UNIVERSITY OF ABERDEEN

Water requirements of malnourished children in extreme hot and dry environments.

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by

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DECLARATION

I declare that this thesis has been composed entirely by myself and it has not been accepted in any previous application for a degree. The work, of which it is a record, has been done by myself. Quotations have been distinguished by quotation marks, and sources of information have been specifically acknowledged.

Jacqueline Conduah Birt

Mother and child at Mao TFC, Tchad.

Abstract

In Sahelian countries such as Tchad, the temperatures in the dry hot season (April-June) consistently exceeds that of body temperature. In such conditions, heat is gained from the environment rather than lost. The only mechanism for heat loss is by evaporation of water. This causes an additional water requirement above that needed for renal excretion of solutes.

Children are particularly vulnerable to heat gain because of their relatively large surface area to weight ratio. Malnourished children in particular may be at a further disadvantage as most of their physiological functions are disturbed. This study examined whether malnourished children exposed to very high temperatures meet their water requirements on a diet of the special milk formula F100.

A field study was conducted that involved giving a dose of deuterium oxide (D_2O) as a tracer, collecting the subsequent urine and taking temperature data (skin, body and environment). The concentrations of D_2O in the urine collected over a 1 week period were used to calculate the total body water and the fractional rate of turnover.

Total body water at 71% of body weight was similar to other studies on malnourished children. However there was a high degree of daily fractional water turnover at 34% due to the high demands of evaporative water loss. Evaporative water loss was estimated using dietary intake and weight gain of the children (1.26L/day) and also by an energy balance model (1.58L/day). Although the average urine osmolalities were high (549mOsm/L), the average renal output suggests an ability to concentrate urine.

This study provides the first data to estimate water requirements of malnourished children exposed to environmental temperatures above their body temperature. The model used for calculating evaporative water loss can be used to calculate water requirements of children exposed to such conditions of heat stress in order to prevent dehydration and hypernatraemia.

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Abbreviations

ACF	Action Contre la Faim
CON	convective heat transfer from air
D_2O	deuterium oxide (heavy water)
ECF	extracellular fluid
EVAP	evaporative heat loss
EWL	evaporative water loss
F100	formula milk (100kcal/100ml)
F75	formula milk (75kcal/100ml)
GI	gastro-intestinal tract
ICF	intracellular fluid
IWL	insensible water loss
MET	metabolic heat production
MUAC	mid upper arm circumference
NGO	Non governmental organisation
RSL	renal solute load
RWL	respiratory water loss
SA	surface area of the body
sa:wt	surface area to weight ratio
TBW	total body water
% TBW/Bwt	% total body water /kg of body weight
TFC	therapeutic feeding centre
TWL	transepidermal water loss
W/H	weight for height

WI	water intake
WL	water loss
WR	water retained

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Chapter 1

Introduction

1.1 BACKGROUND TO STUDY

Human beings like most mammals have physiological adaptations which allow them to maintain internal homeostasis when external conditions dictate otherwise. They work within a narrow temperature range of 35 to 41°C (Open University, 1974). Hence it is not a surprise that many mechanisms interplay in order sustain body temperature. This occurs despite exposure to a wide range of environmental conditions some of which may be extremely hot or cold. In regions of extreme hot and dry conditions, if homeostasis is not maintained, excessive heat may lead to symptoms of heat stress and injury (nausea, tiredness, muscle cramps etc.) which when severe enough can cause death (Prentice *et al*, 1994). In temperate zones, the body normally looses metabolically produced heat to the environment in order to maintain heat balance. Under these circumstances, heat is lost by conduction, convection, radiation and evaporation. In Sahelian countries found sandwiched between the Sahara desert and tropical Africa, the temperatures in the dry hot season (April-June) ranges between 40°C and 50°C in the day with a relative humidity that can be <10%. Such temperatures exceed that of the skin and heat is actually gained by the body rather than lost. In order to dissipate the heat gained, evaporation of water as sweat from the skin is the only effective mechanism (Rowland, 1996). This places an additional requirement for water above that needed for renal excretion of solutes (Weil and Bailie, 1977). As a result this has to be included in the diet or as additional fluids. This study began as an observation that severely malnourished children in Tchad exposed to extreme hot and dry conditions suffered from hypernatraemia typified by very doughy skin and intense thirst due to rise in serum osmolality, a condition which when left untreated may lead to convulsions and brain damage (Freid and Palevsky, 1997). This field study therefore examined whether these malnourished children receive sufficient water in their milk diets to maintain water balance at these extreme conditions. To determine if extra renal losses were compromising their hydration status the study involved measuring total body water (TBW), daily water turnover, urine osmolalities and electrolyte concentrations of malnourished children in the Sahel.

1.2 BACKGROUND OF STUDY AREA

This study was carried out in Mao, Tchad (Figure 1.1) which is about 300km north of N'Djamena, the capital city. The children were all receiving treatment for malnutrition in a therapeutic feeding centre (TFC). Tchad is a landlocked country in the centre of Africa which is in the process of recovering from a civil war. It has one of the poorest levels of statistics in Africa that are used to judge countries against each other. Infant mortality in 1996 was 92 out of 1000 live births and adults have a life expectancy of 47 years (UNICEF, 1998). Malnutrition contributes to the mortality and morbidity of this country. The facilities available in the country for agriculture, education, health and infrastructure are inadequate to meet the needs of the population. It has a relatively small population of 6.5 million for its size. The country has 3 geographical zones, the Sahara desert in the north, the Sahelian belt in the middle and a tropical south. There are 14 administrative regions of which Mao is the capital town for the Kanem district. There are no industries and most of the local population make their living from trading in the markets, livestock or produce from their farms. Daily food typically consists of ground millet or maize cooked to form a solid ball and eaten with different sauces. Meat is expensive so the sauces are usually vegetable based. Most of the ingredients come from farms or are purchased at the local market. Almost all the population of Mao have access to a treated piped water supply from a pumped water tower (Picture 1) which they collect at various points throughout the town (Picture 2). Those in the rural surroundings however have to collect water from wells in the wadis or oasis.

Figure 1.1 Map of Tchad showing Mao (approximately 300km north of the capital city N'Djamena).

Picture 1 Water tower which supplies Mao.

Picture 2 Re-cycled water carrier being filled from the tap in the Mao TFC compound.

1.2.1 MAO, THERAPEUTIC FEEDING CENTRE

The TFC is run by Action Contre la Faim (ACF) a French non-governmental organisation (NGO) who have been in the region since 1994. The TFC is attached to the main hospital for all of Kanem and hence children were referred from all other health centres. Admission criteria for children above 6 months old were severely wasted individuals of weight for height (W/H) at <70% or < -3SD of median NCHS/WHO references and/or a mid upper arm circumference (MUAC) of <11cm (height of >75cm) and/or bilateral oedema (ACF, 1998). Picture 3 shows a patient with marasmus, and Picture 4 shows a patient with kwashiorkor. They stay at the centre with their mothers or guardian until they recover. The initial treatment (Phase I) typically included hydration, prevention of hypoglycaemia and sorting out any infections, vitamin and mineral deficiencies they may have. The patients generally stay for a few days in this phase in a separate room from the others. In this phase the children are fed every 3 hours with a specially prepared formula milk called F-75 (75kcal/100ml). They are fed 130ml/kg/day providing 100kcal/kg/day of energy. Once they have recovered some appetite and have no diarrhoea or vomiting they move to a transitional phase (Phase T) for another couple of days. Here they receive a dilute version of F-100 (100kcal/100ml) at 130ml/kg/day (100kcal/kg/day). Once in Phase II the rehabilitation stage intensive feeding of F-100 is given at 200ml/kg/day (200kcal/kg/day) until their W/H reaches 80%. Those above one years old also receive one meal at lunch time of bouille (porridge) of 280-350kcal. To simplify meal times the quantity of milk were given according to weight classes (table 1.1). In the last stage of treatment Phase III, F-100 was still provided (table 1.1) at 200ml/kg/day (200kcal/kg/day). Porridge is also eaten in this phase and the children eat with their mothers. The children are discharged when their W/H is ≥85% for 2 consecutive weighings. In all the phases except after admission the children stay with their mothers in a locally constructed tent called a 'ferrique' made from mats sewn together (Picture 5). However in the town most people live in houses built with sun dried clay bricks (Picture 6).

Class of weight (kg)	**Quantity given/day (ml/1000kcal)**	**Quantity given/ meal /day (ml)**
<5	1000	160
5 - 7.5	1500	250
7.6-10	2000	330

Table 1.1 Quantity of milk given to the children at the TFC in phases II and III (ACF, 1998).

Picture 3 Marasmic child at Mao TFC (1 year old child).

Picture 4 Child with kwashiorkor at Mao TFC (2 year old child).

1.3 BODY WATER REQUIREMENTS

Water is an ubiquitous compound on which human beings depend for survival. The human body is mainly water and it's availability and maintenance affects our well being. The percentage of the body made up of water is dependent on age, sex and body fat. New born babies have the highest levels of around 80% of body weight (Logan, 1998). By the time children reach puberty there is a differentiation of these levels and females have less body water than males. With increasing age, the values eventually drop for both sexes. The distribution of water is conveniently compartmentalised into that within the cells (intracellular fluid or ICF) and extracellular fluid (ECF). ICF fluid contain high levels of potassium (K) and ECF is rich in sodium (Na) and chloride (Cl) (Logan, 1998).

Picture 5 'Ferrique' or locally constructed tent at Mao TFC.

Picture 6 Typical local buildings in Mao.

1.3.1 WATER REGULATION

Body fluid composition is regulated firstly by factors that affect intake of water (thirst) and secondly by factors that affect renal water loss (anti-diuretic hormone and the function of the kidneys). When there is a restriction of water intake or excess sodium chloride the osmolality of the ECF increases (Vander *et al*, 1994). The sensation of thirst occurs when water enters the ECF from the ICF to equilibrate the osmotic pressure. Small changes in ECF osmolality also leads to immediate increases or decreases of the amount and osmolality of the urine. When there is very little water in the body, the renal tubules reabsorb the fluid. The kidney is however more efficient at getting rid of water. In adults and older children thirst sensations means they are able to supply the water required when hot temperatures demand that they replenish the water lost by sweating. In babies and younger children who are unable to demand extra water it is up to the mothers to do this. There also seems to be a complex inter-relationship between intake and the various output routes of water (figure 1.2). Dietary intake in the form of solid food and drinks are governed by a complex set of biological, psychological and social factors. In addition to the obvious fluid intake and that produced metabolically, most foods have a high water content. The water losses all depend on the level of intake and are affected by environmental forces such as the heat, humidity, type of clothing and exercise level. In temperate conditions water balance is maintained relatively easily by the diet and fluid intake in response to thirst. In extremely hot conditions as experienced in Mao, water balance may not be so straight forward. This is because the body either has to dispose off or store the heat gained from metabolism and also from the environment (through radiation, conduction and convection) in order to maintain body temperature. This additional demand for temperature regulation means that more water is required.

