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Lipoprotein Fractions in Maternal, Cord and Newborn Serum

By

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The lipoprotein (LP) fractions have been estimated in corresponding maternal, cord and newborn blood samples. The total lipoid content of parturients, which is higher than the physiological level, depends below the mean value on the concentration of low density lipoproteins (LDL) and above the mean on the very low-density lipoproteins (VLDL). The quantity of high-density lipoprotein amounted to one third of the total LP in each case. HDL (alpha-LP) in cord blood amounted to 40—50% of the corresponding value for healthy adults, while LDL (beta-LP) to 1/3—1/4 of it. In cord blood, practically no pre-beta fraction is present, while this reaches a well-evaluable level in the newborn's blood on the first day of life.

It is concluded that foetal lipoids arise as a result of the independent and active metabolism of the placenta and the foetus, independently of the maternal physiological hyperlipoproteinaemia. The increase in pre-beta lipoproteins of the newborn on the firstday of life is indicative of the metabolistic switch-over from carbohydrate oxidation to fat oxidation.

Introduction

The lipoids in the blood form an emulsion in aqueous medium. In the serum they are bound as prosthetic groups to protein and circulate as lipoproteins (LP). The main LP classes consist of four principal components, viz. protein, triglyceride, cholesterol and phospholipide. The albumin fraction transporting free fatty acids (FFA), the plasma concentration of which ranges from 6 to 16 mg per 100 ml under physiological conditions, has a significant biological importance. The LPs are labile molecules widely differing in chemical composition. Their classification varies with the separation technique. Their electrophoretic separation on paper, agar or polyacrylamide gel or cellulose-acetate strip is based on the migration properties of the protein component, while their separation by ultracentrifuge makes use of the different density and flotation characteristics of the LP molecules. The LPs amount to 10—15% of the total protein in plasma. By agarose-electrophoresis, the following LP classes can be differentiated.

1. The *chylomicrons* are formed predominantly in the intestinal mucosa and serve the transport of fat. After a fast they are absent from the blood but after food intake they

appear and persist there for several hours. In the circulation they are split by lipoprotein lipase into higher density LP and free fatty acids. Their protein content is 2—2.5% and to 85—90% they consist of triglycerides. This is why their density (0.9 g/ml) is the lowest of all LPs. They measure 0.2—1.5 μ in diameter, their molecular weight is approximately 50 million. At electrophoresis they stay at the starting line ("start fraction").

2. The *pre-beta lipoproteins* migrate together with the alpha$_2$-globulins. They are constituted predominantly by triglycerides (52%). They are the carriers of those triglycerides which are synthetized from the fat deposits by the Golgi apparatus of the liver cells to supply the organism with energy. Thus, while the chylomicrons transport exogenous fat, the pre-beta LPs carry endogenous triglycerides. Their protein content is low (8%) and so is their density ("very-low-density lipoproteins" VLDL).

3. The beta-lipoproteins with their 20% protein content belong to the low-density group ("low-density lipoproteins", LDL). These are produced in the liver and transport cholesterol (40%) and phospholipids (22%). The accumulation of beta LPs points to hypercholesterolaemia. With their low (approximately 2.5 million) molecular weight and small molecular diameter (20 mμ) they do not make the plasma turbid, of their physiological role only so much is known that a free lipoid exchange occurs between them and the membrane of cells which are in contact with serum. Therefore, a certain role is attached to the LDL in preserving the intactness of the cell membranes.

4. The *alpha-lipoprotein* fraction which migrates together with the alpha$_1$-globulins, contains 8% triglyceride and 46% protein. Therefore, its density is high (1.215 g/ml, "high-density lipoproteins", HDL). It is synthetized in the liver, its molecular weight is 250,000, its diameter is the smallest of all fractions, and its serum concentration is practically stable (30—40% of the total LP). Regarding its physiological role, there is an exchange between its lipoid component of the erythrocyte membrane.

One of the most exciting aspects of the subject is the question of the apo-lipoproteins which constitute the protein component of LPs. This protein group has been shown to be widely heterogeneous and, also, that the said passive transport is not its only function. These protein components differ from each other in immunological properties, molecular weight, C and N terminal amino acids, amino acid composition and sequence, and even in the carbohydrate component. Their classification is made difficult by the incessant accumulation of new data. At present, we are far from a uniform nomenclature. It seems that there are three main groups.

(a) A large part of the protein component of the HDL fraction and part of the chylomicron and VLDL fractions is constituted by Apo-LP-A, with a molecular weight of about

20,000. Two sub-groups have been differentiated immunologically.

