Further Insights into the Pathophysiology of Hyperapobetalipoproteinemia: Role of Basic Proteins I, II, III

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Hyperapobetalipoproteinemia (hyperapoB), a familial lipoprotein disorder characterized by an increase in small, dense, low-density lipoprotein (LDL) particles, is strongly associated with coronary artery disease. There are two metabolic defects in hyperapoB: an increased synthesis of a very-low-density lipoprotein in liver, resulting in an overproduction of LDL, and a delayed clearance of postprandial triglyceride and free fatty acids. To date, defects in the apolipoprotein B gene do not appear to explain the hyperapoB phenotype. Defect(s) in the uptake or intracellular metabolism of free fatty acids have been found in cells from hyperapoB patients. Three basic proteins (BPs)—BP I (M, 14 000, pl 9.10), BP II (M, 27 500, pl 8.48), and BP III (M, 55 000, pl 8.73)—were isolated from normal human serum. Compared with normal fibroblasts, cultured hyperapoB fibroblasts incubated with BP I, which appears to be the same protein as acylation-stimulating protein (ASP), showed 50% less stimulation of triglyceride acylation and cholesterol esterification, whereas BP II markedly stimulated cholesteryl ester formation, and BP III caused no difference in response vs normal fibroblasts. However, in cultured normal human macrophages, BP III, but not BP I or BP II, stimulated cholesteryl esterification two- to threefold. BP I, BP II, and BP III may provide new insights into normal metabolism of lipids, lipoproteins, and free fatty acids and the pathophysiology of hyperapoB.

Additional Keyphrases: lipoproteins · apolipoproteins · basic proteins (BP) · fibroblasts · acylation-stimulating protein · cholesteryl ester formation · coronary artery disease

Hyperapobetalipoproteinemia (hyperapoB)6 is a lipoprotein disorder characterized by an increased concentration of the major apolipoprotein apo B and low-density lipoproteins (LDL), and by a significantly low ratio between LDL cholesterol and LDL apo B (1–3). HyperapoB is quite prevalent in patients with coronary artery disease (CAD) (1–3). Such patients may be normolipidemic, hypertriglyceridemic, or (when the number of LDL particles is sufficiently high) hypercholesterolemic (4). The chemical basis for the hyperapoB phenotype resides in an increased number of small, dense LDL particles (3, 4). HyperapoB is clearly familial (5) and, based on studies in several human kindreds (4–6) and in one animal model (7), appears to behave as a Mendelian co-dominant trait. In some kindreds, hyperapoB may reflect the disorder familial combined hyperlipidemia (FCH) (4, 8), a syndrome originally described by Goldstein et al. (9) in survivors of premature myocardial infarction, and characterized by marked hypercholesterolemia, hypertriglyceridemia, or both.

Two metabolic defects have been described in patients with hyperapoB (10–12). In one, there is a significant overproduction of very-low-density lipoproteins (VLDL) in the liver, resulting in an overproduction of LDL (10, 11). In the other, a delayed clearance of postprandial triglyceride is accompanied by an apparent increase in free fatty acids (FFA) and decrease in high-density lipoproteins (HDL) (11, 12).

A better understanding of the biochemical and genetic mechanisms underlying hyperapoB may provide further insight into the pathophysiology of atherosclerosis and premature CAD. This is pertinent because the three major risk factors for CAD—hypercholesterolemia, hypertension, and cigarette smoking—explain only about 50% of the risk of developing CAD (13). Further studies of hyperapoB may shed additional light on the importance of the triglyceride-rich lipoproteins in CAD, namely VLDL and chylomicrons and the products of their lipolysis, VLDL remnants and chylomicron remnants, respectively. Further, it has not been established whether the lower concentrations of HDL cholesterol and of the major apolipoprotein of HDL, apo A-I, that often accompany the hyperapoB phenotype (14) are simply secondary to abnormalities of the metabolism of the triglyceride-rich lipoproteins or are directly involved in the pathophysiology of the disorder. HyperapoB was previously reviewed here two years ago (15); here, we focus on additional work by ourselves and others since that time.

Received October 15, 1990; accepted January 3, 1991.

