The effect of environmental temperature on prandial changes in leucocyte sodium transport in man

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Cellular sodium transport via the Na^+, K^+-ATPase contributes significantly to daily energy expenditure. The effect of a meal on leucocyte Na^+ transport and intracellular electrolytes was therefore investigated in lean normal subjects at room temperatures of 23° and 33°, to determine if the Na pump responds to the need for thermogenesis. In the fasting state, the ouabain-sensitive efflux rate which reflects active Na^+ transport, and the intracellular electrolytes were similar. At 2 h after eating a 4·2 MJ (1000 kcal) meal, the ouabain-sensitive efflux rate constant rose when the room temperature was 23° but not at 33°. The ouabain-sensitive Na^+ efflux rate, an index of active Na^+ transport, rose post-prandially at 23° only. The post-prandial activation of leucocyte active Na efflux in normal subjects was blunted at higher environmental temperatures, when the need for thermogenesis was reduced.

ATPase: Environmental temperature: Leucocytes: Sodium transport

The maintenance of high intracellular potassium and low intracellular sodium concentrations is dependent on Na^+,K^+-ATPase, and this may contribute a variable proportion of resting energy expenditure in man (Ismail-Beigi & Edelman, 1970; Chinet *et al.* 1977; Swaminathan *et al.* 1982). The thermic effect of food and exercise constitutes the remainder of total daily energy expenditure (James & Trayhurn, 1976). Previously, we reported that there was a post-prandial activation of leucocyte active Na efflux in lean humans that was reduced in obesity (Ng *et al.* 1987), and proposed that this may be due to the insulin insensitivity of the obese. If Na transport contributes to dietary thermogenesis, its response to a meal may depend on environmental temperature, for at higher temperatures, the need for thermogenesis would be reduced (Dauncey, 1981) and there may be less activation of active Na efflux post-prandially. In the present study, the effect of a meal on leucocyte Na^+ transport and electrolyte content was, therefore, studied in a group of lean, normal weight humans at two different environmental temperatures, 23° and 33°, to examine the dependency of the fasting Na^+ pump activity and its response post-prandially on the ambient temperature.

SUBJECTS AND METHODS

Materials

Tissue culture medium (TC199) was obtained from Wellcome Diagnostics, Beckenham, Kent, ^{22}Na from Amersham International plc, Amersham, Bucks and ouabain from the Sigma Chemical Co, Poole, Dorset. All other chemicals were purchased from BDH Chemicals Ltd, Poole, Dorset.

Subjects

Ten lean, normal weight subjects (six males, four females) were studied. Their mean age was 47·5 (SE 4·1) years and mean body mass index (weight/height^2) was 22·4 (SE 0·5) kg/m^2. All

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were normotensive and euthyroid, and had no endocrine, metabolic or electrolyte disorders. None were on any medication including the oral contraceptive pill. The study was approved by the Oxford ethics committee.

Subjects arrived at the temperature-controlled laboratory after an overnight fast. The temperature was set at 23° or 33°, and the order of tests on the same subject was randomized. All subjects were lightly clothed in cotton vests and shorts during the studies at both temperatures. A venous cannula was sited and they rested for 1 h at the experimental temperature before any blood sampling. Blood (40 ml) was taken from fasting subjects for plasma insulin, glucose, non-esterified fatty acids, Na^+, K^+ and leucocyte studies.

A 4·2 MJ (1000 kcal) test meal composed of (g/kg) 150 protein, 300 fat and 550 carbohydrate was eaten within 15 min. Blood samples were withdrawn half-hourly, and at 2 h leucocyte Na^+ transport studies were repeated. After 1 week, the same subject was retested with a similar meal at a different room temperature.