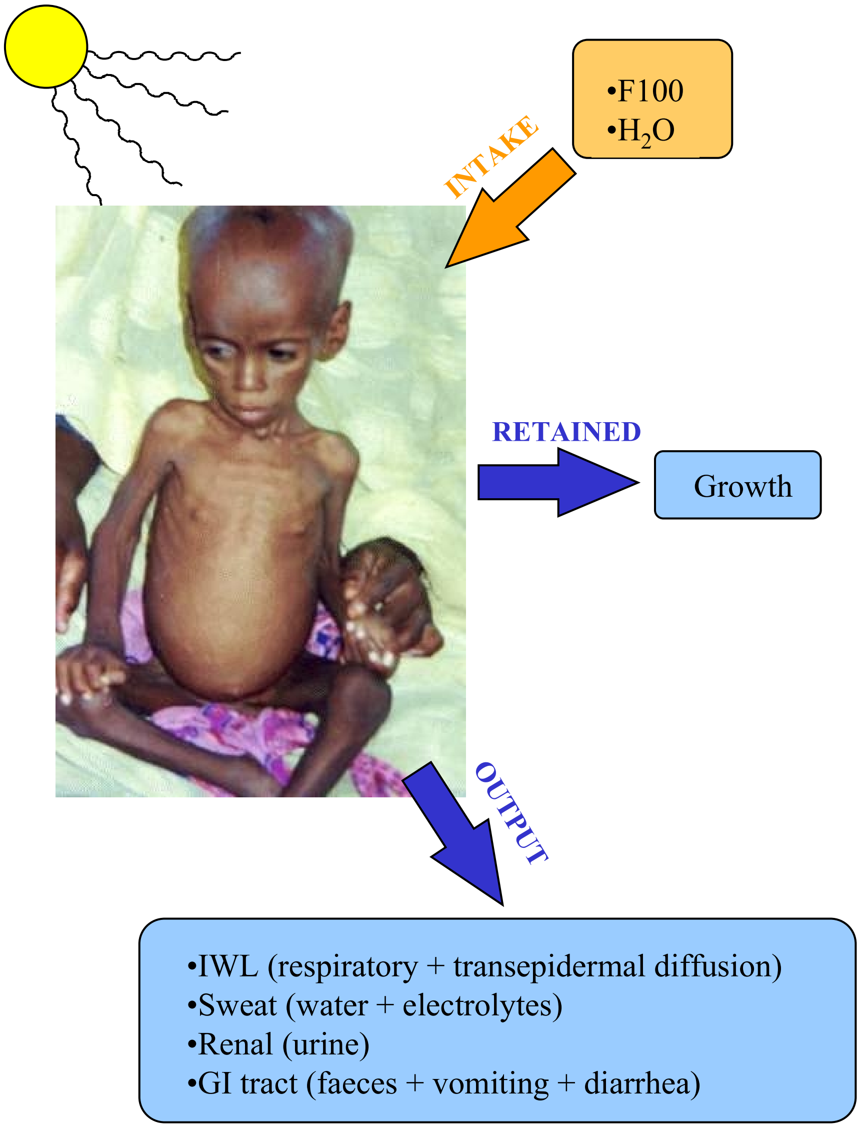

Figure 1.2 Factors that influence water balance in a malnourished child

1.3.2 HEAT REGULATION AND EVAPORATIVE WATER LOSS

Heat is normally lost from the surface areas of the body by the cooling effect of evaporation from the skin. These are largely controlled by homeostatic mechanisms via the nervous system (Open University, 1974; Rowland, 1996). Under normal conditions metabolic heat is dissipated via the lungs and skin by diffusion. These are collectively termed insensible water loss (IWL) consisting of respiratory water loss (RWL) and transepidermal water loss (TWL). Evaporative water loss via sweating begins when these two routes are not sufficient, as in exposure to hot temperatures or exercise. Evaporation depends on the differences between the vapour pressures of the skin and the surrounding environment. Hence in dry heat as experienced in Tchad, evaporation is not hindered and provides a relieving cooling effect. Sweating depends on a number of factors including the number of active glands, the rate of their production and the circulation. When required, blood flow increases and acts as a transporter of heat to carry warm blood from the core of the body to the vasodilated vessels under the skin surface. However this reduces the volume at the core triggering increased heart rate. This combined with excess sweat can make subjects in hot environments susceptible to relative hypovolaemia and dehydration.

1.3.3 ELECTROLYTES AND WATER BALANCE

Heat balance becomes more of a problem if fluids are replaced by electrolyte containing solutions and pure water is lost from the skin and lungs (Gisolfi, 1993; Maughan, 1992). There must be sufficient water remaining to allow for renal excretion of the excess electrolyte. This has to be done without exceeding the child's capacity for renal solute concentration (Fomon, 1974). The formula milks given to malnourished children, F75 and F100 have 75 or 100 kcal/100 ml with a relatively high electrolyte content. Therefore it is possible that water may be a limiting nutrient in Sahelian countries when the milk formulas are used as the diet to treat severe malnutrition. The diets as treatment were deliberately made as concentrated as possible to renourish the children as rapidly as possible. As a comparison, breast milk has been shown to provide adequate fluid for babies without the need for additional water in tropical climates. The urine concentrating capacities of the children tested were not exceeded (Ashraf *et al*, 1993). Almroth *et al* (1990) studied children in the hottest and driest season in India when temperatures reached 40^{o}C and relative humidities of 10 to 35%. He found that 6 to 10 month old breast-fed babies had a mean urinary concentration of 408mosmol/L - well below the capacity for renal concentration demonstrated by other babies of the same age in temperate climates. Breast milk, however, has 70 kcal/100 ml and is relatively low in electrolytes and protein and hence presents a low renal solute load (Wrong, 1996). In contrast the milk given to malnourished children may not be suitable in these hot and dry environments. They were originally formulated and tested in conditions of higher relative humidity and lower environmental temperatures than are encountered in the Sahel. The consequences of trying to maintain body heat means that there is a potential for insufficient water remaining in the body after evaporation. Although with increased sweating there is additional loss of electrolytes, most will be retained and the child may develop hyperosmolar dehydration. This condition occurred in Westernised countries in the 1950s and 60s when mothers made formula feeds that were too concentrated (Taitz and Byers, 1972). It still occurs by accident at present and it carries a particularly high mortality rate.

1.3.4 Body surface area and water balance

The surface area:weight ratio (sa:wt) of the person is related to the rate of gain or loss of heat, the metabolic heat production and the amount of water that is consumed in heat dissipation by evaporative loss. Thus, young children are particularly vulnerable to fluctuations in water requirements. Their higher sa:wt means they have an advantage in heat transfer in places where the mean skin temperatures are higher than that of the environment. They are at a disadvantage when the ambient temperature exceeds skin temperature. The critical temperature where this change occurs seems to be at about 35^{o}C (Rowland, 1996). Above this environmental temperature poor evaporative cooling mechanisms means that children are at a disadvantage. Malnourished children (especially those with defective skin) become even more susceptible to external temperatures due to their even larger sa:wt. Additionally in a malnourished state there is evidence of a defective sweating response, control of peripheral blood flow, renal function and water absorption from the intestine (Alleyne, 1967; Brooke, 1972). This makes these children particularly vulnerable to heat stress and water deficiency due to rapid fluid loss. Brooke *et al* (1974) subjected 12 malnourished babies to heat stress in order to find out if they were unduly affected by heat. At an environmental temperature of 38^{o}C with a relatively low humidity they found that mean rectal temperature increased at a faster rate of 0.75^{o}C/hour in the malnourished state than in the recovered state over the same period. They also found that after recovery the total evaporative heat loss was 44% more than when in the malnourished state.

1.3.5 Models of water loss

Models of water losses have been developed primarily to determine water requirements of healthy adults, athletes, military personnel and new-born infants in incubators (Shapiro *et al*, 1982; Ultman, 1987). Although of potential use in this study the extent to which these models can be extrapolated directly to malnourished children or to more extreme environments is not clear. Direct measurements of these children are necessary to test their applicability. As an example, Wissler's thermal-cardiovascular model (personal communication, 1998) has been used to compute the water requirements of a child weighing 10kg with a 35°C skin temperature at an environment temperature of 45°C. If radiation is assumed to be negligible then the rate of evaporative loss were calculated to be in the order of 1.1L per day (11% of body weight). To this would need to be added the water requirement for renal excretion of solute. In the context that 2% of water loss leads to dehydration and 10% to very severe dehydration requiring immediate resuscitation, such a calculation shows that the water requirement may be very considerable (Open University, 1974). If correct, it would mean the children require a very high water turnover in order to maintain heat balance. If this is not being met then it may mean a re-evaluation of the dilution of the milk formulas currently used and possible consideration of the renal solute load that these diets impose. Hence, direct measurement of body water turnover in a sample of children under these extreme conditions would help to identify if a change in diet is necessary. There has been little published work on water turnover in either healthy or malnourished children. Fusch *et al* (1993) used isotopic techniques to determine water turnover and hence recommendations of water requirements for healthy children.

1.4 STUDY AIMS

The aim of this study was to measure directly the water turnover in children in the TFC in Mao, Tchad. In determining the water requirements, proposals of how the diet formulation should be adjusted to provide a margin of safety for the children can be calculated. The technique employed in the study utilised heavy water or deuterium oxide (D_2O).

1.4.1 HEAVY WATER TECHNIQUE

Heavy water behaves in the body exactly like normal water and is perfectly safe (Schoeller, 1996). The principle of the method is that if a known quantity of D_2O is added to the body pool of water, the subsequent determination of the isotope concentration can be related to the TBW. Unlike water turnover, the technique has been extensively used to measure total body water in premature infants, young children, malnourished children, pregnant women, adults and the elderly (Smith, 1960; Maclennan *et al*, 1981; Davies and Wells, 1994; Leiper *et al*, 1996). These studies have shown that TBW in the malnourished child is increased compared to normal children, even for those non-oedematous cases. This increase has been attributed to the relative rise in ECF volume. Measurement of TBW *in vivo* makes assumptions which may not necessarily be correct. The isotope does not behave ideally as *in vitro* and the assumptions that, when a dose of D_2O is given, there is rapid distribution to all water compartments may not be valid. This means that TBW can also be calculated using two different methods which treat the isotope data in different ways (Davies and Wells, 1994). The plateau method assumes that the tracer isotope reaches equilibration with the body water at a specific time after administration of the dose. The calculation of TBW involves sampling regularly up to and beyond the equilibration time which is normally between 2-6 hours. As there are usually a number of complex physiological processes occurring throughout the body it means that there are additional losses (for instance via IWL) which may therefore not be included in the calculated TBW. The plateau method therefore underestimates the body pool. In the back extrapolation or intercept method the isotope is believed to immediately equilibrate with the body water once the dose is given. This method overestimates the initial isotope concentration and hence underestimates the TBW. Neither of these methods provide the best result and the choice is dependent on the subjects and the situation. In this study the determination of water turnover rates demanded collection of samples over at least a week. Since we did not wish to disturb the treatment of the children in terms of withdrawing meals or fluid intake which is required by the plateau method, the intercept method was chosen. This has been shown to work best in field situations by Salazar *et al* (1993) who compared the two methods in malnourished children in Chile. Blood, urine or saliva are normally used to determine the tracer concentration. In this study urine was chosen as the easiest and non invasive method.

1.5 HYPOTHESIS

The water requirements of malnourished children are not being met at extreme environmental temperatures (above 45°C) through their fluid intake from standard formula milk (F100) used to treat children in less extreme environments, and a more dilute formula is required.

Chapter 2

Methods

2.1 PATIENTS

12 boys aged 12 to 48 months were selected to take part in the study as soon as their condition had stabilised after admission to the TFC. 7 of these were studied again when they had recovered to at least weight-for-height of 73%. The anthropometric data gathered for each patient (coded alphabetically) at the start of each study are summarised in Table 2.1.

				Study 1			Study 2		
Patient	**Age (months)**	**Oedema**	**Height (cm)**	**Weight (kg)**	**W/H %**	**SA (m2)**	**Weight (kg)**	**W/H %**	**SA (m2)**
A	24	no	74.0	7.0	73.09	0.38			
B	24	no	75.0	5.7	58.07	0.36	7.9	82.48	0.41
C	36	no	73.0	6.3	67.50	0.36	7.8	83.58	0.40
D	11	no	71.0	6.2	70.32	0.35	7.4	86.60	0.38
E	12	no	64.0	4.3	63.08	0.28			
F	48	no	84.0	6.6	56.27	0.40	9.2	78.44	0.48
G	24	no	74.0	6.5	67.87	0.37	7.3	76.22	0.39
H	24	yes ***	79.0	8.6	80.36	0.44	8.5	79.43	0.44
J	12	yes**	64.0	5.0	73.34	0.30	5.0	73.34	0.30
L	14	no	75.0	6.7	68.26	0.38			
M	12	no	64.5	5.0	71.80	0.30			
N	12	no	65.5	5.5	75.78	0.32			
Mean	**21**		**71.9**	**6.1**	**68.81**	**0.35**	**7.6**	**80.01**	**0.40**
SD	**12**		**6.4**	**1.1**	**7.01**	**0.05**	**1.3**	**4.54**	**0.05**

*Table 2.1 Anthropometric data of the patients at the start of each study where * reps number of sites of oedema.*

For most of the children the age had to be estimated on arrival at the centre. The study did not in any way interfere with the clinical management and normal procedures of the centre as detailed Chapter 1. The study was explained to the mother of each child and verbal consent obtained by the nurse in charge of the centre. Consent for the study was obtained from the head Ministry of Health official in Mao.

Ethical approval was also obtained from the ethics committee in Aberdeen (Grampian Research Ethics Committee). A translator was utilised for communicating with the mothers.