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Brief Historical Overview

HyperapoB was described in 1980 by Sniderman et al. (1) during a study of 100 consecutive patients undergoing elective coronary arteriography. About half of the patients with coronary atherosclerosis had concentrations of LDL apo B above the upper limit of normal (≥2.4 μmol/L) and concentrations of LDL cholesterol mostly between 2.4 and 3.2 mmol/L (1). The increased concentrations of LDL apo B were similar to those in patients with heterozygous familial hypercholesterolemia (FH), but the FH heterozygotes had LDL cholesterol concentrations usually between 5.16 and 7.75 mmol/L. In a second study, ~80% of 100 survivors of myocardial infarction had LDL apo B >2.4 μmol/L; over half of these survivors also had plasma triglyceride ≥2.26 μmol/L (2), indicating that hyperapoB patients could be either normolipidemic or hypertriglyceridemic. In a third group of patients, those with endogenous hypertriglyceridemia (type IV lipoprotein patterns, i.e., normal concentrations of LDL cholesterol with above-normal triglyceride), cardiovascular disease was five times as prevalent in those whose LDL apo B exceeded 2.6 μmol/L as in those in whom LDL apo B was <1.6 μmol/L (2). A strong, inverse correlation between the concentrations of LDL apo B and plasma triglyceride was found in these type IV patients; for example, those with marked hypertriglyceridemia (≥5.65 μmol/L) had a low concentration of LDL apo B (1.2–1.6 μmol/L), whereas those with moderate hypertriglyceridemia (2.26–5.65 μmol/L) had significant increases in LDL apo B concentrations. These results suggested that it may not be the fasting triglyceride concentration per se that relates to CAD risk, but whether or not the subjects have an increased concentration of LDL apo B (presumably because of an increased rate of apo B synthesis) (2, 14). Moreover, at least some patients with borderline or moderately increased triglyceride content (2.26–5.65 μmol/L) may actually be at significant risk for CAD because they also have hyperapoB, and plasma triglyceride concentrations in that range may be a marker for the disorder.

The clinical relevance of hyperapoB for coronary atherosclerosis was emphasized further in the Montreal Heart Study (16), which found that, 10 years after aorto-coronary artery bypass surgery, an increase in LDL apo B and a decrease in HDL cholesterol were the two strongest indicators of progression of coronary atherosclerosis, both in the native coronary vessels and in the saphenous vein bypass grafts. More recently, Brown et al. (17) conducted a randomized clinical trial of the effect of diet and lipid-lowering agents on regression of coronary atherosclerosis. They selected patients who had (a) an LDL apo B concentration >2.5 μmol/L; (b) coronary artery bypass surgery; and (c) a positive family history of premature CAD. In their preliminary report, lowering the concentration of LDL apo B was the strongest predictor of regression of coronary atherosclerosis.

Characteristics of HyperapoB LDL

The LDL are a heterogeneous group of macromolecules that are ordinarily isolated between salt densities of 1.019 and 1.063 kg/L. HyperapoB LDL, on average, have a smaller molecular mass (usually <2 × 10⁶ Da), a smaller diameter (about 23 nm), and a lower S' (usually <5.0), but a higher hydrated density (~1.055 kg/L) than normal LDL (3, 4). The amount of cholesterol ester in the core of the smaller, denser LDL from hyperapoB patients is less than normal, and this depletion is not ordinarily compensated by an increase in the triglyceride content of the particles. The amounts of phospholipid and unesterified cholesterol in hyperapoB LDL are normal. Both normal and hyperapoB LDL have one molecule of apo B for each molecule of LDL; thus, because the cholesterol ester content is lower, there is a relative enrichment in apo B protein in hyperapoB LDL. These compositional changes are reflected by a low ratio (<1.2) of LDL cholesterol to LDL apo B (however, because of the actual large laboratory variation in apo B values, primarily owing to differences in calibration approach, the absolute LDL cholesterol to LDL apo B ratio may have little meaning). The concentrations of LDL apo B in hyperapoB are not due to the presence of increased Lp(a), a large lipoprotein that consists of one molecule of LDL covalently bound by a disulfide bridge to apo(a), a glycoprotein homologous to plasminogen (18, 19). For example, in the Prospective Cardiovascular Munster (PROCAM) study, Sandkamp et al. (20) found that Lp(a) concentrations in serum were not correlated with the concentrations of apo B or apo A-1.