Leucocyte studies

The leucocytes were isolated by dextran sedimentation as described previously (Ng *et al.* 1987). Cells were loaded with ^{22}Na in TC199 (final specific activity 1110 MBq/l) at 37° for 0·5 h. The leucocytes were then washed once in 20 ml TC199, a procedure that removes 99% of the extracellular ^{22}Na. ^{22}Na efflux was then studied in TC199 at 37° determined over 20 min in the presence and absence of ouabain (Ng *et al.* 1987). Efflux rate constants for Na (ERC;/h) were determined by plotting $\log_e$ cellular radioactivity *v.* time, all determinations having regression coefficients greater than 0·99. The ouabain-sensitive ERC or the fractional turnover rate of intracellular ^{22}Na, represents the active component of Na efflux due to the Na pump, and was calculated as the difference between total and ouabain-insensitive ERC. The coefficient of variation for the ouabain-sensitive ERC in the same subject on different days was 7%.

The other half of the leucocyte pellet was used for determination of intracellular Na and K and was treated identically and concurrently as the half used for ^{22}Na efflux studies so that intracellular electrolyte composition would be similar. The leucocytes were incubated in TC199 at 37° for 0·5 h before centrifuging the cells down and washing them three times in cold (4°) isotonic magnesium chloride buffer ($MgCl_2$ 95 mmol/l, Tris base 5 mmol/l, pH 7·4). This 0·5 h incubation in TC199 allowed the intracellular electrolyte composition to stabilize, for cells isolated by dextran sedimentation at room temperature initially have a high intracellular Na content which falls after 0·5 h at 37° (Simon, 1989). The dry weight of the pellets was determined by heating them in an oven at 80° for 24 h. Nitric acid (1 mol/l) was used to extract the dried pellets, and Na and K measured by flame photometry (Ng *et al.* 1987). The coefficients of variation for intracellular Na and K were 12 and 10% respectively. Na efflux rates (expressed as mmol/kg dry weight per h) were calculated as follows:

$$\text{Na efflux rate} = {}^{22}\text{Na ERC} \times \text{intracellular Na}.$$

The ouabain-sensitive efflux rate is a measure of actual flux of Na through the cellular Na pump.

Other assays

Plasma insulin was measured by radioimmunoassay, glucose by a glucose oxidase (*EC* 1.1.3.4) method and non-esterified fatty acid by the acyl-CoA synthetase (*EC* 6.2.1.3) method (Shimizu *et al.* 1979). Electrolytes were measured on a flame photometer.

Statistics

Leucocyte values at the two different temperatures were compared with a paired Student's *t* test using the Oxstat statistics package (Microsoft Ltd, Reading, Berks) as intracellular Na, ERC and efflux rates show a Gaussian distribution (Ng *et al.* 1987). Plasma glucose, insulin, Na, K, and non-esterified fatty acid concentrations at the two different temperatures were analysed on the Oxford University VAX cluster, using the Statistical Package for the Social Sciences (Microsoft Ltd). A two-factor repeated measures design for analysis of variance was used with temperature and time as within-subject factors. Means with their standard errors are reported, and P values are two tailed and considered significant if $P < 0{\cdot}05$.

RESULTS

The fasting intracellular K and Na concentrations were similar at the two temperatures (Table 1). At 2 h post-prandially, there was a significant rise in both total and ouabain-sensitive ERC at 23° (Table 1). In the same subjects, the same meal at 33° failed to raise total, ouabain-sensitive and ouabain-insensitive ERC significantly (Table 1). However, intracellular Na rose post-prandially by about the same amount at both temperatures (Table 1). When the total, ouabain-insensitive and ouabain-sensitive Na^+ efflux rates were calculated, the fasting ouabain-insensitive efflux rates were similar at 33° and 23° (Table 1). The total and ouabain-sensitive efflux rates at 33° were higher than those at 23°, but these differences did not reach conventional levels of statistical significance. However, there was a significant post-prandial rise in the total and ouabain-sensitive efflux rates at 23° ($P < 0{\cdot}001$, Table 1) but not at 33°. The post-prandial levels were the same at the two temperatures. Intracellular K levels were unchanged by the meal at both temperatures.

