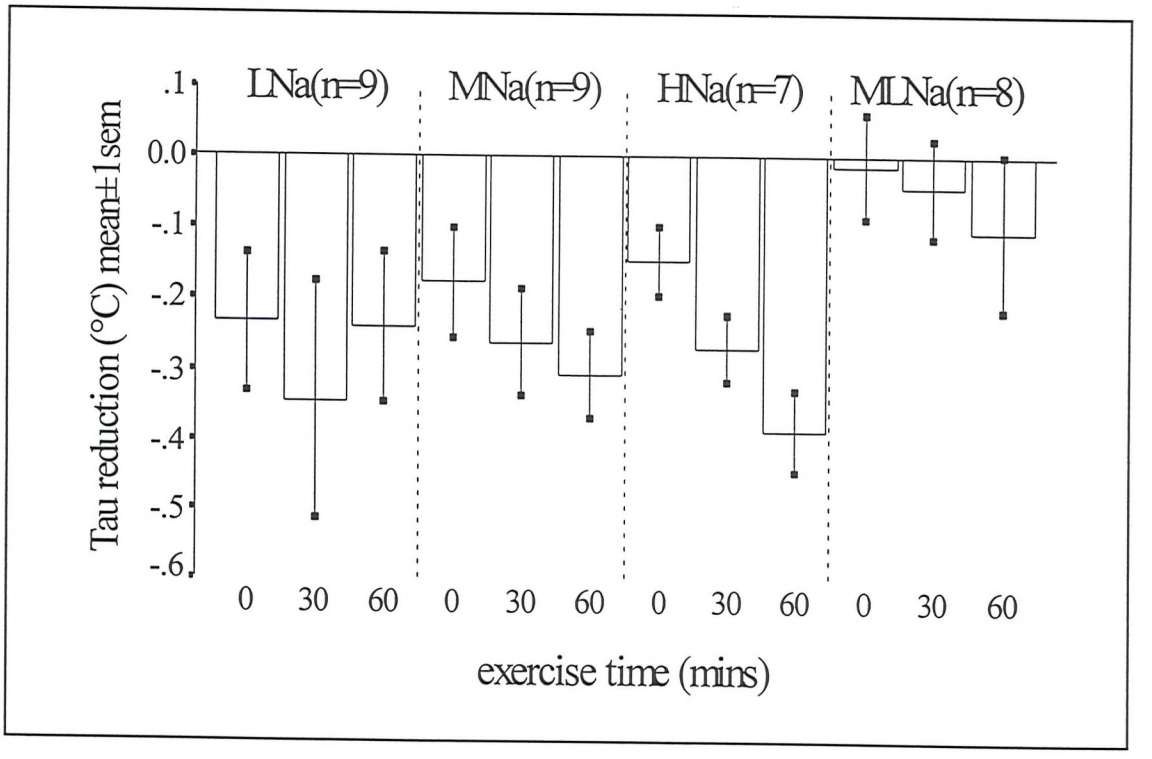
Figure 8.7. Correlation between change in percentage body weight and change in plasma volume (%) on day six.



The relationship between the change in percentage body weight (day 6 relative to day 3) and change in plasma volume (day 6 relative to day 3), indicated a significant ($P<0.05$) direct correlation ($r=0.38$).

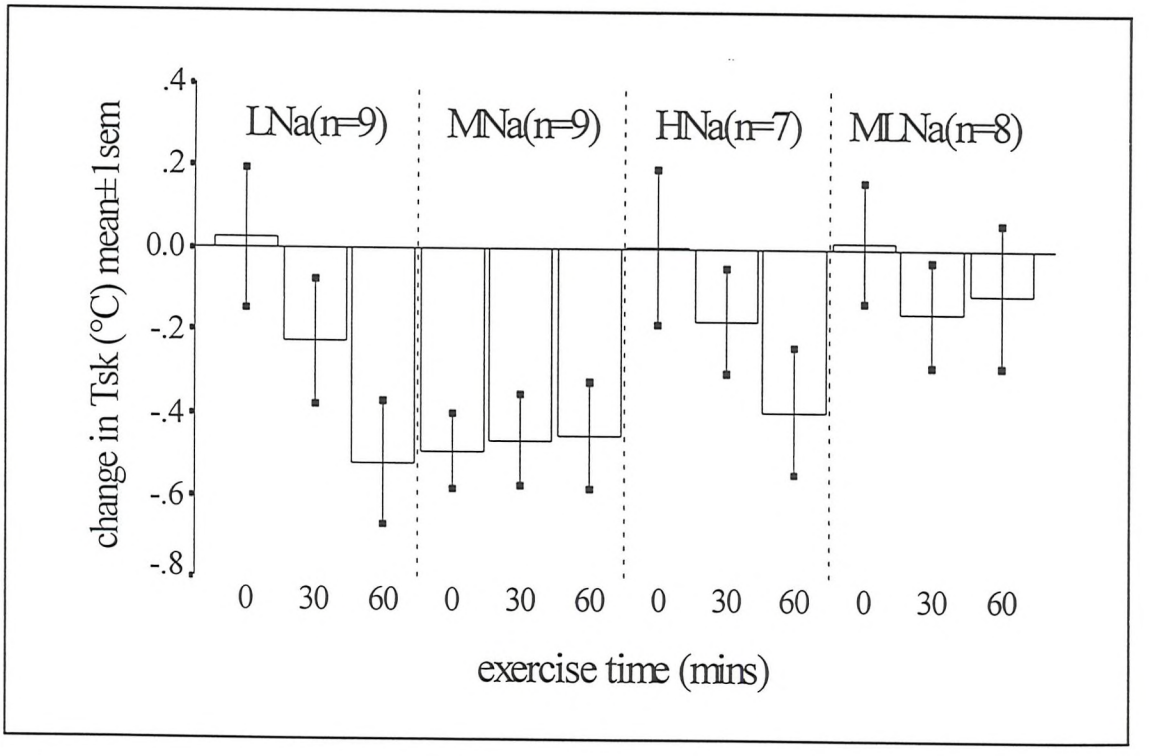
The reduction in aural temperature during the exercise test over the heat exposure period. The aural temperature during exercise at ten minute intervals on day 8 was subtracted from the generally higher profile obtained during exercise on day 4 to give a reduction in aural temperature over the course of the heat exposure. These reductions at 0, 30 and 60 minutes of the step exercise for the MLNa (n=8) subjects were: 0.13±0.07 °C; 0.04±0.07 °C; and 0.11±0.11 °C respectively. For the remaining (n=25), the mean calculated reductions in aural temperature at these time points were: 0.20±0.05 °C; 0.30±0.07 °C; and 0.36±0.07 °C respectively. This reduction at 0, 30 and 60 minutes of the step exercise is presented in Figure 8.8. The reduction in aural temperature in the MLNa condition was compared to that of the remaining 25 subjects by ANOVA of the data at each of the ten minute intervals. This analysis indicated that the reduction in aural temperature (across these time points) was significantly ($P<0.05$) less for the MLNa group compared to the remainder of the subjects (n=25).

Figure 8.8. Reduction in aural temperature (day 4 minus day 8) of the four groups.



The reduction in mean skin temperature during the exercise test over the heat exposure period. The reduction in mean skin temperature between day 4 and day 8 at each of the ten minute time points during the step exercise was calculated. In the LNa condition the mean changes in Tsk were: +0.0±0.2 °C; -0.2±0.2 °C; and -0.5±0.2 °C for the 0, 30 and 60 minute time points. In the MNa condition these values were: -0.5±0.1 °C; -0.5±0.1 °C; and -0.5±0.1 °C. The HNa group: 0.0±0.2 °C; -0.2±0.1 °C; and -0.4±0.2 °C, and the MLNa subjects: 0.0±0.2 °C; -0.2±0.1 °C; and -0.1±0.2 °C. Figure 8.9 presents these reductions in mean skin temperature. There was an indication ($P<0.1$) that the values for MLNa (across these time points) were lower than those of the other groups. In addition the temperature profiles for the MNa group (being almost flat) were shown to differ ($P<0.05$) from those of the other groups.

Figure 8.9. reduction in mean skin temperature (day 4 minus day 8) of the four groups.



Conclusions.

The purpose of this chapter was to focus attention upon how altered dietary sodium intake may have effected changes in the physiological responses to repeated heat exposure, both thermal and circulatory.

The higher body weight of the HNa subjects compared to LNa and MLNa arises from the allocation of subjects to groups on the basis of their estimated BMR. Meeting the higher energy requirements of a large individual whilst maintaining a low sodium intake, and vice-versa for a smaller individual, would have proved impossible using ordinary food items without this pre-selection of subjects. This does not however preclude comparison between the dietary groups and may actually assist in this. The dietary requirement for sodium is independent of sex, age or body weight (COMA, 1991), and so this procedure is unbiased with respect to this factor. Arguably this pre-selection ordered subjects with respect to their habitual sodium intake, (i.e. those who eat less food also ingest less sodium), more so than if the groups had been randomised. This may have reduced the time required to achieve sodium balance during the control period.

The functional outcome of these responses is tolerance of the individual to the heat and their susceptibility to heat illness as this will adversely affect their physical and mental performance. It was therefore of interest to note that only one subject was unable to complete the exercise test and experienced any difficulties, and this was in the low (LNa) rather than the restricted (MLNa) condition. The nature of this heat illness was primarily hyperventilation with consequent symptoms of paraesthesia of the face and fingers. This subject also reported to feel faint and this was probably a consequence of the hyperventilation rather a direct effect of hyponatraemia through sodium depletion; indeed the speed of his recovery would suggest this was the case. Hyperventilation in the heat is commonly reported in cases of heat exhaustion, following increased metabolic heat production, which may occur in the absence of a high environmental heat load (Mustafa et al, 1983). It is, however, possible that

plasma osmolality mediates the thermal stimulus to ventilation at the level of the hypothalamus. Indeed, hyperventilation may be a response to altered osmolality in an attempt to increase evaporative heat loss. The hypothesis that osmolality per se has a direct effect upon thermoregulation is pursued further in Chapter 9, together with a more expansive discussion of heat illness.

In this experimental design whereby food intake was controlled but drinking water was allowed *ad-libitum*, it was important to establish that the subjects were in approximate energy balance. The close agreement between energy intake and expenditure in Figure 8.2 indicated that the subjects were in energy balance, or indeed, a slight positive energy balance state was achieved for the majority of subjects. This would suggest that any acute reductions in bodyweight were a result of fluid loss, and so an indicator of dehydration. The correlation between change in bodyweight and plasma volume on days 6 and 7 also lends support to this argument.

The reduction in percentage body weight on day 4 was numerically lower in the LNa group. Since, body weight was measured at 0700h daily however, this loss was incurred in the previous 24 hours before the heat exposure had commenced. Hence this reduction does not infer a greater initial dehydration, but simply that a steady-state had not been achieved with respect to fluid balance in this group. Rather, it would appear that the MLNa incurred the greatest water debt and as noted above, this correlated with change in plasma volume. The possible reasons for this greater dehydration are discussed further in Chapter 9.

The sodium intake of the moderate and high sodium conditions was sufficient to prevent a deficit in the majority of subjects for most of the trial, whereas as the intakes of the other two conditions were not. What is unknown but of interest, is whether the deficit seen in the MLNa condition persisted until the final day of the trial. The results here indicated that sweat sodium secretion was reduced and that the pattern of that reduction was influenced by sodium balance. The stimulus which caused a reduction in sodium excretion, and the extent to which aldosterone regulates this

response, will be addressed in the following chapter.

The data presented here suggest that the reduction in aural temperature that occurred during heat acclimation was dependent upon the sodium status of the individual, as shown by the difference between dietary groups in the reduction of aural and mean skin temperature. One possible mechanism for this attenuation of reduction in Tau, that of dehydration subsequent to a negative sodium balance, was investigated but no correlation found between this variable and bodyweight or plasma volume. This, and another possible explanation, that of reduced peripheral bloodflow, are discussed in the following chapter.

The impact of the above results can be summarised as follows:

a. Subjects do appear to incur a sodium deficit on heat exposure if ingesting a low sodium diet, although the size of this deficit can be reduced if subjects are accustomed to this level of sodium intake.

b. Sodium balance is more readily achieved if this low sodium intake is incurred prior to heat exposure compared with on heat exposure.

c. A prior low sodium diet does not appear to cause any attenuation of heat acclimation, but neither does there appear to be any potentiation of these responses.

d. In comparison, a restriction of dietary sodium intake does appear to attenuate the reduction in aural temperature associated with heat acclimation.

The implications of the above results will now be discussed in the context of reducing the functional impact of heat exposure upon people who are unacclimatised, so that their thermoregulatory mechanisms are not compromised and acclimatisation proceeds swiftly and effectively.

CHAPTER 9.

DISCUSSION.

