CONCENTRATION OF APOLIPOPROTEIN-E IN HIGH-DENSITY LIPOPROTEINS OF HUMAN BLOOD PLASMA

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Abstract – The aim of this study was to determine apolipoprotein (apo) E concentration in the high-density lipoprotein (HDL) fractions of normolipidemic subjects. ApoE concentrations in total blood plasma and HDL fractions were measured by an immunoturbidimetric method. We observed that the quantitative distribution of apoE among different lipoprotein classes depends on the total plasma apoE concentration: at low total plasma apoE concentration, a substantial amount of apoE was associated with HDL; an increase in total plasma apoE was accompanied by a more equal distribution of apoE among lipoprotein fractions. The concentration of apoE in the HDL fraction was stable and did not depend on the total plasma apoE concentration. Thus, the preservation of a constant concentration of apoE in HDL due to its redistribution among lipoprotein classes is a priority when total plasma apoE concentrations change. This feature should be considered at diagnosis and for the treatment of lipid disorders.

Keywords: Apolipoprotein E, high-density lipoproteins, very low-density lipoproteins, plasma, humans

INTRODUCTION

ApoE is a monomeric glycoprotein with 299 amino acid residues and a 34 kDa molecular mass (Rall et al., 1982). The protein is a structural and functional constituent of plasma chylomicrons and very low-density lipoproteins (VLDL), and their lipolytic degradation products (i.e. chylomicron remnants and intermediate density lipoproteins). ApoE is also found in some subfractions of HDL (Shore and Shore, 1973; Havel et al., 1980; Mahley et al., 1984). ApoE is synthesized in the liver and extrahepatic tissues (brain, kidneys, adrenal glands, spleen, muscles, skin, macrophages, etc.). Plasma apoE is largely liver-derived (Williams et al., 1985; Mahley, 1988).

ApoE plays an important role in lipid metabolism. The main function of apoE is to regulate uptake of lipoproteins from the circulation by receptor-mediated endocytosis (Mahley et al., 1984; Funke et al., 1984). ApoE also participates in the synthesis of some lipoproteins, in the transport and redistribution of lipids among various tissues including the cholesterol transport from peripheral tissues to the liver and in the repair of nervous cells and the vascular wall. Moreover, this protein has been shown in vitro to have antioxidant and anti-inflammatory properties (Eisenberg, 1984; Koo et al., 1985; Mahley, 1988; Hayek et al., 1994; Kelly et al., 1994; Vogel et al., 1994; Mabile et al., 2003; Ali et al., 2005; Pham et al., 2005; Boiko and Kaneva, 2009).

ApoE is a metabolically active apolipoprotein that transfers readily between lipoprotein classes. Initially apoE is secreted with nascent HDL and then transferred on chylomicrons and VLDL (Blum, 1982; Phillips et al., 1983; Luc et al., 1996). Some biological or pathological factors may affect the apoE distribu-
tion among different lipoprotein fractions without changing the total plasma level of apolipoprotein (Siest et al., 1995). These changes in apoE distribution are particularly evident in such pathological states as familial hypercholesterolemia (Gibson et al., 1987), myocardial infarction (Bittolo Bon et al., 1984), coronary artery disease (Barbagallo et al., 2006) and atherosclerosis (Luc et al., 1996). Postprandial variation of apoE concentrations in different lipoproteins fractions is the most common case of physiological change in apoE distribution (Blum et al., 1980). The exchangeability of apoE among lipoprotein particles is critical for lipoprotein metabolism, but despite its importance, the features of this phenomenon have not been clearly defined. Study of apoE distribution among the major lipoprotein classes in the fasting state may be useful for understanding metabolic processes and it has important clinical and diagnostic value.