Table 2 shows that the plasma insulin, glucose and non-esterified fatty acid levels changed with time after feeding at both temperatures ($P < 0{\cdot}0001$, by analysis of variance). There were no significant differences in the response of plasma insulin or non-esterified fatty acid concentrations at 23° or 33°. However, glucose levels were significantly higher at 33° than 23° ($P < 0{\cdot}002$, by analysis of variance). Plasma Na^+ and K^+ were not different at the two temperatures (Table 2).

DISCUSSION

Endothermy may depend on a 'futile' cycle of Na^+ entering cells and subsequently being actively expelled by the Na^+K^+-ATPase, using up ATP in the process (Ng *et al.* 1987). We have previously demonstrated a post-prandial activation of leucocyte Na^+ efflux via the Na pump in lean subjects (Ng *et al.* 1987) and demonstrated a similar increase after intravenous infusion of insulin when plasma glucose concentration was kept normal. This stimulatory effect has also been shown for oral glucose (Ng & Hockaday, 1987; Turaihi *et al.* 1988*a*) in normal subjects. In obesity, the fasting leucocyte Na pump activity is raised (Turaihi *et al.* 1987). Furthermore, the activation of cellular active Na efflux post-prandially or by insulin infusion in an euglycaemic clamp is much reduced in obesity, probably due to insulin resistance (Ng *et al.* 1987), and this may be a possible contributor to energetic efficiency in obesity. In contrast, Turaihi *et al.* (1988*b*) found Na pump activity in anorexia nervosa to be lowered. The Na pump activity is thus dependent on the subject's state of nutrition.

Dauncey (1981) had shown that resting energy expenditure was significantly higher at 22° when compared with 28°, and that after a 2·6 MJ liquid meal, energy expenditure may remain elevated for a longer period at 22° than at 28°. We, therefore, investigated the effect of feeding a large meal on leucocyte active Na efflux at two different temperatures: (1) at

Table 1. *Leucocyte ^{22}Na efflux rate constants (ERC), intracellular electrolytes and Na^+ efflux rates in ten normal subjects before and 2 h after a 4·2 MJ meal at 33° and 23°*

(Mean values with their standard errors)

Ambient temperature...		33°		23°	
		Preprandial	Post-prandial	Preprandial	Post-prandial
ERC (/h)					
Total	Mean	3·37	3·31	3·08	3·32**
	SE	0·12	0·18	0·15	0·15
Ouabain-insensitive	Mean	0·65	0·61	0·68	0·61
	SE	0·03	0·05	0·06	0·05
Ouabain-sensitive	Mean	2·73	2·70	2·39	2·71***
	SE	0·10	0·16	0·11	0·13
Electrolytes (mmol/kg dry wt)					
Intracellular Na	Mean	346	376	326	340
	SE	22	17	19	13
Intracellular K	Mean	34·5	43·7*	31·0	39·5**
	SE	3·8	4·8	3·3	2·9
Efflux rates (mmol/kg dry wt per h)					
Total	Mean	117	148	96	130***
	SE	15	21	12	10
Ouabain-insensitive	Mean	22	26	21	24
	SE	2	3	3	2
Ouabain-sensitive	Mean	96	122	75	106***
	SE	13	19	10	9

Mean values at 2 h after the meal were significantly different from pre-meal levels (Student's t test): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

