The Distribution of Serum High Density Lipoprotein Subfractions in Non-Human Primates

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ABSTRACT

The ultracentrifugal flotation patterns in 1.2 g/ml solvent and ultracentrifugal gradient distribution of high density lipoproteins (HDL) from the primates—human, apes and monkeys—were determined, with emphasis on the gorilla species of apes and rhesus monkeys. Diets for non-human primates were commercial chow, which is low in cholesterol. Molecular weights and protein, cholesterol, phospholipid and triglyceride compositions of various density fractions were determined on human, gorilla and rhesus HDL. The HDL2/HDL3 ratio was determined from the two peaks observed upon flotation in high salt in the analytical ultracentrifuge. The HDL3 of all three species of apes—gorillas (Gorilla gorilla), chimpanzees (Pan troglodytes) and orangutans (Pongo pygmaeus)—was always greater than HDL2, while that of all six species of Old World monkeys—Rhesus (Macaca mulatta), sooty mangabeys (Cercocebus atys), cynomolgus (Macaca fascicularis), stump-tails, (Macaca arctoides) patas (Erythrocebus patas) and African greens (Cercopithecus aethiops)—was less. In addition, the HDL3 concentration in five gorillas was about 15 mg/dl as cholesterol while the HDL2 concentration was 92 mg/dl, much lower and higher, respectively, than humans. HDL2 of gorillas was similar in density and molecular weight to that of humans. The distribution of densities in gorilla HDL was predominantly in HDL2, while rhesus HDL usually, but not always, was unimodal, having a density distribution similar in heterogeneity to human HDL3, but somewhat less dense (peaking at 1.109 vs 1.129 g/ml). The molecular weight of rhesus HDL was about the same as human HDL3 in all three density subfractions and at the peak density. Likewise, the chemical compositions were similar for the subfractions 1.10-1.125 and >1.125 g/ml for rhesus HDL and human HDL3. Consequently most but not all chow-fed rhesus HDL was very similar to human HDL3, but lighter in density.


INTRODUCTION

Fewer detailed studies have been carried out on serum lipoproteins of apes than of monkeys. Apes are more similar to humans in many characteristics, but monkeys are more economical and are easier to adapt to experimental situations. It would be of interest to compare the groups—humans, apes and monkeys. Recently, HDL have assumed importance for their role in plasma cholesterol transport, but efforts to define their precise role have not met with success. In view of the heterogeneity of the high density lipoproteins, we wished to extend the available information to apes and compare the distribution and physical properties of HDL among humans, apes and monkeys. To this end, we have isolated and characterized, under similar conditions, the HDL and density gradient subfractions of gorillas, orangutans and chimpanzees, in addition to performing similar studies on three different species of monkeys: cynomolgus, rhesus and sooty mangabey.

METHODS AND MATERIALS

All animals were fed Purina chow and housed at the Gulf South Research Institute at New Iberia, Louisiana, except for the cynomolgus which were at the University of Arkansas for Medical Sciences at Little Rock. Animals were not fed overnight. Blood was collected and allowed to clot, after which dithionitrobenzoic acid, thimerosal and EDTA were added to the serum. The serum was shipped immediately in ice and arrived iced within 24 hr. Total cholesterol (1), HDL cholesterol (2), triacylglycerol (3) and agarose electrophoresis (4) were carried out on each sample. Human low density lipoproteins (LDL) were samples described previously (5). All but one were male and of ages 47-61.

Purification—HDL was purified by one of two methods. The first was ultracentrifugation between KBr solvents of densities 1.063 and 1.25 g/ml, including a layer of 1.22 g/ml solvent which served to wash the floating HDL, particularly well in the swinging bucket rotor. The other method involved flotation at 1.22 g/ml through a layer of solvent, followed by 6% agarose column gel filtration which

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separates according to molecular size (6). HDL was pure by agarose electrophoresis and analytical ultracentrifugation.

**HDL**$_2$ and **HDL**$_3$—HDL, upon flotation velocity in density 1.2 g/ml KBr at 48,000 rpm in a double sector cell in the Beckman Model E analytical ultracentrifuge, separated into two components, which were well defined. HDL$_2$ and HDL$_3$ were clearly visible as shown in the top photograph of Figure 1. The photographs were enlarged 10X and traced on graph paper. The peak tracings were redrawn to a linear baseline. The two components were estimated by completing tracing of the HDL peak or peaks symmetrically. All area under the HDL peak could be accounted for by two components. A figure illustrating the method has been given previously (7). The relative areas were corrected for the sector shape of the ultracentrifuge cell. No correction was made for the Johnston-Ogston effect since it is about 2-3% (8). There was no relative area change of HDL$_2$ and HDL$_3$ upon dilution. Remixing purified HDL$_2$ and HDL$_3$ yielded the correct ratios.

**Flotation velocity rate** measurements were carried out on 3-10 mg/ml solutions of HDL dialyzed vs 1.2 g/ml KBr solvent containing 0.01% Na azide plus $10^{-4}$M EDTA at pH 7.5. The exact density was measured pycnometrically for each solvent after dialysis. A double sector cell was employed to obtain a baseline. The speed was 48,000 or 47,660 rpm at 25 C and a schlieren bar angle of 60 degrees.

**Equilibrium molecular weights** were carried out at 4-7 C (the exact temperature was measured) in solvents of NaBr containing 0.01% Na azide plus $10^{-4}$M EDTA at pH 7.5 near 1.43 g/ml. (The exact density was measured with a Westphal balance at the temperature used.) Equilibrium was attained in 48 hr at speeds of 10,000-13,000 rpm using 0.15 ml of solvent dialyzed HDL, which had been diluted with solvent to a concentration of 0.3-0.5 mg/ml. Interference optics was used. The reciprocal of the density of the sample was taken as the partial specific volume. This method has been described previously for human HDL subfractions (9) and is similar to that used for LDL (10).

**Density gradients**—In preliminary experiments, density gradients were prepared from a number of salts. In the swinging bucket tubes, gradients have the highest resolution, but require very long equilibrium times. We found KBr to yield steep gradients, particularly at the bottom end. NaCl, on the other hand, could be made nearly linear and ranged over 0.06 g/ml, as from 1.07 to 1.13 g/ml. NaBr was intermediate. Consequently we selected NaCl or NaBr for the density gradients. Gradients were prepared by stepwise layering of 5 salt solutions of NaBr or NaCl with different densities containing 0.01% azide and $10^{-4}$M EDTA in, for

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**FIG. 1.** From top to bottom; A. gorilla #3 HDL containing 57% HDL$_2$; B. gorilla #6 HDL containing 91% HDL$_2$; C. rhesus #7818 HDL; D. human HDL containing 89% HDL$_2$. All were in density 1.2 ± 0.005 g/ml KBr solvent at 48000 rpm, 25 C. Flotation is from right to left. The photographs were taken 80 min after reaching speed. Note the intermediate flotation rate of rhesus HDL. The flotation rates ($S_f$, 1,2) of the four major peaks were (in the same order): 6.6, 6.5, 4.6, and 2.8.