Sodium–lithium countertransport, sodium–hydrogen exchange and membrane microviscosity in patients with hyperlipidaemia


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INTRODUCTION

A cluster of interacting abnormalities including hyperinsulinaemia, insulin resistance, essential hypertension, endothelial dysfunction and abnormal lipid profile have been associated with increased cardiovascular risk [1]. While the exact mechanisms have not yet been fully elucidated, there is good evidence of an interaction between inherited and environmental factors. Combined dyslipidaemia and hypertriglyceridaemia appear to cluster with essential hypertension, insulin resistance and obesity [2], in the 'insulin resistance syndrome'. Despite the inherited component, some investigators believe that the underlying pathology is related to early life influences [3, 4], resulting in subsequent metabolic abnormalities.

Familial hypercholesterolaemia (FH) results in elevated plasma low-density lipoprotein (LDL)-cholesterol levels due to an inherited defect in the LDL receptor gene [5, 6]. Affected individuals develop ischaemic heart disease 10 to 20 years before their normolipaemic peers. It does not appear to be associated with the insulin resistance syndrome [7], and therefore represents a separate phenotype of cardiovascular risk.

Sodium–lithium countertransport (SLC) is thought to represent a sodium–sodium exchanger which can exchange intracellular lithium for external sodium in vitro, thus allowing its activity to be measured. It is present only in erythrocytes, and no clear physiological role has been demonstrated. SLC may be an isoform of sodium–hydrogen exchange (Na+/H+ exchange), a transporter which has a role in erythrocytes and may be involved in membrane structure and function.

Key words: familial hypercholesterolaemia, hypertriglyceridaemia, membrane microviscosity, sodium–lithium countertransport, sodium–hydrogen exchange.

Abbreviations: apo, apolipoprotein; CI, confidence interval; DPH, diphenylhexatriene; FH, familial hypercholesterolaemia; HDL, high-density lipoprotein; HTG, hypertriglyceridaemia; LDL, low-density lipoprotein; PA, phosphatidic acid; pH, intracellular pH; SLC, sodium–lithium countertransport; TMA–DPH, trimethylammonium diphenylhexatriene; VLDL, very-low-density lipoprotein.

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in maintaining intracellular pH and volume, and which may be involved in cell hypertrophy and pathogenesis of hypertension [8, 9]. The association has largely been based on the finding of a similar relationship to insulin in vitro and in vivo in some studies [9, 10]; this has not been confirmed by other groups [11, 12]. Clinical studies suggest that SLC activity is abnormal in conditions associated with increased cardiovascular risk [13–15]. Although neither the protein nor the gene have been characterized, association studies have confirmed that 70–80% of the inter-individual variation in SLC can be explained by genetic factors [16–18]. SLC has been shown to be elevated in patients with essential hypertension [13], with dyslipidaemia exerting an independent effect [15]. In clinical studies standard SLC has been correlated with triacylglycerol levels [14, 19] while some have also found an association with cholesterol [16, 20], and a negative correlation with high-density lipoprotein (HDL) [15, 21]. Other studies of essential hypertension have failed to find any significant relationship between SLC and plasma lipid levels [22].

Some studies examining SLC in the presence of physiological insulin concentrations in vitro have shown a stimulating effect [9], while others have shown no effect [11]. Studies in vivo have suggested a relationship with fasting insulin levels [23], while others suggest that the association is rather with insulin-mediated glucose disposal [24, 25].

Na+/H+ exchange has been demonstrated to be abnormal in a subgroup of patients with essential hypertension [26], while evidence of an association between abnormal activity and hyperinsulinaemia [27] or dyslipidaemia [12] remains controversial. However, Lifton et al. [28] excluded linkage of the NHE-1 gene polymorphism and essential hypertension with a likelihood of odds estimated to be −2 which has been accepted as a significant exclusion of linkage.

Abnormal ion transport in conditions associated with increased cardiovascular risk may be caused by alterations in transporter conformation and turnover rate mediated by alterations in plasma membrane phospholipid or cholesterol composition. Membrane microviscosity has been shown to be increased in hypercholesterolaemia and reduced in hypertriglyceridaemia compared with normolipaemic controls [29]. Microviscosity at the core of the erythrocyte membrane has been shown to correlate positively with SLC in hyperlipidaemia [30], while a relationship has been shown between SLC and microviscosity at the membrane surface in hypertensive patients with a positive family history of hypertension [31].

The current study has been designed to test the hypothesis that both SLC and Na+/H+ exchange kinetic parameters are altered in individuals with FH or hypertriglyceridaemia compared with normolipaemic controls, and may be influenced by membrane microviscosity (affecting turnover of the transporters) or insulin levels (affecting their phosphorylation state). We therefore aimed to characterize individuals with FH, hypertriglyceridaemia and normal controls, and assess membrane function by measuring microviscosity, SLC and Na+/H+ exchange.

