MINIREVIEW

Newly Recognized Lipid Carrier Proteins in Fetal Life (43286)

M. T. RAVI SUBBIAH[1]
Department of Internal Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio 45267

Lipids are carried in plasma mainly in the form of lipoproteins. The protein moieties of lipoprotein particles (called apoproteins) are bound to various lipids in different proportions (1). Five major classes of lipoproteins are known: chylomicrons, very low density lipoproteins, low density proteins, intermediate density lipoproteins, and high density lipoproteins (HDL). Lipoproteins carry virtually all of the cholesterol, triglycerides, and phospholipids in plasma. Extensive literature is available regarding the uptake and metabolism of lipoprotein by cells. Some of this information has been summarized in many excellent reviews (1–4), and will not be covered here.

Free fatty acids (FFA) form a small fraction of plasma lipids consisting of unesterified fatty acids, which are bound to albumin (5). These fatty acids are believed to play an important role in metabolism due to their rapid turnover rate (6). Free fatty acids are generated either by intravascular lipolysis of chylomicrons (7) or by the hydrolysis of triglycerides in the adipose tissue under hormonal control (8, 9). Until recently, albumin was considered the predominant fatty acid carrier in plasma (5). Recently, however, two fetal proteins (i.e., α-fetoprotein and fetuin) have been shown to carry considerable amounts of fatty acids. This brief review will concentrate on the potential role of these fetal proteins in delivering fatty acids to cells, along with a brief review of albumin-mediated fatty acid transport.

Role of Albumin

In the adult plasma, most of the fatty acids appear to be bound to albumin, with a small ionized fraction that remains free in solution and is in equilibrium with the anion (5). Physiologically, the ionized fraction appears to be small enough to not cause injury to cells. Free fatty acids bound to albumin enter the cell in an unbound form either by passive diffusion or receptor-dependent uptake (8). Although cellular uptake of fatty acids has been considered a passive diffusion process, a closer look at the kinetics of fatty acid uptake strongly indicates a role for a receptor-mediated mechanism (9). Since the release of FFA from albumin is considerably slower than the flow of blood through the liver, the efficient hepatic extraction of FFA from the blood cannot be attributed to the simple release of FFA to the water phase and random diffusion across the plasma membrane. Although the presence of membrane albumin-binding protein was suggested, a separate fatty acid-binding protein with strong affinity for fatty acids is now believed to play a major role in fatty acid transport (10). Its role in fatty acid transport was demonstrated using antibody to fatty acid-binding protein, which resulted in nearly 50% inhibition of fatty acid uptake (11). The mechanisms of fatty acid transport through the membrane have not been fully characterized. Fatty acids were shown to enter cultured cardiac cells from the chick embryo through two mechanisms, one involving a saturable process, the other resembling a passive diffusion (12). At fixed concentrations of palmitate or of oleate with increasing concentrations of albumin, this protein increased the rate of palmitate uptake, with a maximum at palmitate/albumin molar ratios between 7 and 10, while it decreased the rate of oleate uptake under all conditions. With all fatty acids, the presence of albumin lowers the proportion of unesterified fatty acids and enhances the proportion of esterified fatty acids recovered in the cardiac cell (12). A similar effect of albumin was also found with hepatocytes and permanent cell lines. The authors suggested a specific role for serum albumin that assumes a dis-

[1] To whom correspondence and requests for reprints should be addressed at Lipid Research Laboratory, University Hospital, M.L. #540, 234 Goodman Street, Cincinnati, OH 45267.

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persing effect of this protein toward dimers (or higher aggregates) of saturated fatty acids and the entry of fatty acids into the cell as monomers.

