AJH 2001; 14:32–37

Relation Between Sodium–Lithium Countertransport and Hypertriglyceridemia in Type V Hyperlipidemia

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Sodium–lithium countertransport (SLC) kinetics were measured in 30 patients with type V hyperlipidemia, 30 patients with type IIB hyperlipidemia on similar treatment, and 30 age- and sex-matched healthy controls. Clinical and laboratory data including basic anthropometry and blood pressure were obtained and blood was taken for detailed lipid biochemistry, glucose, insulin, and leptin measurements. Patients with type V hyperlipidemia were normotensive but more obese than controls, had elevated triglycerides, very low-density lipoprotein, glucose, and insulin; and reduced HDL cholesterol compared with type IIb controls. The median SLC activity (0.23 *v* 0.21 mmol Li^+/L RBC/h) and median maximal velocity (0.33 *v* 0.31 mmol Li^+/L RBC/h) were increased, but not significantly, compared to controls. In patients with type V hyperlipidemia SLC maximal velocity correlated with log triglycerides ($r^2 = 0.853$; $P < .001$) and log very low-density lipoprotein (VLDL) triglycerides ($r^2 = 0.947$; $P < .001$). Sodium–lithium countertransport maximal velocity correlated weakly with the homeostasis model assessment index of insulin resistance ($r^2 = 0.224$; $P = .06$). The sodium affinity of the transporter did not differ between the groups and was independent of any of clinical or biochemical parameter studied. We conclude that VLDL triglyceride is strongly correlated with SLC maximal velocity and activity in patients with type V hyperlipidemia. Am J Hypertens 2001;14:32–37 © 2001 American Journal of Hypertension, Ltd.

Key Words: Sodium–lithium countertransport, familial hyperchylomicronemia, lipoprotein lipase, insulin, hypertriglyceridemia.

Sodium–lithium countertransport (SLC) is a poorly understood membrane transport process.[1] Abnormal behavior of the transporter has been associated prospectively with the risk of hypertension,[2–4] non-insulin-dependent diabetes mellitus,[5] and risk of coronary artery disease.[6–8] Observations of the behavior of the transporter suggest that the kinetic components of the countertransporter show associations between triglycerides and insulin and maximal velocity (V_{max}) and family history and sodium affinity (K_m).[1,9,10] Possible links between plasma lipids and countertransporter maximal velocity thorough the influence of plasma lipids on erythrocyte membrane fatty acid content have been proposed.[9] Only a limited number of studies have examined the interrelationship of SLC and mild dyslipidemia.[11–13] In this study we investigated the effects of hypertriglyceridemia on the behavior of the sodium–lithium countertransporter in patients with type V hyperlipidemia. The results are compared with those obtained from studies in a similarly treated group of polygenic hyperlipidemic patients and untreated healthy controls.

Methods
Patients

The 30 patients recruited for this study included 24 men and 6 women. All presented clinically with hypertriglyceridemia >30 mmol/L (100%), pancreatitis (13%) or eruptive xanthomata (17%), chylomicronemia and elevated very low-density lipoprotein (VLDL)/chylomicron (CM) remnants on lipid ultracentrifugation (100%). Full informed ethical consent was obtained in all patients and they were sampled while receiving lipid-lowering drug therapy. It was deemed unethical to withold treatment for the 3- to 6-month period necessary for return to baseline values for lipids and SLC activity. Lipid-lowering drug

Received December 17, 1999. Accepted May 30, 2000.

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This study was funded by the Special Trustees of the Charing Cross and Westminster Hospitals.

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0895-7061/01/$20.00
PII S0895-7061(00)01228-0

therapy in these patients comprised 200 mg of fenofibrate micronized daily in all but 1 patient who was intolerant of fenofibrate and therefore, was prescribed ciprofibrate. All additional lipid-lowering medications were discontinued for 6 weeks before study.

Basic anthropometric data (height and weight) were collected and blood pressure was measured manually by the same operator on three separate occasions.[7] Alcohol intake was ascertained by asking the patient. Smoking status and clinical data was obtained from the patient histories.