2.2 ANTHROPOMETRIC DATA

- W/H were calculated using NCHS standards (Dibley *et al*, 1987).
- Mean surface area was calculated from three methods (of Dubois and Dubois, 1916; Getan and George, 1970 and Haycock, 1978).
- The weights of most of the children were recorded daily using a hanging scale (Picture 7). Two of the smallest children (M and N) were weighed using a beam balance scale. The scales were calibrated daily with known weights and zeroed each time before use.
- The heights were measured at the start and finish for each study period using a length board (Picture 8).
- The mid-upper-arm circumference (MUAC) were measured at the start and finish for each study using an insertion tape. Any child with a MUAC < 12.5cm is considered to be very thin and undernourished.

Picture 7 Child being weighed using a hanging scale (3 year old child).

Picture 8 Child's length being measured using a length board (2 year old child).

2.3 TEMPERATURE MEASUREMENTS

All temperature measurements were taken 3 times a day (morning, noon and evening).

2.3.1 ENVIRONMENTAL CONDITIONS

A portable handheld microprocessor thermistor thermometer with an accuracy of ±0.4°C and a resolution of 0.1°C was used (HANNA Instruments Ltd, UK) to obtain shade and direct sun light temperatures in the area where the children spent most of their time. Ambient, minimum and maximum temperatures were recorded daily. Maximum and minimum environmental temperatures in the shade were also obtained from the Mao meteorological station. The percentage Relative humidity was obtained 3 times daily from the Mao meteorological station at 6am, 12 noon and 6pm. These were calculated using standard methods from wet and dry bulb thermometers.

2.3.2 BODY AND SKIN TEMPERATURES

Body and skin temperatures were recorded with a digital thermometer. Body temperatures were recorded under the arms (Picture 9) and skin temperatures were taken in 3 positions (head, sternum and spine) with a tape covering the end of the thermometer.

2.4 WATER TURNOVER STUDY

Approximately 2g of deuterium oxide (99.8% D_2O, Sigma Chemicals, UK) was administered to each child on the first day of the study. This was accurately weighed (into numbered vacuutainer tubes) to 4 decimal places in Aberdeen before being transported to the study centre. Immediately before the mid-morning meal, the dose of heavy water was diluted with an approximate equal volume of water and given directly from the tube into the mouth. The tube was thoroughly rinsed with extra water which was also drunk by the child. There were problems in administering the dose of heavy water. Most of the children either dribbled or regurgitated a small portion of the dose.

2.4.1 URINE COLLECTION

For 11 of the children all urine samples were collected by using a plastic urine collector bag (Picture 10) with an adhesive attachment (U-Bag, Hollister Incorporated, USA). A beaker was provided for the oldest child in the study to urinate into. A baseline urine sample was collected prior to administration of the dose of D_2O. Approximately 2 hours after the dose, urine was collected from each of the children and this was used to determine the dilution space and total body water. When possible, for the next subsequent 6 days, urine bags were placed on the children at roughly the same time to collect urine to determine turnover rates. After micturition, samples were removed from the bags weighed and placed into a labelled vacuutainer tube. 100µl of chlorhexidine solution (4% prepared in Aberdeen) was added as a preservative. Urine samples were stored away from direct heat and when in the UK were kept frozen until analysis.

Picture 9 Daily body temperature measurement for TFC records (4 year old child).

Picture 10 Urine bag waiting to be filled! (1 year old child).

2.4.2 Sample preparation for D_2O analysis

Water and deuterium oxide were separated from the solids in each of the urine samples by vacuum sublimation (distillation under reduced pressure) according to the method of Lukaski and Johnson (1985). Essentially this involved placing approximately 3ml of frozen urine sample into the long end of a glass J tube. This was sealed with a rubber stopper and evacuated by inserting a needle attached to a vacuum pump. Once evacuated the short end of the tube was placed into liquid nitrogen in a vacuum flask. The tubes were left for several hours and liquid nitrogen topped up until separation was complete. Once the aqueous phase had been collected in the short end of the tube it was allowed to thaw overnight. The condensate were removed the next day and stored in the fridge or freezer until further analysis.

2.4.3 D_2O analysis by infra-red spectrophotometry (IR)

Deuterium oxide enrichment in the samples was analysed on an infra-red spectrophotometer (Miran IFF, 4 micron filter, 0.2mm path length calcium fluoride cell). Deuterium oxide standards prepared gravimetrically to cover the range 0-750ppm were used to prepare a calibration line for each day. This was used to determine the concentrations of the samples (injected as duplicates) as well as an external standard and an unknown urine sample.

2.5 Electrolytes and osmolality

The electrolyte and solute concentrations were measured in the Department of Clinical Biochemistry. Urine samples were spun at 2500g for 5-10 minutes and the supernatant used to obtain sodium (Na) and potassium (K) concentrations as well as the solute concentration.

2.5.1 ELECTROLYTES

An automatic Flame photometer (Instrumentation Laboratory, 943) was used to obtain the concentrations of Na and K in mmol/L. The instrument was autocalibrated using 100mmol/L solutions of Na and K and zero with distilled water.

2.5.2 OSMOLALITY

A 50µl aliquot of urine was utilised to measure the osmolality of each sample using a cryoscopic osmometer (Osmomat 030, Gonotec). One sample which did not have chlorhexidine added was also measured in order to check the effect of bacterial growth on osmolality.

2.5.3 SPECIFIC GRAVITY

Multistix reagent strips (Bayer Corporation, USA) were used to obtain specific gravity for some of the urine samples. The strip permits determination of urine SG between 1.000 and 1.030 and this correlates within 0.005 with values obtained with refractive index method. These strips effectively measure Na^+ and K^+. There is a cation exchange resin incorporated into the strip which exchanges H^+ for alkaline metals. The strip changes colour based on the pH change from the exchanged H^+. Urea, ammonia, phosphate and creatinine osmolytes are not assessed with the strip.

2.6 DATA ANALYSIS

All calculations and charts were performed using Microsoft excel.

2.6.1 TOTAL BODY WATER CALCULATIONS

Total body water for each patient was calculated from the difference between the baseline and the first sample collected after dosing using the formula of Halliday and Miller (1977).

Assumptions

The dose of D_2O had to be completely consumed without spillage in order to calculate TBW. In most of the children, this was not possible. The values for these children are therefore an overestimate of the TBW. In cases where most of the dose was spilt the children had values that were more than their body weight. Total body water for malnourished children has been found to be between 60-80% of their body weight. In 6 of the children the calculation for % TBW of their weight was more than 80%. Hence the mean value obtained for the others was used to recalculate their TBW.

2.6.2 WATER TURNOVER CALCULATIONS

The concentration of D_2O in the daily samples were used to calculate the turnover of water over the week. The turnover rate comes from the slope (k) of a natural log plot of enrichment values against time.

Assumptions

To calculate water turnover in L/day, it was assumed that the TBW values were accurate.

2.6.3 DIETARY WATER INTAKE CALCULATIONS

The following assumptions were made in order to calculate the water intake:

water intake (WI) = water loss (WL) + water retained (WR)

where

WI = Diet water + non-diet water + metabolic water

WL = Renal loss + evaporative loss + other (faeces, vomiting)

WR = growth

Hence,

water turnover = WI = WL + WR

Energy intake for each child was calculated assuming maintenance energy requirement of 100 kcal/kg/d, and energy cost of growth of 5 kcal/g of weight gain.

Hence,

EI=[weight (kg) x 100 (kcal/kg/d)] + [weight (kg) x weight gain (g/kg/d) x 5(kcal/g)]

F100 water content = 868 ml/L, metabolic water content = 127 ml/L.

Therefore,

total diet water = dietary water intake + metabolic water intake

= (0.868 x EI) + (0.127 x EI)

Non-dietary water intake is assumed to be equal to:

water turnover (L/d) - dietary water (L/d)

2.6.4 RESIDUAL RENAL SOLUTE LOAD

A renal solute load (rsl) of 251mOsm was calculated for F100 milk using the formula of Zeigler and Fomon (1971). Since these children were growing rapidly, it was assumed that a portion of the potential rsl of F100 was being utilised for growth. A weight gain relief on the assumption that each gram of weight gain contains 0.9mOsm (Widdowson and Dickerson, 1964) was applied:

weight gain (g/d) x 0.9 (mOsm) = mOsm retained/child/d

residual rsl = dietary rsl - rsl retained in new tissue

For oedematous children, an adjustment of 0.3mOsm was used for oedema loss from ECF. This calculation assumes all intake based on calculated diet water.

2.6.5 EVAPORATIVE WATER LOSS CALCULATIONS

1 Evaporative loss = turnover - (renal loss + other losses)

2 Wissler's model (Wissler,1998) :

Steady-state energy balance, where rate of metabolic heat production + rate of convective heat transfer = rate of evaporative heat loss:

MET (watts) + CON (watts) = EVAP (watts)

with associated daily water losses:

met (L/d) + con (L/d) = evap (L/d)

$$\text{met (L/d)} = [410 \times \text{body wt (kg)}] / [1000 * 2.372]$$

where 410 kJ is the maintenance energy of a malnourished child, 2.372 is the latent heat of evaporation of water (in kJ/g).

$$\text{CON (watts)} = \text{SA}(m^2) \times \text{heat trans coeff (watts}/m^2\,°C) \times [\text{ env temp - skin temp}]\ (°C)$$

$$\text{Con (L/d)} = [(\text{CON (watts)} \times 86400\ (s)) / 2372] / 1000$$

For Insensible Water Loss (IWL) calculations according to Ultman (1987) see appendix B.

2.6.6 PROBLEMS

It was not possible to collect 24 hour urine samples from these children in order to calculate the non urinary losses. The urine bags remained in place for a few hours only because of excess sweat and the customary use of water to wash after toilet. This meant that the bags had to be replaced several times over a 24 hour period. Extra adhesives were used but none were successful.

2.7 TEMPERATURE EFFECTS ON MORTALITY RATES

The minimum and maximum temperatures in the Phase 1 room (see Chapter 1) have been recorded daily since August 1998. The monthly report of the TFC contains the number of admissions and departures. The number of patients who are cured, die, are transferred to another centre or abandon the centre are recorded. Mean monthly minimum and maximum temperatures were also obtained from the Meteorological station for outside shade temperatures.

Chapter 3

Results

3.1 ENVIRONMENTAL CONDITIONS

The mean shade temperatures (^{o}C) throughout the day were 35.8±2.64 (in the morning), 41.5 ±2.4 (at noon) and 38.46 ±2.23 (in the evening) over the study period. The mean temperature recordings taken at the meteorological station in the town were comparable to that for this study with a mean maximum of 42.0±1.54. The rest of the temperature and humidity data will be found in Appendix C. Figure 3.1 compares the mean shade temperatures of the environment (recordings at the TFC and in the town) with mean body and skin temperatures of all the subjects at noon. The skin and body temperatures are affected by environmental temperature.

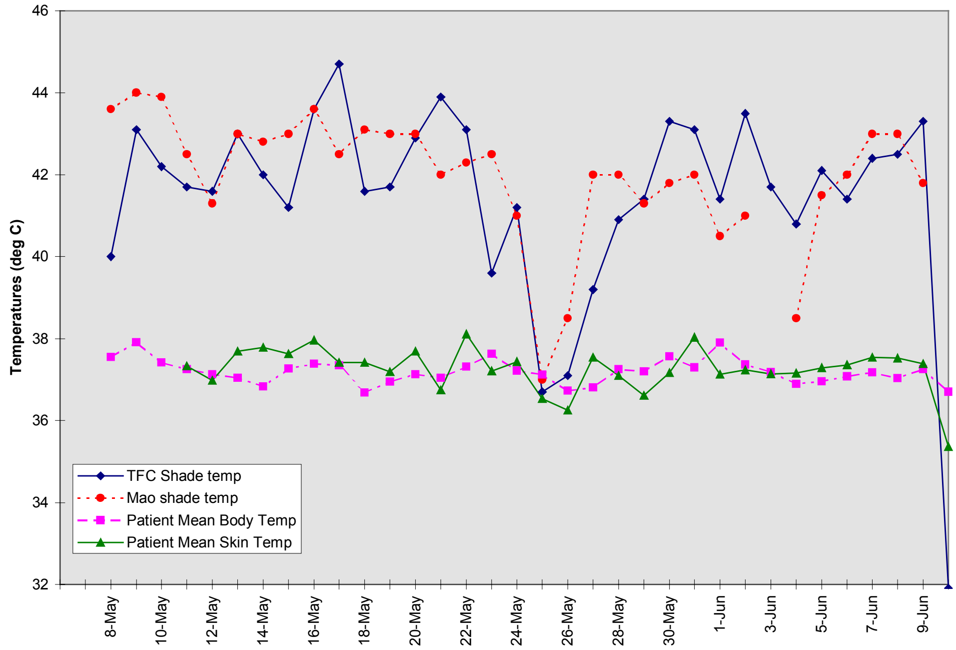

Figure 3.1 Temperature data recorded during the fieldwork in Mao.