23°, which is at the lower limit of the thermoneutral zone for both lean and obese subjects (Blaza & Garrow, 1983) and (2) in a warm (33°) environment where the need for thermogenesis may be much reduced. A difference in response of the cellular Na^+ pump at the two different temperatures may be apparent if the Na pump contributes significantly to thermogenesis, as suggested by previous workers (Ismail-Beigi & Edelman, 1970; Chinet *et al.* 1977; Swaminathan *et al.* 1982). At 23°, a significant post-prandial rise in the active cellular Na^+ efflux rate was found, whereas at 33° where post-prandial heat generation would be superfluous, the meal failed to elicit this cellular Na^+ pump response. Therefore, the thermic effect of food attributable to the change in activity of the Na pump may be reduced at a higher ambient temperature, where a rise in Na pump activity would have been inappropriate. However, in the fasting state, the active component of cellular Na^+ efflux (the ouabain-sensitive efflux rate) was higher at 33° than at 23°, though this did not reach conventional levels of statistical significance, perhaps because of the small number of subjects studied. This raised fasting active Na^+ efflux rate could have blunted any further prandial rise of the efflux rate at 33°, and may suggest that factors controlling the basal preprandial and post-prandial Na^+ efflux rates may differ at the two temperatures studied.

The rise in intracellular Na post-prandially at both ambient temperatures may be due to an increased cellular Na^+ influx, but this was not directly measured in the present study. The increased Na^+ influx could be due to the rise in plasma insulin levels, for Rosic *et al.* (1985) found an increased Na^+ influx via the Na^+/H^+ antiport with secondary activation of the Na^+,K^+-ATPase in skeletal muscle after treatment with insulin.

Table 2. *Plasma glucose, insulin, non-esterified fatty acids (NEFA) and electrolytes after a 4·2 MJ meal at 33° and 23°*
(Mean values with their standard errors)

Time interval (min)...		0		30		60		90		120	
	Temp.(°)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NEFA	33	0·37	0·11	0·16	0·09	0·12	0·07	0·15	0·09	0·15	0·08
(mmol/l)	23	0·31	0·13	0·18	0·09	0·14	0·06	0·16	0·10	0·12	0·06
Glucose	33	5·1	0·4	8·5	1·5	7·3	1·5	6·3	0·7	5·9	0·5
(mmol/l)	23	4·9	0·4	5·9	1·7	5·3	1·4	5·3	0·8	5·2	0·8
Insulin	33	6·7	2·8	88·6	36·4	74·9	36·2	47·9	16·7	38·4	16·3
(mU/l)	23	5·3	2·0	70·0	30·9	54·8	28·2	41·8	20·9	37·4	19·7
K^+	33	3·9	0·3	—	—	4·0	0·3	—	—	4·1	0·3
mmol/l)	23	3·9	0·3	—	—	3·9	0·3	—	—	4·0	0·2
Na^+	33	137	4	—	—	138	4	—	—	138	4
(mmol/l)	23	138	3	—	—	139	4	—	—	139	4

The difference in response to feeding of the leucocyte Na pump at the two temperatures was not attributable to differences in the post-prandial changes of plasma non-esterified fatty acids which are known inhibitors of Na transport (Ng & Hockaday, 1986, 1988). Electrolyte changes were also not contributory. However, although insulin responses were similar, the glucose levels were significantly higher at all time-points after the meals given at 33° (Akanji *et al.* 1987). This may indicate 'arterialization' of venous blood at 33° (Frayn *et al.* 1989), though an alternative explanation may be reduced insulin sensitivity. If the latter were true, it may explain the lack of prandial activation of the leucocyte Na pump at 33°, as we have previously demonstrated that the response was reduced in insulin-resistant obese subjects infused with intravenous insulin and glucose (Ng *et al.* 1987).

In conclusion, the present study and those that examined nutritional influences on the leucocyte Na pump (Ng *et al.* 1987; Ng & Hockaday, 1987; Turaihi *et al.* 1987, 1988*a*,*b*) illustrate the adaptive nature of Na^+,K^+-ATPase to changes in nutrition and ambient temperature. The post-prandial response of the cellular Na pump to a 4·2 MJ meal was blunted at 33° when compared with the same lean subjects at 23°, suggesting that this response of the Na pump to feeding may adapt to the need for thermogenesis. At present, the mechanism of this adaptation is uncertain, although one possibility is a reduced sensitivity to the action of insulin at higher environmental temperatures.