Genetic Studies of HyperapoB

One-third of the offspring of hyperapoB survivors of premature myocardial infarction also had hyperapoB (5). Although the expression of hyperapoB can be delayed until the third decade, it is often expressed in children and adolescents (21, 22). Using quantitative genetic analyses in a large Amish pedigree, Beaty et al. (6) found that the ratio of LDL cholesterol to LDL apo B defined three phenotypes: (a) a very low ratio (<1.0); (b) a moderately low ratio (1.027); and (c) a normal ratio (1.27). Three modes were also found for concentrations of LDL apo B (6). These results suggested a co-dominant mode of inheritance. Researchers from St. Thomas Hospital in London described a new rabbit model for hyperlipidemia, in which the rabbits had either hypercholesterolemia, hypertriglyceridemia, or both hypercholesterolemia and hypertriglyceridemia; these rabbits developed spontaneous atherosclerosis on regular (low-fat) rabbit chow (23, 24). The lipid phenotypes were similar to those found in FCH, and metabolic studies indicated that VLDL was also overproduced in these animals (23). Beaty et al. (7) analyzed the lipid data from this rabbit pedigree and found three modes for triglyceride concentrations (Figure 1). More recently, we have examined the concentrations of plasma apo B in a colony of St. Thomas hyperlipidemic rabbits established at Johns Hopkins. The distribution of plasma apo B concentrations in the Johns Hopkins colony is markedly higher than in normal rabbits; the histograms suggest three modes for concentrations of plasma apo B in the hyperlipidemic rabbits (Figure 2).
Fig. 1. Distribution of concentration of serum triglyceride in 103 rabbits from the St. Thomas Hospital hyperlipidemic pedigree. Ordinate: number of rabbits. Reproduced with permission from Beatty et al. (7)

Fig. 2. Distribution of total apo B concentrations in plasma from 12 normal New Zealand White rabbits (top) and in 38 rabbits from the Johns Hopkins colony of St. Thomas Hospital hyperlipidemic rabbits (JH-STH) (bottom)

Based on the human and animal data, our working genetic hypothesis is that three phenotypes result from the expression of alleles at a single genetic locus: normal (both alleles wild type), hyperapoB (one mutant allele and one normal allele), and FCH (two mutant alleles). Thus, FCH may reflect the marked expression of hyperapoB. Using gradient gel electrophoresis, Austin and Krauss (25) described three phenotypes of LDL size: pattern A, in which the major peak of LDL subclasses had a larger particle size (mean diameter 26.6 nm); pattern B, in which the major peak contained smaller, dense LDL particles (mean diameter 24.8 nm); or an intermediate pattern, in which some characteristics of both patterns A and B were present (mean diameter 25.9 nm). Pattern B has a prevalence of about 15% (25). We have found pattern B and the intermediate pattern in hyperapoB patients (26), and Horowitz et al. (27) recently reported pattern B in patients with FCH. Given the relatively high prevalence of these syndromes, there doubtless is genetic heterogeneity. Indeed, Babirak et al. (28) suggested that the heterozygous state for lipoprotein lipase deficiency may form one subset of FCH. Moreover, a subset of families (six of 30) with FCH showed tight linkage (lod score of 7) between FCH and the ApoAI/CIII/AIV gene complex (James Scott, personal communication).

Apo B Gene and Concentrations of LDL Apo B in Plasma

The concentration of LDL apo B reflects the balance between synthesis and catabolism, processes that are under the influence of a variety of genetic and environmental factors. Environmental factors include the possible effects of dietary saturated fat, cholesterol, and calories (obesity). Genes that regulate the production of triglyceride, cholesterol, and apo B in liver may also influence the synthetic rate of VLDL and its secretion into the circulation. The intravascular metabolism of VLDL may be influenced by the concentrations and activities of lipoprotein lipase and its co-factor apo CII, which determine the rate of lipolysis of triglyceride in VLDL and VLDL remnants. The rate of VLDL catabolism may also be affected by the efficiency with which VLDL remnants are removed from the circulation via binding of E to the LDL (B, E) receptor on the liver. Indeed, the apo E polymorphisms appear to have a major influence on apo B concentration (20). VLDL remnants may also be modified by hepatic triglyceride lipase at the surface of the liver cell, producing LDL, the final byproduct of VLDL catabolism. LDL are then removed from plasma through the interaction of the positively charged residues (lysine and arginine) of apo B with the negatively charged residues of the LDL receptor, predominantly in the liver.