3.2 TOTAL BODY WATER

It is possible to calculate total body water (TBW) using D_2O by two alternative methods, namely the plateau and the back extrapolation as mentioned in Chapter 1. In field studies especially with young children, it is much easier to use the back extrapolation method which does not require abstention from intake. There is an exponential decay of D_2O after administration. If samples are collected after the equilibration period, it is possible to back extrapolate to find the enrichment at the time of dose. Figure 3.2 shows examples of such an exponential decay of D_2O in 2 of the patients studied over the 7 days. In this study back extrapolation to time zero and hence TBW was obtained from the regression equation when x = 0 for all of the children. The enrichment level of D_2O above the baseline was used to calculate the deuterium dilution space using the formula

N = (WA/a)(Sa/(Ss-Sp)

where N = deuterium dilution space

W = mass of water used to prepare standard

a = mass of D_2O used to prepare standard

A = mass of D_2O given as dose to each child

Sa = measured enrichment of standard

Ss = enrichment of urine at zero time after dose

Sp = enrichment of urine before dose (at baseline)

Isotopic fractionation of urine is minimal hence no correction was applied. To include the effects of non-aqueous exchange of labile hydrogen, a correction factor of 0.96 was applied to the dilution space to obtain TBW. The calculated average TBW is shown in Table 3.1.

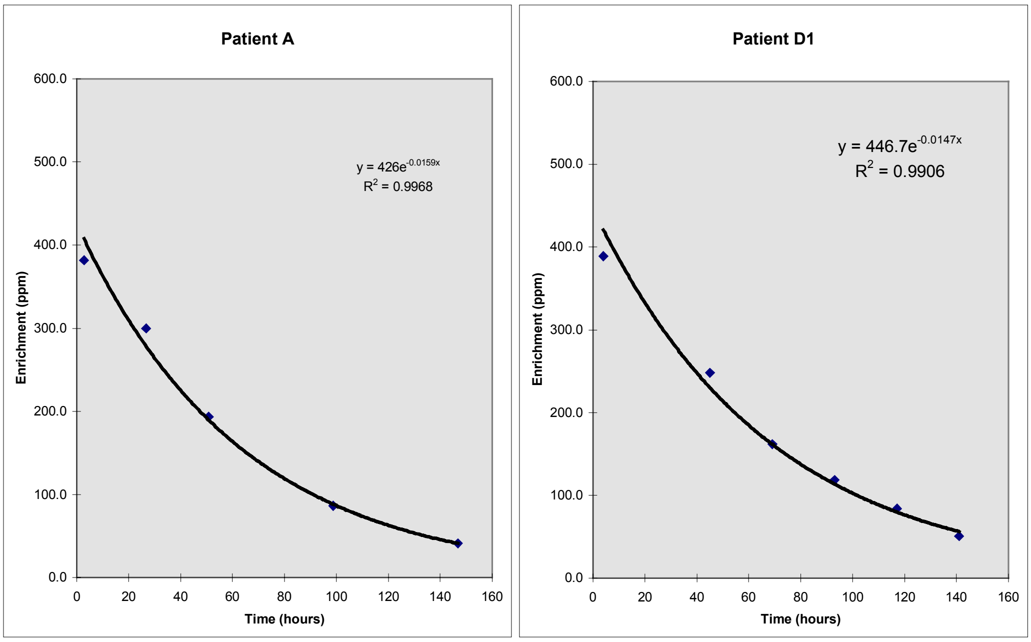

Figure 3.2 Normal plots of two patients (A and D1) showing the exponential decay of D_2O over the course of time. Enrichment refers to the concentration of D_2O as measured by IR spectrophotometry. The regression equation shown on each graph was used to calculate y when x was zero.

The TBW of each child was also calculated using their weight and height at day 1 of the study according to the Friis-Hansen empirical formula (1961) where W is weight in kg and H is height in cm.

$$TBW = 0.135 \times W^{0.666} \times H^{0.535} \text{ (with SD of 8.7\%)}$$

This gave an average TBW for all the children as 4.72L ± 0.83. These are compared in table 3.1 with the values of TBW as calculated for each child from this study. Except in the case of the children with oedema (H and J), all those studied twice decreased their TBW as % of body weight as they recovered. The table also shows that on average 71.28% ± 4.75 of their weight consists of water.

Patient	Initial weight (kg)	TBW (JCB) (L)	% of Body Weight	TBW (FH) (L)	% Body weight
A1	7.00	4.94	70.57	4.93	70.49
B1*	5.70	4.06	71.28	4.33	75.96
B2	7.90	6.34	80.25	5.35	67.70
C1	6.30	4.69	74.44	4.57	72.48
C2	7.80	5.64	72.31	5.26	67.49
D1	6.20	4.71	75.96	4.45	71.80
D2*	7.40	5.27	71.28	4.97	67.16
E1*	4.30	3.07	71.28	3.30	76.74
F1	6.60	4.82	72.95	5.08	76.93
F2	9.20	5.72	62.22	6.33	68.80
G1	6.50	4.77	73.34	4.70	72.25
G2*	7.30	5.20	71.28	5.07	69.51
#H1*	8.60	6.13	71.28	5.86	68.15
#H2	8.50	5.36	63.08	5.82	68.41
#J1	5.00	3.97	79.40	3.65	72.98
#J2*	5.00	3.56	71.28	3.65	72.98
L1	6.70	4.69	70.06	4.83	72.04
M1	5.00	3.43	68.57	3.66	73.28
N1	5.50	3.49	63.51	3.94	71.57
Mean	**6.66**	**4.73**	**71.28**	**4.72**	**71.41**
SD	**1.37**	**0.93**	**4.75**	**0.83**	**3.06**

*Table 3.1 Comparison of TBW of patients as estimated in the study and that of the empirical formula of Friis-Hansen (1961). Water makes up approximately 71% of body weight according to both methods of calculation. Where # represents a child with oedema and * represents the adjusted TBW as described in the text (section 2.6.1).*

3.2 WATER TURNOVER

Even more important in this study is the turnover of water on a daily basis for these children. To calculate the water turnover for each child, the natural logarithm of each enrichment was plotted against time. A straight line was obtained and from the slope (k) of the regression, the turnover rate was calculated per child (table 3.2). The slope value in hours was multiplied by 24 to obtain the daily turnover rates. The average turnover rate (k) was 0.3429 ± 0.0586 per day. This means on average the children in this study were turning over 34% of their body water. Hence there is an average turnover of 1.63L ± 0.45 of water each day. The table also shows the turnover for each child as standardised per unit weight and surface area. On average, 0.24L ± 0.04 of water is turned over par kg per day. When the 7 patients that were studied twice were compared, no statistical significance was found between them except in their weights and surface areas. The p values are reported on the table.

Patient	Turnover/day (%)	TBW (L)	TBW (% of body weight)	Turnover (L/day)	SA (m2)	Turnover/ SA (L/m2/day)	Weight (kg)	Turnover/weight (L/kg/day)
A1	38.16	4.94	70.57	1.89	0.38	4.96	7.00	0.27
B1*	45.12	4.06	71.23	1.83	0.35	5.23	5.70	0.32
B2	28.08	6.34	80.25	1.78	0.41	4.34	7.90	0.23
C1	37.92	4.69	74.44	1.78	0.36	4.94	6.30	0.28
C2	41.76	5.64	72.31	2.36	0.40	5.89	7.80	0.30
D1	35.28	4.71	75.96	1.66	0.35	4.75	6.20	0.27
D2*	29.52	5.27	71.22	1.56	0.38	4.09	7.40	0.21
E1*	22.08	3.07	71.40	0.68	0.28	2.42	4.30	0.16
F1	31.44	4.82	72.95	1.51	0.40	3.75	6.60	0.23
F2	41.28	5.72	62.22	2.36	0.46	5.14	9.20	0.26
G1	36.00	4.77	73.34	1.72	0.37	4.64	6.50	0.26
G2*	28.08	5.20	71.23	1.46	0.39	3.74	7.30	0.20
H1*	34.32	6.13	71.28	2.10	0.44	4.78	8.60	0.24
H2	41.28	5.36	63.08	2.21	0.44	5.03	8.50	0.26
J1	30.00	3.97	79.39	1.19	0.30	3.97	5.00	0.24
J2*	36.72	3.56	71.20	1.31	0.30	4.36	5.00	0.26
L1	30.24	4.69	70.06	1.42	0.38	3.74	6.70	0.21
M1	30.00	3.43	68.57	1.03	0.30	3.43	5.00	0.21
N1	34.32	3.49	63.51	1.20	0.32	3.75	5.50	0.22
Mean (all patients - both studies)	**34.29**	**4.73**	**71.27**	**1.63**	**0.37**	**4.37**	**6.66**	**0.24**
SD (all patients - both studies)	*5.86*	*0.93*	*4.75*	*0.45*	*0.05*	*0.80*	*1.37*	*0.04*
Mean (repeated patients - study 1)	**35.73**	**4.73**	**74.09**	**1.69**	**0.37**	**4.58**	**6.41**	**0.26**
SD (repeated patients - study 1)	*4.94*	*0.71*	*2.88*	*0.28*	*0.04*	*0.53*	*1.11*	*0.03*
Mean (repeated patients - study 2)	**35.25**	**5.30**	**70.22**	**1.86**	**0.40**	**4.66**	**7.59**	**0.25**
SD (repeated patients - study 2)	*6.49*	*0.86*	*6.10*	*0.44*	*0.05*	*0.73*	*1.32*	*0.04*
p (study 1 compared with study 2)	**0.90**	**0.18**	**0.17**	**0.28**	**0.02**	**0.83**	**0.02**	**0.37**

Table 3.2 Daily water turnover of patients expressed as % and standardised per unit weight and surface area.

There are very few studies that have specifically addressed water turnover in children. The turnover rates were compared with that from other studies. However the 34% water turnover is far higher than has been measured in other situations. Two of these studies were used to compare with the present one. The first study by Fusch *et al* (1993) estimated water turnover in German children of normal health exposed to a temperate climate. The second study by Salazar *et al* (1994) on malnourished children recovering from marasmus in Chile under slightly higher environmental temperatures was used to calculate turnover rates. (See Appendix B for method of calculation). Although this paper focused on TBW and the methodologies in use to calculate it, it was possible given the information in the paper to calculate water turnover of these children. Table 3.3 compares the average turnover of water obtained in this study with that of the other two mentioned.

Ages (months)	water turnover rate (L/day)	water turnover rate (L/kg/day)	Reference
12 to 48	1.94 (0.81)	0.240 (0.040)	Tchad (Conduah Birt, 1999)
7 to 12	0.90 (0.21)	0.160 (0.040)	Chile (Salazar *et al* 1994)
10 to 12		0.097 (0.029)	Germany (Fusch *et al*, 1993)
12 to 36		0.064 (0.021)	Germany (Fusch *et al*, 1993)
48 to 72		0.063 (0.017)	Germany (Fusch *et al*, 1993)

Table 3.3 Comparison of water turnover in children from the present study with normal healthy children in Germany and those recovering from marasmus in Chile.

3.3 CONTRIBUTION OF WATER INTAKE TO WATER BALANCE

Most of the children were in Phase II of their treatment so the quantity of milk (dispensed per day) given to each child according to their class of weight could be used to estimate water intake. In the context of a centre in rural Tchad, the dietary intake was therefore calculated from the rate of weight change using a maintenance energy requirement of 100kcal/kg/day and an energy cost of tissue synthesis of 5kcal/kg/day. Table 3.4 shows the results of calculations made for water intake from dietary source. Additional water was assumed to be the difference from water turnover. The calculations does not take into account the losses from spillage of the milk or the extra water given by the mothers and is thus inaccurate. If the dispensed diet had all been consumed the rate of weight change would have been very much higher than that measured. It is clear that a large amount of the dispensed food was either not consumed by the child, taken by the mother herself, spilt, vomited or malabsorbed.