Controls

Thirty age- and sex-matched controls were recruited from healthy volunteers attending Chelsea & Westminster Hospital. The control subjects had no history of coronary disease, hypertension, diabetes, hyperuricemia, or other disorders known to affect lipoprotein profiles. These patients were coinvestigated with the study patients with samples undergoing concurrent analysis.

A separate population of patients with moderate dyslipidemia of Freidricksen type IIB were recruited from the lipid clinic and matched for sex and identical drug therapy and approximately for age. Patients with moderate mixed hyperlipidemia were recruited given the low usage of the drug concerned and its indications in clinical practice.

Separation of Lipoproteins

Blood samples were collected from fasting patients (16 h) into EDTA to give a final concentration of 1 mg/mL. Plasma was separated after centrifugation at 1500 *g* for 10 min at 4°C. Chylomicrons were isolated by precentrifugation at 10,000 *g* for 2 h. Very low density lipoprotein, intermediate density lipoprotein, LDL, and HDL were separated by sequential potassium bromide gradient ultracentrifugation at densities of 1.006, 1.016, 1.030, 1.063, and 1.21 g/L for 16, 16, 18, and 20 h, respectively, using a Kontron Centrikon TI-2070 ultracentrifuge (Watford, United Kingdom) with a 40 well TFT-45.6 rotor at 10,5000 *g* (35,000 rpm) at 4°C.[14]

Biochemical Analysis

Total cholesterol and cholesterol in subfractions was measured by the CHOD-PAP method on a Vitros 950 analyzer (Johnson & Johnson, High Wycombe, United Kingdom). Triglycerides and subfraction triglyceride content were measured after 2-, 5-, and 10-fold dilution using a lipase method on the same analyzer. LDL concentrations were determined by calculation using the Freidewald equation in patients with triglycerides $<$4.5 mmol/L (400 mg/dL) in the control group. Other biochemical parameters including urea and electrolytes, glucose, aspartate transaminase, bilirubin, gamma-glutamyl transferase, urate, and creatine kinase were measured by standard methods on the Vitros 950 analyzer.

Apolipoproteins A1, A2, and B_{100} concentrations were determined by immunoturbidimetry on a Cobas Fara 2 analyzer (Roche, Lowes, United Kingdom) using Dako antisera.[15] Lipoprotein(a) was determined by a similar method after preincubation of the sample with lipase and subsequent centrifugation.[16] The modification has previously been found to be essential to eliminate triglyceride interference with the lipoprotein(a) assay.[16]

Plasma leptin concentrations were measured using an enzyme linked immunosorbent assay (ELISA) kit (Biogenesis) validated using reference sera supplied. Plasma insulin was measured by ELISA using the Boehringer-Mannheim kit for an ES700 automated analyzer with 10% cross-reactivity for pro-insulin and 4% for 22,23 des-pro-insulin. Measurements were validated with a Corning ACS method (Newbury, United Kingdom) with lower cross-reactivity at a later date with no significant alteration in results. Insulin resistance index were calculated using standard formulas from the homeostasis model assessment (HOMA) and from insulin–glucose product.[17,18]

Measurement of Sodium–Lithium Countertransport

Sodium–lithium countertransport activity was measured as sodium-stimulated lithium efflux at 150 mmol/L sodium by established methods.[1,9,10,13] Erythrocytes were loaded with lithium for 3 h and aliquots were added to choline/sodium-containing media. Loaded erythrocytes (9.4 $\pm$ 3.4 mmol/cell/L lithium) were incubated in 10 external media of differing sodium concentrations in the range 0 to 150 mmol/L (0, 15, 30, 45, 60, 75, 90, 112.5, 129, and 150 mmol/L). Isotonicity was maintained by appropriate amounts of choline chloride; incubation media included magnesium chloride (1 mmol/L); glucose (10 mmol/L), ouabain (0.1 mmol/L), and Tris-MOPS pH 7.4 at 37°C (10 mmol/L). Aliquots were removed at 15, 30, and 45 min and used to determine the lithium efflux rate into the supernatant. The kinetic parameters maximal velocity (V_{max}) and sodium affinity (K_m) were derived from lithium efflux rates plotted against the lithium efflux/sodium concentration ratio in an Eadie-Hoffstee plot. Data were fitted by linear regression with K_m and was determined from the slope and V_{max} from the y-intercept. Correlation coefficients of 0.98 were achieved in all cases. Inter- and intraassay variances were 9.8% $\pm$ 6.5% and 5.9% $\pm$ 3.8% for V_{max} and 10.8% $\pm$ 6.0% and 10.2% $\pm$ 5.1% for K_m, respectively.

