CLINICAL RESEARCH

Leucocyte sodium pump activity after meals or insulin in normal and obese subjects: Cause for increased energetic efficiency in obesity?

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Abstract

As cellular sodium pumping is an energy consuming process and differences in the obese may account for their energetic efficiency, leucocyte sodium-22 efflux was studied in obese and normal volunteers both in the fasting state and after a test meal or infusion of glucose and insulin intravenously. The ^{22}Na ouabain sensitive efflux rate constant was significantly higher in obese subjects than normal (mean (1 SD) 2.69 (0.40)/h v 2.35 (0.49)/h). Two hours after a 4.2 MJ (1000 kcal) meal there was an increase in the efflux rate constant from its fasting value in normal weight subjects (2.39 (0.33)/h to 2.71 (0.40)/h) but not in obese subjects (2.65 (0.54)/h to 2.61 (0.58)/h). The rise in ouabain sensitive efflux rates was significantly higher in normal than obese subjects. Both groups showed a rise in intracellular sodium concentrations. The euglycaemic clamp produced similar results.

Feeding or infusion of insulin increases sodium pump activity more in normal than obese subjects. This difference may contribute to any defective dietary thermogenesis in obesity, which may lead to energetic efficiency and a tendency to gain weight.

Introduction

In eukaryotic cells the maintenance of sodium and potassium concentrations against their electrochemical gradients entails the active transport of these ions by the sodium pump. Ob/Ob mice have reduced sodium pump activity in liver and muscle compared with their lean counterparts,^{1,3} and a reduction in this portion of energy expenditure may account for the positive energy balance of Ob/Ob mice. Sodium pump activity has been estimated to contribute up to 40% of energy expenditure at rest in man.^{4,6} Energy

expenditure at rest may account for up to 40% of man's total daily energy expenditure,⁷ the other contributory factors being the thermic effects of food and exercise. A defect in dietary thermogenesis has been reported in obesity,⁸⁻¹¹ and obese people may also show a reduced thermic response to a glucose and insulin infusion¹² that possibly is associated with their insulin resistance.

The purpose of this study was to examine leucocyte sodium pump activity in man. Initial reports of reduced sodium pump activity in erythrocytes from obese subjects compared with normal¹³⁻¹⁵ were not confirmed by later work.^{16,17} The nucleated leucocyte should be more representative of other cells in the body. Leucocyte sodium pump activity was examined in normal weight and obese volunteers at rest, after feeding, and in response to an intravenous glucose and insulin infusion.

Materials, subjects, and methods

All chemicals were Analar. Sodium-22 was from Amersham International, TC 199 (tissue culture fluid) from Wellcome Diagnostics, and ouabain from the Sigma Chemical Co.

SUBJECTS

Fifty volunteers of normal weight (22 men, 28 women; mean age 32.9 (SD 11.4) years; mean body mass index (weight (kg)/height (m) squared) 21.9 (SD 1.9)) and 24 obese subjects (eight men, 16 women; mean age 43.9 (11.9) years; mean body mass index 34.0 (6.5)) were recruited in answer to an advertisement. All were normotensive, and none had any known metabolic, endocrine, or electrolyte disorder. In particular, all subjects were euthyroid. Premenopausal women were studied in the follicular phase. No subject was receiving any drugs, including oral contraceptives. In all cases weight had been stable for at least six months before the study, and none of the obese subjects was taking a weight reducing diet. A dietary survey in 11 normal and 14 obese subjects showed comparable dietary carbohydrate intakes of 225 (SD 66) and 245 (70) g/day, respectively, which were maintained up to the study days. Blood pressure was measured in the right arm in supine subjects after 30 minutes' bed rest using a Hawksley random zero sphygmomanometer by one operator and the average of three readings taken. All subjects gave informed consent and the study was approved by the Oxfordshire ethical committee.

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BLOOD SAMPLES

Subjects were studied after a 12 hour overnight fast and 60 ml blood taken after 30 minutes' bed rest for plasma insulin, glucose, urea, and electrolyte determinations and leucocyte electrolyte and ^{22}Na efflux studies.