(b) Apo-LP-B is the main protein component of the normal LDL fraction but it also figures among the apoproteins of the chylomicron and VLDL fractions.

(c) The Apo-LP-C is found in the protein part of the chylomicron and the VLDL fractions. It is a mixture of three different protein chains, with a molecular weight between 7000 and 10,000. Its main role is to guarantee the stability of low-density molecules of a high triglyceride content not only in the structural sense but also by inhibiting the plasma lipoprotein lipase.

Investigation of Apo-LPs is made difficult by the fact that these protein components migrate from low-viscosity LP molecules to high-viscosity ones, they can be exchanged, and in the new environment their physicochemical and other properties undergo changes. Very little is known about the nature of the protein – lipoid bond and the causes and conditions of its formation and splitting.

Materials and Methods

For the investigations, 12 primiparae or secundiparae standing immediately before delivery were selected. They were all in full health, 18 to 34 years of age, their weight ranged from 61 to 70 kg. The first blood sample was taken after a fast of 6–8 hours; the second, from the umbilical cord during delivery, and the third sample from the term newborn exhibiting no sign of illness, with a birth weight of 2800–4100 g. Eight newborns did not suck before sampling while four of them sucked one or two times. Even in these cases, blood sampling was carried out on an empty stomach.

Determination of total lipoid content. After digestion with sulphuric acid the intensity of colour produced with phosphoric acid-vanillin reagent was measured spectrophotometrically.

Lipoprotein electrophoresis. The sera (10 μl per plate) were run on 0.5% agarose (Calbiochem) with veronal-acetate buffer adjusted to pH 8.6. After fixation with 2.5% trichloroacetic acid, the slides were stained with Sudan black B. The LP fractions were estimated densitometrically, the results are given in percentage and in mg/100 ml; the latter value was based on the total lipoid determination.

Results

1. Parturients

1. It has been shown earlier [1] that the total lipoid value was higher in every parturient than in normal non-pregnant women. The mean was 1316 ± 267 mg per 100 ml. The scattering was wide, the upper limit was twice that of the lowest one. In Fig. 1, the height of the columns

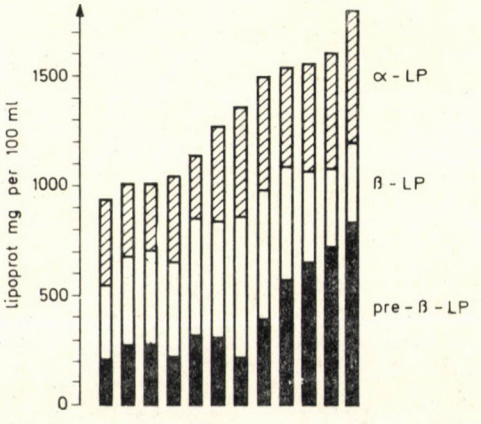

Fig. 1. Distribution of serum lipoproteins of parturients. (Explanation in text.)

shows the total lipoid concentration. The cases are arranged in increasing order of this value.

2. Pre-beta-LP. The compact lower part of columns in Fig. 1 demonstrates that even the blood of fasting parturients contains LP fractions carrying endogenous triglycerides. The mean value for the 12 cases was 418±221 mg per 100 ml, with a fourfold scatter. The value for pre-beta-LP was roughly constant, amounting to 25% of the total lipoid content in the 1000—1400 mg per 100 ml total lipoid concentration range, while the increase in total lipoid above 1400 mg per 100 ml was brought about solely by the increase in the pre-beta fraction. In the last total lipoid value (1800 mg) 47% fell to this component.

3. Beta-LP. The mean value for the 12 cases was 466±105 mg per 100 ml with a twofold difference between the extreme values. This low-density fraction has a decisive part in the development of the total lipoid level (empty part of columns in Fig. 1). Any increase in the value for total lipoid in the range of 1000—1400 mg per 100 ml, is due to the increase in the beta-LP level. At 1400 mg per 100 ml, 49% of the total lipoid consists of beta-LP, but beyond that value its proportion becomes less and less and at the value of 1800 mg per 100 ml it amounts to 20% only.

4. Alpha-LP. The mean value for the high-density fraction was 429± 91 mg per 100 ml in our cases, and amounted to approximately one third of the total lipoid concentration. The change in the various fractions is well illustrated in Fig. 2. The level of alpha-LP is practically constant. That of the beta fraction is growing consistently with the total lipoid value until it reaches the mean. At this point it changes parts with the pre-beta fraction and returns to the starting value.