METHODS

Ethical approval was obtained from the West of Glasgow Ethics Committee and all patients and control subjects gave their informed consent. Forty-eight probands with FH were recruited from the lipid clinics at the Western Infirmary and Glasgow Royal Infirmary, on the basis of elevated serum cholesterol (>7 mmol/l, triacylglycerols <2.5 mmol/l), family history of early cardiovascular disease and presence of xanthoma or xanthelasma either in the individual or a first-degree relative. Thirty-three subjects with either Type IV or Type IIb hyperlipidaemia were also recruited (serum triacylglycerols >3 mmol/l). Secondary hyperlipidaemia was excluded by selecting individuals with normal γ-GT, normal thyroid function tests and no history of diabetes mellitus. Fifty-four normolipaemic controls (cholesterol <5.5 mmol/l, triacylglycerols <2 mmol/l) were recruited from unaffected relatives and spouses of patients with FH and hospital staff.

Subjects attended after an overnight fast. Body mass index was calculated from recorded weight and height. Supine blood pressure was recorded after 5 min rest using a mercury sphygmomanometer.

An 18G venous cannula was inserted into a brachial vein, and approximately 125 ml of blood withdrawn for analysis of platelet microviscosity, erythrocyte SLC, lymphocyte Na+/H+ exchange, lipid analysis [total cholesterol, triacylglycerols, very-low-density lipoprotein (VLDL), LDL, HDL, apolipoprotein (apo) AI, apo B, apo E phenotype and lipoprotein (a)], full blood count, serum biochemistry, fasting glucose and thyroid function tests. Fasting insulin levels were measured in hypertriglyceridaemic and control subjects.

Lipid analysis

Blood was collected in potassium–EDTA (final concentration 1 mg/ml) and plasma was harvested at 4°C by low-speed centrifugation. Aliquots for lipid and lipoprotein were used immediately. Plasma cholesterol, triacylglycerols, HDL-cholesterol, VLDL-cholesterol and LDL-cholesterol measurements were performed by a modification of the standard Lipid Research Clinics protocol [32]. Apo AI and apo B were measured by immunoturbidimetry (Orion Diagnostics, Espoo, Finland), and lipoprotein (a) by ELISA (Innotest, Antwerp, Belgium). Apo E phenotyping was carried out using an adaptation of the method of Menzel and Utermann [33], whereby after isoelectric focusing of delipidated...
plasma, immunoblotting using polyclonal anti-apoE antibody was performed.

Insulin levels

Fasting insulin levels were measured by radioimmunoassay, using the Medgenix Eusia kit (Medgenix, Fleurus, Belgium) which is reported to only cross react with non-circulating pro-insulin and is therefore relatively specific (intra-assay variability, 5.3%; interassay variability, 5.6%).

Sodium–lithium countertransport

SLC was measured in erythrocytes isolated from peripheral blood based on a modification of the method of Canessa et al. [13]. \( K_m \) and \( V_{max} \) were determined according to the method described by Rutherford et al. [34]. Briefly, isolated erythrocytes were incubated in lithium-loading solution (140 mmol/l \( \text{LiCl} \), 10 mmol/l \( \text{Li}_2\text{CO}_3 \), 10 mmol/l glucose, 10 mmol/l Tris–Mops, pH 7.4) at 37°C for 1.5 h. The cells were then washed twice in \( \text{MgCl}_2 \) (285 ± 5 mosmol/kg) and once in choline medium (139 mmol/l choline chloride, 1 mmol/l \( \text{MgCl}_2 \), 10 mmol/l glucose, 10 mmol/l Tris–Mops, pH 7.4, 290 ± 6 mosmol/kg). The cells were then incubated for 6 min at 37°C during the last wash to facilitate pH equilibration. The erythrocyte pellet (300–350 µl) was incubated in either 4 ml of choline medium containing \( 10^{-4} \) mol/l ouabain, or 4 ml of sodium medium (145 mmol/l \( \text{NaCl} \), 1 mmol/l \( \text{MgCl}_2 \), 10 mmol/l glucose, 10 mmol/l Tris–Mops, pH 7.4, 10\(^{-4} \) mol/l ouabain, 290 ± 6 mosmol/kg). The cells were then incubated for 20, 35, 50, 70, 90 and 120 mmol/l \( \text{NaCl} \) medium, with osmolality maintained using choline medium. Cells were incubated at 37°C for 40, 80 and 120 min, and at each time point an aliquot was removed and separated by centrifugation (2000 g for 3 min). The lithium concentration of the supernatant was calculated using an IL943 flame photometer. The rate of lithium efflux for each sodium concentration was calculated from the linear regression of efflux against time. This followed Michaelis–Menten saturation kinetics, and from the Eadie–Hofstee transformation, \( K_m \) and \( V_{max} \) of the countertransporter were calculated. Intra-assay variability for \( K_m \) and \( V_{max} \) was 3.6% and 2.2% respectively, and interassay variability was 8.7% and 7.6% respectively.