Because LDL (B, E) receptor activity in vivo (10) and in vitro (4, 26) appears normal in hyperapoB patients, and because the increased concentration of LDL apo B is secondary to overproduction of VLDL apo B, there has been an interest in determining whether defects in the apo B gene (for example, in the regulatory portion of the gene) may have a role in hyperapoB. Using monoclonal antibodies to apo B, Gavish et al. (29) found that certain epitopes on LDL apo B influenced the concentrations of LDL apo B by about 20%. Coresh et al. (30) recently found that a haplotype for the apo B gene significantly influenced the concentrations of LDL apo B in plasma. Whether the apo B gene is linked to the hyperapoB phenotype has not been determined unequivocally. In one family from the Johns Hopkins CAD study, we did not find linkage between hyperapoB and a mutation in the apo B gene (ApoB-100 Hopkins) that produces a substitution of arginine with tryptophan at amino acid

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residue 4019 (26). Genetic linkage analyses are currently underway with a larger number of families from the Johns Hopkins CAD study to determine whether evidence supports linkage between hyperapoB and haplotypes in the apo B gene.

**Metabolic Defects and Hypothesis of Pathophysiology of HyperapoB**

Two metabolic defects have been described in patients with hyperapoB (Figure 3). In one, overproduction of VLDL leads to overproduction of the dense LDL particles (indicated as LDL heavy in Figure 3). Using monoclonal antibodies to the portion of apo B that is recognized by the high-affinity LDL receptor, Teng et al. (31) found that smaller, denser LDL particles did not react as well as light LDL, suggesting that there may be decreased affinity of hyperapoB LDL for the LDL receptor. Such an effect may be secondary to the rearrangement of apo B on the surface of a cholesteryl ester-depleted particle (Figure 3). These in vitro observations (31) are consistent with in vivo studies in which small, dense LDL did not appear to be cleared from the circulation as quickly as lighter, buoyant LDL in both normal and hyperapoB subjects (10, 11). However, it has been difficult to demonstrate directly a decreased uptake of small, dense LDL by the LDL receptor in cultured human fibroblasts (26, 32)—probably because of the threefold variability in LDL receptor activity in normal cultured fibroblasts (33).

We have also considered the possibility that smaller, denser LDL may be taken up to a greater extent than larger, lighter LDL by an LDL receptor-independent (scavenger) mechanism (Figure 3). Such an effect might facilitate atherogenesis and deposition of cholesteryl esters. For these experiments, we used normal human monocyte-derived macrophages, which have active scavenger receptors but low activities of LDL receptors. Malondialdehyde-altered LDL were used as a positive control to assess the activity of the macrophage "scavenger" receptor, which is unresponsive to down-regulation by LDL (Figure 4). HyperapoB LDL did not promote excessive cholesterol esterification in either normal or hyperapoB human monocyte-derived macrophages (34). There was thus no evidence of hyperapoB LDL being taken up through the scavenger receptor or of a cellular defect in the processing of LDL by the hyperapoB macrophage (Figure 4). Knight et al. (32) found no difference between the binding and degradation of "heavy" and "light" subfractons of LDL in normal cultured monocyte macrophages, which also suggests that heavy LDL is not preferentially taken up by these cells.

The second metabolic defect in hyperapoB apparently involves a delayed clearance of dietary fat through the chylomicron pathway (Figure 3). This defect is associated with an apparent increase in the concentration of FFA in blood (11). One might postulate that such FFA fluxes back to the liver, driving triglyceride production and VLDL synthesis in vivo, but whether such a mechanism operates is not known (Figure 3). The relation between FFA-stimulated VLDL secretion and apo B syntheses is not well understood. Some (35, 36) but not all (37) studies involving cultured hepatocytes have suggested that FFA can induce synthesis and secretion of VLDL apoproteins. Pilot studies by Sniderman et al.

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![Diagram of metabolic pathways](image-url)
Fig. 4. Effect of normal, hyperapoB, and malondialdehyde (MDA) LDL on cholesteryl ester synthesis in cultured normal human monocyte-derived macrophages.