TEST MEAL

Thirteen obese subjects (five men, eight women; mean age 42.9 (13.3) years; mean body mass index 32.0 (3.9)) and 11 normal weight subjects (four men, seven women; mean age 45.5 (13.9) years; body mass index 22.1 (1.6)) were given a test meal of 4.2 MJ (1000 kcal) composed of 15% protein, 30% fat, and 55% carbohydrate. Subjects fasted overnight for 12 hours. A venous cannula was inserted in the antecubital fossa on arrival at the laboratory. After an hour's bed rest four fasting blood samples were taken for insulin, glucose, and non-esterified fatty acid determinations. The meal was consumed within 15 minutes later and blood samples taken at half hour intervals thereafter for two and a half hours. A 40 ml blood sample was taken immediately before and two hours after the meal for leucocyte ^{22}Na efflux and intracellular electrolyte studies.

Body mass index, fasting plasma insulin concentrations, and leucocyte parameters in normal weight and obese subjects. Values are means (SD in parentheses)

	Normal weight group			Obese group		
	Men (n=22)	Women (n=28)	All (n=50)	Men (n=8)	Women (n=16)	All (n=24)
Body mass index (kg/m ²)	22.7 (1.6)	21.4 (1.9)	21.9 (1.9)	32.9 (5.5)****	34.5 (7.1)****	34.0 (6.5)****
Fasting insulin (mU/l)	7.8 (3.1)	3.2 (3.7)	7.4 (3.4)	24.6 (17.9)*	15.6 (8.9)****	18.6 (13.9)****
Leucocyte parameters:						
Intracellular sodium (mmol/kg)	22.1 (5.4)	33.6 (9.4)††††	27.9 (9.5)	22.5 (5.6)	28.9 (6.2)†	26.5 (6.6)
Ouabain sensitive efflux rate constant (/h)	2.42 (0.5)	2.29 (0.49)	2.35 (0.49)	2.61 (0.3)*	2.84 (0.54)**	2.69 (0.4)***
Ouabain sensitive efflux rate (mmol/kg/h)	55.4 (17.5)	82.8 (27.5)†††	69.1 (26.6)	68.6 (27.1)	75.1 (18.9)	72.7 (21.7)

Obese v normal weight subjects: *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.
Women v men: †p<0.05, ††p<0.01, †††p<0.005, ††††p<0.001.

HYPERINSULINAEMIC-EUGLYCAEMIC CLAMP

Eleven obese subjects (five men, six women; mean age 42.3 (13.1) years; mean body mass index 31.2 (2.7)) and 10 normal weight subjects (five men, five women; mean age 43.2 (16.7) years; mean body mass index 22.3 (2.1)) were studied by the euglycaemic clamp technique. All the obese subjects and six of the normal subjects had also been given the test meal.

The method was based on the minimal model of Bergman *et al.*¹⁸ Volunteers reported to the laboratory after an overnight fast and had two venous cannulae inserted, one in each arm, for blood sampling and for the infusion of insulin and 20% dextrose. The distal arm vein used for blood sampling was kept warm by a fan heater. After one hour's rest fasting blood samples were taken for plasma insulin, glucose, non-esterified fatty acid, and leucocyte studies. A primed continuous infusion of human insulin (Eli Lilly) was then started at a rate of 40 mU/m² body surface area/min and blood samples taken every five minutes for glucose measurement on a Beckman glucose analyser. The plasma glucose value was entered into a computer algorithm¹⁸ and the infusion of 20% dextrose altered accordingly to keep the value constant. The glucose infusion rate reached a steady state by 90 minutes, and the 90-120 minute infusion rate was used as a measure of whole body insulin resistance. The plasma glucose concentration during this period had a coefficient of variation of under 5%. This infusion rate divided by the plasma glucose concentration gives the metabolic clearance rate of glucose (ml/kg/min). Blood samples were collected at half hour intervals during the clamp for estimation of electrolyte, insulin, and non-esterified fatty acid values. In addition, plasma insulin concentrations were determined every five minutes from 90 to 120 minutes. Leucocyte efflux and intracellular electrolyte values were measured at rest and at 120 minutes.