In Fig. 3, the total lipoid value has been taken for 100% in each

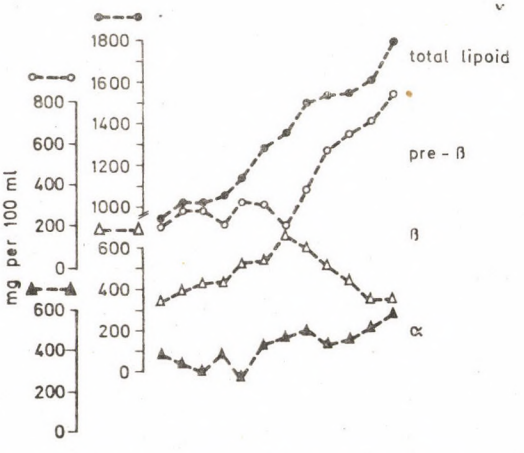

FIG. 2. Under 1400 mg per 100 ml the beta-LP, above that value the pre-beta-LP, are responsible for the individual changes in the total lipoprotein level

case, and the cases have been arranged in the increasing order of pre-beta-LPs. The percentual distribution of the fractions supports the above conclusion in that alpha-LP has a standard value and the percentual distribution of the pre-beta fraction is increasing at the expense of the beta-LP one.

II. Cord blood

1. Total lipoid content. The mean value for the 12 samples was 311±105 mg per 100 ml, one quarter of the serum level of the parturients.

2. The relative values for the fractions can be seen in Fig. 4. The value for the high-density fraction was constant at 125±48 mg per 100 ml, amounting to 40% of the total lipoid. The rest was made up practically by beta-LP (177±57 mg), as the pre-beta fraction was absent in 9 cases and barely detectable in 3 cases.

III. Newborns

1. The total lipoid level was low, 408±108 mg per 100 ml, below the lower limit of healthy adults.

2. Figure 5 shows the relative values for the LP fractions in the increasing order of pre-beta-LPs. The pattern was similar to that seen in Fig. 3. Alpha-LP is a standard fraction also on newborns, with a mean of 146±34 mg. It makes up 36%, while the beta-LP (176±49 mg), 44.7% of the total lipoid level. The pre-beta-fraction was found to be present in the newborns and, in some cases, it even reached the considerable level of 85±49 mg per 100 ml, and a

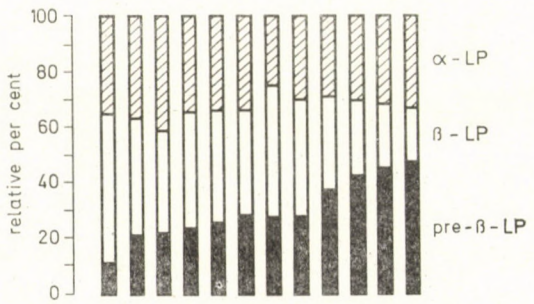

FIG. 3. Relative percentual distribution of data of previous figures

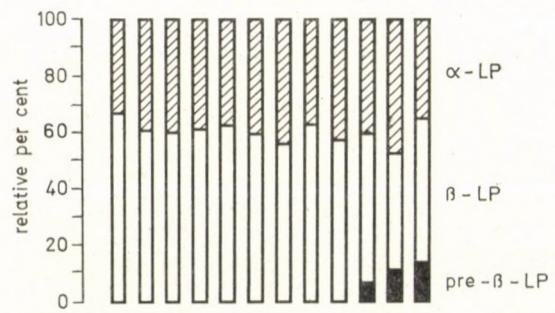

FIG. 4. The lipoprotein fractions in cord blood, are independent of those in the parturient

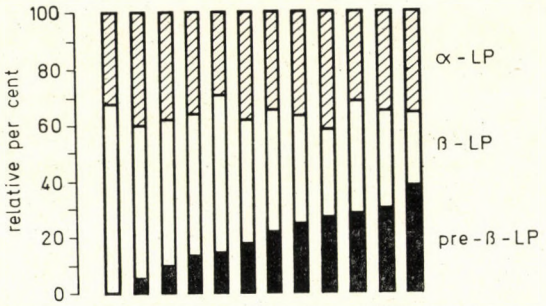

FIG. 5. The pre-beta fraction appears on the first day of life, and increases at the expense of the beta fraction

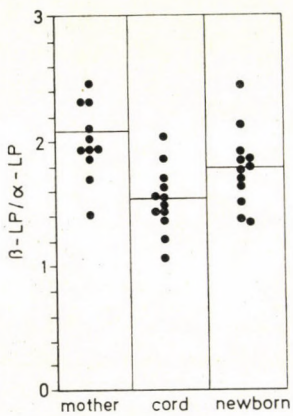

FIG. 6. The beta-LP to alpha-LP ratio in maternal, cord and newborn blood

higher percentual share at the expense of the beta fraction.

