
In numerous clinical situations—e.g., chronic diarrhea, chronic cholestasis, and cystic fibrosis—phylloquinone (vitamin K\textsubscript{1(3)}\textsubscript{(7)}(8)) absorption can be disturbed, in children as well as in adults. The reference interval for phylloquinone in serum of human adults has been described (1-4), but little is known about the reference interval for K\textsubscript{1(3)}\textsubscript{(7)}(8) in serum of healthy children. We measured it in serum of 96 healthy children (40 girls, 56 boys), ages between two months and 19 years. All were Europeans, from various social levels. The children were introduced in the study at the time of a consultation in the Department of Pediatrics of the Hôpital Bicêtre (Paris): consultation in a clinical evaluation before anesthesia for amygdalectomy or for minor surgery such as urethral malformation. In all subjects, coagulation, blood count, and results of liver tests were within normal limits. For quantitative analysis of phylloquinone in the serum samples we used a previously described HPLC procedure (3, 5).

The vitamin K\textsubscript{1(3)}\textsubscript{(7)}(8) values in the children did not follow a gaussian distribution, but an apparent log-normal distribution was confirmed by the Kolmogorov–Smirnov test.

Figure 1 gives the frequency distributions of the values for vitamin K\textsubscript{1(3)}\textsubscript{(7)}(8). The mean value was 337 ng/L. However, with this type of skewed distribution the logarithmic mean is a better parameter. The logarithmic mean for the 96 children was 236 ng/L and the reference interval ranged from 44 to 1272 ng/L, [log x = \pm 2 SD (log x)]. The lowest and highest observed values were 30 and 2075 ng/L, respectively. The logarithmic mean for 40 girls was 227 (40 to 1291 ng/L) and for the 56 boys 242 (46 to 1275 ng/L), both [log x = \pm 2 SD (log x)]. Analysis of these data indicated no age- or sex-related differences.

Finally, we compared the reference interval as obtained for the healthy children population with the reference interval in healthy adults (3). For 50 healthy adults a log-normal distribution also was confirmed, with a logarithmic mean of 247 ng/L (range, 62 to 980 ng/L). For the adult population, the lowest and highest values were 63 and 1035 ng/L, respectively. The logarithmic mean for the 29 adult men was 258 (67-989 ng/L) and for 21 adult women 233 (54-994 ng/L), both [log x = \pm 2 SD (log x)]. Also in adults, no age- or sex-related correlation could be demonstrated for serum vitamin K\textsubscript{1(3)}\textsubscript{(7)}(8). Indeed, there was no significant difference between the values for children and for adults. The significant F\textsubscript{0.05} value for 1 and 144 degrees of freedom, for numerator and denominator, respectively, is 3.90; we obtained an F-value of 0.116.

References

Confusion of Apolipoprotein E Phenotyping by Serum Amyloid A, A. Steinmetz, R. Saile, M. Sefrous, H. J. Parra, and J. C. Fruchart (Service de Recherche sur les Lipoprotéines et l'athérosclérose (SERLIA), Institut Pasteur de Lille, 1, rue du Professeur Calmette, 59019 Lille Cédex, France)

Serum amyloid A (SAA) is an acute-phase reactant mainly associated with high-density lipoproteins (1) but also with very-low-density lipoproteins (VLDLs) or low-density lipoproteins (2). On isofoaming, plasma SAA exhibits mainly two isoforms, SAA\textsubscript{1} and SAA\textsubscript{2}. They differ in only one amino acid; SAA\textsubscript{2} lacks the N-terminal arginine (3).
The VLDL antihuman exhibited homozygous. CFIg. VLDLs ing, and the phenotyping and associate containing the specific 672 subsequent Apolipoprotein E4 serum by 1. SAA three E4 SAA2 apo revealed Note VLDL Immunoblot three the be homozygous specific with heterozygotes the same focusing of apo E4 when apo VLDL protein staining is used to determine phenotypes instead of immunological detection. One should therefore be aware of this phenomenon when interpreting apo E phenotyping results and should always run an apo E4 standard when immunoblotting is not available.

Apolipoprotein (apo) E is a polymorphic apolipoprotein with mainly three isoforms in plasma—E2, E3, and E4—that differ in cysteine and arginine content (4) and are coded for by three alleles e2, e3, and e4. Six phenotypes can be distinguished—three homozygotes (E2/2, E3/3, and E4/4) and three heterozygotes (E2/3, E2/4, and E3/4)—on isofocusing, mainly performed on urea-soluble apolipoproteins from VLDLs (5).

We could demonstrate that coincident with a massive increase in serum amyloid A, the acute-phase proteins associated with VLDLs and are co-isolated with VLDLs by ultracentrifugation. They are urea-soluble apolipoproteins and are separated by isoelectric focusing together with the usual VLDL apolipoproteins, SAA2 almost co-focusing with apo E4 with which it can thus be confused, leading to false phenotyping of apo E when protein staining is used to interpret the phenotypes. After Coomassie Blue staining, the SAA-containing VLDL may be looked at as an apo-E4-containing pattern. A more reliable procedure for discriminating the proteins focusing at the apo E4 position would seem to be the transfer of apolipoproteins, after isofocusing, to nitrocellulose sheets and their identification by use of specific antibodies to apo E. As shown in Figure 1, apo E4 and SAA2 almost co-focus. In the Figure they are discriminated by specific antibodies both to apo E and SAA. Initially the VLDL phenotyped for apo E from lane B was seen with a pronounced band in the apo E4 position, interpreted as an apo E4 homogenous pattern. Isofocusing of plasma and subsequent immunoblotting revealed that the band found in the apo E4 position consisted both of apo E4 and SAA (Figure 1, B, C). The plasma was thus found to be apo E3/4 heterozygous. An additional component of the band is thus clearly identified as SAA2 by specific antibodies to SAA. In some other cases, apo E4 was reported when it actually was not present, e.g., in VLDLs from plasma in lane A of Figure 1.

Recently Stuyt et al. (6) reported on the co-focusing with apo E4 of additional proteins in VLDL from patients with acute pancreatitis. Our results demonstrate that one of these co-focusing proteins can be serum amyloid A isoform SAA2, which could help explain the different reports (7, 8) on apo E4 frequencies in type V hyperlipoproteinemia. We show here that immunological identification of apolipoproteins avoids the possible confusion of SAA2 with apo E4.

We (9) and others (10), by setting up immunoblotting procedures for phenotyping of apo E, have seen the possible confusion of the apo E pattern with serum amyloid A when apo VLDL protein staining is used to determine phenotypes instead of immunological detection. One should therefore be aware of this phenomenon when interpreting apo E phenotyping results and should always run an apo E4 standard when immunoblotting is not available.

References