High-Density Lipoprotein (HDL), HDL₂, and HDL₃ Cholesterol Concentrations Determined in Serum of Newborns, Infants, Children, Adolescents, and Adults by Use of a Micromethod for Combined Precipitation Ultracentrifugation

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Cholesterol (C) concentrations in the two major subfractions of high-density lipoproteins (HDL₂-C and HDL₃-C) in sera from both sexes, ages ranging from newborns to adults, were measured by use of a micromethod for combined precipitation–ultracentrifugation. Sera were obtained from 91 boys, 68 girls, 15 healthy men, and 14 women. The HDL₂-C concentration was higher in women than in men; the HDL₃-C concentration was similar in these two groups. This sex-related difference, generally seen in adults, was found to begin at ages 11–15 y. The value of HDL₂-C in females increased with age in a stepwise manner, whereas that in males increased up to ages 6–10 y but tended to decline thereafter. The HDL₃-C concentration was higher in the adults than in the children. This micromethod for separating operationally defined HDL subfractions is of value for lipoprotein research in children.

Additional Keyphrases: reference values · pediatric chemistry

A high concentration of high-density lipoprotein cholesterol (HDL-C) in serum is well known to be associated with a lower risk of coronary heart disease (CHD) (1). Separation of HDL into its two major subfractions, HDL₂ and HDL₃, has revealed that the cholesterol carried by the former (HDL₂-C) is particularly related to the negative risk of CHD (2). Atherosclerosis is well known to begin early in life (3), and there is increasing interest in the early primary prevention of atherosclerosis. However, conventional ultracentrifugal separation (4) of HDL subfractions requires a large sample volume and is time-consuming; therefore, it is not suitable for epidemiological studies in children. Methods based on precipitation with polyaniyons have been developed, but the optimal conditions for the specific separation of the two major HDL subfractions have not yet been determined (5).

To date, the HDL₂-C concentration has not been evaluated systematically in the pediatric age group. The micromethod for combined precipitation–ultracentrifugation described by Eyre et al. (6) separates operationally defined HDL₂ and HDL₃ in a small volume of serum sample and in a relatively short time (3.5 h). In the present study, we used this method to measure HDL₂-C and HDL₃-C in sera from both sexes, ages ranging from newborns to adults.

Materials and Methods

Preprandial serum specimens were obtained from 91 boys, 68 girls, 15 healthy men (mean age 28 y), and 14 women (mean age 28 y), none of them hyperlipidemic. Cholesterol was measured manually by an enzymatic method, with the “Cholesterolase C” reagent (Eiken Chemical Co., Tokyo, Japan), and spectrophotometrically in a Model CL 720 spectrophotometer (Shimadzu, Kyoto, Japan). We separated HDL₂ and HDL₃ according to the method of Eyre et al. (6), modified as follows.

Place 100 μL of a 315 g/L aqueous solution of potassium bromide into a cellulose propionate “Airfuge” tube (Beckman Instruments, Palo Alto, CA), and evacuate the solvent. After heparin–Mn²⁺ precipitation of the serum sample (6), transfer 175 μL of supernatant fluid to the tube, mix thoroughly to give a final density of 1.125 kg/L, and ultracentrifuge (we used a Beckman Airfuge with an A-100/18 rotor as described in (6)). When left for 15 min at room temperature in a vertical position, the lower margin of the supernate will be >9 mm from the bottom of the tube, as defined by presatining with Fat Red 7B (Beckman). To remove the HDL₃ fraction, gently aspirate 110 μL of the infranatant fluid (the volume corresponding to the 9-mm height measured as above) with a Hamilton syringe, placing the blunted tip at the center of the bottom of the tube. Measure cholesterol concentration in the whole supernate (HDL₂-C); then calculate the HDL₂-C by subtracting HDL₃-C from HDL-C.

The selectivity of the separation of HDL₂ and HDL₃ by this method was confirmed electrophoretically (7). Intra-assay CVs (n = 6) for HDL₂-C and HDL₃-C were 3.4% and 5.9%, respectively.

Statistical significance was determined by the method of least significant difference, calculated after one-way analysis of variance.

Results

Table 1 lists, by sex, the values for HDL-C, HDL₂-C, and HDL₃-C in all age groups studied. HDL-C was significantly higher in both girls ages 11–15 y and women than in their male counterparts, and this was solely ascribable to the differences in HDL₂-C concentrations. There was no significant sex-related difference in the younger age groups. Males, HDL₂-C gradually increased up to ages 6–10 y and then declined slightly to the value for adults. In contrast, there was a stepwise increase in HDL₂-C in females up to adult age. HDL₃-C in males was unchanged up to ages 11–15 y, but the value for men was higher than those for the pediatric age groups. In females, the HDL₃-C concentration was lower in newborns than in infants (ages 1–11 months), unchanged thereafter up to ages 11–15 y, and then slightly increased again. The sum of both subfractions, HDL-C, increased with age in females from newborns to adults and also in males up to ages 6–10 y. However, the change in males from ages 6–10 y to adult age was not

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3 Nonstandard abbreviations: HDL, high-density lipoproteins; C, cholesterol; and CHD, coronary heart disease.

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unidirectional, reflecting a change in HDL$_{L-3}$-C opposite that of HDL$_{L-2}$-C.