Fig. 6 shows the density distribution and the mean beta to alpha ratio. The fact that, in addition to the beta fraction, the numerator includes the pre-beta values, explains the arrangement of mean values.

DISCUSSION

According to the extensive investigations of VAN DUYNE and HAVEL [1a], during the first trimester of pregnancy no change occurs in the serum lipids and lipoproteins as compared to the period before pregnancy. The HDL is the first fraction to increase by about 25% in the second trimester. In the third trimester the amount of LDL increases to about the same extent and VLDL is also increasing sharply, to more than twice the non-pregnant value. An increase in phospholipids, cholesterol (by about 30%) and triglycerides (by 100%) also takes place in the second half of pregnancy. All these values take a certain time to return to normal after delivery. The VLDL fraction becomes normal sooner, while the HDL fraction which was the first to increase, takes about 6 weeks for this.

No acceptable explanation has been offered for the increase in either the total lipoid level or the individual LP fractions. VON STUDNITZ [10] suggested that the increase in HDL in the second trimester may be caused by the increased oestrogen production. Concerning the increase in LDL and VLDL in the last third of pregnancy, WALKER [11] assumed a dietic change which might result in a positive calorie balance.

The increase in the VLDL fraction deserves special attentions. Under physiological conditions, in non-pregnants, an increase in the fasting value of this fraction is to be expected if for some reason there are no sufficient carbohydrate reserves to cover the energy demand, and instead of carbohydrate, fat must be oxidized. However, RANDLE et al. have shown that this switch-over of the metabolism may occur in the opposite manner, too. If the serum triglyceride and mainly the free fatty acid level is increasing for some reason, the increased supply of fat leads to its increased consumption which suppresses glucose utilization even if the carbohydrate supply is sufficient. A similar metabolic situation might occur at the end of pregnancy. Further studies are, however, needed to clarify these points and especially the reaction of the developing foetus.

In a previous paper we have discussed the practically independent behaviour of the total LPs in the parturients and in cord blood, in other words, the lack of VLDL in cord blood when this fraction constitutes half of the mother's total LPs. It should be remembered that the placenta lets through selectively certain fatty acids and even intact lipoid molecules. However, the processes taking place in the placenta with the aim to synthetize fatty acids and lipoids to satisfy the demands of the foetus have a greater importance. The almost exclusive source of energy of foetal metabolism is glucose, and this is also the basis of the glycogen and fat deposits [7]. Consequently, the foetus does not have to synthetize giant molecules like VLDL with a 5-to-50-million molecular weight to cover its direct energy demand, and it is clear that the pre-beta LP of the mother cannot pass across the placenta. Therefore, what remains in cord blood are the HDL and LDL fractions. As the HDL has a standard value of 40%, the same must be the case with LDL. According to a recent paper [9], the composition of LDL in cord blood is different from the LDL found in adult blood: not only is its concentration lower, but it contains less cholesterol and triglyceride, and more protein.

In the first hours of extrauterine life the basic processes of metabolism of the newborn are obviously continuing from the point which means the end of intrauterine life: the energy is supplied by carbohydrate oxidation. The quantity of the latter is, however, limited, as the glycogen stores of a mature newborn amount to only 1% of its body weight, so that it cannot meet the first-day demand of approximately 50 kcal/kg. One of the well-known signs indicating the rapid decrease in carbohydrate content is the hypoglycaemia developing in the first hours of life. It may be assumed that in this situation the growth hormone, the production of which is enhanced by hypoglycaemia, starts a mobilization of depot fat, from which triglycerides are then produced in the liver. The latter are transported to the site of utilization

by the VLDL. This would explain the appearance of this fraction and its increase on the first day of life, even before the first sucking. One of the metabolic properties of extrauterine adaptation is the temporary change from the predominantly carbohydrate oxidation to a predominantly fat oxidation. This is indicated by the decrease in the respiratory quotient [6], the increase in the blood level of free fatty acids and glycerol [3, 5] and the appearance of ketone bodies [4]. The period of increased fat oxidation then lasts until the gradual setting of nutrition physiologically equilibrates the energy household of the newborn.

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