The fatty acid-binding protein present in the plasma membrane is believed to be a 40-kDa protein that binds to fatty acids but not to cholesterol or phospholipids (10, 11). Apart from plasma membrane fatty acid-binding proteins, a distinct family of fatty acid-binding proteins has been identified in a variety of tissues (13, 14). These proteins are present in a high concentration in tissues (2–5% of cytosolic protein) with one or two binding sites (13). It is presumed that fatty acids carried to the plasma membrane by albumin bind to plasma membrane by a fatty acid-binding protein, which could further be transferred to cytosolic fatty acid-binding proteins, either to sites of triglyceride synthesis or for mitochondrial oxidation.

Role of Fetuin

Fetuin is an acidic glycoprotein present in the fetal plasma of mammals and is known to be a major protein of fetal bovine serum (15). Fetuin is believed to be responsible for some of the growth-promoting effects of fetal serum in cell culture (16), although some studies have suggested that other associated proteins such as α_2-macroglobulin and platelet-derived growth factor might be responsible for the noted effects (17, 18). This is further complicated by the fact that chromatography of fetuin preparations yields many bands, which represent either aggregates or isoforms of the molecule (19, 20). Studies of α-fetoprotein (another fetal glycoprotein) indicated that long-chain fatty acids are bound to this protein (21). Because fetuin (distinct from α-fetoprotein) has been identified in fetal plasma of humans and other mammals (22, 23), we investigated whether any lipids are bound to the fetuin molecule. Our studies indicate that fetuin prepared by Pederson's method (15) and used widely in cell culture media is associated with a lipoprotein-like particle floating at a density similar to that of high density lipoprotein upon ultracentrifugation (24). This particle carries a considerable amount of lipid (i.e., cholesterol, cholesteryl esters, triglycerides, and small quantities of fatty acids). This particle eluted in the range of very low density lipoproteins on agarose chromatography while it floated at the density of HDL. This discrepancy might be due to differences in particle size when compared with plasma HDL. Total lipids amounted to nearly 33% of the molecule, giving it a density similar to that of HDL. The binding of lipids to the protein does not appear to be simple hydrophobic binding (analogous to fatty acid binding to albumin or α-fetoprotein), since these lipids could not be removed by charcoal extraction and remained with the fetuin fraction after ultracentrifugation. Furthermore, the presence of a lipid-containing particle does not appear to be due to simple contamination with plasma lipoproteins, since the delipidated lipoprotein particle did not yield any major bands corresponding to know apolipoproteins. The major protein band associated with the lipids gives a band with the same molecular weight as fetuin, which suggests that a portion of fetuin or an isoform might bind lipids. Association of a lipoprotein-like particle was noted in fetuin prepared by Pederson's method (15), but not with fetuin isolated by Spiro's method (25). The method of Spiro involves ethanol extraction, which is likely to remove the lipids and perhaps is responsible for the absence of lipoprotein-like particles associated with the molecule.

Further studies were done in our laboratory on the effect of fetuin on exogenous fatty acid incorporation into rabbit fetal aortic smooth muscle cells and human skin fibroblasts (26). This study demonstrated for the first time that in the presence of fetuin, incorporation of exogenous fatty acids into cellular triglycerides is markedly accelerated using two cell types and three different preparations of fetuin. When compared with albumin, which is believed to be the major carrier of fatty acids (5) in adult plasma, fetuin was nearly 50 times more efficient in this process. This critical role of fetuin was demonstrated by increases in (i) radioactive fatty acid incorporation, (ii) cellular content of triglycerides, and (iii) by strong staining with Oil Red O and electron transmission microscopy. To determine whether the differential of fetuin and albumin in fatty acid incorporation was due to the differences in their binding to fatty acid, studies were done on the relative binding capacity of the two proteins. Although the available unbound fatty acid in the presence of fetuin was greater than with albumin, critical experiments done at fatty acid to protein ratios that would yield identical unbound fatty acid levels still showed a 500% increase in fatty acid incorporation when cells were exposed to fetuin. This suggests that independent of binding differences between the two proteins, fetuin still has a stimulating effect.

