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FREE FATTY ACIDS ALTER THE ELECTROPHORETIC MOBILITY OF SERUM LIPOPROTEINS

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When normal human serum was analysed after addition of free fatty acids (FFA: oleic acids), the lipoproteins showed abnormal mobility on agarose-gel electrophoresis. β- and α-lipoproteins from the serum with a FFA concentration of 2.04 μEq/ml or more migrated with a mobility showing a more negative charge than native ones. Although the ultracentrifugal profile of the lipoprotein cholesterol was not altered with the excess-FFA, FFA were detected in the lipoprotein fractions as well as in free proteins. The lipoproteins isolated from the excess-FFA serum also showed abnormal mobility on agarose-gel electrophoresis.

These results suggest that, in the serum containing excess-FFA, FFA attach themselves to the lipoproteins and alter their charge.

INTRODUCTION

Free fatty acids (FFA) are essential substance for various tissues as a source of energy. However, excess-FFA have been known to exert detrimental effects on cell membranes, and to inhibit a variety of metabolic reactions. And high levels of FFA have been well discussed in the pathogenesis of coronary heart disease (1).

Recently, some investigators have focused their attention on the chemically modified lipoproteins, saying that it is these, rather than normal lipoproteins, that lead to cholesterol ester accumulation in the cells of atherosclerotic reaction (2-4). However, there is no evidence that such modifications of lipoproteins occur in vivo.

We observed previously that the serum lipoproteins from a patient with a high level of FFA showed abnormal mobility on the electrophoresis. So we predicted that FFA modified the nature of the lipoproteins, and that this conventional substance is a candidate playing a hazardous role in lipoprotein metabolism in vivo.

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To evaluate the prediction described above, we first studied the influence of FFA on the nature of serum lipoproteins in vitro.

MATERIALS AND METHODS

Serum. Blood samples were obtained from normal humans after overnight fast. Serum was separated after standing at room temparature for 3 hours. Serum of 20 subjects was pooled to use for the experiments (FFA: 0.34 μEq/ml).

FFA addition. Oleic acids (P-L Biochemicals Inc., U.S.A.) was used as FFA. After addition of FFA, the samples were shaken vigorously and left at room temparature for 3 hours, then subjected to the analysis.

Agarose-gel electrophoresis. Agarose-gel electrophoresis was performed using the apparatus of Pol E-Film System (Pfizer, Diagnostic Div., U.S.A.) and commercial agarose plates (Universal Electrophoresis Film, Corning ACI) containing 1.0% agarose. Electrophoresis was carried out in MOPS-Li buffer (Nipponshoji, Co., Ltd., Japan) at room temparature for 50 min. Detection of lipoproteins was done by staining cholesterol with TC-Diformazan method using Staining Reagent-N for TC-fraction (Nipponshoji, Co., Ltd., Japan) (5).

Ultracentrifugation. A RPS-27-2 rotor was used in a Hitachi Model 65P7 Ultracentrifuge (Hitachi Koki Co., Ltd., Japan) for fractionation of lipoproteins. The density of the sample (10 ml) was adjusted to 1.30 g/ml by adding solid NaBr. The sample was overlayed with 14 ml of NaBr-solution (d = 1.06) and 10 ml of distilled water. All solutions contained 1 mM EDTA. Centrifugation was carried out at 25,000 rpm (av RCF 87,700 x g) for 20 hours at 15°C. The rotor content was collected in 2.0 ml fractions. Each fraction was assayed for density and dialysed against 0.15 M NaCl overnight. The lipoproteins fractionated by the ultracentrifugation were concentrated with Centriflo CF25 (Amicon, U.S.A.) to appropriate strength for the electrophoretic analysis.

Chemical analysis. Total cholesterol (TC) and FFA were determined by employing established enzymatic methods (6,7).

RESULTS

Fig. 1 shows the influence of FFA on the behavior of serum lipoproteins in the agarose-gel electrophoresis. β-lipoproteins migrated to the abnormal region on the gel when the FFA concentration of the sample was 2.04 μEq/ml. α-lipoproteins also showed altered mobility at the FFA concentration of 2.04 μEq/ml. The abnormal mobility of these lipoproteins, indicating a more negative charge than native ones, became remarkable as the FFA-concentration increased. Pre-β-lipoproteins were not detected in this method due to the small amount present.