Introduction.

This thesis has examined whether dietary sodium intake can be manipulated in such a manner as to enhance the normal responses to heat acclimation. Specifically, the question posed within the central hypothesis was:

> *Can a low sodium intake prior to heat exposure stimulate aldosterone secretion and effect subsequent electrolyte and fluid balance in a manner which improves thermoregulation in the heat during the acclimation period?*

To the physiologically näive this strategy has an element of risk, being counter to current advice, and intuition; sodium losses are known to increase upon heat exposure and any resulting sodium deficit might be expected to exacerbate thermoregulation. For those who are aware of the concepts of sodium balance and heat adaptation however, this manoeuvre presents a way in which the acclimation process might be potentiated or accelerated, reducing the period of heat acclimation.

The current advice from the Committee on Medical Aspects of Food Policy (COMA, 1991) relating to sodium supplementation states that the need for sodium supplementation (above the Dietary Reference Value of 70 mmol/d) is not established, although in the short term (i.e. during acclimatisation) additional sodium may be required. This advice is essentially unchanged since that given by Leithead and Lind (1964), and has not been revised despite a number of considerable advances in knowledge. This perceived requirement for salt tablets during heat acclimatisation has become dogma, and there has been little new research into sodium requirements in the past thirty years until recently (Armstrong et al, 1993; Francesconi et al, 1993). Those studies which have been conducted in the intervening period have concentrated upon the endocrinological aspects without reference to the application of the findings to whole-body sodium requirements.

This is the first study to systematically investigate the effects of low, moderate and high sodium intakes upon thermoregulation during heat acclimation in controlled conditions in the past thirty years. With respect to the above central hypothesis and the current dogma regarding sodium requirements in the heat, the results presented in this thesis have shown that:

a. unacclimatised men ingesting a low sodium (70 mmol/d) diet did not experience any symptoms of heat illness or sodium depletion despite exposure to considerable heat stress (40°C) for ten hours each day;

b. total sodium losses were reduced during the initial days of heat exposure in subjects who had a low sodium (LNa) intake prior to heat exposure, compared to subjects who received no prior low sodium (MLNa);

c. no thermal dysfunction was detected in these low sodium (LNa) subjects compared to moderate (170 mmol/d) and high (340 mmol/d) sodium intakes;

d. sodium restriction (MLNa) upon heat exposure resulted in thermoregulatory responses indicative of a compromised extracellular fluid volume when compared to those same responses in subjects who received a low sodium diet (LNa) prior to heat exposure;

e. plasma aldosterone concentration was increased in response to a state of negative sodium balance (although not significantly so). When combined with subsequent heat exposure, this negative sodium balance potentiated plasma aldosterone secretion to elicit a significant elevation of plasma aldosterone;

f. a low sodium intake maintained at 70 mmol/d prior to and during heat exposure did not give rise to any symptoms of heat illness, nor did a sudden

restriction of sodium from a normal intake (170 mmol/d) to this low level. This restriction did, however, appear to compromise thermoregulation, by impairing the expansion of plasma volume which accompanied the heat acclimatisation process;

g. The increase in sodium retention by the sweat glands as acclimation developed, measured indirectly by whole-body washdown and estimation of sodium secretion, was independent of the sodium balance status of the subjects.

The above conclusions raise a number of pertinent issues which will be discussed in this chapter. Firstly, if it is accepted that aldosterone secretion can be manipulated by dietary sodium intake, what is the mechanism for this increase and how does this response vary in a temperate as opposed to a hot environment - i.e. how is the response potentiated? Secondly, what effect does an elevated plasma aldosterone concentration have upon sodium balance, and how is this achieved? Thirdly, what effect, if any, does this raised aldosterone response have upon non-electrolyte changes in heat acclimation such as plasma volume expansion, increased heart rate and thermoregulation?

Taken overall, the above results suggest that the advice regarding supplementation remains correct, i.e. no supplement is required on a moderate (170 mmol/d) intake (although this "moderate" intake is previously undefined). In the light of the above conclusions, however, this advice could be reappraised and improved by including more quantitative information. Thus the data from this thesis suggest that:

1. personnel ingesting a low (70 mmol/d) sodium intake require no further supplementation if performing only light activities for short periods, in this instance an estimated Physical Activity Ratio of 1.4 (COMA, 1991);

2. restriction of sodium intake in the heat is likely to impair heat acclimatisation;

3. ingestion of a low (70 mmol/d) sodium diet prior to heat exposure will stimulate aldosterone secretion, which will reduce overall sodium loss during subsequent heat exposure, but will not prevent an initial negative sodium debt.

This chapter also addresses the relevance of these research findings for the military given the increased desirability of rapid deployment of forces and the need to attain acclimatisation rapidly with the minimum risk of heat illness. Thus the military operational relevance of these data will be discussed in the context of any potential benefit or disadvantage of dietary sodium manipulation and current policy regarding salt supplementation.

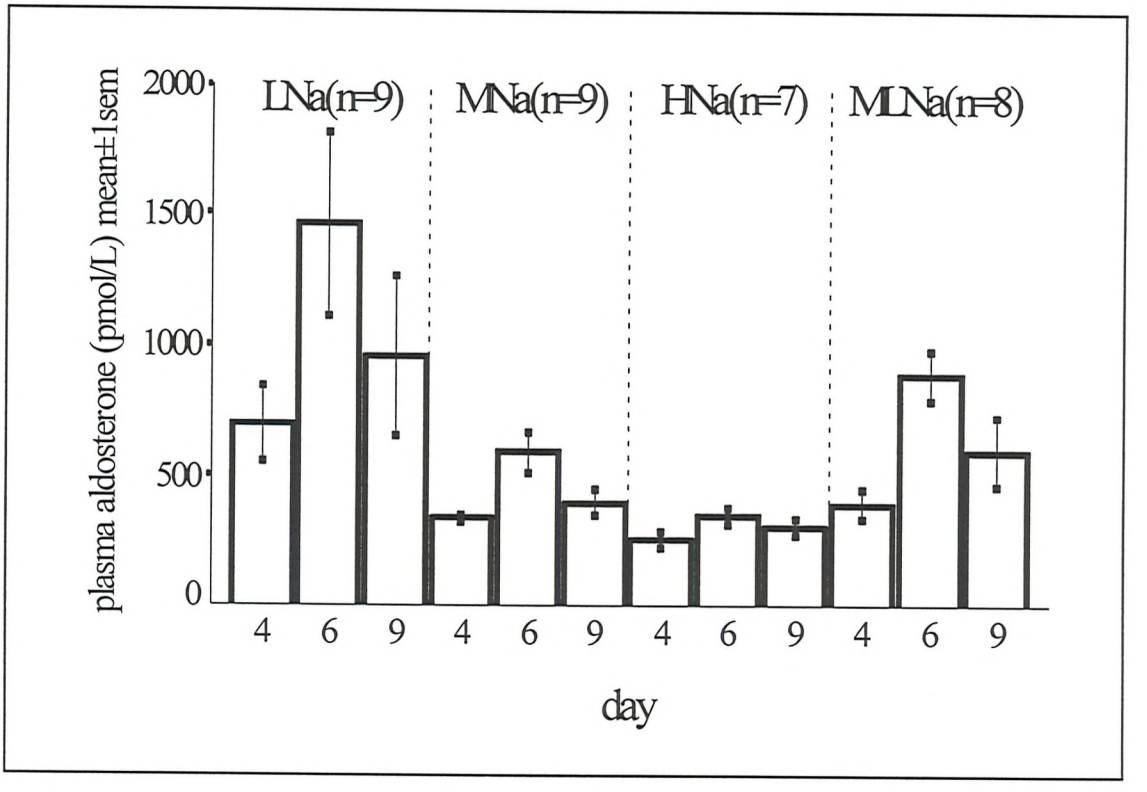
Studies regarding the requirement for sodium were conducted during the first half of this century, many by or on the behalf of the military, in a drive for appropriate dietary guidelines for working in the heat. These investigations have necessitated considerable commitment and time due to non-automated techniques, the difficulties of preparing low sodium diets, and the lengthy duration over which such studies were conducted. This effort has not resulted in a full resolution of the issues, limited perhaps by the desire for an expedient solution to heat illness and the available knowledge at that time. Hence earlier advice regarding salt supplementation, which has survived to this day, was formulated before the concept of sodium balance was widely appreciated and when adequate environmental and dietary controls were difficult to achieve. Indeed, many "classical" studies (McCance, 1938; Ladell et al, 1944; Robinson et al, 1950) were conducted before the discovery, isolation, and understanding of aldosterone, and prior to research of the changes that occur in sweat composition during sweating. It is unsurprising therefore, that this thesis re-visits many of these earlier issues, with a perspective which is based upon a re-evaluation of sodium and fluid homeostasis pertaining to thermoregulation.

The effect of a reduced sodium intake upon aldosterone, during and prior to heat exposure.

To become heat acclimatised, the sodium conserving responses of the kidney and sweat glands need to be initiated and sustained. The involvement of the adrenal cortex in restoring and maintaining sodium balance following heat exposure is a key factor in heat acclimation. It was this response, therefore, which was of particular interest to these experiments, and a key hypothesis was that aldosterone secretion could be stimulated both during and prior to heat exposure by ingestion of a low sodium diet.

During heat exposure it was clear that plasma aldosterone secretion increased following dietary sodium restriction prior to heat exposure (Figure 7.6), compared to normal sodium intake. Furthermore, the secretion of plasma aldosterone during heat exposure was dependent upon the level of sodium intake, being potentiated in the LNa group and attenuated by a high intake of sodium. This is shown in Figure 9.1 which combines the data from Chapters Six and Seven; the highest plasma concentration was achieved on day 6 (after 48 hours of heat exposure) in all conditions. The size of this aldosterone response was inversely related to sodium intake and presumably sodium balance at that instance. It follows that this response can be potentiated by a low sodium intake. Post-hoc Scheffé testing between all four groups revealed no significant differences on day 1. On day 3 the mean value was higher ($P<0.05$) for the LNa group compared to the other groups, and higher ($P<0.05$) than either the HNa or MNa on day 4 and day 6. The HNa and MLNa values were also dissimilar ($P<0.05$) on day 6. Assuming aldosterone to be critical to plasma volume expansion, then this response may positively influence subsequent thermal balance during the heat acclimation process.

Figure 9.1. The concentration of aldosterone in the four conditions upon exposure to heat. LNa (n=9); MNa (n=9); HNa (n=7); MLNa (n=8).



Note how the size of the aldosterone response was inversely proportional to the level of sodium intake (i.e. was lowest for HNa and highest for the LNa) and was potentiated by prior low sodium (i.e. the response of LNa compared to MLNa).

Increased aldosterone secretion in response to heat exposure was first suggested by Hellman et al (1956), who measured an increased urinary excretion of aldosterone. Luetscher & Axelrad (1954) were the first to objectively measure increased urinary aldosterone excretion following sodium deprivation. Since then, several groups have confirmed these findings and others which demonstrate an increased aldosterone secretion occurs in response to: physical activity (Costill et al, 1976; Geyssant et al, 1981); dehydration (Francesconi et al, 1985); heat exposure (Kosunen et al, 1976; Follenius et al, 1979); and sodium deprivation (Smiles & Robinson, 1971).

