INTRODUCTION

In normal subjects, chylomicron- and VLDL-remnants are rapidly removed from the circulation by means of receptor-mediated endocytosis in the liver or conversion into low density lipoprotein (LDL) (Brown et al., 1981). The apolipoprotein E (apo E) present on lipoprotein remnants plays a central role in the hepatic metabolism of remnant particles as this apolipoprotein is recognized with high affinity by the hepatic receptors involved in remnant uptake (Sherill et al., 1980).

As determined with isoelectric focusing human apo E can be separated into three major isoforms i.e. E2, E3 and E4 and a number of minor glycosylated isoforms (Utermann et al., 1977; Zannis and Breslow, 1981). Amino acid sequence analysis has established that the three major apo E isoforms differ by single amino acid substitutions (Rall et al., 1982a). Apo E3 is the most commonly occurring or wild type form. Apo E4 is supposed to be derived from E3 by a Cys → Arg substitution at position 112 and is designated as E4 (Cys → Arg). At present three forms of apo E2 have been described E2 (Arg → Cys), E2 (Arg → Cys) and E2 (Lys → Gin).

In addition, other variant forms of apo E are discovered i.e. E3 (Ala → Thr, Ala → Pro), E3 (Cys → Arg, Arg → Cys) and E1 (Gly → Asp, Arg → Cys), the latter being one charge unit more negative than E2 (Innerarity et al. 1984). Besides the above described apo E variants with known amino acid substitutions other apo E variants have been described (Yamamura et al. 1984a, Yamamura et al. 1984b, Ghiselli et al. 1984, Havel et al. 1983). Except for apo E4, the above mentioned variants of apo E represent the underlying major defect in familial type III hyperlipoproteinemia due to a more or less pronounced defect of these variants in binding to the hepatic lipoprotein receptors (Rall et al. 1982b; Schneider et al. 1981).

Most type III hyperlipoproteinemic patients are E2/E2 homozygotes. We found a patient (C.V.) with type III hyperlipoproteinemia but with E3/E3 phenotype. We wondered whether the apo E3 from this patient was defective in binding to the LDL receptor. Therefore, apo E3 from patient C.V. was isolated by preparative SDS polyacrylamide gel electrophoresis, complexed with phospholipid vesicles according to method A described by Schneider et
al. (1981) and tested for competing with \(^{125}\)I-LDL for degradation by HeLa cells at 37°C. From the results shown in Fig. 1 it is obvious that this apo E3 is defective in binding to the LDL receptor albeit to a lesser degree than apo E2. In addition, apo E3 isolated from the probands mother and one sister (Fig. 1) also appeared to be defective in binding to the LDL receptor as compared with apo E3 derived from a apparently healthy subject with phenotype E3/E3. This defective apo E3 is denoted as apo E3-Leiden.

After cysteamine treatment both normal apo E3 and apo E2, that contain respectively one and two cysteine residues, will migrate at the position of apo E4 upon isoelectric focusing. In Figure 2 it is shown that after treatment with cysteamine apo E3-Leiden does not focus on the E4 position indicating that apo E3-Leiden does not contain any cysteine residues or that in apo E3-Leiden cysteine residues are not accessible for reaction with cysteamine.

To investigate the possibility that the apo E3-Leiden isoform represents a sialylated derivative of apo E4 (Cys\(_{112}\) + Arg), VLDL from patient C.V. was subjected to neuraminidase treatment. As evaluated by isoelectric focusing, treatment of VLDL from patient C.V. with neuraminidase had no effect on the major E3 band but only on the minor bands at position E2 and E1 (Fig. 3). This observation is identical to that which is found after treatment of normal apo E3 with neuraminidase.

In order to perform a family study we tested the mother, three sisters and three brothers of the proband (patient C.V.). All members tested, except one sister and one brother, had elevated triglyceride, plasma cholesterol and apo E levels. The density gradient profiles were remarkably uniform in the affected family members. They all showed the presence of intermediate density lipoproteins (IDL) and, in contrast with most type III hyperlipoproteinemic patients, LDL was relatively high. The density gradient profiles of the normolipidemic brother and sister did not show the presence of IDL. Isoelectric focusing of apo VLDL of the family...