\( \text{Na}^+ / \text{H}^+ \) exchange

\( \text{Na}^+ / \text{H}^+ \) exchange was measured according to the method of Carr et al. [12]. Fifty millilitres of venous blood collected into acid–citrate–dextrose buffer was separated by a modification of the method of Boyum [35]. After dilution with an equal quantity of physiological salt solution buffer, the blood was layered onto a sterile Lymphoprep solution [sodium metrizoate (9.6%)/Ficoll (5.6%) mixture]. After centrifugation at 400 g for 30 min at room temperature, the thin lymphocyte layer at the density interface was collected and resuspended in a large volume of physiological salt solution buffer gassed with 5% \( \text{CO}_2 \) (pH 7.4, 37°C). After two further washes, the lymphocytes were incubated for 30 min with bis(carboxyethyl)carboxyfluorescein acetoxyethyl ester (5 µmol/l at 37°C). After centrifugation and resuspension in physiological salt solution buffer (20 min, 37°C), aliquots were used to measure resting pH (resting fluorescence was recorded over 40 s as the ratio of emissions at 530 nm from alternating excitations at 495 and 440 nm (slit widths 5 nm and 2.5 nm respectively)). A further aliquot was resuspended in a \( K^+ / \text{Hepes/nigericin} \) medium (140 mmol/l \( \text{KCl} \), 10 mmol/l \( \text{Hepes} \), 6 µmol/l \( \text{nigericin} \)). pH calibration was performed using \( \text{nigericin} \) to abolish the transmembrane pH gradient, and small aliquots of potassium hydroxide (0.1 mol/l) to effect intracellular pH (pHi) changes in the suspension (external pH range 6.2–7.6). Extracellular pH was measured with a pH microelectrode. The relationship established between pHi and fluorescence ratios was sigmoidal, but did not differ significantly from linearity between pHi 6.2 and 7.6. The correlation coefficient for least-squares linear regression across this range was always greater than 0.995.

Intracellular acidosis (pHi 6.2) was induced using a \( K^+ / \text{Hepes/nigericin} \) buffer (140 mmol/l \( \text{KCl} \), 10 mmol/l \( \text{Hepes} \), 4 µmol/l \( \text{nigericin} \)). After centrifugation the cells were resuspended in a similar buffer, but with \( \text{nigericin} \) replaced by non-esterified fatty acid–BSA (5 g/l) to scavenge residual ionophore. Aliquots (100 µl) were placed in a series of cuvettes to which was added 2.9 ml of Hepes buffer (pH 7.4) with sodium concentrations of 140, 70, 30, 20, 10 and 0 mmol/l, maintaining osmolality with choline. The first 20 s of pH recovery after addition of sodium is linear, and this was measured for each sodium concentration. A least-squares linear regression model converted the rate of recovery to the rate of change of pH using the calibration relationship. Interassay variability was 5.6%, intra-assay variability 10.8%.

The values of \( V_{max} \) and \( K_m \) were obtained from a least-squares linear regression using the Hanes linear transformation for first-order kinetics:

\[
\frac{[\text{Na}]}{\text{Rate of charge of pH}} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \times [\text{Na}]
\]

At any given pHi,

\[
\text{H}^+ \text{ efflux rate} = \text{buffering capacity} \times \text{rate of change of pHi}
\]

Buffering capacity was measured according to the method of Roos and Boron [36]. Briefly 1 mmol/l
ammonium chloride is added to acid-loaded cells (pH 6.2) which produces a change in pH of approximately 0.2 units. It is assumed that the pKₐ for the dissociation of ammonia is unchanged in the intracellular environment and that NH₃ (not NH₄⁺) is freely permeable across the cell membrane. Buffering capacity was calculated as described by Carr et al. [12]. The coefficient of variation for the buffering capacity measurement was 7.8%.