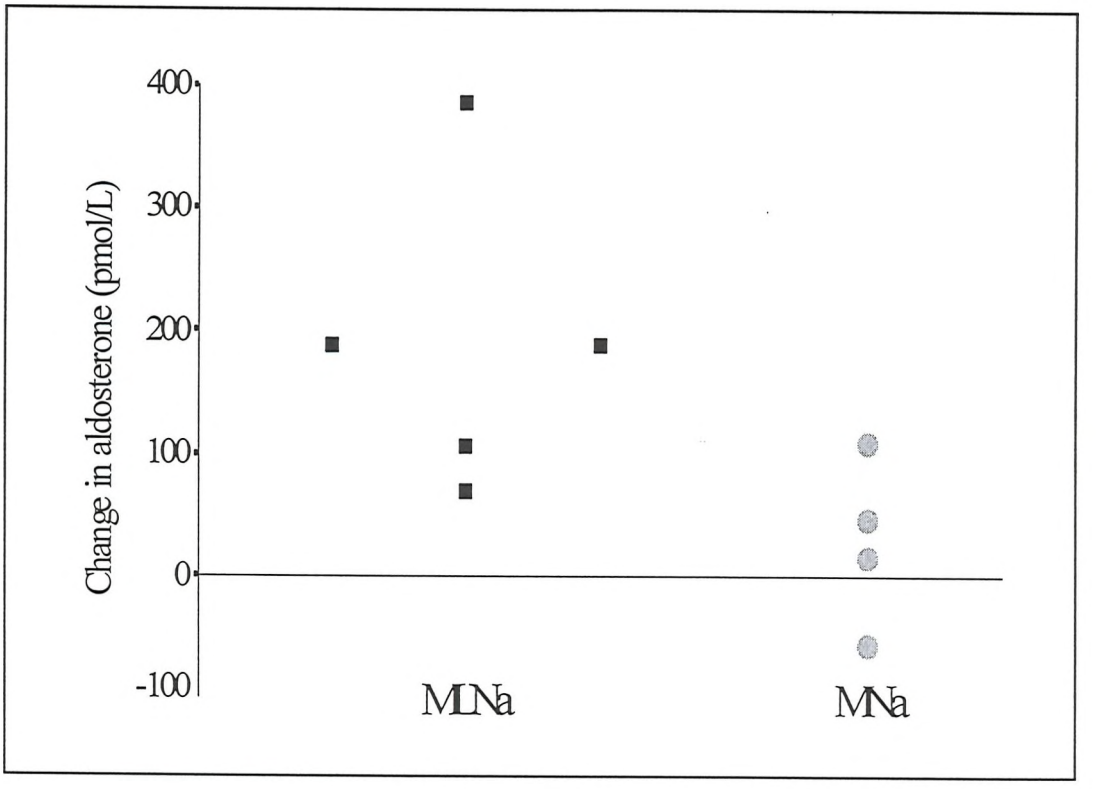
Few studies, however, have investigated the combined effects of heat and sodium deprivation. One of the earliest studies to attempt this, was that of Streeten et al (1960). These authors studied four men on the same sodium restricted diet before and after heat exposure and measured urinary aldosterone excretion. They noted that the excretion of this hormone persisted long after the subsidence of other steroids and therefore concluded that this response was dissociated from the control of corticotrophin. In these early experiments, urinary aldosterone was assayed by the sodium excretion of adrenalectomised rats injected with human urine extract. This technique presented a number of difficulties, which reduced the reliability of the data and prevented correlation of this indirect functional bioassay with changes in sodium human excretion.

The present investigation has used plasma aldosterone concentration rather than urinary excretion, as a measure of aldosterone secretion. This has the advantage of being a direct measure although plasma concentration is determined not only by changes in the rate of secretion, but also metabolic clearance. Thus one criticism of short-term heat exposure studies utilising exercise to stimulate heat acclimation, is that hepatic blood flow is reduced to compensate for the increased metabolic demands of the working muscles and vasodilation of the cutaneous circulation. Hence a reduction in hepatic blood flow, by more than 25% during hyperthermy, is a contributory factor to the reduced clearance and concomitant rise of plasma concentration (Collins & Few, 1979). These experiments, however, have combined

the minimum period of exercise with a lengthy heat sojourn for all dietary conditions, allowing comparison between sodium replete and deplete states on successive days at the same ambient temperature.

The hypothesis that restriction of dietary sodium stimulates aldosterone secretion prior to heat exposure was partly substantiated by the results of this thesis. In the temperate environment there was no significant increase in aldosterone secretion in response to a reduced sodium intake as judged by the between-group comparison for each day. Nevertheless, the maximum values for aldosterone concentration (Table 5.5) were higher during the days of restriction, compared to either the control phase or those of the control (MNa) group. An alternative analysis is to compare the plasma aldosterone concentration on day four (the last blood sample taken before the sodium restriction commenced) to day six, 48 hours later (i.e. the same time period as for the LNa group prior to heat exposure). The increase between these two days was significantly ($P<0.05$) greater for restricted sodium group. That one or two individuals in the MLNa group were more susceptible to this stimulus is apparent from Figure 9.2. This Figure shows the increase in aldosterone concentration over this 48h period for the individuals in these two groups. As can be seen, three of the MLNa subjects showed a relatively higher increase in plasma aldosterone concentration, whereas a reduction was observed in one of the MNa subjects. During heat exposure The nine LNa subjects demonstrated a significant rise in aldosterone concentration on day three (just prior to heat exposure) compared to day one (Figure 7.6) to a mean level of approximately 650 pmol/L.

Figure 9.2. Change in aldosterone concentration (day 6 minus day 4) for the restricted condition (MLNa; n=5) and control condition (MNa; n=4) in the temperate environment.



Note that three of the MLNa subjects incurred a higher increase in plasma aldosterone concentration whereas one of the MNa subjects incurred a reduction.

In contrast to the LNa subjects, the rise in aldosterone for the MNa (170 mmol/d) and HNa (340 mmol/d) groups upon heat exposure was delayed and smaller (see Figure 9.1), in agreement with the clinical data of Luetscher & Axelrad (1954). This data also supports the hypothesis of Collins and Weiner (1968), who asserted that salt deprivation in temperate conditions induced a significant increase in aldosterone excretion, which could be reduced by salt loading and greatly magnified by salt depletion.

Taken together, these results would indicate that a restriction of dietary sodium intake does stimulate secretion of aldosterone, although the degree of this response varies considerably between individuals. The reasons for this variation may be previous long-term (i.e. longer than the three day control period) sodium status and the underlying level of atrial natriuretic peptide. These results are in agreement with those of Smiles and Robinson (1971). These authors reported an increased urinary excretion of tetrahydroaldosterone (THA), the major metabolite of aldosterone, during sodium deprivation. THA excretion increased by 3-6 times the control levels over a seven day period when five subjects were acclimatised on a low sodium (30 mmol/d). In other words, a sudden reduction in sodium intake or increase in sodium losses, sufficient to effect a net negative sodium balance, will stimulate the secretion of aldosterone, which acts to conserve body sodium.

The mechanism for increased aldosterone secretion.

The mechanism whereby aldosterone secretion is stimulated by repeated heat exposure to restore sodium balance has been disputed: a negative sodium balance (presumably sensed via a reduction of plasma sodium concentration) has been suggested as the stimulus (Collins & Weiner, 1968); alternatively, it has also been argued that a negative balance is unnecessary, and that the stimulus is the combination of exercise and heat, via renin-angiotensin activity (Finberg et al, 1974; Davies et al, 1981).

The former hypothesis was not supported by previous research (Finberg & Berlyne, 1977) which reported that aldosterone secretion in the heat was increased in subjects on a low sodium diet without a reduction in plasma sodium concentration. In Chapter 5 of this thesis a restriction of dietary sodium in temperate conditions resulted in a net sodium deficit being incurred for approximately three days before sodium balance was restored (Figure 5.2). During this interim phase it was clear that body weight, and thereby total body water was reduced, and this was assumed to be a result of a net negative fluid balance (i.e. fluid intake minus urinary volume) during this period. Thus these results support the hypothesis that restriction of dietary sodium in a temperate climate produces a net negative sodium balance, and that this reduces extracellular volume (as estimated by body weight). These responses, however, did not appear to stimulate a significant increase in plasma aldosterone, although the concentration of this hormone did rise. Furthermore, in the heat exposure studies, plasma sodium concentration fell below the normal clinical range in the LNa group on days 7, 8 and 9 and in the MNa group on day 9, whereas aldosterone secretion appeared to peak prior to this fall in concentration. Also, plasma aldosterone was significantly elevated prior to heat exposure in the LNa subjects without firstly reducing ECF volume as detected by weight loss or change in plasma volume. Thus these data do not support the hypothesis that negative sodium balance stimulates aldosterone secretion via changes in plasma sodium concentration.

In contrast, the data in this present study concur with the view that aldosterone is stimulated by lowered sodium delivery to the distal tubule, detected within the glomerular filtrate or by the macula densa, and mediated by renin and angiotensin. Note that this may be in response to heat exposure, exercise, which reduce the effective circulating blood volume, or dietary depletion of sodium. (Lote, 1994; Rowell, 1986). The data in the hot (LNa) experiments, where no reduction in ECF volume occurred but nevertheless aldosterone concentration was increased prior to heat exposure, would support this view. This putative mechanism could be similarly applied to the temperate MLNa subjects who also experienced a reduction in plasma volume to stimulate the volume or low-pressure baroreceptors. The reduced effective

circulating volume mechanism explains observations of increased aldosterone in response to acute heat exposure (Follenius et al, 1979), and dehydration (Francesconi et al, 1985).

In other words, it is suggested that the renin-angiotensin response is a common mechanism whereby heat, exercise and dietary sodium restriction can stimulate aldosterone secretion, primarily through changes in ECF volume but also, possibly in response to a reduced sodium concentration in the glomerular filtrate. These afferent stimuli appear to be integrated in a compound rather than additive manner. Hence heat exposure and a sodium deficit together, yielded a greater response than might be expected from the summation of these individual stressors.

Does the aldosterone response require a sodium deficit?

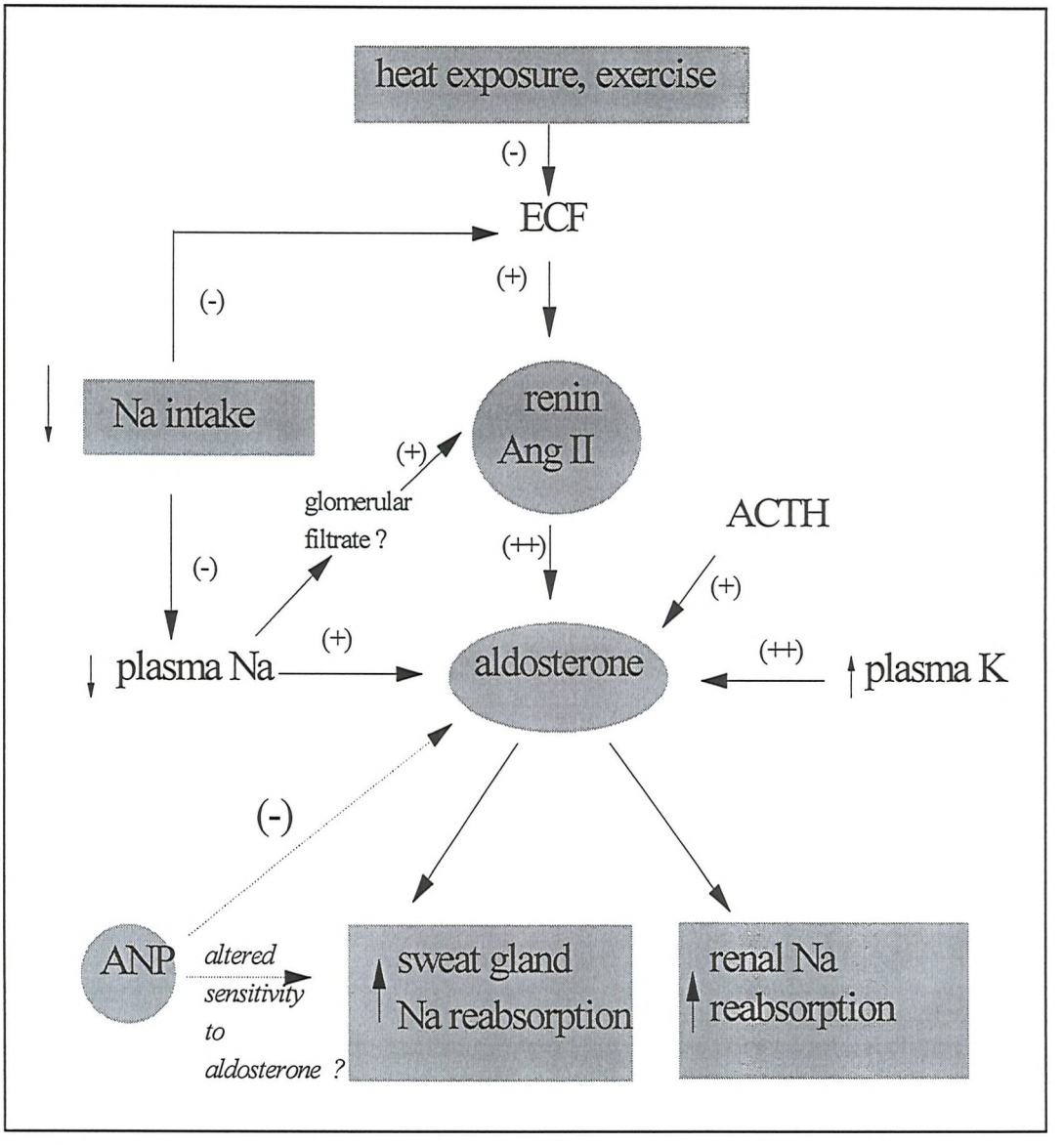
This question can be broken into two parts: firstly is a sodium deficit required in order to stimulate an increased secretion; and secondly, what is the effect of sodium balance upon this response? From the non-dietary stimuli cited in the preceding section, it is clear that the answer to the first question is "no." Thus it is unsurprising that Finberg & Berlyne (1977) reported increases in aldosterone secretion during a brief (30 minute) exercise period in the heat without a significant sodium deficit being incurred.