Patient	rate of weight gain (g/kg/day)	energy intake (kcal/day)	total dietary water (ml)	additional water (ml)
A1	22.4	1486	1478	407
B1*	25.1	1284	1278	554
B2	6.3	1040	1035	745
C1	27.2	1487	1480	299
C2	9.2	1137	1131	1224
D1*	6.9	834	830	831
D2*	15.4	1311	1305	251
E1*	0.0	430	428	250
F1*	30.3	1660	1652	-138
F2	4.7	1134	1129	1234
G1	11.0	1007	1002	714
G2	0.0	730	726	734
H1*	-15.0	217	216	1888
H2*	-1.7	779	775	1439
J1	0.0	500	498	693
J2*	-8.6	286	284	1023
L1*	6.4	884	880	540
M1	-5.7	357	355	673
N1	2.6	621	618	581
Mean (all patients - both studies)	**7.2**	**905**	**900**	**734**
SD (all patients - both studies)	*11.8*	*409*	*407*	*467*
Mean (repeated patients - study 1)	**12.2**	**999**	**994**	**692**
SD (repeated patients - study 1)	*16.5*	*523*	*521*	*621*
Mean (repeated patients - study 2)	**3.6**	**917**	**912**	**950**
SD (repeated patients - study 2)	*7.8*	*346*	*344*	*404*
p (study 1 compared with study 2)	**0.17**	**0.63**	**0.63**	**0.37**

Table 3.4 Water intakes of the children calculated from energy intake and weight gain where additional water is assumed to be administered independently by the mothers (calculated as turnover - dietary water).

3.4 CONTRIBUTION OF RENAL SOLUTE LOAD TO WATER LOSS

Very little of the water turnover (1.63L ± 0.45/day) must be left for urinary losses if the only mechanism for heat loss at these temperatures is by evaporative loss. Urine concentrations of the children should therefore be very high. There is a general tendency to concentrate the urine on a daily basis. This is however not reflected in the mean values calculated for each child over the week of the study and hence in the overall mean of 549±222mOsm/L. The lowest urine concentration was seen in the child with kwashiorkor. 10 children had concentrations above 400mOsm/L and 4 had values above 800mOsm/L. Table 3.5 shows the renal components of water balance.

Patient	residual rsl (mOsm)	urine osmolality (mOsm/L)	renal water output (L)
A1	230	609	0.38
B1*	192	341	0.56
B2	215	696	0.31
C1	217	622	0.35
C2	220	934	0.24
D1*	170	299	0.57
D2*	225	279	0.80
E1*	107	441	0.24
F1*	235	527	0.45
F2	245	569	0.43
G1	187	837	0.22
G2	182	934	0.20
H1*	93	330	0.28
H2*	199	259	0.77
J1	125	612	0.20
J2*	84	465	0.18
L1*	182	404	0.45
M1	115	397	0.29
N1	142	881	0.16
Mean (all patients - both studies)	**177**	**549**	**0.37**
SD (all patients - both studies)	*50*	*223*	*0.19*
Mean (repeated patients - study 1)	**174**	**510**	**0.38**
SD (repeated patients - study 1)	*50*	*198*	*0.15*
Mean (repeated patients - study 2)	**196**	**591**	**0.42**
SD (repeated patients - study 2)	*53*	*280*	*0.27*
p (study 1 compared with study 2)	**0.27**	**0.30**	**0.67**

Table 3.5 Renal outputs of the children calculated from urine osmolality and residual renal solute load.

No correlation was found between the osmolalities of all the children with their % water turnover. Renal solute load (RSL) of F100 milk given to the children in this study was estimated using the Zeigler and Fomon (1971) method and adjusted for growth to give residual RSL. The average urine osmolality over the week for each child was used along with the residual RSL to calculate evaporative water loss (EWL). EWL as main component of extra renal loss (ERL) was calculated from total water intake minus renal water. Table 3.6 shows the values compared with that obtained by the Wissler equation which provides an estimated value for EWL (see section 3.5.1).

Patient	water turnover (L/day)	evap loss from water (L/day)	Wissler evap loss (L/day)
A1	1.89	1.51	1.59
B1*	1.83	1.27	1.35
B2	1.78	1.47	1.86
C1	1.78	1.43	1.54
C2	2.36	2.12	1.82
D1*	1.66	1.09	1.44
D2*	1.56	0.75	1.68
E1*	0.68	0.43	1.01
F1*	1.51	1.07	1.64
F2	2.36	1.93	2.15
G1	1.72	1.49	1.62
G2	1.46	1.26	1.80
H1*	2.10	1.82	2.01
H2*	2.21	1.45	2.00
J1	1.19	0.99	1.20
J2*	1.31	1.13	1.17
L1*	1.42	0.97	1.57
M1	1.03	0.74	1.24
N1	1.20	1.04	1.37
Mean (all patients - both studies)	**1.63**	**1.26**	**1.58**
SD (all patients - both studies)	*0.44*	*0.42*	*0.31*
Mean (repeated patients - study 1)	**1.69**	**1.31**	**1.54**
SD (repeated patients - study 1)	*0.28*	*0.29*	*0.26*
Mean (repeated patients - study 2)	**1.86**	**1.44**	**1.78**
SD (repeated patients - study 2)	*0.44*	*0.47*	*0.31*
p (study 1 compared with study 2)	**0.28**	**0.49**	**0.03**

Table 3.6 Water turnover versus the two calculated evaporative water losses in L/day.

3.5 CONTRIBUTION OF EVAPORATIVE WATER LOSS

As mentioned in the introduction to the thesis (Chapter 1), there are many influences on water turnover. Of these evaporative water loss (EWL) consisting of insensible water losses (IWL) and sweat are difficult to measure. As a result there are many formulas mostly derived from experimental work on babies, athletes and soldiers that are used to calculate IWL, sweat loss and hence total EWL in order to estimate fluid requirements specifically for these groups. None of these formulas seem particularly suitable for the patients in this study as the body compositions and activities are very different. Such a computational model by Ultman (1987) which was originally designed for use on new born babies to estimate IWL was estimated for the children in this study (mean 0.74±0.04g/h/kg). The calculations were derived for babies at different temperatures to those found in this study and hence their use here may be a violation of the formula.

3.5.1 CALCULATION OF EWL FROM WISSLER'S EQUATION

Using Wissler's thermal-cardiovascular model (personal communication) the evaporative loss required to maintain body temperature by these children was calculated. This formula is a scaled down version of a model developed for adults. This uses a steady state energy balance of

Rate of evaporative loss = Rate of metabolic heat production + Rate of convective heat transfer from air

(See Methods section for calculations). Table 3.6 shows the values obtained for individual patients. A mean value for EWL was estimated at 1.58 ± 0.31L/day.

3.6 ELECTROLYTES

Figure 3.3 shows the sodium and potassium levels averaged for each child over the week that they were studied. In most cases the sodium and potassium as well as the urine concentration increased for those studied on two occasions.

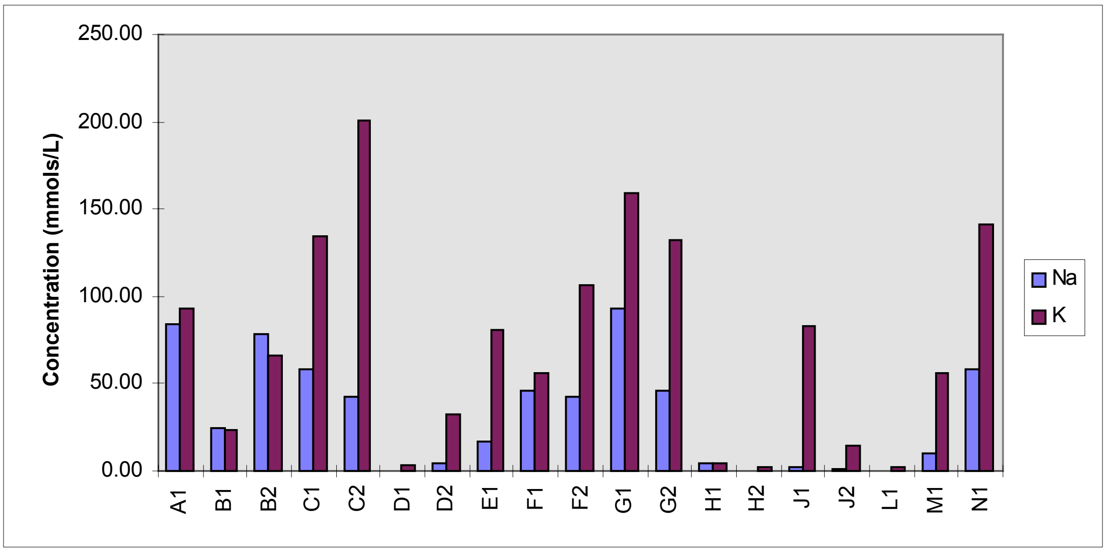

Figure 3.3 The relationship between sodium (Na) and potassium (K) measured in the urine of the patients.

3.7 ENVIRONMENTAL TEMPERATURE V MORTALITY

The impact of environmental temperatures on mortality of the children in the centre was studied by collating the maximum and minimum temperatures taken over the last year. All the patients were initially admitted to the Phase I room. Here the temperatures were recorded with the monthly minimum and maximum staying relatively constant at 28 and 30°C respectively. The external shade temperatures obtained from the meteorological station were also used to compare with the mortality data. No significant correlation was found between mortality and either the indoor temperatures (r^2 = 0.1127) or with the external temperature(r^2 = 0.5223). The correlation plots are shown in Figure 3.4.

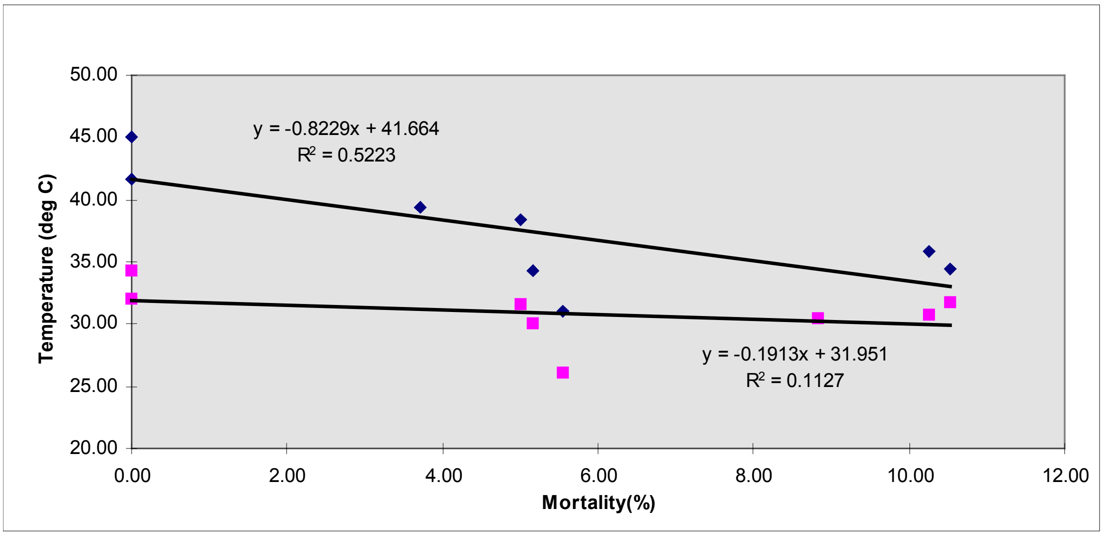

Figure 3.4 Relationship between environmental temperature and mortality

Pictures 11 to 14 show the weight change for two patients (both two years old) after treatment at the TFC.

Picture 11 Two year old malnourished patient 3 days after admission to TFC.

Picture 12 Same patient fully recovered after 20 days at TFC.

Picture 13 Two year old severely malnourished child 3 days after admission to TFC.

Picture14 Same patient just before discharge from the TFC, after 35 days treatment.

Chapter 4

Discussion

4.1 WATER REQUIREMENTS

Evaporative water loss is the only way of sustaining body temperature and maintaining water balance when environmental temperatures exceed that of the skin. Such condition occur regularly in regions such as Mao. The critical elements for coping in these conditions include physiological and behavioural (such as seeking shade cover, type of clothing worn, work performed etc.) responses. Children as mentioned in the introduction (Chapter 1) are at a disadvantage when it comes to responding appropriately to maintain homeostatic balance. Malnourished children in addition have disturbances in every physiological function that may be required for heat and water balance. Hence renourishing the children in these situations requires careful attention to not only their energy intake but also to the potential effects the diet may have on water requirements at these extreme temperatures.