**MATERIALS AND METHODS**

**Subjects and sampling**

The cross-sectional study of 36 apparently healthy men was conducted. The median (25%; 75%) values for the subjects’ age and body mass index were 31 (29%; 36%) years and 24.7 (21.8%; 25.4%) kg/m². The studied subjects had normolipidemia (total cholesterol <5.5 mmol/l; triglycerides <1.8 mmol/l) and did not receive any lipid-lowering drugs. All participants were considered as being free from serious and chronic illnesses at the time of the recruitment. Each subject gave written informed consent for participating in the study, which was approved by the ethics committee of Institute of Physiology, Komi Science Center, Ural Branch of Russian Academy of Sciences.

A single blood sample of 5 ml was taken by rapid venipuncture with minimum stasis in the morning after an overnight fast of 12-13 hours. The samples were collected into vacutainers (Becton Dickinson BP). Blood samples were centrifuged; plasma was placed into Eppendorf microcentrifuge tubes and stored at -20°C until analysis.

**Lipid measurements**

Plasma total cholesterol and triglyceride concentrations were measured by enzymatic methods on a Power Wave-200 automated spectrophotometer (Bio-Tek Instruments, USA) with commercially available kits (Chronolab, Switzerland). HDL-cholesterol concentration was determined by assayng the cholesterol in the supernatant obtained after precipitation of apoB-containing lipoproteins with phosphotungstate/magnesium chloride.

**ApoE measurements**

ApoE concentrations were determined in the total plasma and in the supernatant containing HDL (HDL apoE) after precipitation of apoB-containing lipoproteins. The amount of apoE in the HDL fraction (% of apoE in HDL) was expressed as a percentage of the total plasma apoE level. ApoE concentration was measured by immunoturbidimetric method using a kit from Chronolab (Switzerland).

The samples were analyzed immediately after thawing at 37°C in a thermostatic bath. Measurement of each sample was carried out in duplicate and the mean was calculated. Absorbance of all samples was measured on the Power Wave-200 automated spectrophotometer (Bio-Tek Instruments, USA) at 340 nm.

**Statistical analysis**

Statistical analysis was performed with Statistica 6.0 (Statsoft, Tulsa, USA). Variables are presented as median and interquartile range (25th and 75th percentiles). The total plasma and HDL apoE levels in participants were compared across apoE quartiles using the Kruskal-Wallis test. A value of P<0.05 was accepted as statistically significant.

**RESULTS**

Total plasma and HDL apoE concentrations are presented in Table 1, together with the concentrations of other plasma lipids, the subjects’ age and body mass.
indices. The plasma concentrations of total cholesterol, triglycerides and HDL-cholesterol were in the normal range. The median of the total plasma apoE concentration for the whole group was 2.42 (2.14; 3.34) mg/dl, with values ranging from 1.38 to 4.38 mg/dl. The concentration of apoE in the HDL varied from 1.03 to 1.96 mg/dl. Thus, the range of variation of the total plasma apoE concentration was much wider than for HDL apoE. This causes a large variability among individuals in the percentage of plasma apoE associated with HDL.

The subjects were divided into quartiles on the basis of total plasma apoE concentrations (Table 2). The total plasma concentrations of apoE in the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> quartiles were <2.14, 2.14-2.42, 2.43-3.34 and >3.34 mg/dl, respectively. All the quartiles contained nine subjects. Median values for the concentration of HDL apoE were not significantly different across the quartiles despite there being significant differences in the total plasma apoE concentration (caused by the design of the research). Conversely, the percentage of apoE in HDL changed significantly across the quartiles. The amount of apoE in HDL was inversely associated with the total plasma apoE concentration. A higher percentage of apoE in HDL was observed in the lowest quartile. The amount of apoE in HDL in the subjects of this quartile varied from 56.9 to 98.1%. Subjects in the highest quartile had a lower percentage of apoE in HDL (from 32.4 to 54.1%). The median value for the percentage of apoE in HDL decreased from 78.6% in the lowest, to 41.8% (P<0.001) in the highest quartile. Thus, it is likely that the substantial amount of apoE at the low total plasma apoE level was associated predominantly with the HDL fraction. The increase in total plasma apoE concentration was accompanied by a more equal distribution of apoE among the lipoprotein classes. Meanwhile, the concentration of apoE in the HDL fraction in the subjects was stable and did not depend on the total plasma apoE level.