LEUCOCYTE STUDIES

 ^{22}Na Efflux determinations

^{22}Na Efflux rate constant determinations were as described.¹⁹ Briefly, leucocytes were separated from 40 ml blood by dextran sedimentation.²⁰ Half of the leucocytes were used for ^{22}Na efflux studies. These leucocytes were loaded with ^{22}Na in TC 199, specific activity 1110 MBq/l, and after centrifugation the pellet washed once in TC 199. Efflux was measured in TC 199 at 37°C with and without ouabain 100 µmol/l, aliquots of cell suspension

being spun down every four minutes for 20 minutes. The radioactivity from the cell pellets was counted on a gammacounter till more than 10 000 counts above background were recorded. The total and ouabain insensitive efflux rate constants were determined by linear regression of log_e cellular radioactivity against time. The ouabain sensitive efflux rate constant is the difference between the two and represents sodium pump activity.²¹ The coefficients of variation (by the method of duplicates, where coefficient of variation=(sum of squared differences/2n)^{0.5}/mean) for 13 subjects on two different days one week apart were 13.3% (ouabain insensitive efflux rate constant) and 7% (ouabain sensitive efflux rate constant). There is no difference in ouabain sensitive efflux rate constants between polymorphonuclear and mononuclear leucocytes.²²

Intracellular electrolytes

The other half of the isolated leucocyte pellet was resuspended in TC 199 and kept at 37°C for half an hour. The cells were then spun down and washed thrice with ice cold isotonic magnesium chloride buffer (MgCl₂ 95 mmol/l, trometamol (TRIS) base 5 mmol/l, made up in Analar water, pH 7.4 with HCl). This washing does not lead to substantial loss of intracellular sodium

over 30 minutes (unpublished observations); and as all washing was completed within 15 minutes there was minimal loss of intracellular sodium. Furthermore, the extracellular fluid contaminating every pellet was likely to contribute less than 0.1% of any sodium (and less for potassium) measured finally after the three washes (unpublished observations). Pellets were then weighed to the nearest microgram and dried to constant weight in an oven at 80°C for 24 hours. Dry weight is about 25% of wet weight. Pellets were dissolved in nitric acid 0.1 mol/l, lithium nitrate 15 mmol/l being used as internal standard. Concentrations of Na⁺ and K⁺ were measured by flame photometry and intracellular electrolytes expressed as mmol/kg dry weight. In 13 duplicate determinations a week apart the coefficient of variation was 11% for Na⁺ and 9.2% for K⁺. We have found that there is no significant difference in the intracellular concentrations of Na⁺ and K⁺ between polymorphonuclear and mononuclear leucocytes (n=12).

Efflux rates were calculated as the product of the intracellular sodium and efflux rate constant and expressed as mmol/kg dry weight/h.

OTHER ASSAYS

Insulin concentrations were measured by radioimmunoassay.²³ Plasma electrolyte values were determined by flame photometry and glucose concentration by the glucose oxidase method on a Beckman analyser. Plasma non-esterified fatty acid determinations were by the acylcoenzyme A synthetase method.²⁴ Incremental plasma insulin area and decremental plasma non-esterified fatty acid areas were calculated from the plasma concentration-time curves.

STATISTICS

As efflux rate constants and electrolyte values showed a Gaussian distribution comparisons were by Student's *t* test, paired and unpaired as appropriate. Efflux rates, intracellular sodium concentrations, and ouabain sensitive efflux rate constants before and after the meal or euglycaemic clamp were also compared by analysis of variance with repeated measures to determine the contribution of sex and obesity to the changes described. Correlations reported for the meal and insulin clamp studies are Spearman correlation coefficients as numbers were relatively small. Results are expressed as mean and standard deviations (SD), and two tailed *p* values are quoted.