**Measurement of platelet microviscosity**

Microviscosity was measured according to methods previously established in our laboratory [37]. Platelet-rich plasma was obtained from whole blood collected into 3.9% citrate and centrifuged at 250 g for 8 min at 37°C. After addition of EGTA the cells were centrifuged for 10 min at 250 g, then resuspended in platelet buffer (140 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l MgCl₂, 10 mmol/l glucose, 20 mmol/l Hepes, pH 7.3, at 37°C). Platelet count was adjusted to 2.5 x 10⁹ using a Coulter counter. Cells were then incubated with the fluorescent probes trimethylammonium diphenylhexatriene (TMA-DPH), diphenylhexatriene (DPH) and phosphatidic acid (PA) for 10 min (15 min for DPH) at 37°C in platelet buffer. Measurements of fluorescence polarization were performed in a fluorimeter with computer-controlled excitation and emission polarizers (Perkin-Elmer LS-50). The fluorescence intensity (I) is measured at excitation and emission wavelengths of 350 nm and 430 nm, respectively. Anisotropy (A) is calculated according to the equations:

\[
A = \frac{(I_v - GI_{vh})/(I_v + 2GI_{vh})}{G = I_{hv}/I_{hh}}
\]

where the first subscript is the excitation orientation and the second is the emission orientation: v, vertical; h, horizontal. G is the correction factor for the optical system (evaluated before each anisotropy measurement). Inter- and intra-assay variabilities for the three probes were as follows: TMA-DPH, 3.6% and 0.5%; DPH, 10.8% and 7.0%; PA, 3.3% and 3.0%.

**Statistics**

Multiple regression analysis was carried out to assess the influence of the group, age and sex on each measurement, which allowed for any confounding effects of the latter two variables. The individual effects of age and sex were investigated with Pearson product-moment correlations and two-sample t-tests respectively. Partial correlation coefficients, given age and sex, were calculated between pairs of measurements where appropriate. Within each model, pairwise comparisons were calculated using Tukey’s method to allow for multiple testing. Parameters which were not normally distributed were logₑ transformed before further analysis and the corresponding confidence intervals are presented in terms of ratios of geometric means [38]. Descriptive statistics are expressed as median (interquartile ranges) except where otherwise stated.

**RESULTS**

Demographic parameters of the three groups are shown in Table 1. In view of the differences in median age and ratio of males and females between the groups, it was necessary to correct routinely for age and sex.

<table>
<thead>
<tr>
<th>Table 1. Demographic parameters, cholesterol and triacylglycerols of the three groups. Data are given as medians (interquartile ranges).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Sex (%MF)</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
</tr>
</tbody>
</table>

Biochemical measurements, blood pressure and body mass index

Biochemical variables and blood pressure of the three groups are shown in Table 2.

In keeping with our selection criteria, LDL levels were higher in FH patients than in patients with hypertriglyceridaemia (HTG) or control groups. VLDL was significantly higher in patients with HTG than in patients with FH or controls (P<0.001), even after allowing for the effects of age and sex. HDL levels were lower in the HTG group, and were significantly different between females and males (means ± SD: females 1.27±0.36 mmol/l; males 0.98±0.26 mmol/l; P<0.001; 95% confidence interval (CI) for female–male mean HDL: 0.176 to 0.392), but were not influenced by age (r = 0.021, P = 0.914).

Apo AI levels were higher in FH and HTG groups than in controls (P = 0.038), after age and sex correction. Individually, sex influenced apo AI levels (means ± SD: females 1.3±0.2; males 1.2±0.2; P = 0.004, 95% CI: 0.037 to 0.191), whereas age did not (r = 0.203, P = 0.598). Apo B levels were lower in controls compared with patients with either FH or HTG (P<0.001). For this measurement, the important confounder was age (r = 0.393, P = 0.027); sex did not have a significant effect (P = 0.54). Apo E phenotype was similar in the three groups (P = 0.0782), with the overall distribution being as follows: E2/E2, 2.8%; E3/E2, 12.9%;
Table 2. Biochemical variables and blood pressure in patients with FH, HTG and normal controls. Data are expressed as medians (interquartile ranges), and Tukey's 95% CIs have been used to express differences between the groups. Measurements marked * were log transformed before use in multiple regression, so that CIs are for ratios of group geometric means; P-values are given for differences between groups from multiple regression analysis (correcting for age and sex).