Statistical Analysis

Results were analyzed using a commercial spreadsheet package (Microsoft Excel and SPSS; SPSS Inc, Chicago, IL). Linear regression was used to determine SLC kinetics and lipid fraction correlations. Population data was analyzed by ANOVA when normally distributed and otherwise by the nonparametric Mann-Whitney U test. Multivariate analysis was conducted for sodium–lithium countertransport kinetics with patient demographic and clinical biochemical

Table 1. Clinical characteristics of patients with type V hyperlipidemia, patients with polygenic mixed hyperlipidemia on similar drug treatment, and controls

Parameter	Type V	Polygenic	Controls
Sex (M/F)	24/6	24/6	24/6
Age (yr)	50.1 ± 9.7	54.0 ± 7.8	49.6 ± 10.0
Blood pressure systolic (mm Hg)	127 ± 14	135 ± 21	115 ± 16
Blood pressure diastolic (mm Hg)	80 ± 7.8	83.2 ± 12	75 ± 8.5
Alcohol use (U/wk)	10 ± 10	15 ± 9	10 ± 10
Cigarette smoking	1/0	3/0	0/0
Diabetes mellitus (M/F)	6/1	0/0	0/0
Body mass index (kg/m^2)	27.9 ± 2.9	27.5 ± 4.8	24.2 ± 2.6

characteristics by multiple linear regression after transformation of non-normally distributed parameters. A value of $P <$ .05 was considered significant.

Results

Clinical data on the patient and control groups is shown in Table 1 and biochemical data are presented in Table 2. All type V patients remained hypertriglyceridemic compared to the healthy controls and patients with polygenic hyperlipidemia. Initial presentation triglycerides varied from 1150 to 12,830 mg/dL (12.6 to 145.0 mmol/L) in these patients and had been reduced by treatment to 146 to 4310 mg/dL (1.65 to 48.7 mmol/L) (with an average improvement of 3.2-fold; range 0.4 to 19.8). Six type V patients were diet-controlled diabetics but none were hypertensive. All other patients and controls were euglycemic. Systolic and diastolic blood pressure were slightly elevated in both patients with type V and in polygenic hyperlipidemic patients (P = .05) compared to healthy controls. Both groups were significantly more obese than controls (P = .05). Plasma triglyceride, cholesterol, insulin, and glucose concentrations were elevated in patients compared to controls. Patients with polygenic hyperlipidemia had elevated total and LDL cholesterol and LDL/apolipoprotein B concentrations despite fenofibrate therapy and elevated HDL, apolipoprotein A1 and A2 concentrations because of fibrate therapy compared to healthy controls. Patients with type V hyperlipidemia had elevated triglycerides ($P <$ .001), cholesterol ($P <$ .001), VLDL ($P <$ .001), glucose (P = .02), insulin (P = .008), and insulin–glucose product (P = .003) or HOMA index (P = .003), but not insulin:glucose ratio and reduced HDL (P = .04) compared to the polygenic drug-treated group. Plasma leptin was slightly, but

Table 2. Biochemical characteristics and sodium–lithium countertransport kinetics in patients with type V hyperlipidemia, polygenic hyperlipidemic patients on similar drug therapy, and controls

