of the degree of peripheral vasomotor tone) were monitored for 1 h before and 2 h after the injections. In some experiments oxygen consumption ($\dot{V}_{O_2}$) was also measured.

Results. The effect of L-aspartic acid was to evoke changes which increased heat production and conservation and decreased heat loss, and this caused a rise in T_{re}. This general pattern was consistently seen but the extent of the changes depended on the ambient temperature (T_a). At 0° and 7 °C T_a 500 nmoles · kg^{-1} L-aspartic acid exerted little effect on RF which was already low, or on skin blood vessels which were fully constricted but it produced an increase in heat production, signalled by a rise in oxygen consumption, and a rise in T_{re}. Following the injection of 500 nmoles · kg^{-1} at 20 °C T_a, RF fell, the ears vasoconstricted and T_{re} rose. $\dot{V}_{O_2}$ was not monitored. A dose of 100 nmoles · kg^{-1} produced similar effects at this T_a but the changes were smaller and short lived. At T_a 40 °C, RF was of the order of 240 breaths · min^{-1} but on injection of 500 nmoles · kg^{-1} L-aspartic acid it immediately fell to 30–50 breaths · min^{-1} This resulted in a steep rise in T_{re}. After 20–30 min, RF rose above the pre-injection rate and T_{re} declined towards the normal value. At any one T_a the intensity of the changes produced by 500 nmoles · kg^{-1} L-aspartate was very similar to those produced by an ICV injection of 800–1000 nmoles · kg^{-1} L-glutamic acid but the effects did not last as long. The changes produced by L-aspartic acid at T_a 7°, 20° and 40 °C were greatly attenuated by the prior injection of 20 nmoles · kg^{-1} atropine sulphate; a dose of 40 nmoles · kg^{-1} atropine sulphate (tested at 20° and 40 °C T_a only) almost completely abolished the action of 500 nmoles · kg^{-1} L-aspartic acid.

These results suggest that injected L-aspartic acid, like injected L-glutamic acid, exerts an effect on the hypothalamic pathways between cold sensors and heat production (and conservation) effectors. The fact that the effect of both amino acids is blocked by atropine sulphate may indicate that their site of action is at, or before, the postulated[4,5] cholinergic synapse on this pathway. Such an interpretation should, however, be accepted with caution since there is evidence that atropine can block amino acid receptors as well as muscarinic receptors[6]. Caution is also necessary in accepting these results as evidence of a physiological role of endogenous aspartic and glutamic acid in thermoregulation but they suggest such a possibility.

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Lipoprotein lipase activator deficiency in very low density lipoproteins in rat nephrotic syndrome

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Summary. Marked urinary loss of lipoprotein lipase activator in experimental rat nephrotic syndrome may be partly responsible for its deficiency in plasma very low density lipoproteins.

Normally, circulating triglycerides which are primarily transported in the very low density lipoproteins (VLDL) are metabolized to glycerol and fatty acids by 2 important processes. These are a) an enzyme system of lipoprotein lipases (LPL) and b) a group of apolipoproteins which are constituents of the protein moiety of VLDL. The C apolipoproteins play a major role in the activity of LPL with apolipoprotein CII (apoCII) as the principal activator peptide. C apolipoproteins are transported in high density lipoproteins (HDL) and VLDL. VLDL contain B and C apolipoproteins. C apolipoproteins disassociate from VLDL during their catabolism, are transferred to HDL, and the VLDL is converted at least in part to the B apoprotein containing low density lipoproteins (LDL)[2]. HDL acts as a 'reservoir' of apoCII activator peptide[3]. Exogenous and endogenous hypertriglyceridemia is associated with redistribution of apoCII from HDL to chylomicrons[3] and VLDL[4]. The long half life (8–10 h) of C peptides compared to the relatively short half life of B apoprotein of VLDL (10 min) suggests that C peptides cycle between HDL and VLDL and chylomicrons[5,6].

We have previously studied an experimental rat model of hyperlipoproteinemia[7]. In these rats, the nephrotic syndrome (proteinuria, edema and hyperlipoproteinemia) was induced with a single injection of puromycin aminonucleoside. The model disease in rats mimics the disease in humans in that severe atherosclerosis occurs in association with the hyperlipoproteinemia[8,9]. In these nephrotic rats we have shown a) urinary loss of HDL, b) activation of LPL in vitro with hydrolysis of triolein to fatty acids and glycerol and c) an alteration in the composition of urinary HDL compared to its composition in the plasma. Based on these observations we have hypothesized that urinary loss of HDL and activator peptides in the nephrotic syndrome may be associated with an inadequate supply of activator for newly formed VLDL[7]. In order to test this hypothesis the following experiment was performed in which were included measurements of plasma VLDL activator activity and urinary loss of LPL activator in the puromycin aminonucleoside nephrotic syndrome rat model.

Materials and methods. 14 Sprague-Dawley rats, 240–280 g in weight were used. In 7 rats nephrotic syndrome was induced with a single jugular vein injection (under ether anesthesia) of the aminonucleoside of puromycin 6-dimethylamino-9 (3-amino 3′-deoxy-D-B-ribofuranosyl-purine), 10 mg/100 g rat weight as described previously[7]. 7 control rats were given a single jugular vein injection of saline. The rats were placed in metabolic cages. 7 days after injection, the rats were fasted except for water and urine was collected over 17 h. Blood samples in EDTA (1 mg/ml) were obtained by intracardiac puncture and plasma separated at 4 °C by centrifugation. The urine and plasma from each group of 7 rats were separately pooled so as to obtain sufficient material for analysis.