The second question is a more complex issue, which demands that the stimuli for aldosterone secretion are sensitive to, or dependent upon, dietary sodium intake. It was evident in the present studies that heat exposure brought about an increased aldosterone secretion in all dietary groups exposed to the heat, and that there was an interaction between diet and heat exposure which resulted in the lowest secretion for the HNa group and greatest for the LNa group (Figure 9.1). This is in contrast with Follenius et al, (1979) who reported that acute heat exposure produced similar increases in plasma aldosterone concentration regardless of differences in sodium intake. Note however that these authors were examining responses to acute exposure, and that direct comparison is therefore inappropriate.

Kosunen et al (1976) investigated the effects of short duration heat exposure (in a sauna) on plasma sodium and aldosterone concentration. They observed that although plasma aldosterone peaked thirty minutes after the heat exposure and returned to baseline within two hours, the consequent increase in plasma concentration and haemodilution endured for the following two days. This suggests that the effects of aldosterone may outlast the hormone or that heat stress elicits sodium retention by another mechanism. It may be that the cumulative effect of aldosterone with each period of heat exposure produces and maintains the acclimated state. This hypothesis may also explain the results of Kirby and Convertino (1986) who demonstrated a reduced plasma aldosterone after ten days with an increased sodium reabsorption and therefore argued that plasma aldosterone sensitivity was increased with time. Such a conclusion is important to the present study as it implies that sodium intake maintained at a habitually low level may stimulate aldosterone secretion, without the need to repeatedly restrict dietary sodium intake further.

In summary, the precise stimulus for dietary / heat exposure mediated aldosterone secretion remains unresolved; a change in renal haemodynamics could be effected by a reduction in the circulating extracellular fluid volume, a reduced plasma sodium concentration or a reduced glomerular filtrate sodium concentration. Reduced sodium intake appears to be just one stimulus to plasma aldosterone secretion that has a modulatory function rather than being the primary stimulus to control. A putative model of how these factors may stimulate aldosterone secretion in the heat is presented in Figure 9.3.

Figure 9.3. Putative model of factors affecting aldosterone secretion and conservation of sodium at the sweat gland.



Regulation of sweat sodium reabsorption: does sweat sodium sparing only occur in sodium deficient states?

Previous work has associated the adaptation of the sweat glands with the observed total body sodium deficit (Robinson et al, 1950; Smiles & Robinson, 1971), although this has been disputed (Davies et al, 1981). In the present study a similar reduction in sweat sodium secretion (25-30 mmol over the five day heat exposure period) was observed in all three dietary groups in the heat (low LNa; moderate MNa; and high HNa), with no evidence of an interaction between dietary sodium intake and duration of heat acclimation. This would suggest that this mechanism is independent of the amount of sodium ingested and occurs without firstly incurring a salt deficit, in agreement with Davies et al, (1981).

The physiological adaptation of the sweat glands is reportedly dependent upon an increased secretion of aldosterone (Collins & Weiner, 1968), but the results here do not support this theory. These data revealed significantly higher levels of aldosterone secretion in the LNa, (even prior to the heat exposure period) and in the MNa group on day 6, but no similar significant increase was seen in the HNa group. Sodium retention, however, appeared to be equally effective in all three of these groups. Furthermore, in each group the highest mean secretion of plasma aldosterone was achieved on day 6, whereas the greatest reabsorption of sodium presumably occurred after this day - although no sweat collections were conducted on day 6 for comparison. Thus adaptation of the sweat gland to conserve sodium does not appear to be solely dependent upon aldosterone secretion nor a sodium deficit.

One of the main areas of contention within this field of experimentation is whether or not sodium sparing by the sweat glands is mediated exclusively by aldosterone, or whether this hormone has a permissive action which allows other factors such as sodium balance to exert their control. Thus whilst McCance (1938), Taylor et al (1943), and Smiles & Robinson (1971) have reported that sodium reabsorption is greatest in sodium deficient states, Davies et al (1981) concluded that the increased

sodium conservation was attributable to repeated exercise in the heat in the absence of any sodium deficit.

In the present studies, the comparison of results in Chapter 8 from day 4 to day 8 (Figure 8.2) indicated a similar reduction in sweat sodium excretion over the period of heat exposure, irrespective of dietary sodium intake. This suggests that although the absolute secretion of sodium via this route was dependent upon the total sodium available, the adaptation of the sweat glands was similar following heat exposure to reduce this loss by 30-50%. In addition, overall mean values for sodium balance for the groups (Figure 8.3) indicate that the MNa and HNa groups did not incur a sodium deficit. Nevertheless, some adaptation of the sweat glands still appears to have occurred. Thus, these data do not support the hypothesis that a state of negative sodium balance must exist before sweat sodium retention occurs.

Sweat secretion.

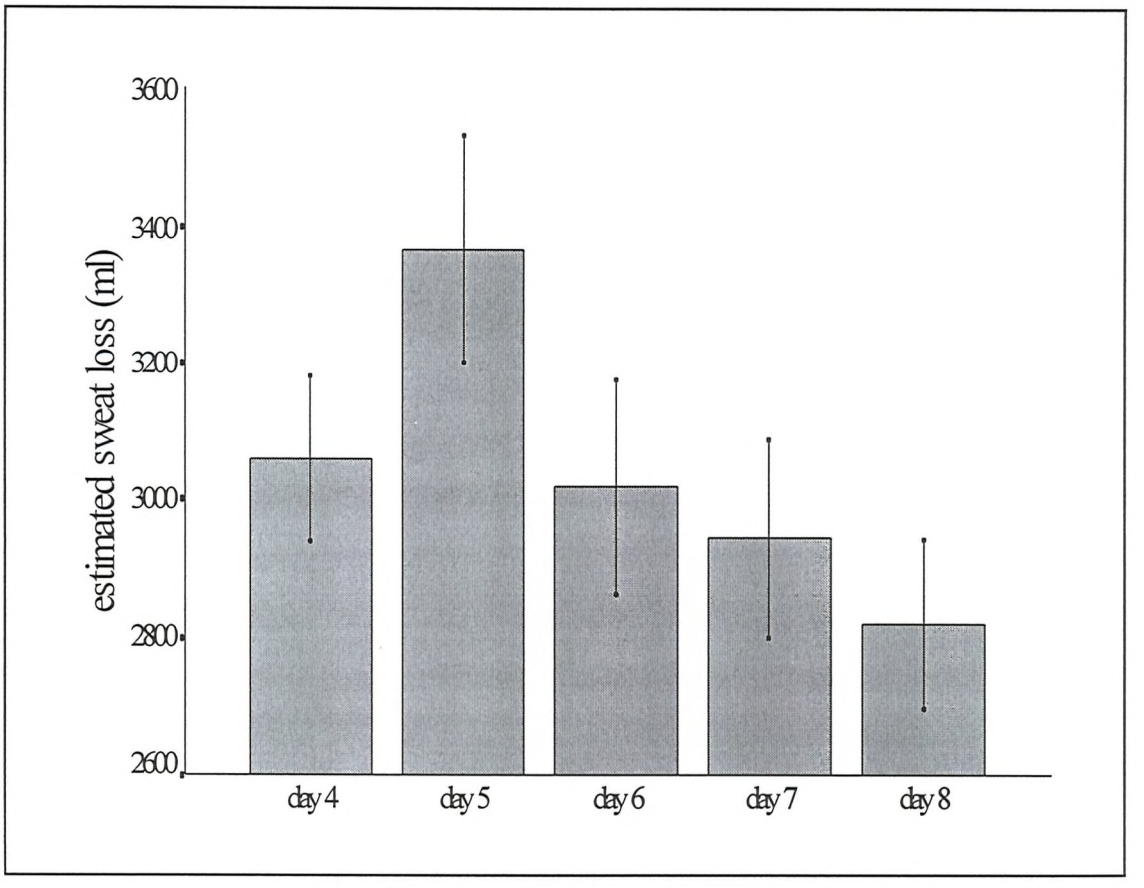
One criticism of the above conclusion is that the absolute amount of sweat sodium from the washdown process has been examined and not the sweat concentration, due to methodological difficulties which are discussed below. Assuming, however, that either sweat rate remained constant or increased (as expected in heat acclimation), then this reduction in sweat sodium must reflect a more dilute sweat being secreted. Thus the production of a more dilute sweat should have occurred in all dietary conditions, irrespective of sodium balance. The assumption of increased sweat rate is discussed at greater length below.

A primary response to heat acclimatisation is the increased sweat production in response to elevated core temperature. In the current study, the lack of an increase in sweat production during the exercise period may be ascribed to one, or a combination of the following: the sensitivity of measurement; the relatively short duration over which this measure was performed; and the reduced central drive for sweat production. This last factor is indicated by the day-to-day decline in core

temperature (see Figure 6.9 for example). In a review of sweat gland training (Collins et al, 1965) it has been asserted that increased rate of sweating observed with heat acclimation may be a transient phenomenon which may not necessarily always accompany acclimatisation, or may be unimpressive. This is thought to be particularly the case when heat acclimatisation occurs in a hot-wet environment, as opposed to hot-dry conditions (Fox et al, 1967) due to the greater sweat suppression by hidromeiosis (Brebner & Kerslake, 1963; Kerslake, 1972). The most logical explanation, however, is that an increased sweat production was not observed due to the decrease in body temperature during acclimation in these conditions of constant environmental stress. If controlled hyperthermia had been the stimulus, then this adaptation may have been apparent.

It was intended at the outset of these experiments to estimate sweat losses during the twelve hour washdown periods by difference in weight, allowing for all intake of foods and fluid, and losses of urine, stools, evaporative and metabolic losses. Thus the precise body weight and fluid/food balance status was recorded at the start and end of each washdown period. In attempting these calculations, however, the assumption of a steady-state of fluid balance from day-to-day, upon which this calculation depends, (Robinson & Robinson, 1954) was found invalid and the reliability of this method of calculation questioned. For example, food eaten at mealtimes prior to the washdown period would not have been fully metabolised and hence resulted in metabolic weight loss estimated within the calculation. A simpler approach of estimation based upon body weight change corrected for fluid intake and urinary output only was therefore adopted. Mean daily estimates calculated in this manner for all 33 subjects exposed to the heat are shown in Figure 9.4. Analysis of variance of these data revealed no significant ($P=0.11$) trend with duration of heat exposure. Thus, the above assumption that changes in absolute sweat sodium secretion reflect altered sweat composition was supported by these data.

Figure 9.4. Daily sweat loss (mean±1sem) estimated from fluid balance, urinary loss and changes in body weight during heat exposure (n=33).



Renal escape from aldosterone.

The gradual increase in urinary sodium excretion on days 6, 7 and 8 is of interest. Similar rises following pronounced reductions in urinary excretion have been reported previously (Armstrong et al, 1993). It is suggested that this secondary increase in urinary excretion occurred in response to the delayed reabsorption by the sweat glands; the renal tubules respond to an elevated aldosterone secretion in 6-12 hours, whereas increased reabsorption by the sweat glands is only fully accomplished 1-2 days later (Collins & Weiner, 1968). Thus on day 8 both groups of subjects exhibited a reduction in urinary sodium reabsorption which was balanced by an increase in reabsorption by the sweat glands, hence total sodium excretion remained unchanged. This "escape" phenomenon of the renal tubules from the effect of aldosterone is well known and indicates that sodium balance has become re-established by the greater retention of sodium at the sweat glands.