Parameter	Type V	Polygenic	Controls	P
Triglycerides (mg/dL)	520 (146–4310)	181 (78–830)	163 (75–198)	<.001
Cholesterol (mg/dL)	266 (189–702)	227 (185–300)	216 (120–281)	<.001
VLDL triglyceride (mg/dL)	519 (146–2265)	44 (9–380)	44 (10–136)	<.001
LDL cholesterol (mg/dL)	109 ± 54	150 ± 31	115 ± 27	.39
HDL-cholesterol (mg/dL)	28.5 ± 11.0	53.6 ± 19.6	42.5 ± 11.6	.04
Apolipoprotein A1 (mg/dL)	123 ± 28	161 ± 31	135 ± 33	<.001
Apolipoprotein A2 (mg/dL)	48 ± 13	60 ± 11	51 ± 15	<.001
Apolipoprotein B (mg/dL)	134 ± 33	139 ± 42	122 ± 33	.98
Lipoprotein (a) (mg/dL)	19 (4–149)	23 (4–235)	18 (4–39)	.26
Glucose (mg/dL)	92.8 (73.8–288)	86.5 (67.0–131)	84.6 (63.1–114)	.02
Fasting insulin (IU/L)	13.7 (4.9–50.9)	10.4 (4.6–28.6)	9.8 (4.3–18.8)	.008
Insulin-glucose product	74 (23–332)	48 (21–171)	45 (18–140)	.003
HOMA	3.28 (1.02–14.7)	2.13 (0.92–7.60)	1.65 (0.78–3.40)	.003
Leptin (IU/L)	6.48 (2.3–67.0)	6.67 (1.21–38.7)	5.80 (1.82–27.6)	.6
SLC activity (mmol Li$^+$/L RBC h)	0.23 (0.12–0.87)	0.21 (0.07–0.50)	0.22 (0.04–0.68)	.44
SLC V_{max} (mmol Li$^+$/L RBC h)	0.33 (0.12–1.02)	0.31 (0.09–0.76)	0.36 (0.05–0.66)	.53
SLC K_m (mmol/L)	57.9 (15.4–112)	71.0 (24.7–126)	65.2 (6.3–291)	.47

VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HOMA = homeostasis model assessment; SLC = sodium–lithium countertransport

Statistical comparisons are between polygenic hyperlipidemic and type V patients. Non-normally distributed data are quoted as median and range.

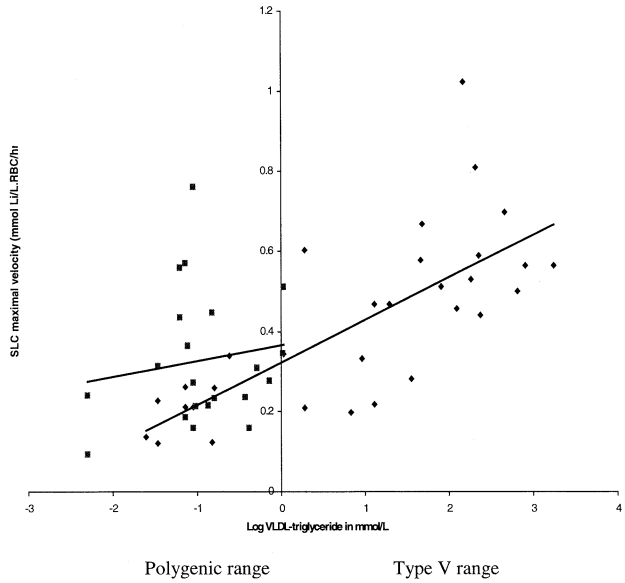

FIG. 1. Relationship of sodium–lithium countertransport (SLC) maximal velocity with $\log_e$ very low-density lipoprotein triglycerides (VLDL) (in mmol/L) for patients with polygenic and type V hyperlipidemia.

not significantly, reduced in patients with type V hyperlipidemia compared to controls in contrast to body mass index.