Although it is clear that fetuin stimulates the synthesis of triglycerides, the mechanisms involved are not known. Whether the stimulation of triglyceride synthesis is mainly due to the entry of fatty acids into cells or whether fetuin might directly stimulate lipogenic enzymes is not known. Four of the key enzymes involved in the triglyceride synthesis are: (i) glycerophosphate dehydrogenase, which is involved in glycero-P synthesis (27); (ii) glycerophosphate acyl transferase (28), catalyst for the first step in the formation of phosphotidic acid; (iii) phosphotidic acid phosphohydrolase (29), which forms diglycerides; and (iv) diacylglycerol acyl transferase, a specific enzyme for triglyceride synthesis. The temporal relationship between the initial ratios of fatty acid entry and the changes in these enzyme activities as influenced by fetuin needs to be investigated. Furthermore, in the fetal plasma, fetuin co-exists with two

other plasma proteins involved in fatty acid transport, *i.e.*, albumin and α-fetoprotein. How fetuin will alter fatty acid uptake and metabolism in the presence of these carriers will also need to be investigated using concentration ratios as they exist in fetal plasma. Finally, the eventual changes in free fatty acid and total fatty acid concentration and composition of specific tissues as influenced by fetal proteins is not known.

Further studies indicated that fetuin may also play a role in removing cholesterol from cells. Cholesterol transport from the cells to plasma lipoproteins is believed to occur predominantly in unesterified form (30). Two types of cholesterol efflux are known to occur, (i) one involving diffusion through membranes, and the other (ii) acceptor mediated (31). Of the potential acceptors, HDL (HDL3, in particular) and complexes of apoA1 with phospholipids are the most efficient (32). Smaller HDL particles with a prebeta mobility have been identified that appear to be involved in cellular cholesterol efflux (33). Most cells possess receptors for HDL (34) that increase after cholesterol loading (35). Cholesterol available for efflux is determined by the cholesterol ester cycle, which in turn is determined by cellular uptake (via low density lipoprotein receptors) and esterification of cholesterol and its subsequent hydrolysis by neutral cholesterol esterase (36). Cholesterol metabolism of cells can be conveniently investigated by the uptake and esterification of labeled cholesterol incorporated into low density lipoproteins (37). One of the intriguing findings during the studies on the effect of incubating fetuin with cells was that despite the association of significant cholesterol and cholesteryl ester with fetuin, no changes in cellular cholesterol content were noted, while the triglyceride content increased markedly (see preliminary studies). This suggested the possibility that fetuin might have a role in removing cholesterol from cells. Preliminary studies (38) on the effect of fetuin on the removal of [^{14}C] cholesterol (prelabeled in fibroblasts and HepG2 cells by loading cholesterol liposomes) showed that fetuin (especially the supernatant fraction (24) of fetuin) markedly induced efflux of [^{14}C]cholesterol from cells (as efficiently as HDL) and that this effect was linear with time and concentration. This supernatant fraction significantly reduced the cholesterol content of fibroblasts. These studies suggested that fetuin might have a dual function (i.e., delivery of fatty acids to cells and removal of cholesterol from cells). Cellular cholesterol efflux has been shown to be influenced by the nature of fatty acids (38) and the physical state of cholesteryl ester inclusions (39). Stein *et al.* (38) noted that in HepG2 cells cultured with linoleic acid, efflux of cholesterol into the media was decreased when compared with cells treated with oleic acid. Since fetuin appears to be an efficient carrier of fatty acids, its effect on fatty acid delivery might play a role in the subsequent release of cholesterol into the media. It is possible that the induction of lipogenesis by fetuin may be related to its effect on cholesterol efflux, since a recent study (40) suggested that induction of cholesterol efflux by apoA1 was related to the accumulation of diglycerides by cells. Fetuin might facilitate the removal of cholesterol from cells by HDL through such mechanisms. Furthermore, whether fetuin influences the activity of key enzymes involved in cholesterol esterification and hydrolysis (41) is not known.