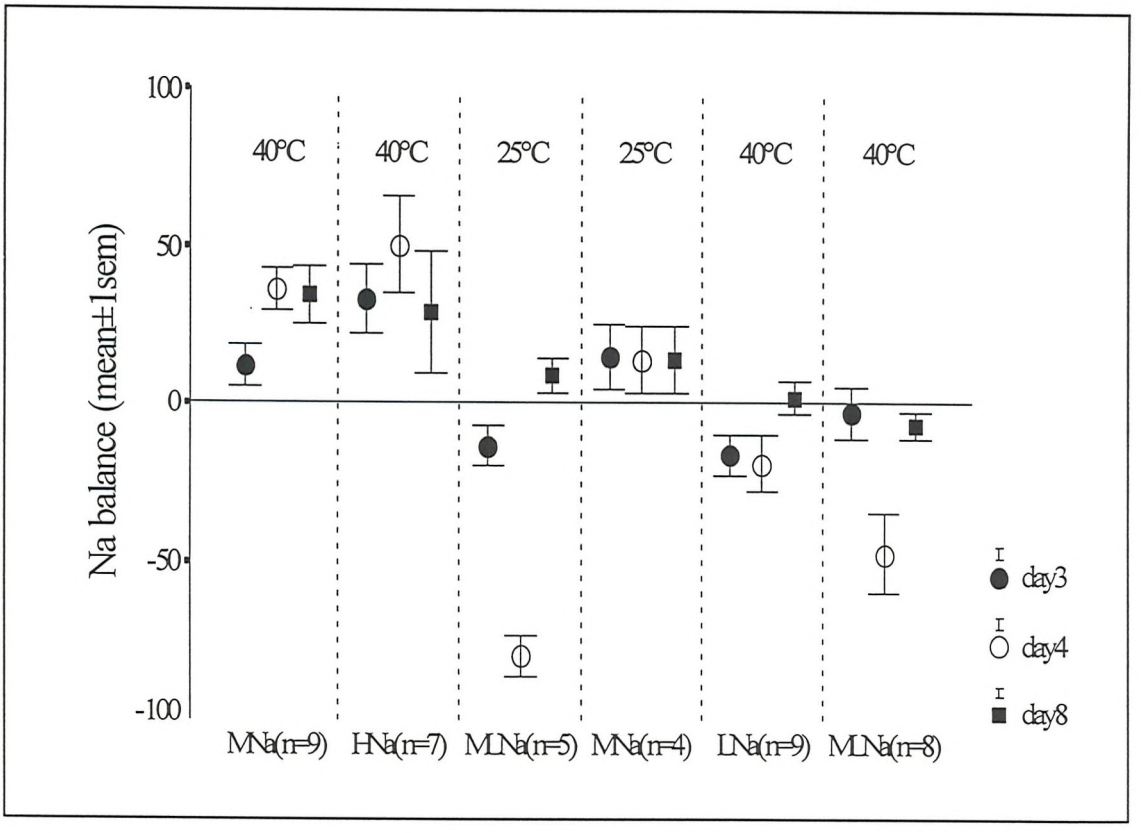
Sodium and fluid balance.

Sodium balance.

In a hot climate, prior reduction of sodium intake (LNa) potentiated the aldosterone response such that sodium losses were attenuated. This was apparent from the reduced *total* sodium deficit of the LNa group compared with the MLNa group as shown in Figure 8.3. A significantly lower reabsorption in the restricted (MLNa) compared to the prior low (LNa) diet was only revealed when urinary and sweat losses were combined to yield net total sodium balance (Figure 7.4). Analysis of this variable indicated that the pattern of sodium deficit during the heat sojourn differed between these two conditions; the total sodium deficit of the MLNa was greater on the initial day of heat exposure compared to that of the LNa group. These differences are shown in Figure 9.5 which presents the net sodium balance for all the conditions. It is interesting to note in this figure that the largest sodium deficits were experienced by the restricted groups (MLNa) on day four (the first day of this dietary restriction)

and that this deficit was greater in the temperate environment compared to heat exposure. A possible explanation for this disparity in sodium balance whilst ingesting an identical dietary intake, is that renal reabsorption of sodium was stimulated by a potentiated secretion of aldosterone in the heat, whereas in the temperate environment reduced dietary sodium intake was the only stimulus.

Figure 9.5. Mean net total body sodium balance of each of the groups on days 3, 4 (first day of heat exposure) and 8 (last day of heat exposure).



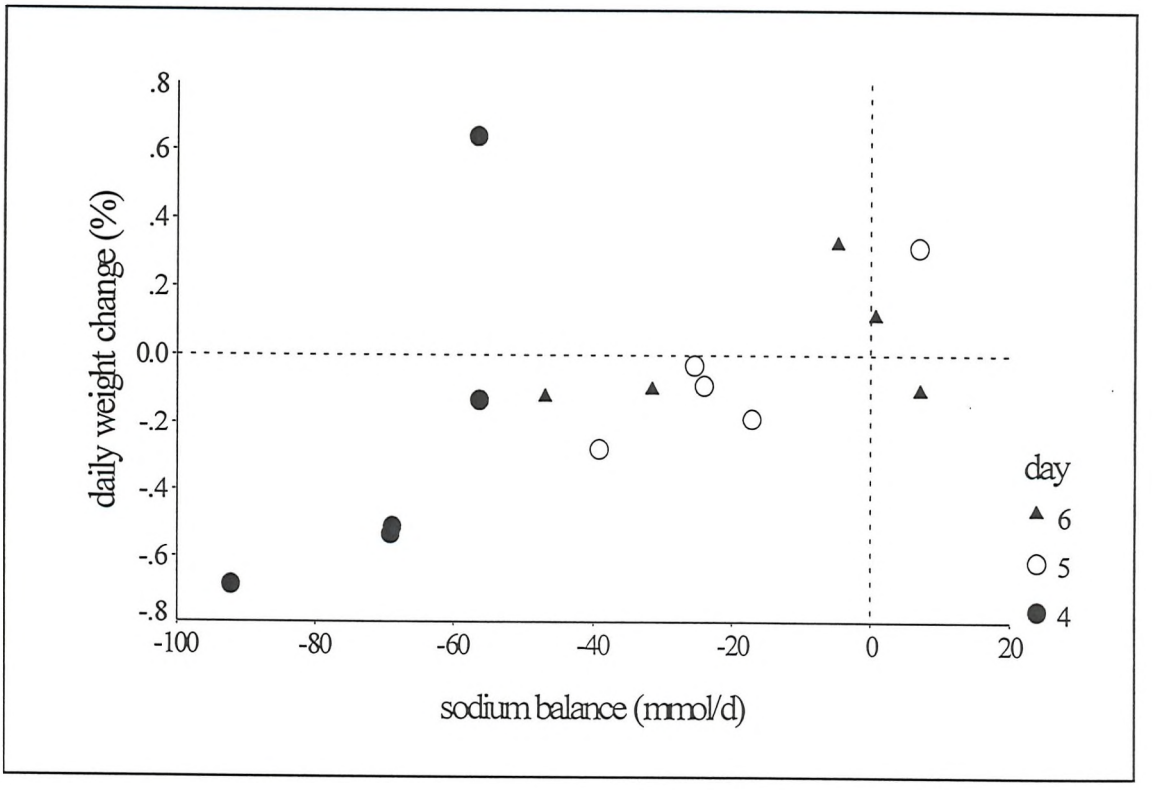
Note that the temperatures indicated are for all three days in the case of the temperate exposure conditions (25°) and for days 4 and 8 only in the hot exposures.

Fluid balance.

The reduction in bodyweight experienced by the restricted (MLNa) group in Chapter 5 (Figure 5.5) occurred within 24 hours of commencing the restricted diet. The fall in urinary sodium excretion commenced within the same 24 hour period, and reached its lowest level within 72 hours. The cumulative loss of urinary sodium during this time was a mean of 103 mmol of sodium whereas the MNa group were in a net positive balance over the same period by an average of 87 mmol of sodium. A sodium deficit of 103 mmol is equivalent to 700 mls of extracellular fluid or approximately one percent of bodyweight. The median reduction in bodyweight over the first two days of heat exposure was 0.5% of weight on day 3 (see Figure 5.5), or from a mean bodyweight of 78.97 kg (on day 3), approximately 395 mls of fluid. The percentage body weight data Chapter 5, together with the calculated sodium balance (intake minus urinary loss) is presented in Figure 9.6. This figure shows the change in sodium deficit over this initial period of dietary restriction (days 4, 5 and 6) for these subjects (n=5) in relation to the day-to-day change in bodyweight. A trend of sodium balance and change in bodyweight is apparent with both variables becoming less negative with time, the respective deficits being corrected within 48 hours in three of the five subjects. These trends in sodium deficit and weight loss are likely to be dependent; fluid being lost from the extracellular space or plasma volume in order to maintain osmolality, as reported by McCance (1936).

The limitations of this method of estimation of fluid balance were indicated in Chapter 5. Errors in the estimation of faecal, insensible water and metabolic weight losses, together with the assumption of a steady-state upon which this estimation rests, severely limit the validity of this method of calculation of fluid balance and hence also estimated sweat loss from fluid balance.

Figure 9.6. Daily sodium balance of the restricted sodium group versus daily change in percentage body weight.



Note the progressive increase in sodium balance (i.e. becoming less negative) from day-to-day appeared to be correlated with an improvement (i.e. smaller reduction) in body weight.

Effects of dietary sodium restriction upon plasma volume, thermoregulation and consequent heat acclimation.

Expansion of plasma volume.

Senay et al (1976) suggested that plasma expansion was the most critical adaptation in heat acclimatisation. Previously plasma expansion has been attributed either to sodium and water retention, (Wyndham et al, 1968) or to protein movement from the interstitial space to the vasculature (Senay, 1979). More recently, Nielsen et al (1993) concluded that the mechanism for the increased plasma compartment may be an isosmotic volume expansion caused by an influx of protein to the vascular compartment, *and* a sodium retention induced by a significant increase in aldosterone.

In the present study, during heat exposure the sodium restricted (MLNa) group of subjects incurred a slight reduction in plasma volume whereas all the other groups showed a small increase. Subsequent haematocrit and haemoglobin changes indicated that plasma volume expansion later occurred to recover this initial drop in the MLNa subjects, to a degree which was similar to that observed in the LNa and MNa groups. Furthermore, the restricted subjects sustained the greatest reduction of bodyweight, indicating that this plasma expansion must have occurred by a greater transduction of fluid from the intracellular space, rather than from additional total body water.

The above changes of bodyweight and plasma volume indicated a reduction of total body water and expansion of the ECF volume at the expense of the intracellular space. The same restricted diet in the temperate climate, however, produced a reduction of bodyweight (assumed to be total body water) within 48 hours, which was matched by a reduced plasma volume (albeit only by within-group statistical analysis). Thus, with no environmental heat load, plasma volume appears to contract in order to maintain plasma osmolality. This suggests that volume homeostasis is secondary to maintenance of plasma osmolality until such point that the effective circulating volume is critically compromised. It would also follow, therefore, that

thermoregulation in the heat is one such critical factor, since the extracellular compartment space was maintained or expanded during heat acclimation.

Thermoregulation.

In the heat exposure experiment, the MLNa group incurred the smallest reduction in aural and mean skin temperatures over repeated exposures, indicative of a reduced ability to dissipate heat, or a greater heat production in these subjects. As the environmental conditions were identical for all heat exposures and the metabolic heat production was approximately the same, this latter explanation is unlikely. Thus it may be assumed that the heat loss (by radiation, conduction, convection and evaporation) of the subjects was impaired.

In this environment whereby the dry bulb temperature exceeded that of the skin, the thermal gradient for heat transfer by radiation, convection and conduction was reversed such that the individual gained heat from the surroundings. Thus the only operative route for heat loss was via sweat evaporation. In addition to the lower onset and increased sensitivity of the sweating response following acclimatisation, it has also been demonstrated that peripheral bloodflow is also higher, for a given core temperature (Fox et al, 1963). Both of these responses increase heat flux and thus reduce the thermoregulatory stress upon the acclimatised individual in the heat. When dehydrated, sweat production (Pearcy et al, 1956; Sawka et al, 1985; Sawka et al, 1992) and peripheral circulation (Nadel et al, 1980) are known to be attenuated. These two factors are clearly connected by the total body water available, and it is this factor which is critical to the maintenance of thermal conductance and evaporative heat loss.

The interdependence of plasma volume expansion and thermoregulation during acclimation in the present study.

In the context of the present heat exposure experiments, it is hypothesised that firstly the MLNa incurred a sodium deficit, and a concomitant diuresis and / or reduced fluid intake to maintain plasma osmolality at the expense of a reduced extracellular fluid volume. Following this adaptive reduction, evaporative heat loss was reduced, and to a lesser extent thermal conductance (during the transition period to the cooler evening and overnight hours). The critical period during heat acclimatisation is the initial days of exposure before any expansion of plasma volume can occur. For the extracellular fluid volume to be further compromised by a natriuretic/diuretic drive at this time could be critical to thermoregulation in the heat.

It is of interest that the MLNa group appeared to experience a similar increase in plasma volume after the initial 24 to 48 hours of heat exposure (Figure 8.6). After this initial reduction, the expansion appears to occur at a faster rate, to achieve a similar level by day eight in comparison with the MNa and LNa groups. This suggests that the transfer of interstitial protein (Senay 1975; Senay et al, 1976) and water to the vascular volume associated with the plasma expansion was altered by the subjects' sodium and fluid balance state. One possible explanation for this may involve AVP in a non-renal response to influence the movement of fluid in and out of the vascular compartment. Thus in the present experiment, hypohydration may have stimulated AVP release (although no increase plasma sodium concentration was detected) which then augmented the translocation of protein to the intravascular space. This modulatory effect of AVP is supported by Khokhar et al (1976) who demonstrated an increase of plasma volume as a result of AVP infusion at a concentration below that thought to have a direct pressor action.

The putative direct action of plasma osmolality upon thermoregulation.