Cells were grown for seven days in the presence of normal human type AB serum and then switched to lipoprotein-deficient media with or without the indicated LDL for 24 h, at which time the incorporation of [1-14C]oleate into cholesteryl esters was determined (32). Data are presented as mean ± 1 SD.

Identification of Acylation Stimulatory Activity

During studies of fatty acid metabolism in normal cultured human fibroblasts and adipocytes, Cianflone et al. (39) identified in human plasma a factor that significantly stimulated triglyceride synthesis in these two cell types. During attempts to purify further the acylation stimulatory activity, Cianflone et al. (40) observed that this effect was due to a protein having a basic pI. This basic protein was subsequently isolated and characterized and called acylation-stimulating protein (ASP) (41). The molecular mass of ASP is 14 000 ± 400 Da and its pI is 9.0 ± 0.3. In normal human fibroblasts, ASP stimulates oleate incorporation into triglyceride by about twofold the rate in cells incubated without ASP (41).

Work in our laboratory has focused upon the study of acylation stimulatory activity in normal human and hyperapoB fibroblasts. Using preparative isoelectric focusing, we isolated a mixture of basic serum proteins (42). Preparative SDS-PAGE purified three basic proteins from this mixture (Figure 5). The M, and pI of these proteins were, respectively, basic protein I, 14 000 and 5.10; basic protein II, 27 500 and 6.48; and basic protein III, 55 000 and 8.73 (42).

Characterization of Basic Proteins I, II, and III from Human Serum

The three basic proteins have different amino acid compositions (42); Figure 6 depicts the major differences. Basic protein I has about twice as much arginine as basic proteins II and III and three times as much cysteine as basic protein III (basic protein II has no cysteine residues). Basic protein II is enriched in proline. Basic protein III has half as much histidine as basic proteins I and II but two- to threefold more methionine. Basic protein III also is more enriched in serine than are basic proteins I and II (Figure 6). These basic proteins are relatively enriched in nonpolar amino acids (42). The anomalous behavior of the basic proteins that we observed on Sephadex or polyacrylamide column chromatography may be related to the tendency of these nonpolar residues to interact, causing the proteins to self-associate in aqueous buffers. To account for their high pI, most of the Asx and Glx residues of these basic proteins are probably in their amidated form.

By its reaction with an antiserum to ASP (gift from K. Cianflone and A. Snidman) and Western blot analysis, basic protein I appears to be identical with ASP (42). This agrees with the similar amino acid compositions, molecular masses, and pI values of the two proteins that have been observed in both laboratories (41, 42). The high content of cysteine in ASP and basic protein I may...
produce intermolecular association via disulfide bridges. Cianfone et al. (41) found that some of the cysteine residues in the oxidized form may be essential for ASP activity because, when ASP was purified in buffers containing β-mercaptoethanol, the activity of ASP was lost. Basic proteins I, II, and III appear to differ from SCPβ and FABP, two major sterol-carrier and fatty acid-binding proteins (41–43).

Effect on Oleate Incorporation in Normal Human Fibroblasts

We found (42) that basic proteins I, II, and III all manifested acylation stimulatory activity in normal human fibroblasts, and that these proteins stimulated the incorporation of oleate into triglyceride, cholesteryl esters, and phoeholphospholipids, the order of stimulation being basic protein I > basic protein II > basic protein III (42). This stimulatory activity was concentration-dependent and saturable (Figure 7), suggesting a rate-limiting process. While studying the effects of the basic proteins in hyperapoB cells, we observed a 50% decrease in the incorporation of oleate into triglyceride in the presence of basic protein I, but a markedly greater stimulation of the incorporation of oleate into cholesteryl esters in the presence of basic protein II (Figure 7).

To substantiate these observations further, we studied cultured human fibroblasts from six normal subjects and from six unrelated patients with familial hyperapoB (each proband had at least one similarly affected first-degree relative) (42). The results of this experiment are summarized in Figure 8. There were no significant differences between the normal and hyperapoB cell lines when no basic serum proteins were added to the cell culture medium (42). In contrast, for acylation stimulation of oleate incorporation into triglyceride with basic protein I, the hyperapoB cells had a 50% deficit (Figure 8, left). Basic proteins II and III promoted no difference in triglyceride synthesis between the normal and hyperapoB cells. In marked contrast, stimulation of oleate incorporation into cholesteryl esters was significantly increased (up to ninefold) by basic protein II in hyperapoB cells (Figure 8, right), whereas the effect of basic protein I on oleate incorporation into cholesterol esters was significantly depressed. Again, no differences were seen for basic protein III. These data provide further evidence that basic proteins I, II, and III are different.