<table>
<thead>
<tr>
<th>Variable</th>
<th>FH</th>
<th>HTG</th>
<th>Controls</th>
<th>P</th>
<th>FH versus HTG</th>
<th>FH versus controls</th>
<th>HTG versus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL (mmol/l)</td>
<td>6.1 (1.6)</td>
<td>3.7 (2.3)</td>
<td>2.1 (1.2)</td>
<td>&lt;0.001</td>
<td>(2.22, 3.49)</td>
<td>(2.54, 3.74)</td>
<td>(-0.37, 0.94)</td>
</tr>
<tr>
<td>VLDL* (mmol/l)</td>
<td>0.6 (0.6)</td>
<td>2.06 (1.7)</td>
<td>0.4 (0.3)</td>
<td>&lt;0.001</td>
<td>(0.24, 0.44)</td>
<td>(1.18, 2.21)</td>
<td>(3.60, 6.85)</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio*</td>
<td>1.2 (0.5)</td>
<td>0.8 (0.2)</td>
<td>1.2 (0.4)</td>
<td>&lt;0.001</td>
<td>(0.19, 0.51)</td>
<td>(-0.14, 0.17)</td>
<td>(-0.50, -0.17)</td>
</tr>
<tr>
<td>Lipoprotein (a)*</td>
<td>6.8 (2.4)</td>
<td>7.9 (2.1)</td>
<td>4.0 (1.7)</td>
<td>&lt;0.001</td>
<td>(0.77, 1.06)</td>
<td>(1.45, 1.96)</td>
<td>(1.38, 2.19)</td>
</tr>
<tr>
<td>Apo A (g/l)</td>
<td>1.3 (0.2)</td>
<td>1.3 (0.2)</td>
<td>1.2 (0.3)</td>
<td>0.038</td>
<td>(-0.16, 0.07)</td>
<td>(-0.02, 0.19)</td>
<td>(0.01, 0.25)</td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td>1.4 (0.3)</td>
<td>1.6 (1.5)</td>
<td>0.9 (1.3)</td>
<td>&lt;0.001</td>
<td>(-0.28, 0.03)</td>
<td>(0.43, 0.73)</td>
<td>(0.55, 0.87)</td>
</tr>
<tr>
<td>Lipoprotein (a)* (mmol/l)</td>
<td>2.94 (0.24)</td>
<td>2.3 (0.29)</td>
<td>2.90 (0.23)</td>
<td>0.198</td>
<td>(0.81, 4.21)</td>
<td>(0.48, 2.26)</td>
<td>(0.24, 1.30)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>146 (21)</td>
<td>146 (16)</td>
<td>134 (19)</td>
<td>&lt;0.001</td>
<td>(-10.60, 5.40)</td>
<td>(4.31, 19.49)</td>
<td>(6.29, 22.71)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>88 (16)</td>
<td>92 (14)</td>
<td>82 (14)</td>
<td>&lt;0.001</td>
<td>(-9.40, 1.54)</td>
<td>(-8.84, 9.54)</td>
<td>(2.67, 13.89)</td>
</tr>
<tr>
<td>Fasting insulin* (m-unit/dl)</td>
<td>5.3 (0.7)</td>
<td>5.5 (1.0)</td>
<td>5.2 (0.4)</td>
<td>0.082</td>
<td>(-0.50, 0.07)</td>
<td>(-0.19, 0.32)</td>
<td>(-0.01, 0.57)</td>
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<tr>
<td>Fasting glucose (mmol/l)</td>
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</table>

Body mass index was higher in both FH and HTG groups compared with controls after correction (P = 0.01), but was only weakly influenced by age (r = 0.258, P = 0.057). Fasting insulin was measured in 29 individuals with HTG and 29 normolipaemic age- and sex-matched controls. As values were not normally distributed the data were loge transformed before further analysis. Insulin levels were higher in patients with HTG than in controls (P = 0.011).

Similarly, fasting glucose levels appeared to be higher in the HTG group compared with either the FH group or normal controls but this was not a significant difference (P = 0.082). The glucose/insulin ratio was not different between the two groups [HTG, 0.53 (0.46); controls, 0.59 (0.3), P = 0.395].

Sodium–lithium countertransport

Using a multiple regression model corrected for age and sex, individuals with hypertriglyceridaemia had a higher SLC \( V_{max} \) than normal controls (P = 0.005, see Fig. 1). The 95% Tukey CI for the ratio of HTG to control geometric means was (1.08, 1.68). In addition, there was a significant sex difference in SLC \( V_{max} \) (95% ratio CI: 0.73 to 0.98, P = 0.015).

E3/E3, 60.2%; E4/E2, 2.8%; E4/E3, 20.4%; E4/E4, 0.9%. This is similar to the distribution found in the West of Scotland population [39].

Lipoprotein (a) was not normally distributed, and was therefore loge transformed for further analysis. Levels tended to be lower in HTG than in FH or control groups, but this did not reach significance (P = 0.198). Although used to correct this analysis, neither age nor sex correlated individually with lipoprotein (a) levels.

Both the FH and HTG groups had significantly higher systolic blood pressures than the control group (P < 0.001), even after allowing for the strong relationship between systolic blood pressure and age (r = 0.546, P < 0.001). Age was also an important covariate for diastolic blood pressure in both the FH (r = 0.301, P = 0.044) and control groups (r = 0.608, P < 0.001), but not in the HTG group (r = 0.159, P = 0.41). After correcting for this effect, diastolic blood pressure was still significantly higher in the HTG group than either the FH or control groups. In addition, both systolic and diastolic blood pressure correlated with loge triacylglycerols, given age and sex (systolic blood pressure: partial r = 0.271, P = 0.003; diastolic blood pressure: partial r = 0.258, P = 0.005).