An alternative or parallel mechanism for the modification of thermal responses to heat is that of hyperosmolality (Fortney et al, 1984; Harrison et al, 1978). According to these authors plasma osmolarity *per se* can have a direct effect on the rate of rise of core temperature, in the absence of hypovolaemia. Fortney et al (1984) reported that when dehydrated subjects were infused with saline to restore plasma volume, core temperature during a subsequent heat tolerance test was significantly higher than during a control test. These authors attributed this to an increase in the thresholds for vasodilation and sweating. Presumably this modified response was effected either by a direct neural action of the osmoreceptors to the hypothalamus, or an indirect effect of AVP at this site of temperature regulation. In a later paper by these authors (Fortney et al, 1988), however, rather less emphasis was placed upon this putative response when the authors were unable to counter the increase in osmolality produced by moderate exercise. Furthermore infusion of 3% saline, as used the earlier trials, did not significantly modify sweating or core temperature responses.

In summary, in the present experiments the most likely reason for the "impaired" thermoregulatory responses seen in the MLNa group was a reduction in plasma volume. Although there was no evidence of differential rate of sweat loss over this period, this is the most logical cause of the reduced heat dissipation. A reduced peripheral blood flow due to this reduction in blood volume may have occurred, but in this environment (40°C) would have had no effect on heat loss during the daytime. Sweat rate may have also altered in response to changes in plasma osmolality, although changes in plasma sodium were not evident, perhaps as this was measured before rising and not at the time of the heat tolerance test. Further work is required to clarify the mechanism whereby the hypothalamus senses alterations in body fluid compartments.

Heat illness.

Other studies of salt requirements in the heat have reported heat exhaustion, cardiovascular intolerance and impaired thermoregulation. In comparison with the "low" (70 mmol/d) sodium diet in the present study, Taylor et al (1943) investigated ten subjects on a 104 ± 35 mmol/d sodium diet, with a control (pre-heat exposure) period lasting two days and a heat exposure of three and a half days. Exercise (two 60 minute periods of intermittent moderate intensity treadmill exercise) was performed each day. Subjects on this sodium intake became dehydrated (2.5% of bodyweight) despite ad-libitum drinking water, and incurred an estimated sodium deficit of 146 mmol in the first 24h period. Note however, that these authors assumed all weight loss to be attributed to the extracellular space and then used the normal plasma sodium concentration to calculate sweat sodium loss on this basis.

Streeten et al (1960) examined four subjects on a diet which excluded virtually all sodium (35 mmol/d) during heat exposure (32°C to 38°C for seven hours a day for between 15 and 23 days). Sodium balance in two subjects was restored within two days of heat exposure and they suffered no symptoms of heat illness other than a single muscle cramp. The other two subjects suffered severe cramp, nausea and vomiting and were weak and exhausted within seven days. These authors cite the responsiveness of the sweat glands (i.e. heat acclimatisation status) as the critical factor differentiating these two groups in determining man's ability to avoid heat illness. This implies that it is the sodium balance of the individual that will determine the appearance or otherwise of heat illness symptoms. Thus one explanation for the absence of any exercise impairment in the recent study of Hargreaves et al (1989) was that this is due to adaptation of the sweat glands, following adherence to a low (50 mmol/d) sodium diet for two weeks prior to the study. This conclusion supports the above argument for sodium reduction rather than supplementation.

None of the subjects in this present study suffered symptoms of heat illness which could be ascribed to salt deficiency. This is in agreement with the findings of

Armstrong et al, (1993), who fed a diet of 137 mmol/d of sodium prior to heat exposure and then reduced this to 68 mmol/d for half of their subjects on exposure to heat. These subjects were forcibly rehydrated with a volume of water equivalent to their sweat loss and urine each hour during the heat phase. Despite the forced rehydration of the subjects in this study, significant weight loss was incurred in the restricted sodium group 48 hours after heat exposure commenced, a similar latency period and amount (1-1.5%) as in the present study. Interestingly, Armstrong et al also reported a significant increase in plasma sodium for their restricted sodium subjects from 137 mmol/L to 140 mmol/L during heat exposure, and a smaller increase in plasma volume compared to the non-restricted control group. There were no differences in sweat production.

There are several important similarities between the study of Armstrong et al (1993) with the present thesis despite the different methodologies used. Firstly, the expansion of plasma volume attributable to heat acclimation appeared to be delayed by sodium restriction in the heat (although not by prior sodium reduction). Secondly, sodium restriction in the heat caused a significant reduction in body weight after a short latency period, indicative of negative fluid balance. Thirdly, both studies have shown that combined sodium restriction and heat exposure potentiated aldosterone secretion for several days.

Thus it would appear that sodium restriction in the heat attenuates the cardiovascular responses to repeated heat stress (i.e. plasma volume expansion), and this may, under certain circumstances, lead to impaired heat acclimation. This present study found significant differences between the dietary sodium groups with respect to measures of body temperature during exercise, but only if the restricted subjects (n=8) were compared to all the other heat exposure conditions (n=25). This must have been in response to the acute reduction in sodium in the MLNa subjects rather than the low sodium intake per se, since the LNa were not similarly affected. It may be argued that this "protection" was endowed by effects of earlier and increased aldosterone secretion, acting to conserve sodium, so permitting plasma expansion. The above

sequence of events supports the hypothesis that the expansion of plasma volume is dependent upon the availability of sodium in the extracellular fluid space.

In summary, these data support the concept of change in plasma volume as being the critical factor in heat acclimatisation and subsequent thermoregulation. Accordingly, restriction of dietary sodium during heat exposure will negate the expansion of plasma volume and impair thermoregulation, but not necessarily to the point of heat illness. In contrast, restriction of sodium prior to the heat exposure will potentiate aldosterone, which appears to facilitate the isosmotic expansion of plasma volume upon which the heat acclimation process is dependent.

Changes in heat acclimation attributable to manipulation of sodium: salt supplements or a prior reduction of dietary sodium intake ?

It has been hypothesised previously in this thesis that supplements may reduce the effectiveness of sweat sodium conservation during heat acclimatisation as suggested by several groups (McCance, 1938; Weiner & Van Heyningen, 1952; Robinson et al, 1950). Thus an individual dependent upon salt supplements may not acclimatise fully, or so rapidly as one who has experienced a salt deficit. This may have important consequences if intake of sodium via salt supplements or food is suddenly prevented. In contrast, it has also been hypothesised that reduced dietary intake of sodium will potentiate the aldosterone mediated response of sweat sodium conservation, and therefore augment the heat acclimation process.

Supplementation.

The advice to supplement dietary sodium intake arises from the assumption that excretory losses are significantly increased on heat exposure; an assumption that will depend upon the environmental heat stress, metabolic work rate, and the pre-existing sodium status of the individual. It would appear from the present study that at low work rates, sodium losses were not so high as to induce symptoms of heat illness.

Thus, the advice not to supplement unacclimatised men (c. below) appears correct under the conditions of this present study (light activity in a hot/dry environment).

From the percentage change in plasma volume (Figure 8.6) it would appear that the overall increase of the HNa group was lower (approximately 4% on day 8) compared to the other dietary conditions, including the restricted (MLNa) group (approximately 7-8% on day 8). Indeed, the HNa appeared to suffer a slight reduction in this measure on day 6, suggestive of an impaired fluid balance. Excessive intake of sodium can reduce thermal tolerance if not accompanied by an increased fluid intake (Dasler et al, 1973; Wyndham et al, 1973). This HNa group also demonstrated the second smallest reduction in aural and mean skin temperature, second only to the MLNa group as discussed above. These results support the hypothesis of a high plasma sodium concentration adversely modifying thermoregulation. The earlier study by Taylor et al (1943) however, investigated the cardiovascular and temperature adjustments to heat exposure, in an attempt to characterise heat acclimatisation and identify those individuals who may be susceptible to heat illness. These authors commented that doubling salt intake (from an average of 260 mmol/d of sodium in their experiments) did not influence the response or rate of acclimatisation to heat.

Adverse effects of supplementation upon fluid balance

Leithead and Lind (1964) gave general advice which concurred with this view of supplementation, on the assumption that usual sodium intake is generally adequate to offset any losses through sweating. Additionally they stated that no extra salt should be given when water is in short supply, which acknowledges that the minimum water requirement is increased if all of the additional salt load is to be excreted. Thus although the kidney has a remarkable capacity to concentrate the urine, this is a finite response. Hence in exceptional circumstances of inadequate water replacement, obligatory urinary output may compromise hydration.

According to Ladell et al (1944) some British soldiers in Libya became polyuric after

prolonged heat exposure with urinary volumes exceeding 10 litres per day. Knochel and Reed (1987) have suggested that the high total sodium intake (estimated at 350 mmol/d) of Servicemen taking salt supplements and ingesting an estimated 146 g of protein daily caused a solute challenge; they considered that the estimated total solute load (in excess of 1500 mosm) would have disturbed the thirst mechanism and so explain these symptoms of polyuria.

Effect of sodium supplementation upon body potassium.

A further possible negative effect of supplementation has been reported by Dasler et al (1973), who indicated that excessive salt intake increased the excretion of potassium, calcium and phosphate. The subjects in which these biochemical changes were elicited also demonstrated a reduced work capacity, which was ascribed to a cardiovascular impairment. Symptoms of weakness, fatigue and lethargy have been reported and ascribed to hypokalaemia elsewhere (Knochel and Vertel, 1967). These authors postulated that potassium depletion occurs via two routes: sweating, since although sweat volume increases potassium contraction remains unaltered; and renal wasting, in response to a greater sodium potassium exchange.

The hypothesis of Dasler et al (1973), above, ignores the suppression of aldosterone secretion by salt loading, as evidenced by the HNa condition in this present study, who experienced no marked changes in plasma potassium nor urinary potassium excretion. Thus, any reported symptoms relating to potassium depletion may not necessarily be a direct result of salt loading and require further investigation.

Sodium restriction.

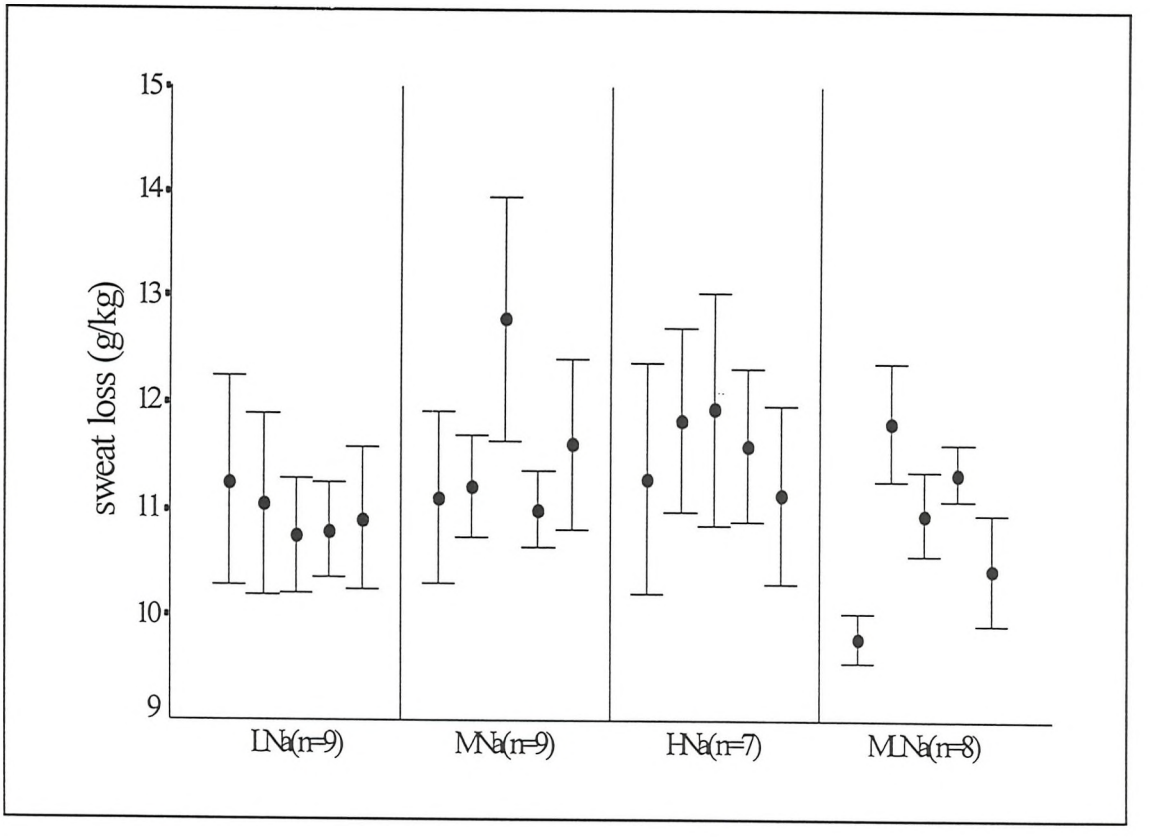
Collins (1966) suggested that increases in aldosterone under conditions of negative sodium balance may conceivably retard the development of heat acclimation. This followed observations that hypodermic administration of methacholine (to induce non-thermogenic sweating), with aldosterone, was associated with a significant

reduction in urine flow; whereas spirolactone (an aldosterone antagonist), caused a significant diuresis. A comparable reduction in sweat rate was also observed on the day of aldosterone administration and the following day. Collins suggested that the urinary flow reduction was partly due to an increased secretion of AVP in response to renal conservation of sodium. For a similar mechanism to apply to the sweat glands, however, would necessitate a direct action of AVP on sweat gland activity. The work of Taylor et al (1943) could be interpreted as providing indirect evidence for such an action. This group observed that subjects on a low sodium diet during heat acclimation secreted the same volume of sweat as those on a moderate intake on the first day of heat exposure, but on the second and third days sweat volume in the restricted group was 11% and 6% smaller.