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REFERENCES

Akanji, A. O., Bruce, M., Frayn, K., Hockaday, T. D. R. & Kaddaha, G. M. (1987). Oral glucose tolerance and ambient temperature in non-diabetic subjects. *Diabetologia* **30**, 431–433.

Blaza, S. & Garrow, J. S. (1983). Thermogenic response to temperature, exercise and food stimuli in lean and obese women, studied by 24 h direct calorimetry. *British Journal of Nutrition* **49**, 171–180.

Chinet, A., Clausen, T. & Girardier, L. (1977). Microcalorimetric determination of energy expenditure due to active sodium–potassium transport in the soleus muscle and brown adipose tissue of the rat. *Journal of Physiology* **265**, 43–61.

Dauncey, M. J. (1981). Influence of mild cold on 24 h energy expenditure, resting metabolism and diet-induced thermogenesis. *British Journal of Nutrition* **45**, 257–267.

Frayn, K. N., Whyte, P. L., Benson, H. A., Earl, D. J. & Smith, H. A. (1989). Changes in forearm blood flow at elevated ambient temperatures and their role in the apparent impairment of glucose tolerance. *Clinical Science* **76**, 323–328.

Ismail-Beigi, G. & Edelman, I. S. (1970). Mechanism of thyroid calorigenesis: role of active sodium transport. *Proceedings of the National Academy of Sciences, USA* **67**, 1071–1078.

James, W. P. T. & Trayhurn, P. (1976). An integrated view of the metabolic and genetic basis for obesity. *Lancet* **ii**, 770–773.

Ng, L. L., Bruce, M. A. & Hockaday, T. D. R. (1987). Leucocyte sodium pump activity after meals or insulin in normal and obese subjects: cause for increased energetic efficiency in obesity? *British Medical Journal* **295**, 1369–1373.

Ng, L. L. & Hockaday, T. D. R. (1986). Non-esterified fatty acids may regulate human leucocyte sodium pump activity. *Clinical Science* **71**, 737–742.

Ng, L. L. & Hockaday, T. D. R. (1987). The effect of oral glucose on the leucocyte sodium pump in normal and obese subjects. *Clinical Endocrinology* **27**, 345–353.

Ng, L. L. & Hockaday, T. D. R. (1988). The effect of Intralipid infusion on the human leucocyte sodium pump in vivo. *British Journal of Nutrition* **60**, 49–55.

Rosic, N. K., Standaert, M. L. & Pollet, R. J. (1985). The mechanism of insulin stimulation of (Na^+,K^+)-ATPase transport activity in muscle. *Journal of Biological Chemistry* **260**, 6206–6212.

Simon, G. (1989). Is intracellular sodium increased in hypertension? *Clinical Science* **76**, 455–461.

Shimizu, S., Inoue, K., Tani, Y. & Yamada, H. (1979). Enzymatic microdetermination of serum free fatty acids. *Analytical Biochemistry* **98**, 341–345.

Swaminathan, R., Burrows, G. & McMurray, J. (1982). Energy cost of sodium pump activity in man: an in-vivo study of metabolic rate in human subjects given digoxin. *IRCS Clinical Biochemistry* **10**, 949.

Turaihi, K., Baron, D. N. & Dandona, P. (1987). Increased leucocyte Na-K ATPase in obesity: reversal following weight loss. *Metabolism* **36**, 851–855.

Turaihi, K., Baron, D. N. & Dandona, P. (1988*a*). Effect of glucose intake on ^{86}Rb influx and [^{3}H]ouabain binding. *Metabolism* **37**, 171–174.

Turaihi, K., D'Souza, V., Wakeling, A. & Dandona, P. (1988*b*). Diminished [^{3}H]ouabain binding and ^{86}Rb influx by leucocytes in anorexia nervosa. *Metabolism* **37**, 486–490.

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