Body mass index was higher in both FH and HTG groups compared with controls after correction (P = 0.01), but was only weakly influenced by age (r = 0.258, P = 0.057). Fasting insulin was measured in 29 individuals with HTG and 29 normolipaemic age- and sex-matched controls. As values were not normally distributed the data were loge transformed before further analysis. Insulin levels were higher in patients with HTG than in controls (P = 0.011). Similarly, fasting glucose levels appeared to be higher in the HTG group compared with either the FH group or normal controls but this was not a significant difference (P = 0.082). The glucose/insulin ratio was not different between the two groups [HTG, 0.53 (0.46); controls, 0.59 (0.3), P = 0.395].
Triacylglycerols were positively correlated with SLC \( V_{\text{max}} \) on a loge scale across the population studied (partial \( r = 0.341, P = 0.001 \); Table 3). In a subgroup analysis the correlation was significant within both the control and FH patient groups, but not within the HTG group.

In keeping with the results for triacylglycerols, VLDL also correlated with SLC \( V_{\text{max}} \) (partial \( r = 0.190, P = 0.047 \)), as did the cholesterol/HDL ratio (partial \( r = 0.282, P = 0.003 \)). Insulin initially appeared to significantly correlate with \( V_{\text{max}} \) (partial \( r = 0.363, P = 0.008 \)), but after exclusion of one very high insulin value the correlation no longer reached significance (partial \( r = 0.145, P = 0.316 \)). Fasting glucose correlated with SLC \( V_{\text{max}} \) (partial \( r = 0.258, P = 0.010 \)), whereas the glucose/insulin ratio did not (partial \( r = 0.095, P = 0.538 \); Table 3).

There were no differences in SLC \( K_m \) between the three groups studied (Fig. 1), and no evidence of an effect of sex or age. \( K_m \) was not significantly correlated with systolic or diastolic blood pressure in this study (systolic: \( r = 0.035, P = 0.72 \); diastolic: \( r = 0.03, P = 0.75 \)), nor with any of the lipoprotein parameters measured.

**Na\(^+\)/H\(^+\) exchange**

There were no differences in \( K_m \) between the patient groups (Fig. 2) and age or sex effect. \( K_m \) did not correlate with any of the lipid parameters, or with insulin or glucose. There were no differences in Na\(^+\)/H\(^+\) exchange maximal proton efflux rate (\( V_{\text{max}} \)) due to age, sex or diagnostic group, and correlations with all continuous covariates were too small to reach significance.

**Membrane microviscosity**

At the core of the membrane, microviscosity as recorded by the fluorescent probe DPH was decreased in individuals with HTG when compared with normal controls [anisotropy units: HTG, 0.220 (0.035); controls, 0.238 (0.035); 95% CI: -0.0358 to -0.0001]. When patients with HTG and FH were compared, there was a significant difference in DPH anisotropy [0.220 (0.035) compared with 0.244 (0.019); 95% CI 0.005 to 0.0043; Fig. 3].

In the combined groups, DPH was negatively correlated with both loge triacylglycerols (partial \( r = -0.286, P = 0.003 \)) and with VLDL (partial \( r = -0.242, P = 0.014 \); see Table 3. DPH was not influenced by sex (95% CI: -0.0074 to 0.0174) or by subject age (\( r = 0.077, P = 0.429 \)).

Microviscosity measurements at the outer or inner surfaces of the membrane (TMA-DPH and PA respectively) did not significantly differ between the groups. The results were not normally distributed with a large number of values at the mode (Fig. 3). PA, however, was related to age (\( r = 0.207, P = 0.030 \)).

There were no correlations between SLC and Na\(^+\)/H\(^+\) exchange parameters, or between the two transporters and microviscosity measurements. We did not find a significant correlation between SLC

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**Table 3. Partial correlation coefficients of SLC \( V_{\text{max}} \) and DPH anisotropy with relevant variables, correcting for age and sex**