In the present study, there were no significant differences between the groups with respect to sweat secretion (as estimated from the daily fluid balance), nor was there any significant effect upon urinary output. Further examination of the sweat secretion during exercise (g per kg of body weight) in the heat did not yield any significant differences between dietary groups, although the sweat secretion for the MLNa group on day 4 was the lowest of all the groups (Figure 9.7). Thus the present study would not support this hypothesis. A simpler explanation for the changes in urine and sweat production reported by Collins (1966) may rest in the experimental conditions since neither fluid nor sodium intake were controlled.

In summary, with respect to sweat secretion and the retention of sodium by the sweat glands, it would appear that the balance of the evidence supports reducing the level of sodium intake prior to heat exposure, in preference to giving supplements. The effect of these alternative strategies upon subsequent disposition to heat illness is discussed in the following section.

Figure 9.7. The mean (±1sem) sweat loss (g/kg) during the one-hour exercise period of each of the four groups during the heat exposure days (day 4 to day 8).



Fluid intake.

It has been suggested that water intake rather than sodium is the key to effective thermoregulation and acclimation (Noakes et al, 1988; Sawka, 1992). Thus if additional sodium is required then this could be administered by the use of beverages containing sodium and possibly other electrolytes. This strategy would have the advantage of ensuring more complete rehydration, in circumstances of acute dehydration (Nose et al, 1988). High concentrations of sodium reduce palatability, hence sodium concentrations in the rehydration beverage formulations are approximately 25 mmol/L (Maughan, 1991). If beverages were the only source of sodium then approximately seven litres of fluid would be required to maintain the sodium balance of an individual.

The rate of rehydration is likely to be dependent upon the electrolyte composition of the beverage as it affects intestinal absorption. Additional sodium has been variously reported to increase fluid absorption (Davis et al, 1987), or to have no significant effect upon the gastric emptying of electrolyte-carbohydrate beverages (Rehrer, 1990; Rehrer et al, 1993). These disparate results may be explained by the confounding effects of additional carbohydrate within the beverage. Availability of this ingested fluid as it relates to extracellular fluid volume is likely to be critical in terms of performance during the period of heat exposure, particularly if exercising.

As noted above, beneficial effects of additional sodium within the rehydration beverage have been reported (Nose et al, 1988; Nielsen et al, 1986; Maughan & Leiper, 1995). This greater volume is attributable to a restoration of plasma osmolality at normovolaemia with additional sodium, whereas plasma concentration is restored at a reduced plasma volume with water only, due to an increased diuresis. Thus it is advisable to add sodium to drinks when rapid rehydration over a short period of time is necessary. It must be remembered, however, that the data supporting the positive benefits of electrolyte beverages relates to circumstances of acute exercise in the heat, typically lasting two or three hours. The conclusions from

these acute studies cannot necessarily be applied to longer periods of heat acclimatisation over several days when ingesting a normal sodium intake from food. Hence the need for longer studies such as the present investigations which examine the adaptive responses to chronic heat exposure under circumstances of controlled dietary sodium intake. The data in this present study suggests that during longer periods of heat exposure where sodium intake is maintained in the diet, any additional sodium in excess of requirements would be excreted. Thus the comparable effectiveness of electrolyte versus non-electrolyte replenishment strategies to replace sweat losses in any twenty-four hour period is therefore likely to be similar.

Conclusions and implications of results.

Conclusions.

In summary, with regard to the decision to supplement dietary sodium during heat exposure or to restrict it prior to the event, the following conclusions can be stated:

a. The response of increased aldosterone is effected by several mechanisms, primarily angiotensin II through the indirect effects of exercise and heat exposure, but also potassium, ACTH, and sodium. Manipulation of sodium intake *per se* may be sufficient to stimulate increased aldosterone secretion, but this effect is potentiated by the combined stressors of sodium depletion and heat.

b. The sodium retaining ability of the sweat glands is affected by aldosterone directly, (as indicated by injection with methacholine for example), but it would appear that this is of little consequence in practice. The present study found that when the skin is repeatedly exposed during heat stress, sodium retention by the sweat glands was similarly elicited in subjects irrespective of their sodium balance status.

c. Total sodium losses were reduced during the initial days of heat exposure in subjects who have a low sodium (LNa) intake prior to heat exposure.

d. No dysfunction was detected in any of the subjects. Thermoregulatory and plasma volume measures in this study suggest, however, that the plasma volume expansion associated with heat acclimatisation is critically affected by sodium intake. Thus those subjects in which sodium intake was restricted upon heat exposure (MLNa) exhibited thermoregulatory responses indicative of a compromised extracellular fluid volume.

Implications.

The implications of the above are clear with respect to sodium restriction; this must not occur if thermoregulation is to be optimally maintained. They are less clear with regard to whether a strategy of salt supplementation or prior restriction should be enforced. Adoption of one strategy or another may be dependent upon other circumstances and therefore several recommendations are proposed depending upon the likely level of heat stress, physical activity and availability of fluids and electrolytes.

From this present study it would appear that if the average sodium intake of the general population was reduced to the level advised by COMA (1991), then this would not result in any impairment of heat acclimatisation; rather the individual would simply adapt to this lower level. For the tourist, where a long duration of physical activity is unlikely, a period of reduced sodium intake prior to heat exposure would be unlikely to have any adverse effects. Indeed, the potentiated aldosterone response such a strategy would initiate could assist in the heat acclimatisation process.

If the environmental heat load or physical activity level is higher than that simulated in these experiments, as is possible for the military, then it is likely that personnel would become sodium depleted in the initial days of heat acclimatisation and that

supplements would therefore be necessary if personnel show symptoms of salt depleted heat illness. Supplementation prior to heat exposure, however, would not be advised since this would attenuate aldosterone secretion, and may cause detrimental effects. Furthermore, the risk of sodium depletion following supplement intake, as might occur in Military Operations, is an even greater hazard than if personnel are not given supplements. For the Services, therefore, the advice should be a low sodium (70 mmol/d) diet would present no hazard to heat acclimatisation, although salt supplements may be necessary at higher work intensities in the heat, if and when symptoms of heat illness become apparent.

In the case of the athlete, acclimatised to the heat through endurance training, then sodium losses are unlikely to exceed intake over the course of a day because of improved sodium retention by the sweat glands. Additional sodium in rehydration beverages is required if this is the only source of sodium during the event (i.e. no meals or snacks are taken), since this is likely to speed the rate of intestinal absorption and ensure a fuller restoration of plasma volume than if water only is drunk. Athletes should not risk incurring a sodium deficit by suddenly reducing their dietary intake of sodium as might happen through loss of appetite prior to competition. If, however, sodium intake is lowered gradually from a level typical of a UK adult to the Dietary Reference Value, then there is no reason why sodium balance should not be maintained through enhanced reabsorption.

The above recommendations are a logical extension of the hypothesis that if whole body sodium balance is perturbed, then adaptive responses will be initiated through the action of aldosterone to restore that balance. This will presumably occur in all aldosterone sensitive organs, not just the renal tubules and sweat glands but also the salivary glands and colon. In accordance with this "adaptive reduction" point of view, the subsequent effects of this altered sodium status upon hydration and thermoregulation need to be recognised and evaluated. Hence the requirement for a whole-body systems approach to this investigation.

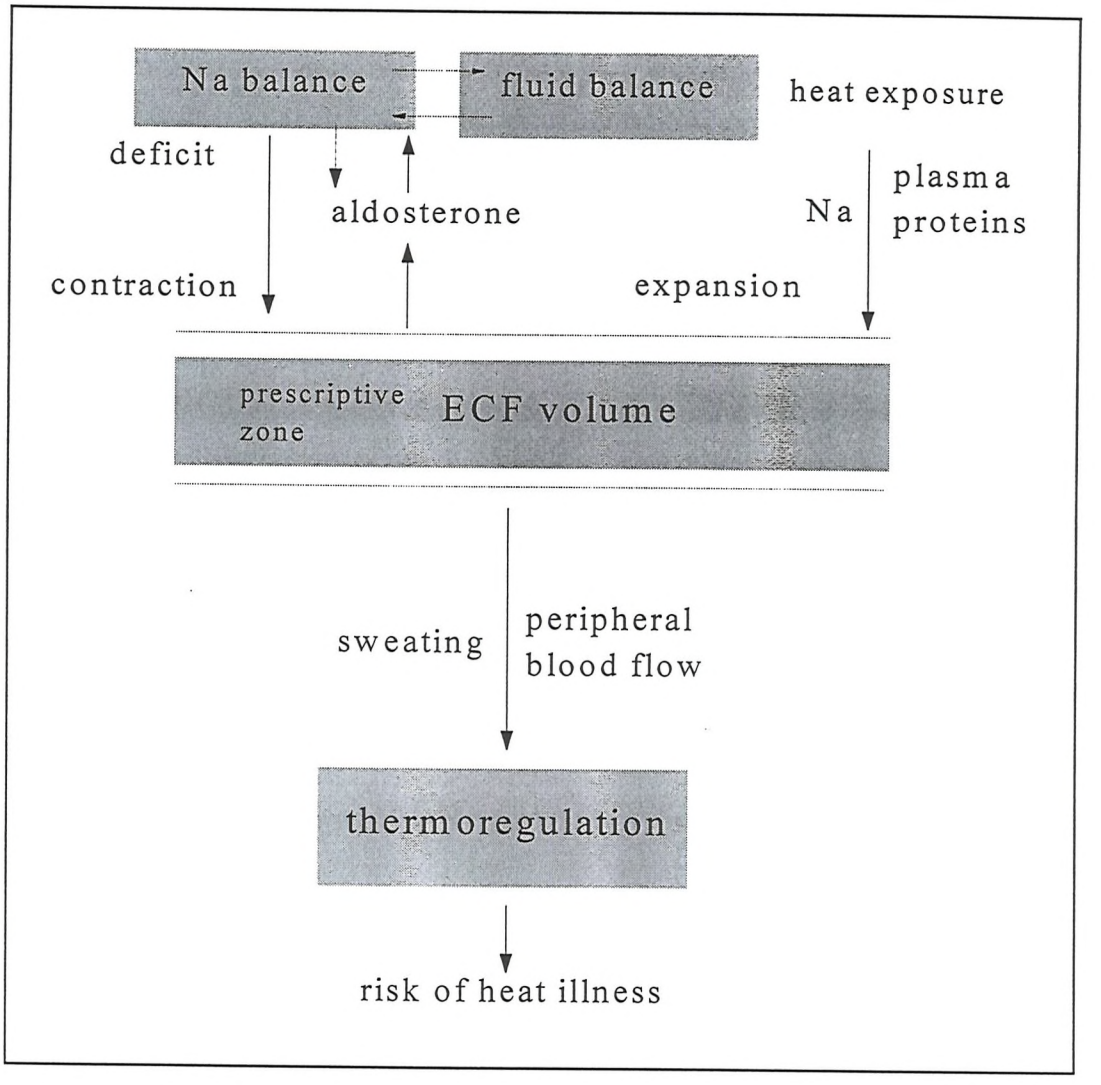
Revision of the working hypothesis.

In the context of the overall model (Figure 1.2) described at the introduction of this thesis it would appear that in response to a reduced intake of dietary sodium two effects occur: an increased aldosterone secretion and a reduced extracellular fluid volume to maintain plasma osmolality. This second response may be considered a reductive adaptation; if it occurs during heat stress, when there is a further opposing stimulus to expand plasma volume, then this latter response is compromised and may then adversely effect thermoregulation by impaired heat dissipation. At this stage, the increased secretion of aldosterone and subsequent effect of this hormone to conserve sodium appears to be a critical over-riding factor in this thermoregulatory process. Thus the response (alterations of plasma volume) which is elicited from a combination of opposing stressors (sodium deficit versus sodium retention and / or influx of plasma proteins) may ultimately depend upon the mediating influence of aldosterone secretion.