Sodium–lithium countertransport activity and V_{max} were not significantly different in patients with type V hyperlipidemia compared to controls and polygenic hyperlipidemic patients. In patients with polygenic hyperlipidemia log total triglyceride correlated only very weakly with SLC V_{max} ($r^2 = 0.322$; $P = .08$) and not with log VLDL triglycerides ($r^2 = 0.15$; $P = .21$) or SLC activity. However, in patients with type V hyperlipidemia, SLC activity correlated weakly with log total triglyceride ($r^2 = 0.302$; $P = .05$) but strongly with log VLDL triglycerides ($r^2 = 0.853$; $P < .001$). Similarly, SLC V_{max} correlated with log-transformed triglyceride concentration ($r^2 = 0.359$; $P = .05$) and more strongly with log-transformed VLDL triglyceride concentration ($r^2 = 0.947$; $P < .001$) in patients with type V as opposed to type IIb hyperlipidemia (Fig. 1). Multiple regression and analysis of variance confirmed that VLDL triglycerides accounted for 97% of the variance in SLC V_{max} because of triglycerides. In contrast a negative correlation was noted with lipoprotein(a) ($r = -0.293$; $P = .05$) in patients with type V hyperlipidemia. Weak correlations ($r^2 = 0.181$ and 0.224; $P = .13$ and $P = .06$) were seen with the HOMA index in patients with hyperlipidemia but not in controls. No correlation was observed with the HOMA index of β-cell function. There was no significant difference in K_m between the groups and no correlation of this variable with any biochemical parameter studied.

Discussion

The aim of the study was to identify the separate contributions of plasma triglycerides and insulin resistance on the expression of SLC by studying an extremely hypertriglyceridemic patient group with moderate-to-severe insulin resistance. Previous studies have shown that the kinetics of SLC correlate with cardiovascular risk factors particularly hypertension,[1,2,4,8,9] the metabolic syndrome (insulin resistance),[1,5] diabetes,[5] ethnic origin,[19] and to be altered in patients with established coronary arterial disease.[6,7] Although many studies have been conducted on this transporter its exact mechanism of operation and the link between its altered behavior and disease remains obscure. Some studies have suggested that the V_{max} of the countertransporter is determined by the fatty acid content of the membrane, namely by triacylglycerols, resulting in alterations of phospholipid profile[20] and exposure of sulfhydryl groups.[3] Yet in patients with hypertension or polygenic mixed hyperlipidemia it has always been thought that the mechanism might involve insulin sensitivity rather than be related to triglyceride concentration, although this remains controversial.[21–23]

It is difficult to separate the effects of chronic hyperinsulinemia from those of triglycerides on SLC in patients with insulin resistance or diabetes as insulin is fundamental to the pathogenesis of both disorders. However, a rare group of patients with lipoprotein lipase dysfunction or deficiency show a marked hypertriglyceridemia (Freidricksen type I or V hyperlipidemia) with lesser degrees of impaired glucose tolerance or diabetes. These patients present with hypertriglyceridemia and sometimes pancreatitis or eruptive xanthomata. They can have a reduced adipose tissue mass in severe cases and it might be expected that these patients would thus have reduced plasma leptin compared to controls. These patients are insulin resistant when plasma insulin and glucose are measured and many proceed to develop non-insulin-dependent diabetes, but the degree of hypertriglyceridemia far exceeds the change in insulin sensitivity in these patients in contrast to patients with diabetes where the converse is usually true. Therefore, patients with severe hypertriglyceridemia form an ideal group in whom to separate the effects of triglycerides and insulin resistance on the countertransporter. It was considered unethical to discontinue lipid-lowering therapy in these patients for sufficient time for SLC activity and plasma lipids to stabilize at baseline values, therefore, patients were studied on treatment. The fibrate drugs commonly used in the treatment of hypertriglyceridemia often improve insulin resistance and markedly alter the lipid profile. This fact necessitated the inclusion of an additional control group of patients with moderate triglyceride elevation (type IIB) who had been prescribed fibrate therapy to allow comparison of the effects of triglycerides and not drug treatment on SLC. In patients with type V hyperlipidemia triglyceride and VLDL triglyceride concentrations were reduced by a factor of 0.4 to 19.8 from presentation values, but remained high. As expected, the effects of fenofibrate were seen in polygenic hyperlipidemic patients when compared with controls and pretreatment values in this group: an elevation in HDL cholesterol, apolipoprotein A1 and A2 concentrations, and a reduction of LDL cholesterol. Otherwise, the polygenic hyperlipidemic patients were well matched with the healthy controls.