This study examined whether the F100 milk given to the children at Mao TFC was sufficient to meet their water requirements. In extreme heat, there is a high water turnover, which imposes a major stress on the individual. This is mainly in the form of evaporative loss. The resulting high output of evaporative loss has to be matched by an equal or greater amount of water intake. Without replacement from dietary sources and/or metabolic water, life cannot be maintained. It was expected that if the F100 milk formula, given to the severely malnourished children in this study, was not meeting their water balance requirements, then they would show any one or combination of the following conditions:

- abnormally low TBW level with a high % turnover
- high evaporative water loss as sweat and IWL
- low volume of renal output with high osmolalities
- high skin and body temperatures
- dehydration
- signs of hypernatraemia/hyperosmolality.

The results obtained from this study indicate that the children indeed have high levels of evaporative water loss. This in turn, affects their water turnover rate (34%). Their TBW appears to be comparable with other studies on malnourished children (~71% of body weight). They have very high urine osmolalities (549mOsm/L) but their renal output appears to be average. Their skin and body temperatures are affected by ambient temperatures, but none of the children had fever (increased core temperature) during the study. Except for one case of clinical hypernatraemia, none of the children appeared to suffer from dehydration.

4.2 TOTAL BODY WATER

Heavy water was used to determine some of these influences on water requirements. The first determination from this was the total body water (TBW) of each of the children. TBW as a percentage of body weight (%TBW/Bwt) remains relatively stable in healthy individuals. In malnourished children, the changes associated with body composition means that TBW is also affected. Many studies on malnourished children have assessed TBW (Waterlow, 1992). They show that recovered and control subjects have approximately 63% of body weight consisting of water. However, in the malnourished state, reported values range from 60% to as much as 84% (Smith, 1960; Drinkman *et al*, 1965), with a mean of 71% from this study. Some of the studies with the highest values have been conducted on oedematous children (Smith, 1960). Nevertheless the general consensus is that %TBW/Bwt is about 10% higher in malnourished children than healthy individuals of similar age (Fjeld *et al*, 1989). Patrick *et al* (1960) concluded from analysing a number of these studies that during initial recovery there was a rise in %TBW/Bwt (possibly due to the deposition of glycogen in ICF), followed by a fall to normal levels as the weight of the patients increased to normal. If this is the case then most of the children in this study were recovering remarkably well. This is clearly shown in figure 4.1 which compares the TBW in subjects that were studied twice. Nearly all the levels in study 2 were lower than the earlier ones. The exception was child B, who was the most severely malnourished patient in the study, and whose %TBW/Bwt rose by 9%. This may be because he was in an earlier stage of catch-up growth when the second study was performed (Patrick *et al*, 1978). The fall in %TBW/Bwt in the children with oedema (H and J) did not fall more than any of the others. The formula of Friis-Hansen (1961) was also used to estimate TBW. He calculated that 1-2 year olds have approximately 58.7 %TBW/Bwt, which is in good agreement with other studies. The values obtained from this study correlates well with the predicted values of Friis-Hansen's model. This is not surprising as the formula uses the child's weight and height. Thin individuals from the formula have a higher % body weight as water.

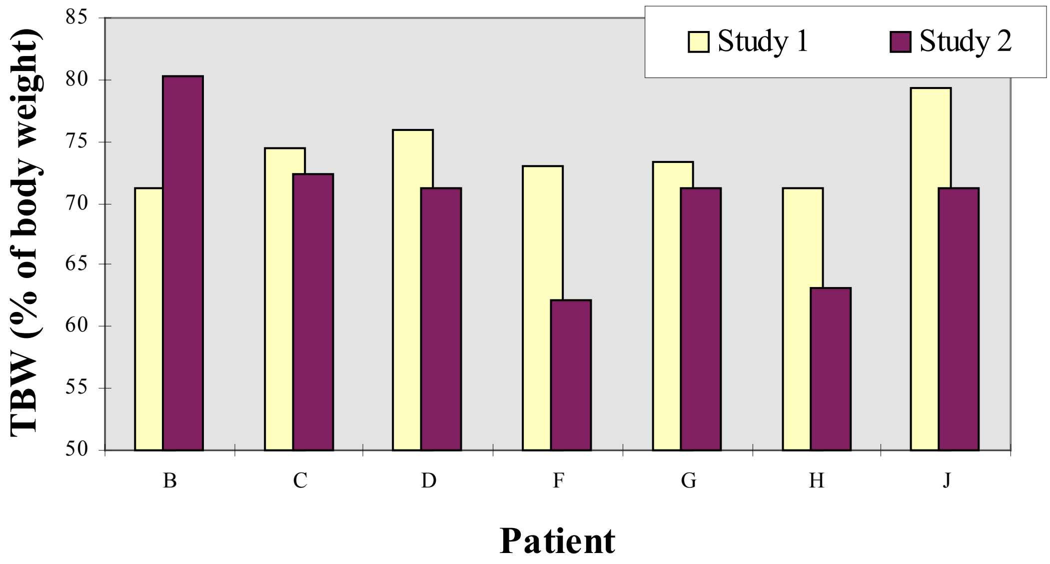

Figure 4.1 TBW as percentage of body weight in patients that were studied on two occasions. Patients H and J had oedema.

4.2.1 Errors in use of method

Even though equilibration of D_2O is assumed to be complete within 2 hours of the dose, the back extrapolation method to calculate TBW has been shown to produce values that agree with other techniques such as the Friis-Hansen (1961) model and the reference values of Fomon *et al* (1982). This is dependent on accurate dosing and minimisation of factors that might contribute to errors. Failure to consume all the weighed dose leads to a higher than expected TBW. This was suspected for six of the measurements in this study. On some occasions, the %TBW/Bwt was higher than actual body weight! The exchange of labile hydrogen with deuterium means that TBW, and hence turnover, are overestimated when using the dilution principle by about 1-5% (Leiper *et al*, 1999). In conditions of high ambient temperature, it is possible that isotopic fractionation may contribute to errors. In the dry heat of Mao, with relative humidity averaging 22% over the study period, the diffusion of water to the body is limited, but loss of D_2O may be a problem. If this is unduly affected, the concentration of heavy water remaining in the body, will lead to an underestimation of TBW. The calculations used have not been corrected for fractionation via evaporative loss in this study. Although evaporative loss was found to be very high, it was assumed that errors from spillage of the dose are the dominant errors in the measured TBW in the present study.

4.3 WATER TURNOVER

In this study the % mean turnover on a daily basis was 34%. This is very much higher than in other studies where turnover has been measured in children. However, there is no information from malnourished children exposed to such high temperatures to compare with this data. The errors introduced from TBW may also influence the water turnover expressed in absolute terms (i.e. as L/day but not when expressed as a fractional turnover rate). Nevertheless, there is a tendency for great inter and intra individual variation in turnover. There appears to be no significant difference ($p>0.05$) in the turnover of the subjects that were studied twice. The effects of ambient temperature may play a major part in the lower turnover levels obtained for healthy German children (Fusch *et al*, 1993). However the surface area of skin exposed to the environment may itself differ markedly with behavioural changes induced by environmental temperatures.

4.4 HIGH EVAPORATIVE WATER LOSS (EWL)

Nearly all children can concentrate their urine to 1000mOsm/L (Wrong, 1996) without water balance problems occurring. However this suggests a lack of water intake or a high output via ERL. From the turnover rates obtained it would seem unlikely that intake is a problem in this case. The turnover rates are high due to a high output via evaporative loss. Since this type of loss is unusual in other children (to this degree), it would appear to be related to the environmental temperature. Figure 4.2 shows a graph comparing average skin and environmental temperatures taken at noon with 7 day running average of the percentage water turnover. The turnover seems to be at its lowest when the temperature is also at its lowest.

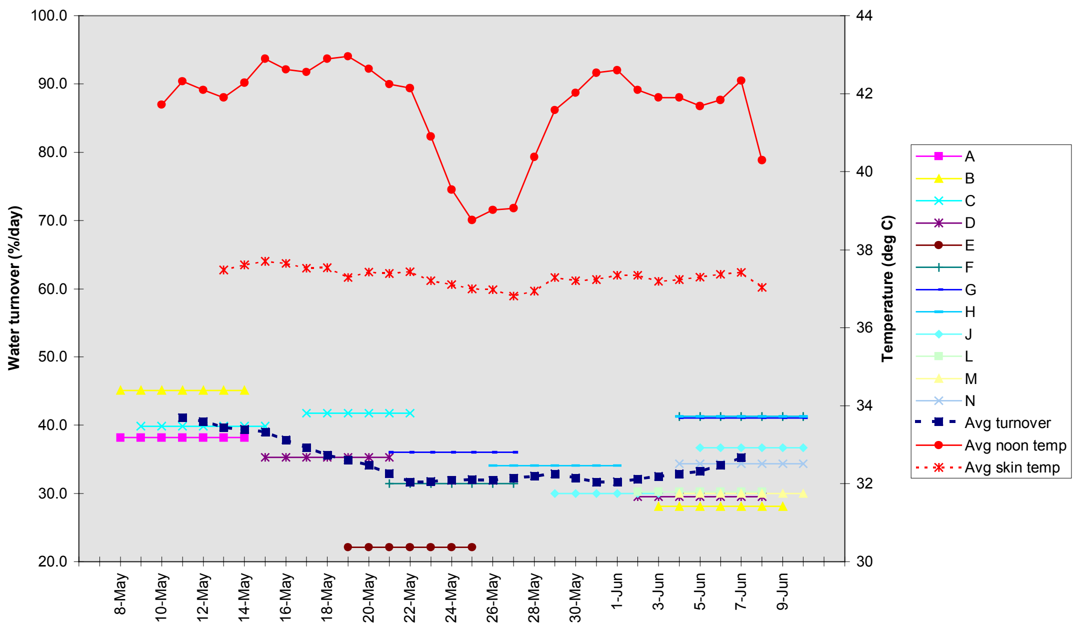

Figure 4.2 Relationship between skin and environmental temperatures in the afternoon with % turnover of water per day.

In full term infants, evaporative water loss ranges between 30-70 ml/kg/day (Bergman *et al* 1974). However, this has been shown by Darrow *et al* (1954) to be elevated by 50 to 100% when ambient temperature rises. The sa:wt ratio are even greater in malnourished children than they are in normal children and may exacerbate the convective/conductive heat gain from the environment. In a malnourished state, body temperature was has been shown to be more susceptible to ambient temperature Brooke *et al* (1974). They showed that malnourished children in Jamaica exposed to heat stress increased their rectal temperature at a rate of 0.75°C/hour compared to 0.34°C/hour when recovered. The chart in figure 4.3 shows the correlation between water turnover and surface area of children in the study (r = 0.7, p<0.01). There is an increasing turnover with SA.

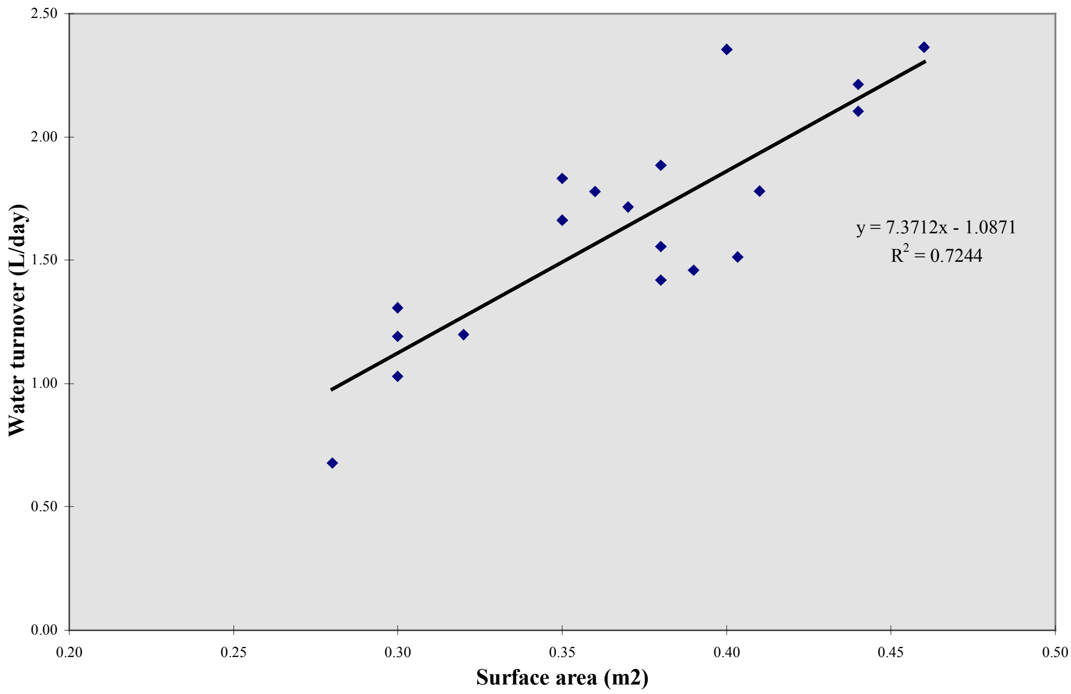

Figure 4.3 The relationship between water turnover and body surface area.