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>Correlation coefficient (( r ))</th>
<th>( P )</th>
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</thead>
<tbody>
<tr>
<td>Loge SLC ( V_{\text{max}} )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loge triacylglycerol</td>
<td>112</td>
<td>0.322</td>
<td>0.001</td>
</tr>
<tr>
<td>VLDL</td>
<td>109</td>
<td>0.190</td>
<td>0.047</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio</td>
<td>111</td>
<td>0.282</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL</td>
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<td>0.075</td>
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<tr>
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<td>0.153</td>
<td>0.122</td>
</tr>
<tr>
<td>Weight</td>
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<td>0.142</td>
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<tr>
<td>Fasting glucose</td>
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<td>0.010</td>
</tr>
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<td>Glucose/insulin ratio</td>
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<td>0.538</td>
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<tr>
<td>Insulin</td>
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<td>0.316</td>
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<tr>
<td>Systolic blood pressure</td>
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<td>0.214</td>
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<tr>
<td>Diastolic blood pressure</td>
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<td>0.133</td>
<td>0.175</td>
</tr>
<tr>
<td>DPH anisotropy</td>
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<tr>
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<tr>
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<tr>
<td>Diastolic blood pressure</td>
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<td>-0.284</td>
<td>0.003</td>
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**Fig. 2. Scatter diagrams of lymphocyte Na\(^+\)/H\(^+\) exchange maximal proton efflux rate (\( V_{\text{max}} \)) (top panel) and \( K_m \) (bottom panel) in patients with FH, HTG and in normal control subjects**
Sodium transport and membrane microviscosity

The aims of this study were to compare plasma lipids, SLC and Na⁺/H⁺ exchange kinetic parameters and membrane microviscosity in patients with FH, combined hyperlipidaemia or primary HTG and normolipaemic controls. In view of the controversy surrounding the exact relationship of SLC to Na⁺/H⁺ exchange, we wished to correlate the activity of the two transport systems, and assess the influence of membrane microviscosity on their kinetic parameters. This is the first study to simultaneously measure these three indices of membrane structure and function, utilizing the kinetic parameters of SLC.

The patient groups were divided on the basis of their hyperlipidaemia. It has been shown that patients with FH do not have a higher incidence of obesity, hypertension and insulin resistance than the general population [7]. Combined hyperlipidaemia is likely to have a polygenic origin and we have confirmed that these individuals have higher blood pressure, body mass index and fasting insulin than normolipaemic controls. Most studies of SLC have measured only standard activity (i.e. the rate of lithium efflux into 140 mmol/l sodium solution minus the rate of efflux into 0 mmol/l sodium solution). More recent studies have utilized kinetic parameters, i.e. sodium affinity (Kₘ) and maximal rate of activity (Vₘₐₓ), to yield more useful information [26, 34, 40]. We have found an elevated SLC Vₘₐₓ in individuals with HTG when compared to normolipaemic controls. This confirms the findings of Rutherford et al. [41] who found a correlation between Vₘₐₓ and triacylglycerols, and also previous studies which measured only standard SLC [15, 30]. Across the whole group SLC Vₘₐₓ correlated with logₑ triacylglycerol levels: within the FH group higher activity was related to higher serum triacylglycerol levels. Variable effects of plasma lipids on membrane lipid composition in different individuals have been demonstrated [30, 42], suggesting that factors other than plasma lipid levels may also be important. A correlation between SLC activity and triacylglycerols has been shown in normal individuals [14, 22, 43], and in hypertension [15], diabetes mellitus [24] and diabetic nephropathy [44].

The values we have obtained for SLC Vₘₐₓ are higher than those obtained in some studies [27], but comparable to those found by Carr et al. [31]. This may be due to a higher Kₘ in the population studied, although methodological variation cannot be excluded. Canessa et al. [45] have adapted their original SLC methods to obtain a 'true' saturation of the transporter by increasing the intracellular osmolality to 600 mosmol using the ionophore nystatin, and measuring lithium efflux in extracellular sodium concentrations of up to 300 mmol/l. We chose to measure SLC in more physiological sodium concentrations of up to 150 mmol/l as saturation kinetics appeared to be obtained in most individuals under these conditions.

We failed to demonstrate a relationship between serum cholesterol and SLC Kₘ or Vₘₐₓ, despite showing an increase in membrane microviscosity in FH compared with HTG individuals. Some studies have suggested a relationship between serum cholesterol and SLC activity [20, 21], and membrane cholesterol enrichment in vitro has been shown to result in a reduction in SLC activity or a reduction in passive lithium leak [46]. Other studies in vivo

\[
\text{log}_e V_{\text{max}} \text{ and DPH (partial } r = -0.048, P = 0.652) \text{, allowing for age and sex.}
\]
have failed to show any relationship [15, 22, 42], and a study of the effect of lipid-lowering drugs on SLC in patients with hyperlipidaemia only demonstrated a fall in SLC activity in individuals who had a concomitant fall in serum triacylglycerols [47].