Our results agree with a previous study in showing a correlation of countertransporter V_{max} with both log triglycerides[12] and also VLDL triglycerides, but differ in not finding any clear relationship with glucose or insulin and in the lack of difference in SLC parameters between control and patient groups. The patients in the previous study[12] comprised a similar, but far less severely affected, hyperinsulinemic hypertriglyceridemic (type IV) cohort of 33 patients with a fasting triglyceride concentration of 424 ± 256 mg/dL (4.8 ± 2.9 mmol/L) and cholesterol of 258 ± 96 mg/dL (6.7 ± 2.5 mmol/L). In contrast to the previous study, SLC activity was not elevated in our patients compared to drug-treated or normal controls. Blood pressures were also markedly lower (127/80 mm Hg *v* 146/92 mm Hg), therefore the elevation in SLC activity in the previous study could have been confounded by the presence of hypertension. One other study has also noted an elevation of SLC activity in 9 patients with type IV/V hyperlipidemia but complete blood pressure data are not available from that study and many were on antihypertensive medications.[24] Our patients, like those in the other studies that have shown a correlation of SLC activity with insulin resistance, had elevated VLDL triglyceride and cholesterol concentrations even on maximal tolerated therapy.[12,21] The findings of a lack of strong correlation of SLC with parameters associated with insulin resistance in patients with type IV hyperlipidemia[12] are similar to the results of this study of type V in which the HOMA index[17,18] rather than insulin-to-glucose ratio was used as the index of insulin sensitivity. In patients with type V hyperlipidemia the extremely elevated free fatty acids may affect insulin secretion directly more than is usually seen and this may account for the lack of clear correlation of SLC activity and V_{max} and measures of insulin resistance in this group of patients. The discrepancies between the results in the two studies including patients with increased VLDL could be from the small number of patients studied or differences in selection of patients or controls. Yet, the lack of correlation of SLC activity with measures of insulin sensitivity and euglycemic clamp data has been noted in other studies in both normoglycemic and diabetic patients,[25–27] therefore, the relationship of SLC activity with insulin resistance is controversial.

Previous studies noted a nonsignificant inverse trend for correlation of lipoprotein(a) and SLC V_{max} that did not reach significance.[12] In contrast, there was a significant correlation in this study ($r^2 = -0.293$; $P = .05$). Lipoprotein(a) is cleared by the VLDL receptor,[28] which is responsible for most chylomicron and VLDL uptake in these patients, therefore the inverse correlation could be from simple outcome of competition for this receptor site. However, triglycerides can interfere with the quantification of lipoprotein(a),[16] therefore an artifactual increase in lipoprotein(a) caused by high triglycerides cannot be wholly discounted.

Previous studies have demonstrated a correlation between countertransporter activity and body mass index.[1,29] The logarithm of plasma leptin concentration is a good correlate of total adipose tissue mass and body mass index and correlates with insulin resistance[30] and thus, would be expected to correlate with the countertransporter V_{max}. In patients with homozygous severe lipoprotein lipase deficiency, adipose tissue mass is reduced but this effect was not seen clearly in this study as the patients were probably heterozygotes. The correlation between plasma leptin concentration and body mass index was similar to that seen in other studies ($r^2 = 0.62$; $P < .001$).[30] Countertransporter activity and V_{max} did not correlate with body mass index or logarithm of plasma leptin concentration in this population, although the patients were mildly overweight compared to the control population.

This study demonstrates that although SLC activity and kinetic parameters are similar in polygenic hyperlipidemic patients and those with type V hyperlipidemia, they seem to show associations with different factors related to alterations in SLC activity. In patients with type V hyperlipidemia, there is a strong association of SLC V_{max} with log VLDL triglycerides and little with the HOMA measure of insulin resistance. In contrast, in patients with polygenic hyperlipidemia, there is little association of SLC V_{max} with either VLDL triglycerides or insulin resistance. This implies that other factors, lacking in type V hyperlipidemia, modulate SLC V_{max} in patients with polygenic hyperlipidemia and supervene the relationship with triglycerides. The role of candidate factors to explain this effect including HDL cholesterol and lipoprotein lipase activity remains to be determined.

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