Results

Obese subjects had significantly higher systolic blood pressures than the normal group (127 (14) v 117 (11) mm Hg; $p < 0.002$) but diastolic pressures were similar (obese group 79 (9) mm Hg, normal group 76 (7) mm Hg; NS). The table shows the leucocyte intracellular sodium concentrations and ouabain sensitive ^{22}Na efflux rate constants and efflux rates in the normal and obese subjects stratified by sex. Obese subjects had significantly higher ouabain sensitive efflux rate constants than normal subjects, which was true whether men and women were analysed separately or together. Though there was no significant difference in intracellular sodium concentrations between obese and normal subjects, concentrations were higher in women, which may account for the higher ouabain sensitive efflux rate in normal women, as there was no significant difference in the ouabain sensitive efflux rate constant between the sexes. Ouabain insensitive efflux rate constants were significantly higher in normal compared with obese subjects (0.66 (0.25) v 0.53 (0.14)/h; $p < 0.009$).

Responses to the test meal differed between the two groups. In the 11 normal subjects after two hours there was an increase from the fasting value in the total ^{22}Na efflux rate constant (3.06 (0.44) to 3.32 (0.47)/h; $p < 0.003$) and the ouabain sensitive efflux rate constant (2.39 (0.33) to 2.71 (0.40)/h, $p < 0.0001$; fig 1). The 13 obese subjects did not show any increase from the fasting value (total efflux rate constant 3.22 (0.55) to 3.26 (0.58)/h, ouabain sensitive efflux rate constant 2.65 (0.54) to 2.61 (0.58)/h; fig 1). Intracellular potassium remained unchanged with feeding in both groups. Intracellular sodium concentrations rose from the respective fasting values in both groups (normal group 30.7 (9.9) to 38.1 (9.8) mmol/kg, $p < 0.004$; obese group 25.5 (6.6) to 31.0 (6.0) mmol/kg, $p < 0.008$). The increase in ouabain sensitive efflux rates was greater ($p < 0.02$) in normal subjects (from 74.2 (30.1) to 102.9 (29.5) mmol/kg/h; $p < 0.0001$) than the obese (67.7 (23.2) to 81.5 (24.6) mmol/kg/h; $p < 0.009$; fig 2). Passive (ouabain insensitive) efflux rates were unchanged from the fasting value in normal subjects (20.5 (8.9) to 22.9 (6.9) mmol/kg/h; NS) but an increase from the fasting value was found in the obese group (14.6 (4.8) to 20.1 (7.2) mmol/kg/h; $p < 0.02$).

By analysis of variance the rise in ouabain sensitive ^{22}Na efflux rate and efflux rate constant was significantly lower in the obese ($p < 0.03$ and $p < 0.003$, respectively) with no significant interaction with sex. Overall, women had a higher leucocyte intracellular sodium concentration ($p < 0.002$) but there was no significant contribution from sex to the rise in intracellular sodium with the meal.

Insulin and glucose incremental areas were smaller in normal than obese subjects (insulin area 83.5 (39.0) v 190.0 (110.5) mU/l/h, $p < 0.001$; glucose area 0.93 (1.82) v 2.33 (0.94) mmol/l/h, $p < 0.03$). Decremental non-esterified fatty acid areas were similar (normal 0.30 (0.18) v obese 0.35 (0.22) mmol/l/h). The change in leucocyte ouabain sensitive efflux rate constant with feeding was significantly and negatively correlated with body mass index ($r_s = -0.56$; $p < 0.004$) and insulin incremental area ($r_s = 0.53$; $p < 0.008$).

FIG 1—Effect of test meal on leucocyte ^{22}Na ouabain sensitive efflux rate constant in normal and obese subjects. Only rise in ^{22}Na ouabain sensitive efflux rate constant in normal subjects was significant ($p < 0.0001$). ● = Fasting. ▲ = Two hours after meal. ○ = Mean (bars are SD).

FIG 2—Effect of test meal on leucocyte sodium ouabain sensitive efflux rates in normal and obese subjects. Rise in sodium ouabain sensitive efflux rates with meal was significant in both normal ($p < 0.0001$) and obese ($p < 0.009$) subjects. ● = Fasting. ▲ = Two hours after meal. ○ = Mean (bars are SD).