This revised model is schematised in Figure 9.8 in which ECF volume is indicated to have a central role. The ECF volume is regulated in response to alterations in sodium and fluid balance and the changes in intravascular plasma protein or sodium flux initiated by repeated heat exposure. Depending upon the balance of these separate stimuli, ECF volume can be expanded or reduced. When expanded, as in heat acclimatisation, peripheral blood flow and sweating are potentiated such that thermoregulation is achieved at a lower body temperature. When the ECF volume is reduced however, these responses are attenuated and thermoregulation is impaired, increasing the likelihood of heat illness.

Arguably, each of the key components to this model, (sodium balance, fluid balance, ECF volume and thermoregulation) are inter-dependent homeostatic mechanisms which operate within a prescriptive zone. Beyond this prescriptive zone, a stimulus results in a reductive adaptation, and in extreme circumstances, profound dysfunction.

Figure 9.8. Revised model for the working hypothesis and a putative mechanism of sodium balance upon aldosterone secretion.



The extracellular fluid (ECF) volume is regulated (by osmoregulation) within a prescriptive zone. This zone is extended to the zone of accommodation by plasma volume expansion during heat acclimatisation, but reduced by a sodium deficit. The balance of these two stressors governs the ECF volume and subsequently modulates thermoregulation. When the ECF volume is compromised, impairing thermoregulation, heat illness may result.

Experimental critique and future investigations.

This study has been conducted in a controlled environment whereby the ambient temperature and vapour pressure has been reproduced for all the heat exposures, and the temperate condition experiment. Thus a lengthy heat sojourn was the main stimulus for heat acclimatisation. This has avoided the confounding effects of exercise training which is known to initiate similar adaptive responses. Indeed much of the confusion regarding the endocrine responses to heat acclimation has arisen due to the use of prolonged bouts of physical activity. In contrast, this study has used the minimum amount of physical activity to avoid any reduction in basal metabolism that may arise from inactivity.

An alternative approach to heat acclimation has been to utilise immersion in warm water. This however, is not without drawbacks; immersion is known to cause a diuretic and natriuretic response following increased venous return, stimulated by the hydrostatic pressure upon the body during immersion (Epstein, 1992). One criticism of the present study however, is that whilst the environmental heat stress was constant, the degree of hyperthermia was reduced as heat acclimation progressed, making it difficult to measure and evaluate altered sweat sensitivity.

The three day control phase appeared to be sufficient to ensure that subjects were "in-balance" prior to heat exposure, as indicated by the separation of the dietary condition at the end of this period (Figure 6.1 and Figure 7.2). This outcome could be predicted from previous work (Armstrong et al, 1987; Taylor et al, 1943; Collins et al, 1971; Robinson et al, 1950) and the theoretical calculation (Simpson, 1988). The subjects were also shown to be in energy balance (Figure 8.1), thus the regression equations for BMR from Schofield (1985) and the physical activity ratio of 1.4 appear to have been appropriate for the estimation of energy expenditure in this instance.

Sweat sodium secretion was estimated by the technique of whole-body washdown. This has advantages overall the local sweat collection methods of using capsules.

Previous studies such as that by Taylor et al (1943) have utilised sweat collection "gauntlets." These and similar sweat bag devices can only give a result for regional sweat loss. More importantly, this figure is likely to be affected by the local environmental conditions within the collection bag (increased ambient vapour pressure and temperature) which will initially increase sweat production as temperature rises, but then cause suppression of sweating as the sweat remains unevaporated on the surface of the skin leading to hydromeiosis (Brebner & Kerslake, 1963; Kerslake, 1972). These disadvantages are overcome by the use of whole-body washdown technique.

In these investigations the estimation of sweat concentration as opposed to twelve hour sweat sodium secretion, has not been assessed. This was due to the difficulties encountered regarding the measurement of sweat production, estimated from weight loss (corrected for all other sources of weight loss and gain including metabolic and respiratory losses) over the 12h washdown period. This would have been easier if the 12h period had not included two mealtimes, and if it had been synchronised with the collection of urine or faeces. One possibility for future trials is to reduce the period of the collection to four or six hours. In this present protocol this change of period for sweat production would have limited the duration of the heat exposure on that day or necessitated additional days for sweat collection. Capsules could only be considered if: there was a suitable regression between collection sites and whole body sweating; and if the air space within the capsule could remain unsaturated (i.e. by use of the ventilated capsule technique).

An alternative experimental design which avoids some of these problems can be suggested. A suitable protocol would divorce the sweat collection (pre- and post heat exposure) from the heat exposure period, and utilise an intermittent moderate intensity exercise task in the heat, sufficient to maintain constant hyperthermy and sweat production for three or four hours. Sweat collection by whole body washdown could then be conducted over shorter periods (4 hours) as described. One further advantage of this design is that a similar sweat-test collection period could be introduced prior to

the first day of the dietary control phase, enabling pre-existing sweat sodium concentration and sweat production to be determined.

A further measure, that of total body water would have been a useful adjunct to plasma volume changes reported in this thesis, since this appears to have been a critical factor. Indeed, measurements of total body water were made by deuterium dilution. A good correlation was found between this measure and total body water estimated from skinfolds thickness prior to heat exposure. This is shown for 25 of the subjects who experienced heat exposures (groups LNa; MNa and HNa; Figure 9.9). When the dilution was repeated following the heat exposure, however, inconsistent changes were observed in a number of subjects (alterations in estimated TBW which were inconsistent with the observed changes in body weight). The reasons for this are unclear but may relate to an increased period for equilibration of deuterium following plasma volume expansion by heat acclimation. Certainly, Marken-Lichtenbelt et al (1994) recommend a period of 10 hours rather than the 8 hours used in these studies.

The accuracy of estimation of plasma sodium and sodium losses in this study was lower than anticipated, triplicate measures having a coefficient of variation of less than 2% for only 75% and 90% of plasma and urine samples respectively. To address this concern, 5% was subtracted from the balance data for the LNa and MLNa groups, and added to the MNa and HNa conditions (see Chapter 8, page 141) to minimise the differences between these groups. Following this procedure, ANOVA revealed similar differences ($P<0.01$) between the overall sodium balance (across both days) with respect to diet, with similar differences between the groups on day 4 as before (HNa higher ($P<0.05$) than either LNa or MLNa). However, there were no significant differences between the four groups on day 8. Thus allowance for possible measurement errors of this nature does not alter the conclusion that sodium balance was not maintained in the LNa and MLNa. Consideration of errors of this magnitude however, does weaken the earlier argument for the process of sodium retention being hastened when ingesting a low sodium intake.

Figure 9.9. Total body water calculated by deuterium dilution and lean body mass prior to heat exposure (n=25).

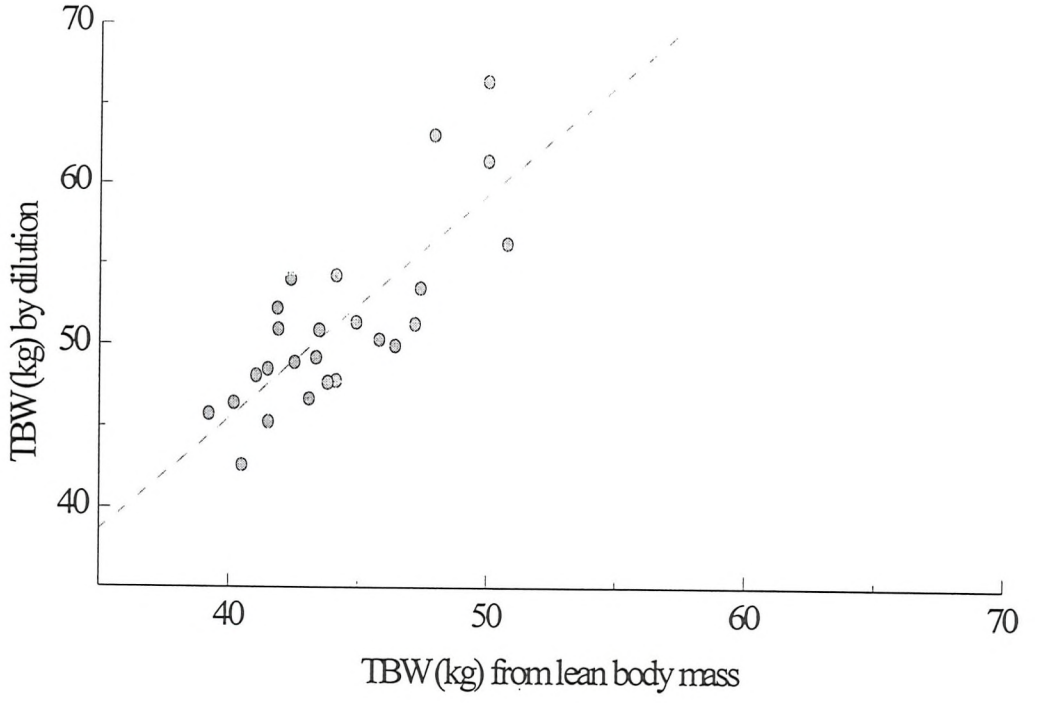
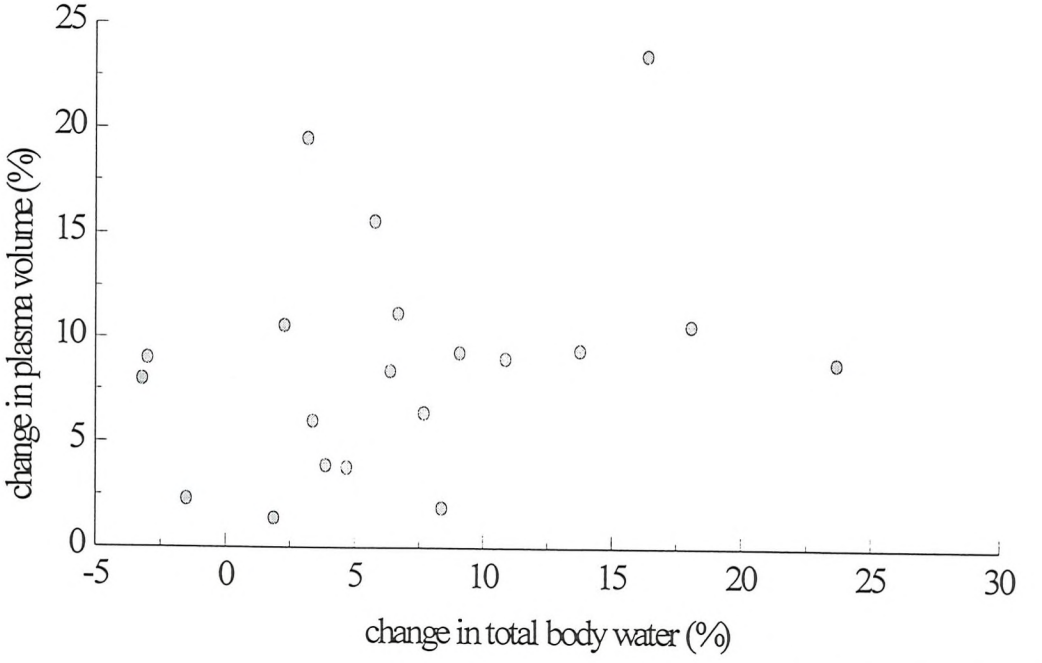


Figure 9.10. Change in plasma volume versus change in deuterium dilution space over the heat exposure period (days 4 to 8) n=20.



day 8 compared to day 4.

Future investigations.

A central issue in this thesis is the effect of sodium status upon the expansion of plasma volume during heat acclimation. The mechanism for this remains undetermined and requires further investigation. Of military relevance is the subsequent effects of impaired heat acclimatisation, through whatever cause, upon thermoregulation. It would therefore be of benefit to extend these experiments to higher work loads to cause a greater sodium depletion. The effects of additional sodium and other electrolytes when added to rehydration fluids is also of military importance, and in particular any effect upon the rate of rehydration. Of concern to the public health policy for reducing salt in the diet is the effect of a habitually low sodium intake. The specific question of interest being whether habitually low sodium intake causes a reduction in sweat sodium losses upon heat exposure and subsequent acclimation through an increased sensitivity to aldosterone. These experiments therefore need to be extended to address adaptation to longer-term dietary sodium manipulation.

Concluding remarks.

Claude Bernard first realised the central role of the "milieu interieur" in providing the correct internal environment for the body's physiological control systems to function. This thesis has attempted to extend that concept of homeostasis beyond electrolyte transport to the integration of whole body systems; specifically the interdependence of thermoregulation and volume homeostasis as determined by sodium balance through endocrine control. The concept of sodium homeostasis as the "milieu interior" can be viewed as a zone of stability which the stressor must exceed in order to stimulate an adaptive response. This study would suggest that the maintenance of this sodium "milieu" may define the outcome of such an adaptation.

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