The effects of EWL are assumed to increase at a rate of 10% for each °C rise in body temperature. Correlation of mean skin and body temperatures of the children in this study was found with water turnover (figure 4.2). Evaporative water loss via the skin leads to a very high loss and affects fluid balance and temperature control (Rutter, 1996). Hence to calculate EWL, either water balance or energy balance may be used. EWL via the skin makes up two thirds of extrarenal losses and was calculated in this study in two very different ways. Firstly the difference between water turnover and urine volume taking into account retention from growth was used to calculate total evaporative water on the assumption that it makes up most of the extra renal losses (1.26L/day±0.42). Secondly the Wissler model was used to determine EWL (1.58L/day±0.31). A comparison of the patients who were studied twice showed that the Wissler equation was significant ($p<0.05$) but this was not the case with the first method. This may well be due to errors associated with the calculation (from dietary water intake and residual renal solute load).

4.5 RENAL OUTPUT

When fluid intake approximates that of requirement, water excreted as urine is determined by the solutes in the urine (rsl) and renal concentrating ability. Renal solute load is very important for the purposes of feeding malnourished children, as their impaired kidney function has been shown to have water and electrolyte disturbances (Alleyne, 1967). In a study by Alleyne (1967) the urinary concentrating capacities of 32 malnourished children were found to be impaired. On recovery all functions improved considerably. Hence, under conditions of high evaporative water loss, as in Mao, the F100 milk formula has to provide as low an osmolar load to the kidneys as possible. Most of the children in the study were found to have a high urine osmolality (549 ± 222). This shows that they were actively conserving water. Urine osmolalities in healthy infants have almost as wide a variation which develops to adult values (up to 1200 mOsm/kg) by the time they are 1 year old. In the patients studied there is evidence to suggest that they can concentrate their urine to high levels. It is not clear if this is due to lack of fluid intake, the effect of high evaporative water losses, or if it is their normal levels. Golden, (1998, unpublished) has made theoretical calculations which predict that F100 milk volumes with high renal solute load diminish the range of IWL that can be tolerated. Unlike breast milk, small changes in IWL requires greater urine osmolalities in order to maintain water balance. The mean urinary volume obtained was 0.37L ±0.19. However, this estimate does not take into account faecal water loss. Although faecal water loss has been demonstrated to be very low at about 10g/kg/day (Golden and Golden, 1981), excessive diarrhoea may greatly increase this volume. Water losses channelled through the high evaporative values (1.26L/day and 1.58L/day) seen in these children may explain the high osmolalities. Plasma osmolality is normally about 300mOsm/kg. If the children in the study have urine osmolalities of this order, then their mean urine output (isosthenuric water output) increases to 0.59L +/- 0.17. This would mean they require an additional 0.22L ± 0.19 to achieve isosthernuria.

4.6 CONCLUSIONS

The present study provides the first data to estimate the water requirements of malnourished children exposed to environmental temperatures above body temperature. The energy balance equations used by Wissler, with an assumption of a diffusive heat gain by the body of 7 Watts/m^2 ^oC temperature difference seem to provide a reasonable model for the measured turnovers. Therefore this approach can be used to estimate the water requirements for children exposed to different degrees of temperature stress, and diets modified accordingly to prevent dehydration or hypernatraemia.

References

Action Contre La Faim (ACF). Update on the nutritional protocols. December 1998.

Alleyne GAO. The effect of severe protein calorie malnutrition on the renal function of Jamaican children. Paediatrics, 1967; 39 (3): 400-411.

Almroth S and Bidinger P. No need for water supplementation for exclusively breast-fed infants under hot and arid conditions. Trans. Roy. Soc. Trop. Med and Hygiene 1990; 84: 602-604.

Ashraf RN, Jalil F, Aperia A and Lindbald BS. Additional water is not needed for healthy breast-fed babies in a hot climate. Acta Paediatr. 1993; 82: 1007-11.

Brinkman GL, Bowie MD, Friis-Hansen B and Hansen JDL. Body water composition in kwashiorkor before and after loss of edema. Pediatrics, 1965; 36 (1): 94-103.

Brooke OG. Influence of malnutrition on the body temperature of children. B. Med. J. 1972; 5 February.

Brooke OG and Salvosa CB. Response of malnourished babies to heat. Arch. Dis. Child. 1974; 49: 123-127.

Coile E, Ayoub E and Raile R. Hypertonic dehydration (hypernatremia): the role of feedings high in solutes. Pediatrics 1958; 22: 5.

Darrow DC, Cooke RE and Segar WE. Water and electrolyte metabolism in infants fed cow's milk mixtures during heat stress. Pediatrics, 1954; 14: 602-617.

Davies PSW and Wells JCK. Calculation of total body water in infancy. Eur. J. Clin. Nutr. 1994; 48: 490-495.

Dibley MJ, Goldsby JB, Staehling NW and Trowbridge FL. Development of normalized curves for the international growth reference: historical and technical considerations. Am. J. Clin. Nutr. 1987; 46: 736-748.

Du Bois D and Du Bois EF. A formula to estimate the approximate surface area if height and weight are known. Arch Intern Med 1916; 17: 863.

Fjeld CR, Schoeller DA and Brown KH. Body composition of children recovering from severe protein-energy malnutrition at rates of catch-up growth. Am. J. Clin. Nutr. 1989; 50: 1266-75.

Fomon SJ. Infant nutrition. 1974 W. B. Saunders Company, USA.

Freid LF and Palevsky. Hyponatraemia and Hypernatraemia. In The Medical Clinics of North America- Renal Disease Ed Saklayen MG. WB Saunders Co. USA, 1997; 81 (3) : p585-609.

Friis-Hansen B. Body water compartments in children: changes during growth and related changes in body composition. Pediatrics, 1961; 28 (2): 169-181.

Fusch C, Hungerland E, Scharrer B and Moeller H. Water turnover of healthy children measured by deuterated water elimination. Eur. J. Pediatr. 1993; 152: 110-114.

Getan and George Cancer Chemo. Res. 1970; 54: 225.

Gisolfi CV. Water requirements during exercise in the heat. In National Academy of Sciences: Nutritional needs in hot environments, National Academy Press, Washington D.C. USA, 1993; p87-96.

Golden BE and Golden MHN. Dietary zinc percentage output and composition of faeces. In trace element metabolism in man and animal 4 Ed Howell M, Gawthorne JM and White CL. Australian Arch Sci, 1981; p73-6.

Golden MHN. Theoretical model for the effects of insensible water losses on urine osmolalities for children on F100 milk formula. Unpublished 1998.

Halliday D and Miller AG. Precise measurement of total body water using trace quantities of deuterium oxide. Biomed Mass Spectrom 1977; 4: 82-7.

Haycock GB, Schwartz GJ and Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children and adults. J. Pediatrics, 1978; 93 (1): 62-66.

Leiper JB, Carnie A and Maughan RJ. Water turnover rates in sedentary and exercising middle aged men. Br. J. Sports Med. 1996; 30: 24-26.

Leiper JB, Maughan RJ and Prastowo SMP. Effect of fasting during Ramadan on water turnover in men living in the tropics. In press, 1999.

Logan RW. Fluid, electrolyte and acid-base disturbances. In Campbell AGM and McIntosh N (eds): Forfar & Arneil's Textbook of Pediatrics. Churchill Livingstone (division of Pearson Professional Ltd), 1998.

Lukaski HC and Johnson PE. 1985. A simple inexpensive method of determining total body water using a tracer dose of D_2O and infrared absorption of biological fluids. Am J Clin Nutr. 1985; 41: 363-70.

MacLennan AH, Millington G, Grieve A, McIntosh JEA, Seamark RF and Cox LW. Neonatal body water turnover: A putative index of perinatal morbidity. Am. J. Obstet. Gynecol. 1981; 139 (8): 948-952.

Maughan RJ. Fluid and electrolyte loss and replacement in exercise. In Williams C and Devlin JT (eds): Foods, nutrition and sports performance. E & FN Spon, 1992.

Open University. Science: A Third Level Course - Physiology of Cells and Organisms (12 - Thermoregulation in the new-born mammal. 13 - Adaptation to the external environment: temperature). The Open University Press, Milton Keynes, UK. 1974.

Patrick J, Reeds PJ, Jackson AA, Seakins A and Picou DIM. Total body water in malnutrition: the possible role of energy intake. Br. J. Nutr. 1978; 39: 417-424.

Prentice AT, Garmel GM and Auerbach PS. Environment-dependent sports emergencies. In The Medical Clinics of North America, WB Saunders Co, USA, Ed McKeag DB 1994; 78 (2): 305-25.

Rowland TW. Developmental exercise physiology. Human Kinetics, Champaign, IL, USA, 1996.

Rutter N. The immature skin. Eur. J. Pediatr. 1996; 155 (suppl 2): 818-820.

Salazar G, Infante C and Vio F. Deuterium equilibration time in infant's body water. Eur. J. Clin. Nutr. 1994; 48: 475-481.

Schoeller DA. Hydrometry. In: Roche AF, Heymsfield SB and Lohman TG (eds). Human Body Composition. Human Kinetics, USA, 1996.

Shapiro Y, Pandolf KB and Goldman RF. Predicting sweat loss response to exercise, environment and clothing. Eur J Appl Physiol 1982: 48: 83-96.

Smith R. Total body water in malnourished infants. Clin. Sci. 1960; 19: 275-285.

Taitz LS and Byers HD. High calorie / osmolar feeding and hypertonic dehydration. Arch. Dis. Child 1972; 47: 257.

Ultman, JS. Computational model for insensible water loss from the newborn. Pediatrics, 1987; 79 (5): 760-765.

UNICEF (United Nations Children's Fund). The state of the world's children (focus on nutrition). 1998.

Vander AJ, Sherman JH and Luciano DS. Human Physiology (the mechanisms of body function) - Chapter 16 'The kidneys and regulation of water and inorganic ions'. McGraw-Hill inc., USA, international edition, 1994.

Waterlow JC. Protein-energy malnutrition, 2nd ed. Edward Arnold (division of Hodder & Stoughton), 1992.

Weil WB and Bailie MD. Fluid and electrolyte metabolism in infants and children: A unified approach. 1977 Grune and Stratton Inc., USA.

Widdowson EM and Dickerson JWT. Chemical composition of the body. In Comar CL and Bronner F (eds): Mineral Metabolism Vol II Part A, New York, Academic Press, 1964 p1.

Wissler G. Model for evaporative water loss. Personal Communication, 1998.

Wrong O. Water and monovalent electrolytes. In Garrow JS and James WPT (eds): Human nutrition and Dietetics, Churchill Livingstone, 1996.

Ziegler EE and Fomon SJ. Fluid intake, renal solute load and water balance in infancy. J. Pediatr 1971; 78: 561-8.

Appendix A

ORIGINAL PROJECT PROTOCOL

Water requirements of malnourished children in extreme hot and dry environments.

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Prof. M. Golden (University of Aberdeen – supervisor)
Prof. R. Maughan (University of Aberdeen – supervisor)
Claudine Prudhon (ACF-Paris – supervisor)

This research will be carried out in Tchad and Aberdeen.

Introduction and background

The body requires a stable internal environment for integrity of normal processes despite exposure to extreme external conditions. Under normal temperatures, the body looses heat, produced metabolically, to the environment to maintain heat balance. In Sahelian countries such as Tchad, the temperatures in the dry hot season (April-June) often exceeds 40°C. Last year, it was as high as 47°C with a relatively low humidity of <15%. Under normal circumstances, heat is lost by conduction, convection, radiation and evaporation. When the environmental temperature is above skin temperature heat is actually gained by radiation, conduction and convection rather than lost, and the only mechanism for heat loss is by evaporation of water. This causes an additional requirement for water (to dissipate heat) above that needed for renal excretion of solutes (Weil and Bailie, 1977). The additional water required will be related to the amount of heat that is generated metabolically, which has to be disposed of and the rate of heat gain from the environment through radiation, conduction and convection. These are all related to the environmental temperature and humidity on the one hand and to the dietary intake, rate of growth, respiratory rate and fever on the other. The surface area:volume ratio of the person is related to the rate of gain or loss of heat, the metabolic heat production and the amount of water that is consumed in heat dissipation by evaporative loss. Thus, young children are particularly vulnerable to fluctuations in water requirements. In a malnourished child, there is also a relative increase in the body surface area to volume ratio, which exacerbates the situation. Furthermore, there is evidence of defective sweating response, control of peripheral blood flow, renal function and water absorption from the intestine, which will make the malnourished child particularly vulnerable to heat stress and water deficiency.