Fig. 2 shows the influence of FFA (3.6 μEq/ml) on the ultracentrifugal profile of the lipoprotein cholesterol. No difference was observed in the banding pattern of the lipoproteins from the excess-FFA serum compared to that of normal serum (Fig. 2b). However, a considerable amount of FFA was detected in the lipoprotein fractions of the excess-FFA serum (Fig. 2a).

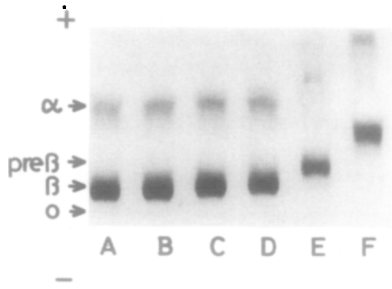

Fig. 1 Influence of FFA on the electrophoretic mobility of the serum lipoproteins. Agarose-gel electrophoresis and cholesterol staining were performed as in the text. 30 µl of each sample was applied. A: normal serum B-F: serum with FFA added to concentrations of 1.02, 2.04, 4.00 and 5.40 µEq/ml, in this order. The arrows indicate the migration position of each class of lipoproteins. O: origin +,-: electrodes

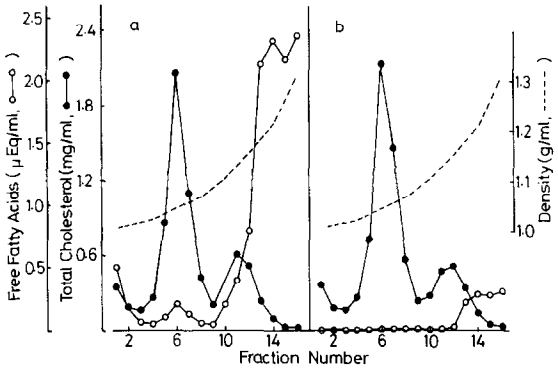

Fig. 2 Influence of FFA on the ultracentrifugal behavior of the serum lipoproteins. Ultracentrifugation was carried out as in the text. The fraction number 1-2, 5-7 and 11-13 were regarded as VLDL, LDL and HDL, and subjected to the analysis. a: excess-FFA serum b: normal-FFA serum

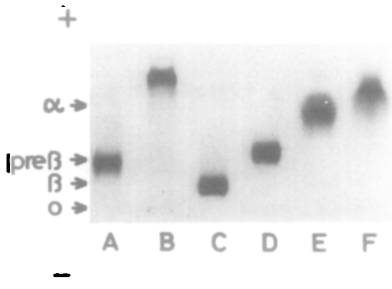

Fig. 3 Agarose-gel electrophoresis patterns of the lipoproteins isolated from the excess-FFA serum. A, C and E were VLDL, LDL and HDL from normal-FFA serum. B, D and F were VLDL, LDL and HDL from excess-FFA serum. Approximately 40 µg of cholesterol (in 30-40 µl) of each fraction was applied to the gel. The symbols at the left side mean the same as in Fig.1.

Fig. 3 shows the agarose-gel electrophoresis of the lipoproteins isolated by ultracentrifugation as in Fig. 2. All classes of lipoproteins from the excess-FFA serum showed abnormal mobilities, indicating a more negative charge than those of normal-FFA serum. The difference in migration was greatest in very low density lipoproteins (VLDL), and least in high density lipoproteins (HDL), with low density lipoproteins (LDL) in between.

DISCUSSION

It is well known that serum-FFA level changes according to physiological and pathological conditions. A large amount of knowledge has been accumulated concerning the detrimental effects of excess-FFA on membrane function and on metabolic functions (1).

The experiments reported here demonstrate that FFA alter the charge of all classes of lipoproteins. These are observations concerning serum containing an unusual high level of FFA. However, we have found that such levels of FFA were achieved frequently in patients on hemodialysis using heparin for anticoagulant, and the lipoprotein from them showed abnormal mobility on agarose-gel electrophoresis (to be published).

Recently, chemically modified lipoproteins, i.e., acetylated, glutaraldehyde and malondialdehide-treated LDL, were shown acquiring a more negative charge than native ones and making macrophages to accumulate cholesterol ester (2-4).

As the lipoproteins from excess-FFA serum were also shown with a more negative charge than native ones, we are anxious that abnormal lipoproteins may exert a detrimental effect on a variety of biological functions.

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