In contrast to the study of Carr et al. [12] who found a correlation between Na+/H+ exchange maximal proton efflux rate (Vmax) and cholesterol in normal individuals, we found no group differences or correlations with any of the Na+/H+ exchange parameters measured. In cultured lymphoblasts Na+/H+ Vmax was found to be elevated in cholesterol-depleted cells, and inhibited in cholesterol-enriched cells [48]. The extreme effect of these membrane alterations in vitro is greater than that seen in vivo.

The mechanism whereby triacylglycerol levels affect SLC Vmax is unknown, but may involve alterations in phospholipid composition locally affecting transporter turnover or availability for binding. Although the current study showed that SLC Vmax was affected by serum triacylglycerol levels, and that microviscosity at the core of the membrane was lower in patients with HTG than in patients with FH or controls and also inversely correlated with serum triacylglycerols, we did not demonstrate a direct relationship between SLC Vmax and microviscosity. This is in contrast to a study by Dowd et al. [30] which demonstrated significant correlations between SLC Vmax and DPH anisotropy in erythrocyte membranes, but not between anisotropy and lipids. The differences may be due to the cell preparation or patient groups used. Patients with hypertension have been shown to have altered membrane microviscosity [49], with serum triacylglycerols correlating with DPH anisotropy. They also have an altered erythrocyte membrane cholesterol distribution, with a lower total cholesterol but cholesterol enrichment of the inner leaflet [50]. Hypertensive patients with a positive family history have higher erythrocyte membrane TMA-DPH anisotropy when compared with normotensive controls, and have an inverse correlation between SLC Km and TMA-DPH anisotropy [31]. This suggests that in essential hypertension, where SLC is recognized to be largely genetically determined [16, 17], there may be a relationship between membrane composition, fluidity and conformation of the protein moiety. Supportive evidence comes from the work of Thomas et al. [40], who suggest that altered conformation resulting in exposure of a sulphhydryl group in essential hypertension may explain the altered activity found in this group of patients.

Neither SLC nor Na+/H+ exchange Km or Vmax correlated with blood pressure in this study, despite the fact that blood pressure was elevated in patients with HTG and in patients with FH compared with normal controls, even after correcting for age and sex. Patients were not selected on the basis of personal blood pressure or family history of hypertension. A proportion of patients was on antihypertensive therapy, which may also have affected our results.

It has been suggested that SLC activity may reflect post-translational modification of an erythrocyte Na+/H+ exchanger and that these changes may be influenced by insulin levels [9]. Two distinct subgroups of hypertensive patients have been described, one with a normal SLC and enhanced Na+/H+ exchange Vmax, and another with elevated SLC but normal Na+/H+ exchange Vmax and low Hill coefficient [26]. Pontremoli et al. [10] have found that insulin in vitro causes an increase in both SLC and Na+/H+ Vmax, while others have found an opposite effect on SLC Vmax [11], or no relationship between fasting insulin levels or insulin in vitro and Na+/H+ Vmax [12, 51].

In the current study, fasting insulin levels were measured in 29 individuals with HTG, and in age- and sex-matched controls. While fasting insulin levels were elevated in the HTG group (who also had an increased body mass index, systolic and diastolic blood pressures and reduced HDL compared with controls), the correlation between SLC Vmax and insulin levels or glucose/insulin ratio did not reach statistical significance. We therefore failed to confirm a relationship between SLC Vmax and insulin levels in our subjects, and within the HTG group the significant correlations were with lipid parameters and fasting glucose levels. While some studies have shown a correlation of SLC with fasting insulin in vivo [23], others suggest that the correlation is between elevated SLC and insulin-mediated glucose disposal [24, 25, 27]. There was no evidence of a relationship between Na+/H+ exchange and fasting insulin or glucose levels.

In view of the controversy as to whether SLC is a mode of Na+/H+ exchange in erythrocytes [9, 52], we wished to compare the two transporters simultaneously. We did not find any correlation between Na+/H+ exchange in lymphocytes and SLC in erythrocytes. We accept there may be differences in the exchanger between the two cell types, but a recent study comparing both transport systems in erythrocytes of normotensive and hypertensive individuals found that, despite an elevated Vmax of Na+/H+ exchange and elevated standard SLC in hypertensive patients, the two parameters were not significantly correlated [53].

Thus, we have confirmed elevated SLC Vmax in our hypertriglyceridaemic population which is independent of other components of the insulin resistance syndrome. Elevated cholesterol does not appear to have an effect on SLC in vivo. Altered SLC activity has been suggested to be an intermediate phenotype of cardiovascular risk in several previous studies [9, 14, 15, 27]. Whether this is due to modulation by insulin receptor activity, or rather reflects a more fundamental disorder of membrane structure which predisposes to the insulin resistance syndrome but can be influenced by plasma lipids, remains to be fully elucidated.
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REFERENCES