The hyperinsulinaemic-euglycaemic clamp produced similar plasma insulin concentrations in normal (69.7 (8.4) mU/l) and obese subjects (77.9 (21.1) mU/l). A higher metabolic clearance rate of glucose was found in the normal compared with obese subjects (9.86 (3.27) v 3.24 (1.34) ml/kg/min; $p < 0.0001$), reflecting the insulin resistance of obesity. The clamped plasma glucose concentration was slightly lower in the normal than obese subjects (5.0 (0.28) v 5.5 (0.58) mmol/l; $p < 0.02$). Plasma non-esterified fatty acid concentrations fell to the same extent in the normal (non-esterified fatty acid area 0.51 (0.23) mmol/l/h) and obese subjects (0.46 (0.47) mmol/l/h). In the 10 normal subjects rises from the fasting values in the total and ouabain sensitive efflux rate constants were found after two hours (3.52 (0.54) to 3.84 (0.55)/h ($p < 0.05$) and 2.74 (0.42) to 3.16 (0.47)/h ($p < 0.004$), respectively). No such responses were found in the obese group (total efflux rate constant 3.35 (0.54) to 3.47 (0.59)/h (NS); ouabain sensitive efflux rate constant 2.70 (0.38) to 2.79 (0.48)/h (NS)). Intracellular potassium concentrations remained unchanged in both groups, but intracellular sodium rose significantly from fasting values (normal group 32.6 (10.0) to 39.1 (11.3) mmol/kg ($p < 0.008$); obese group 27.9 (9.3) to 32.9 (8.5) mmol/kg ($p < 0.02$)). As a result the change in ouabain sensitive efflux rates was greater ($p < 0.05$) in the normal subjects (88.8 (26.9) to 123.8 (39.1) mmol/kg/h; $p < 0.002$) than in the obese subjects (76.0 (28.6) to 92.8 (31.1) mmol/kg/h; $p < 0.02$) without significant change in the passive ouabain insensitive efflux rates in either group.

By analysis of variance the change in ouabain sensitive efflux rate constant with the clamp was lower in the obese group ($p < 0.03$). There was no interaction of sex with the change in ouabain sensitive efflux rates or efflux rate constant. The change in ouabain sensitive efflux rates with the clamp, though lower in obese subjects ($p < 0.08$), did not reach conventional levels of significance.

The metabolic clearance rate of glucose during the euglycaemic clamp was highly correlated with the body mass index ($r_s = 0.83$; $p < 0.0001$) but not with the change in leucocyte ouabain sensitive or insensitive efflux rates or intracellular sodium concentration. It was significantly correlated with the change in the ouabain sensitive efflux rate constant ($r_s = 0.48$; $p < 0.03$). Only in the obese subjects was the metabolic clearance rate of glucose directly related to the intracellular potassium concentration ($r_s = 0.68$; $p < 0.03$), the correlation being non-significant in normal subjects ($r_s = 0.36$).

Discussion

Reduced sodium pump activity has been found in animal models of obesity.^{1,3} There is controversy over this in human obesity, with reports of reduced,^{13,15} raised,¹⁶ or unchanged¹⁷ activity of the sodium pump in erythrocytes. In nucleated human cells there is agreement that sodium pump activity is raised in muscle,²⁵ liver,²⁶ and leucocytes²⁷ in the obese. We have found that the leucocyte ouabain sensitive efflux rate constant is raised and the ouabain insensitive efflux rate constant significantly depressed in obesity.

This suggests an increased fractional turnover rate of the sodium pump, though the lack of difference in the ouabain sensitive efflux rates implies that changes in intracellular sodium have limited persistence of any differences in efflux rates that may have existed initially. We did not find a lowered intracellular sodium concentration, which may have been because of either (a) the variability in measuring this parameter or (b) an increased influx of sodium over the long term in obesity, which may be related to the hyperinsulinaemia.²⁸ We have assumed that the sodium pump of leucocytes reflects the activity of other nucleated cells²⁹ and that there is no difference in the efficiency of the process between normal and obese subjects. If so then the lack of difference in the fasting leucocyte ouabain sensitive efflux rates between normal and obese subjects cannot explain the tendency to gain weight in obesity.