Discussion

In the original report on micro-ultracentrifugation (6), the mean value for HDL$_{L-2}$-C in men was one-fifth that of HDL$_{L-3}$-C. In contrast, in the present study the mean values for HDL$_{L-2}$-C in most groups were similar to or a little higher than those for HDL$_{L-3}$-C. The absolute mean value for HDL$_{L-2}$-C in men in our series was threefold that in their (6) series, and our mean HDL$_{L-3}$-C value was a little lower than theirs. When a similar method was evaluated in other previous series, the value of HDL$_{L-2}$-C was threefold (9) and twofold (9) of that in the original report (6). It is well known that there is a wide variability in values for HDL$_{L-2}$-C and HDL$_{L-3}$-C obtained in different laboratories for normal individuals (10). The normal values for both HDL$_{L-2}$-C and HDL$_{L-3}$-C in men that we obtained here are similar to those reported for Japanese (11) and for white persons (12). Therefore, the discrepancy between our values and those in the original report (6) does not appear to be race related or method related.

The HDL-C concentration is well established to be higher in premenopausal women than in men, and this is solely attributed to the difference in the HDL$_{L-2}$-C concentration, the mean HDL$_{L-3}$-C value being similar for the two sexes (13). HDL$_{L-2}$-C concentration is well known to be modulated by sex hormones (14), and some have suggested that this sex-related difference in HDL$_{L-2}$-C generally found in adults is developed during sexual maturation. According to the general concept that androgens decrease and estrogens increase HDL-C, several large-scale epidemiological studies were performed previously in an attempt to reveal the change in the HDL-C concentration in relation to hormonal or physiological maturation in adolescents (15, 16). However, they failed to observe a consistent, unidirectional change in each sex.

No previous study has investigated the age-related change in HDL$_{L-2}$-C and HDL$_{L-3}$-C from the newborn period to adulthood. The present study not only confirmed the general finding in adults, but also revealed that a similar, and smaller, sex-related difference was also the case as young as ages 11–15 y. Thus, we could demonstrate the differential age-related changes in cholesterol concentrations in individual major HDL subfractions in each sex. Among the changes observed here, the dichotomy of the changes in HDL$_{L-2}$-C and HDL$_{L-3}$-C in males ages 6–10 y and older obscured the age-related change in total HDL-C.

The present results suggest that separation of HDL$_{L-2}$-C and HDL$_{L-3}$-C in large-scale epidemiological studies in adolescents contributes to further elucidating the changes in HDL metabolism in response to sexual maturation. Although the present data are still preliminary, the technique used here is certainly of value for lipoprotein research in children.

References


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Development and Validation of an Automated, Enzyme-Mediated Colorimetric Assay of Salicylate in Serum

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This salicylate-specific assay can be adapted for use with most discrete analyzers, for rapid emergency or routine testing with small serum or plasma sample volumes and a single calibration. The basis of this method is as follows: salicylate monoxygenase (EC 1.14.13.1) converts salicylate to catechol in the presence of NADH; the catechol then reacts with 4-aminoephanezine under alkaline conditions, catalyzed by manganese ions, to produce a red dye. Incorporation of an NADH-regenerating system, involving glucose and glucose dehydrogenase, into the enzyme reagent ensures that the working reagent is stable for more than two weeks. The standard curve is linear over the drug concentration range 0.0 to 5 mmol/L. The CV was <4% over 20 days. Results correlated well with those by the Trinder colorimetric method and an HPLC method. We saw no interference by any of 80 drugs we tested at therapeutic concentrations or by endogenous compounds in serum.

Additional Keyphrases: Trinder method, HPLC compared, toxicity, emergency procedure

Salicylate assay should be available in all clinical chemistry laboratories, such requests usually being related to the confirmation and assessment of overdose (1). The most commonly used technique, colorimetry, involves formation of a purple complex with ferric salts in an acid medium (2). Some of the interferences in the method can be removed by precipitating the serum proteins, but this step precludes complete automation of the assay.

Specific methods described have either required HPLC (3) or have involved use of the enzyme salicylate monoxygenase (4–7). With this enzyme, salicylate can be quantified either by monitoring the NADH consumed (4, 5) or by detecting the catechol produced (6). Because salicylate monoxygenase also acts on salicylate analogs, thereby consuming NADH, and because this enzyme possesses inherent NADH oxidase activity (necessitating a reagent blank), the latter approach offers superior specificity, detecting neither the major metabolites of salicylate nor salicylate analogs.

In the enzymatic method described by Chubb et al. (6) salicylate is converted to catechol, which is detected by formation of an indophenol dye with 4-aminoephene under alkaline conditions. Because this reaction requires a high pH, three reagents are required (4-aminoephene being stable only under acidic conditions). The method cannot be adapted to common analyzers because of the constraints imposed by the use of three reagents.

Here we describe a modification of the enzymatic procedure that can be adapted to most analyzers. Our major objective was to find a colorimetric method for catechol that would be compatible with a single- or two-stage reaction.

Materials and Methods

Salicylate monoxygenase (EC 1.14.13.1) was purified from Pseudomonas cepacia (ATCC 29351) as described by Hammond et al. (7). Glucose dehydrogenase (EC 1.1.1.47) from Bacillus megaterium was obtained from Genzyme Biochemicals Ltd., Haverhill, Suffolk, U.K. "Trizma" base [tris(hydroxymethyl)methylamine], EP[(2-hydroxyethyl)piperazine-3-propanesulfonic acid], AMP (2-amino-2-methyl-1-propanol), AMPD (2-amino-2-methyl-1,3-propanediol), TAPS [tris(hydroxymethyl)methyl-3-amino propane-sulfonic acid], and glycylglycine buffers, NADH, 4-