**DISCUSSION**

The functional properties of lipoproteins depend on the apoprotein constituents that enter their structure. The amount of structural apoproteins (apoA, apoB) in lipoproteins is stable, whereas the distri-
bution of metabolic apoproteins (apoC, apoE etc.) among lipoprotein fractions may greatly vary (Castro and Fielding, 1984). It is believed that the change in apoprotein content of lipoproteins plays a key role in the initiation of many physiologically important metabolic processes.

In normolipidemic subjects, the majority of apoE in the plasma is associated with HDL (Blum et al., 1980; Phillips et al., 1983; Fredenrich et al., 1997). The current literature states that the HDL apoE concentration varies from 39% (in the total plasma apoE concentration of 6.67±1.92 mg/dl) (Avogaro et al., 1983), to 64% (in the total plasma apoE concentration of 4.65±0.27 mg/dl) (Gibson et al., 1987). We did not find any mention of a higher HDL apoE concentration in the literature. Our results indicate that the amount of plasma apoE associated with HDL may achieve 98%. This was observed at low total plasma apoE concentrations, i.e. practically the whole amount of apoE when total the plasma apoE concentration was low, was associated with HDL. This primary accumulation of apoE in HDL provides a stable concentration of apoE in these fractions, even at lowest total plasma apoE concentrations. In the investigated subjects, the amount of apoE in the HDL fraction varied in a narrow range, from 1.03 mg/dl (in the total plasma apoE level of 1.49 mg/dl) to 1.96 mg/dl (in the total plasma apoE level of 3.72 mg/dl). This suggests that preservation of a constant concentration of apoE in HDL when total plasma apoE concentrations are low is a priority and is ensured due to the reduction of the amount of apoE in other lipoprotein classes. A fact confirming the importance of preservation of a constant concentration of apoE in HDL is that the decrease of the total apoE concentration by statin therapy in patients with hyperlipidemia occurs as a result of its removal from VLDL and IDL fractions, while the concentration apoE in HDL does not practically change (Tvorogova et al., 1998).

The decrease of apoE in HDL and the simultaneous increase of apoE in apoB-containing lipoproteins is observed in some disease states, such as atherosclerosis and after myocardial infarction (Bittolo Bon et al., 1984; Siest et al., 1995). ApoE-rich apoB-containing lipoproteins are associated with atherosclerosis because macrophages actively endocytose these proteins and transform them into foam cells (Kameda et al., 1984; Bates et al., 1987). Thus, the accumulation of apoE-rich apoB-containing lipoproteins and the decrease of apoE concentrations in the HDL fraction may represent additional factors that could promote and initiate the atherosclerotic process and coronary heart disease (Wilson et al., 1993; Barbagallo et al., 2006).

A redistribution of apoE among lipoproteins occurs in a number of physiological processes, for example in the development of alimentary lipemia. In alimentary lipemia, the total plasma level of apoE did not change, although a major transfer of this apoprotein from HDL to triglycerides-rich lipoproteins was apparent (Blum et al., 1980; Siest et al., 1995). Some authors have suggested that the apoE in HDL acts as a reservoir, from which ApoE can be rapidly dispatched on other lipoproteins in response to metabolic changes (Blum et al., 1980; Falko et al., 1980)

In conclusion, the lipoprotein distribution of apoE in men depends on the total plasma apoE concentration. At low total plasma apoE concentration, a substantial amount of apoE is concentrated in the HDL fraction, whereas at higher total plasma apoE concentrations, an equal distribution of apoE among the major lipoprotein-density classes is observed. The concentration of apoE in the HDL fraction remained stable and did not depend on the total plasma apoE concentration. Maintenance of apoE associated with HDL appears to preserve optimal functional properties of these lipoproteins.

REFERENCES