VLDL was isolated from plasma by ultracentrifugation using a L5-50 Beckman ultracentrifuge and a 40.3 rotor for 22 h[10]. The supernate from the 1st ultracentrifugation was layered with 0.15 M sodium chloride in 0.01 M EDTA and respun again for 22 h. VLDL protein[11], total plasma cholesterol and triglycerides, were quantitated using Lipid

Pooled plasma and urine data in saline treated (control) and nephrotic syndrome rats

Group	Plasma Total cholesterol (mg/dl)	Total triglycerides (mg/dl)	LPL activator (units/ml)	Plasma VLDL LPL activator VLDL protein (units/mg)	Urine Total cholesterol excretion (mg)	Total activator excretion (units)
Control rats	63	50	68	86	0.63	70
Nephrotic rats	287	266	99	46	6.79	343
Value in nephrotic rats as percent of control rats	456	532	146	53	1070	490

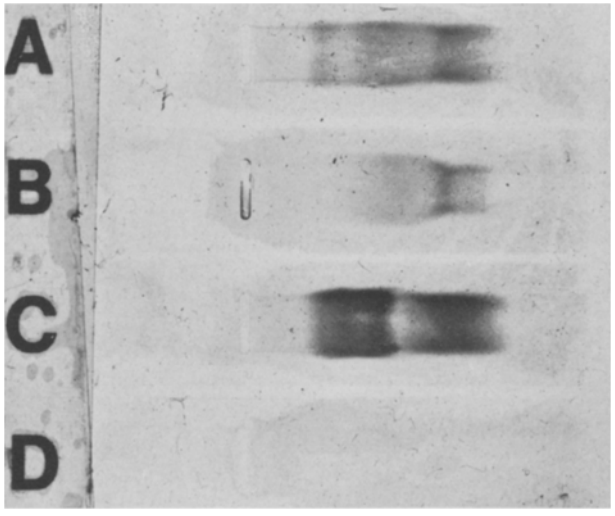
Research Clinic's methodology[12]. Lipoprotein electrophoresis was performed using agarose agar[13]. The lipoprotein lipase activating property of plasma and urine was quantified in vitro[4]. Urine was dialyzed against 0.15 M sodium chloride prior to activator measurement. Quantitative measurements reported are the mean of at least 2 duplicate values.

Results. In rats with nephrotic syndrome, there was a striking increase in total plasma cholesterol and triglyceride (table) (approximately 4.6 and 5.3fold respectively). Plasma activator rose 1.5fold. The ratio of plasma LPL activator: total plasma triglyceride in control and nephrotic rats was 1.36 and 0.37 respectively. This represents a fall to 27% in this ratio in nephrotic rats compared to controls. Activator activity per mg of isolated VLDL protein dropped to almost half the control value. Urinary excretion of activator and cholesterol was approximately 5 and 11 times that in the control rats respectively.

Lipoprotein electrophoresis in agarose agar (figure) of urine from nephrotic rats revealed a band with alpha lipoprotein mobility when concurrently electrophoresed with the plasma lipoproteins. Non migrating lipid staining material was observed at the origin. No band was detectable in the lipoprotein electrophoretograms of control rat urine. Plasma lipoprotein electrophoresis revealed intense staining in the beta, pre-beta and alpha bands.

Discussion. This study shows that in this rat nephrotic syndrome model, the LPL activating property of VLDL protein is almost halved compared to control values. This is associated with a marked urinary excretion of LPL activator. These 2 observations lend support to the hypothesis that urinary loss of LPL activator may in part be responsible for the lowered LPL activating property of VLDL protein in this species. The urine electrophoretograms shows that alpha lipoproteins are excreted. Previously we have also shown that when urine from nephrotic rats is fractionated by density gradient ultracentrifugation, cholesterol is detected only in the high density (1.063–1.210) range. This study shows that in nephrotic rats, HDL cholesterol excretion was twice that of LPL activator. This observation suggests that part of the LPL activator may be re-absorbed during passage of HDL in the nephron. This suggestion is supported by our previous observation[7] that the composition of plasma HDL is different from urine HDL in this nephrotic syndrome model. Although mechanisms for the renal alteration of HDL structure in the nephron are unknown, tubular maxima of reabsorption may exist for HDL or its components. The kidney is an active organ in catabolism of apolipoprotein AI, the major component of HDL[14]. The possibility of a LPL inhibitor in nephrotic urine cannot be ruled out.

Total plasma LPL activator increased only 1.5fold whereas total plasma triglycerides increased 5.3fold in nephrotic rats. This may be explained by a) renal and urinary losses of LPL activator as discussed above and b) the presence of LPL inhibitors in plasma from nephrotic rats a possibility for which there is no evidence.



Agarose agar electrophoretograms of rat plasma and urine stained for lipoproteins. Samples were applied in the troughs. Anode and cathode terminals of electrical field are on the right and left respectively. *A* Saline control rat plasma. *B* and *C* Urine and plasma from nephrotic syndrome rats respectively. *D* Saline control rat urine.

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