Finally, recent studies (42) noted that bovine fetuin has a 70% homology in cDNA sequence to human α2.HS protein, which is also found in the fetus. Whether α2.HS protein can carry out some of the properties associated with fetuin is still not clear. Whether fetuin and α2.HS protein possess the same or different receptors in cells, and how their receptors relate to fatty acid-binding proteins, is still to be determined.

Role of α-Fetoprotein

α-Fetoprotein (AFP) is a serum protein, present in the early stages of development of mammals, that virtually disappears in adult life (43). This protein is mainly synthesized by the fetal liver and the yolk sac (44, 45), and the intracellular presence of AFP in other fetal tissues, for the most part, appears to be due to the extraction of this protein from extracellular sources (46–49). The ability to internalize AFP is lost when cells attain a certain degree of differentiation, but the ability has been shown to reappear in some cultured neoplastic cells (50, 51). Studies have also shown the selective accumulation of radiolabeled AFP in spontaneous mouse mammary neoplasms (52, 53). These studies suggested that AFP, like other serum proteins, might bind to specific membrane receptors and be subsequently internalized by receptor-mediated endocytosis. Indeed, Villacampa *et al.* (54) provided experimental evidence demonstrating the presence of such surface receptors for AFP on human breast carcinoma cells.

The biological role of AFP during embryogenesis has not been completely elucidated, but it could be related to the property, shared by AFP of different species, of binding polyunsaturated fatty acids, mainly docosahexaenoic (22:6) and arachidonic (20:4) acids (55, 56). AFP, like serum albumin, possesses two to three high affinity-binding sites for unesterified fatty acids (57). The molar ratio of fatty acid to protein in AFP ranges between 1 and 3, while that of albumin is <1. Consequently, under physiological conditions, the saturable component of the transfer of fatty acid bound to AFP should be more prevalent (58). For this reason, it could be a physiological carrier of free fatty acids in serum during development.

In an elegant study, Uriel *et al.* (59) demonstrated that fatty acids bound to AFP may enter cells by a two-

component process. One was a diffusible, nonsaturable process wherein net uptake was a function of the fatty acid to AFP molar ratio and was linearly related to the concentration of arachidonic acid in the medium. The second was a saturable process evident when the molar ratio of arachidonic acid to AFP was fixed and the concentrations of the ligand and the protein both varied.

Extending the studies documenting the existence of specific AFP receptors at the surface of the malignant cells (50), Gueskens (60) showed that the protein is endocytosed via coated pits, vesicles, and receptosomes, while AFP is recycled back to the cell surface. This pathway appears to be similar to that of transferrin (an iron-binding protein) endocytosed by cells through specific membrane receptors (61).

The exact role of plasma fatty acid carriers in the receptor-mediated fatty acid transfer is not clear. It might involve the interaction of the protein-fatty acid complex with the specific receptors followed by the release and transfer of fatty acid to fatty acid-binding protein. This working hypothesis does not exclude the possibility that protein-fatty acid complexes may directly enter cells via endocytosis. The binding of fatty acids and its delivery to cells may form an important event in defining the biological role of AFP during embryogenesis. Since AFP carries polyunsaturated fatty acids and these acids form important components of phospholipids, especially in brain tissues, the transport and cell delivery of long-chain fatty acids could be the major physiological activity of AFP. This function can be critical during early embryonic growth, at a time when AFP is present in plasma at a high concentration.

Summary

An attempt is made in this review to summarize new findings that propose the direct involvement of two fetal proteins (i.e, fetuin and α-fetoprotein) in fatty acid transport and delivery to cells. The presence of these proteins at a high plasma concentration in fetal life and the critical need for fatty acids during development strongly support this hypothesis. Tissue and cell specificities involved in the uptake of these fetal proteins and the mechanism of fatty acid delivery awaits further research.

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