We have also found a raised intracellular sodium concentration in women, whether obese or of normal weight. No sex difference in leucocyte sodium values has previously been reported.²⁰ This increase in intracellular sodium largely accounted for the raised efflux rate in normal women. The differences in efflux rate constant and intracellular sodium values were not due to differences in the proportion of polymorphonuclear and mononuclear leucocytes as they have similar ouabain sensitive efflux rate constants and amounts of intracellular sodium.

In this initial part of the study the obese subjects were significantly older than the normal controls. Age has been reported to be associated with a lowering of erythrocyte sodium pump activity.³⁰ If this is also true for leucocytes then a larger difference in the fractional turnover rate (²²Na ouabain sensitive efflux rate constant) of the sodium pump might have been detectable in a younger obese population.

Ingestion of protein, carbohydrate, and fat is associated with an increase in energy expenditure—the thermic effect of food. In obesity some workers have found a reduced dietary thermogenesis for mixed meals or glucose,^{8,11} which others have not confirmed.³¹⁻³⁷ Differences in energy expenditure measuring techniques, the duration of observation after the meal, how the results are expressed, or the size and nature of the energy load may account for these discrepancies. When 24 hour energy expenditure is studied in a respiration chamber a defect in dietary thermogenesis in the obese is detectable.³⁷ The thermic effect of food may depend on several processes—for example, the energy cost of storing food, the energy cost of substrate cycles, the increase in sympathetic nervous activity leading to an increase in energy expenditure, and any change in sodium pump rate. The sodium pump may participate in a “futile” cycle, as any sodium entering the cell has to be extruded against a gradient, which is energetically costly.⁴⁵

We have previously shown that ingestion of oral glucose leads to an increase in the leucocyte ²²Na ouabain sensitive efflux rate constant in normal but not obese subjects.³⁸ In this study there was an increase in the ouabain sensitive efflux rate constant, efflux rates, and intracellular sodium in normal subjects two hours after a meal. This means that there was an increase in sodium influx and that sodium pump activity was also activated. The similarity of this postprandial response to that during the insulin clamp suggests a role for insulin in these processes. Indeed, insulin has been found to increase sodium influx in muscle²⁸ and also to stimulate the sodium pump in various tissues.^{19,39,40}

On the contrary, in the obese, though there was an increase in intracellular sodium, there was no increase in the ouabain sensitive efflux rate constant. The increase in ouabain sensitive efflux rates was smaller than in the normal subjects and was presumably due to the rise in intracellular sodium. Likewise, there was a rise in the passive efflux rate of sodium. Thus there is a failure of the sodium pump to respond as in normal subjects, which may partly account for the defective dietary thermogenesis in obesity. Such a defect in the postprandial activation of cellular sodium pumping may be associated with the insulin resistance of obesity.

We tested the hypothesis that the smaller postprandial rise in sodium pump activity in obesity was associated with insulin resistance by infusing insulin and glucose into normal and obese subjects by the hyperinsulinaemic-euglycaemic clamp technique. Raising insulin acutely in normal subjects led to an increase in

intracellular sodium concentrations, ouabain sensitive efflux rate constant, and efflux rates. In obesity the same findings as with the meal were obtained—that is, there was an increase in intracellular sodium but no increase in ouabain sensitive efflux rate constant and therefore a smaller increase in the ouabain sensitive efflux rate of sodium than normal. The thermic effect of insulin and glucose infusions has been shown to be lower in obesity,¹² which may partly be due to a reduced response in cellular sodium pumping to insulin. The thermic defect is reversible by increasing plasma insulin concentrations⁴¹ but it is not known whether the defect in activation of cellular sodium pumping in obesity may be so reversed.