Heat balance becomes more of a problem if fluids are replaced by electrolyte containing solutions and pure water is lost from the skin and lungs. There must be sufficient water remaining to allow for renal excretion of the excess electrolyte. This has to be done without exceeding the child's capacity for renal solute concentration (Fomon, 1974). If insufficient water remains the electrolytes will be retained and the child will develop hyperosmolar dehydration. This condition occurred in Westernised countries in the 1950s and 60s when mothers made formula feeds that were too concentrated. It carries a particularly high mortality rate.

The diets used for the treatment of the severely malnourished were deliberately made as concentrated as possible. This was thought to be safe in order to promote rapid weight gain and recovery from wasting. Additionally, these diets have been formulated and tested in conditions of higher relative humidity and lower environmental temperatures than are encountered in the Sahel. Breast milk has 70 kcal/100 ml and is relatively low in electrolytes and protein. In comparison F75 and F100 used to feed malnourished children have 75 or 100 kcal/100 ml with a higher electrolytes and protein content. This raised the possibility that water is the limiting nutrient in Sahelian countries when the milk formulas are used as the diet to treat severe malnutrition. Similar considerations apply to Oral Rehydration Solutions.

That this is a practical problem emerged from analysis of data from therapeutic feeding centres (TFC) run by Action Contra la Faim (ACF) in Mali and Tchad, where higher than expected mortality occurred during the hot season last year. On clinical examination, a number of children in the TFC were found to be hypernatraemic. In these children the symptoms produced were doughy skin, loss of consciousness and convulsions, followed by death.

Models of water losses have been developed and have been used to determine water requirements (Shapiro *et al*, 1982; Ultman, 1987). These are based on measurements at moderate temperatures in healthy adults and in infants in incubators. However, the extent to which these models can be extrapolated directly to malnourished children or to more extreme environments is not clear, and direct measurements are necessary to test the applicability. Our collaborators have used Wissler's thermal-cardiovascular model, and the data of Rowland (1996), to compute the water requirements of a child weighing 10kg with a 35°C skin temperature at an environment temperature of 45°C. If radiation is assumed to be negligible then the rate of evaporative loss were calculated to be in the order of 1.1L per day. To this eleven percent of body weight would need to be added the water requirement for renal excretion of solute. In the context that 5% of water loss leads to dehydration and 10% to very severe dehydration requiring immediate resuscitation, such a calculation shows that the water requirement may be very considerable. If correct, it would mean a re-evaluation of the dilution of the milk formulas currently used and possible consideration of the renal solute load that these diets impose. The only way in which to determine if these computations are approximately correct is to make direct measurement of body water in a sample of children under these extreme conditions. These data will then be used to determine the water requirements and to recommend how the diet formulation should be adjusted to provide a margin of safety for the children.

Hence the aim of the study is to measure directly the water turnover in children in the TFC in Mao, Tchad using heavy water or deuterium oxide (D_2O). Heavy water behaves in the body exactly like normal water and is perfectly safe (Schoeller, 1996). The technique has been widely used to measure total body water and turnover in premature infants, young children, pregnant women, adults and the elderly (Maclennan *et al*, 1981; Davies and Wells, 1994; Leiper *et al*, 1996).

Hypothesis

The water requirements of malnourished children are not being met at extreme environmental temperatures (above 45°C) through their fluid intake from standard formula milk (F100) used to treat children in less extreme environments, and a more dilute formula is required.

To achieve this and other objectives, the following will be carried out;

- Measure directly the water turnover in a sample of children in the therapeutic feeding centre (TFC) in Mao, Tchad using heavy water.
- Measure body, skin and environmental temperatures and humidity to generate models to predict insensible water losses, sweat loss and renal concentrating ability in such patients.
- Using data gathered over the last year of maximum and minimum temperatures to assess the impact this has on mortality rates.

Design and Methods

Ethical approval will be sought from both the Ministry of Health in Tchad and the ethics committee in Aberdeen (Grampian Research Ethics Committee). Verbal consent will be obtained from the mother or guardian of each child after the project has been explained by a translator.

Water turnover

8 boys (used because of ease of urine collection) aged 6-59 months will be selected from the TFC and given the heavy water as soon as their condition has stabilised in phase 1. A further 8 boys will be measured at the start of phase 2. The study will not interfere in any way with the clinical management of the children. They will receive their normal milk-based diet every 4 hours. A baseline urine sample will be collected and then 1g of heavy water (99.8% D_2O) administered. This will be accurately weighed in Aberdeen, sealed into vials and taken to Tchad. The heavy water, approximately 0.1g/kg body weight, will be given directly into each child's mouth before the first feed of the morning of day 1. After administering the heavy water,

urine will be collected between 1-4 hours of equilibration. For the subsequent 6 days, where possible, urine will be collected at the same time in the morning. Urine will be collected directly into special bags which will be attached to the body by adhesive. Samples will be removed from the bags as soon as possible after micturition and stored in storage bottles. These will be stored away from direct heat and chlorhexidine added to prevent bacterial growth. They will be returned to the UK for laboratory analysis (vacuum distillation of each sample followed by infra-red spectroscopy).

The urine specific gravity will be measured by multistix and osmolality calculated. The sodium and potassium will be measured by flame photometry and urea by the nitroprusside reaction.

The water turnover will be repeated after the child has regained weight to be more than 85% weight for height.

Body conditions

Body temperature and skin temperature will be measured with a thermocouple thermometer twice daily.

Samples of sweat will be taken from each child during the study for measurement of sweat electrolyte concentration.

Dietary intake (recorded for each feed given by staff)

Environmental conditions

Maximum and minimum environmental temperature will be measured daily.

The ambient temperature and relative humidity will be measured twice daily.

Anthropometry

Age.

Weight in kg

Height/length in cm

MUAC

Temperature effects on mortality rates

These will be taken from the records at the TFC over the last two years.

Exclusions

Girls will be excluded from the study due to the difficulties of collecting urine without contamination from faeces. Any child who vomits or has severe diarrhoea within the equilibration time after dosing with heavy water.

Outcome measures and data analysis

The urine obtained from baseline and heavy water concentrations in the first morning sample will be used to calculate total body water and lean body mass. Daily water turnover will be estimated by using daily sample collections to obtain a semi-log plot. Linear decay rate can then be calculated from this.

Mean values will be obtained for body and environmental temperatures and used in model calculations of non-urine water losses. Since there are no models for malnourished children at these conditions, IWL will be calculated using models for new born babies as in Ultman, 1987.

Calculations of weight for height and weight gain over the study will be performed.

Anticipated Problems

In hot conditions sweat will be produced in great excess and the adhesives on the urine bags may become wet and detach. However if the bags are changed regularly and the area of attachment is thoroughly cleaned with soap and water and dried, this can be avoided.

Timings of urine collection are important and have to fit into the feeding regime already in place in the centre and also the micturition of the children.

If the children are dehydrated they will not be able to produce sufficient urine on a regular basis each day. The analysis requires a minimum of 5ml of urine per sample.

Storage and transportation of samples will have to be done with care to prevent bacterial growth and losses.

Potential benefits

It is hoped that direct measurements will give accurate water requirements at these conditions so that fluid intake for these children can be adjusted accordingly.

Equipment List

Most of these will already be in use in the TFC in Chad or available in Aberdeen.

Heavy water and tubes to store and transport them to Tchad
One use urine bags (~160)
24 hour urine bags (~ 60)
Electronic balance for weight of urine
Beakers
Dropping pipettes
Urine storage bottles (~180)
Preservative (Chlorhexidine)
Storage box/bag for samples
Filter paper
Polythene bags
Adhesive tape
Stadiometer
Salter scale/balance

Tape measure

Multistix for measuring specific gravity

Thermometer capable of measuring max and min

Hygrometer for humidity

Distillation apparatus

Infra-red spectrophotometer

Flame photometer

Protocol in English and French?

Headed letter explaining study in English and French?

Hardback note book for record keeping

References

- Davies PSW and Wells JCK. Calculation of total body water in infancy. Eur J Clin Nutr 1994; 48: 490-5.

- Fomon SJ. Infant nutrition. 1974 W. B. Saunders Company, USA.

- Leiper JB, Carnie A and Maughan RJ. Water turnover rates in sedentary and exercising middle aged men. Br J Sports Med 1996; 30: 24-6.

- Maclennan AH, Millington G, Grieve A, McIntosh JEA, Seamark RF and Cox LW. Neonatal body water turnover: A putative index of perinatal morbidity. Am. J. Obstet. Gynecol. 1981;139:948-52.

- Rowland TW. Developmental Exercise Physiology. 1996 Human Kinetics Illinois, USA.

- Schoeller DA. Hydrometry. Human Body composition. Ed: Roche AF, Heymsfield SB and Lohman TG. 1996, Human Kinetics, USA.

- Shapiro Y, Pandolf KB and Goldman RF. Predicting sweat loss response to exercise, environment and clothing. Eur J Appl Physiol 1982: 48: 83-96.

- Ultman JS. Computational model for insensible water loss from the newborn. Pediatrics 1987; 79: 760-5.

- Weil WB and Bailie MD. Fluid and electrolyte metabolism in infants and children:A unified approach. 1977 Grune and Stratton Inc, USA.

Appendix B

INSENSIBLE WATER LOSS MODEL

Calculation using Ultman's computational model (1987).

$$IWL = RWL + TWL$$

where:

$\log_{10}p_a = 7.09161 - 1668.21/ (228 + T_a)$

$\log_{10}p_s = 7.09161 - 1668.21/ (228 + T_s)$

$RWL = [0.411 + (0.000968*T_a)] - [(7.40*RH)*(p_a/(10133 - RH*p_a)$

$TWL = k(A/W) [p_s - (RH/100)p_a]$

$k = k_o \exp [9.119 - 2809/(273 + T_s)]$

$k_o = 1.5 [1 + (2*1.3 - 3) / (2 + PA)]$

where:

T_a and T_s are ambient and skin temperatures (^oC); RH, relative humidity (%); W, weight; PA ,postnatal age (days); IWL, RWL and TWL, respiratory, transepidermal water loss (g of water/h/kg). k, k_o, mass transfer coefficients (g/h/m2/kPa); A, surface area (m^2); ps, pa, equilibrium water vapour pressure (kPa) of p_s and p_a at T_s and T_a (kPa).

WATER TURNOVER CALCULATION

Example of calculation of water turnover using data from Salazar *et al*, 1994.

Child 1 (Boy 1)

where the following information was given by the authors:-

TBW from intercept method = 3.556L

Water for dose dilution (W_o) = 0.1583L

Dose (D) = 2177.3mg

Enrichment at time zero = E_o

$$TBW = (D/E_o) - W_o$$

Hence $E_o(ppm) = 2177.3/(3.556 + 0.1583) = 586ppm$

The concentration of D_2O was estimated from the graph at times (t = 200, 400, 600 and 950 mins). The average of these values was used in the turnover comparisons.

E.g.

At t = 600mins (0.416 days) E(t) = 530ppm obtained from graph.

$$\text{Hence } E_{(t)} = E_o \exp^{(-kt)}$$

$$(E_{(t)} / E_o) = \exp^{(-kt)}$$

$$\mathrm{Ln}\,(E_{(t)} / E_o) = -kt$$

$$k = [-\mathrm{Ln}\,(E_{(t)} / E_o)] / t$$

$k = [-\mathrm{Ln}\,(530 / 586)]/0.416 = 0.24$.

The table overleaf shows all the calculations for each of the 4 boys in the study.

Appendix C Raw data

TEMPERATURE AND HUMIDITY DATA

SUBJECT DATA

ANTHROPOMETRIC CALCULATIONS

Appendix D - Calculations

LABORATORY ANALYSIS DATA

ENRICHMENT PLOTS (NORMAL)

ENRICHMENT PLOTS (NATURAL LOG)

EVAPORATIVE LOSS CALCULATION TABLES

EVAPORATIVE LOSS CALCULATION USING WISSLER MODEL

URINE ELECTROLYTES AND OSMOLALITY

ENVIRONMENTAL TEMPERATURE V MORTALITY DATA