Hypertension is more common in obesity and is associated with insulin resistance and hyperinsulinaemia.⁴² If an acute effect of insulin is to increase intracellular sodium in vascular smooth muscle then vascular tone might increase owing to raised intracellular calcium.⁴³ As activation of active sodium efflux is less in obesity, the increased intracellular sodium from energy intake or insulin should persist longer, possibly with a deleterious effect on blood pressure in the long term. Energy restriction without salt restriction has a hypotensive effect in obesity.⁴⁴

An additional factor suggested by our insulin clamp studies may be a relation between intracellular potassium and insulin sensitivity in obesity. A significant correlation between the metabolic clearance rate of glucose and intracellular potassium was found in obese but not normal subjects. It is known that both glucose tolerance and insulin secretion are impaired in potassium depletion.⁴⁵ Both glucose tolerance and insulin secretion are improved by potassium supplementation.

In summary we have shown, firstly, that though there may be no difference in the leucocyte sodium pump activity (when expressed as absolute efflux rates) in obesity, increased activity may have been present as the ouabain sensitive efflux rate constant is increased. If sodium pumping contributes substantially to energy expenditure equality accords with a normal resting energy expenditure (when expressed per unit of lean body mass) in obesity, though absolute measures are raised.⁴⁶ Secondly, eating a meal increases cellular sodium pump activity in normal people but less so in obese subjects. The rise in obese subjects is purely due to an increase in intracellular sodium, probably via an increase in sodium influx, which also leads to an increased passive efflux rate. The postprandial responses of the leucocyte sodium pump are reproduced by insulin and glucose infusions.

We put forward the hypothesis that in human obesity a defect in the responses of the cellular sodium pump to feeding or insulin may contribute to any defective dietary thermogenesis. Whether this defect existed before weight gain or results from it is unknown, but in the obese it would produce greater energetic efficiency. Though such a defect in the postprandial activation of the sodium pump is small, it might cumulatively lead to positive energy balance over a long period. The ability of insulin to increase intracellular sodium concentrations may also be the basis of the association between hyperinsulinaemia, hypertension, and obesity.

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Free radicals and Dupuytren's contracture

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Abstract

The concentration of substrate expressed as hypoxanthine capable of reacting with xanthine oxidase to release superoxide free radicals (O₂⁻) was measured in control and Dupuytren's contracture palmar fascia. In Dupuytren's contracture palmar fascia the concentration of hypoxanthine was six times that of control and was greatest in "nodular" areas. Xanthine oxidase activity was also detected in Dupuytren's contracture palmar fascia.

These results suggest a greater potential for hypoxanthine-xanthine oxidase generated oxygen free radical formation in Dupuytren's contracture than in control palmar fascia. Production of free radicals may be an important factor in the pathogenesis of Dupuytren's contracture. The benefit of allopurinol in the management of Dupuytren's contracture and other fibrotic conditions may thus be explained, as allopurinol binds to xanthine oxidase and prevents release of free radicals.

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Introduction

Dupuytren's contracture affects the palmar fascia, which becomes thickened and shortened and may lead to disabling fixed flexion deformities of the fingers. The prevalence in white populations is between 4% and 6%, rising to 20% in men over 65,¹ and is higher in diabetics, particularly when retinopathy is present, but lower in patients with rheumatoid arthritis.^{2,3}

The cause is unknown but may include localised ischaemia of the palmar fascia.^{4,5} During ischaemia adenosine triphosphate is broken down, increasing the amount of the purine bases hypoxanthine and xanthine and converting xanthine dehydrogenase to xanthine oxidase^{6,7} (fig 1). Xanthine oxidase, located in the endothelial cells of small vessels,⁸ catalyses the conversion of both hypoxanthine to xanthine and xanthine to uric acid. Both reactions produce superoxide free radicals (O₂⁻) and hydrogen peroxide (H₂O₂). Superoxide free radicals, hydrogen peroxide, and their degradation products may damage various tissues and alter vascular permeability.⁹⁻¹¹ In animal studies allopurinol (a competitive inhibitor of xanthine oxidase) and free radical scavengers such as superoxide dismutase and catalase limited the damage associated with acute ischaemia.^{12,13} Preliminary clinical results suggest that allopurinol may improve Dupuytren's contracture.¹⁴

To see whether free radicals might be important in the pathogenesis of Dupuytren's contracture we have measured the concentration of substrates able to react with exogenous xanthine oxidase to produce superoxide free radicals in Dupuytren's and control palmar fascia. These substrates are most likely to