Fig. 7. Effect of increasing concentration of serum basic protein I (top panels), basic protein II (middle panels), and basic protein III (bottom panels) on incorporation of [14C]oleate into cellular lipids in hyperapoB and normal fibroblasts

We studied one normal and one hyperapoB cell line. Fibroblasts were grown to confluence in serum-containing media (42). After 24 h in serum-free media, cells were changed to F12 media containing [14C]oleate–albumin (42). Basic proteins I, II, and III in increasing concentrations were added to the cell lines; after the cells were incubated for 6 h, they were harvested and analyzed for incorporation of [14C]oleate into triglyceride (left panels), cholesteryl esters (middle panels), and phospholipids (right panels) (42)

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Lipid, Lipoprotein, and LDL Apo B Concentrations and Effect of Basic Proteins on Oleate Incorporation into Cellular Lipids

We next examined whether there was any relationship between the concentrations in plasma of total cholesterol, total triglyceride, LDL cholesterol, LDL apo B, and HDL cholesterol in the six normal subjects and the six patients with familial hyperapoB and the incorporation of [14C]oleate into triglyceride and cholesteryl esters by basic proteins I and II in fibroblasts from these subjects (42). We found a significant inverse correlation between the rate of oleate incorporation into triglyceride in cultured fibroblasts in the presence of basic protein I and the concentrations of total cholesterol, total triglyceride, LDL cholesterol, and LDL apo B in plasma (Figure 9, left). In contrast, there was a positive correlation with the concentration of HDL cholesterol (Figure 9, left). We obtained similar findings for the effect of basic protein I on oleate incorporation into cellular cholesteryl esters (data not shown). These data indicate that basic protein I may have a role in the pathophysiology of hyperapoB, particularly in relation to its pleiotropic phenotype: higher concentrations of total cholesterol, triglyceride, and LDL apo B, but lower concentrations of HDL (see also above).

For basic protein II, the effects correlated in the opposite direction from those seen for basic protein I (Figure 9, right). The effect of basic protein II on the rate

Fig. 8. Effect of basic proteins I, II, and III on Incorporation of [14C]oleate into triglyceride (left) and into cholesteryl esters (right) in cultured fibroblasts from six normal subjects and six unrelated patients with familial hyperapoB (42)

Data represent mean (+ 1 SD). Fibroblasts were grown to confluence in serum-containing media, changed to serum-free media for 24 h, and then incubated for 6 h in F12 medium with basic proteins I (0.43 μmol/L), II (0.21 μmol/L), or III (0.11 μmol/L), or without protein.

Fig. 9. Correlations between the effect of basic protein I on incorporation of [14C]oleate into triglyceride (left panels) or of basic protein II on incorporation of [14C]oleate into cholesteryl esters (right panels) in six normal and six hyperapoB fibroblasts and the concentrations of total cholesterol, total triglyceride, LDL cholesterol, LDL apo B, and HDL cholesterol

Data are drawn from those presented by Kwiterovich et al. (42)
of oleate incorporation into cellular cholesteryl esters was significantly and positively correlated with the concentrations of total cholesterol, LDL cholesterol, and LDL apo B in plasma (Figure 9, right); trends also indicated a positive correlation with plasma triglyceride and a negative correlation with HDL cholesterol, but these did not reach statistical significance. Thus, the greater stimulation of cholesterol esterification with basic protein II in hyperapoB cells was correlated with higher plasma concentrations of LDL cholesterol and LDL apo B. These results suggest that basic protein II may affect the plasma concentrations of LDL through a different mechanism than basic protein I does. One possibility is that basic protein I has its effect primarily on peripheral cells (such as adipocytes), where there is a decreased incorporation of FFA in hyperapoB (Figure 3). It will be particularly of interest to determine whether basic protein II abnormally stimulates FFA uptake and (or) apo B production in liver cells; apo B production may be driven by cholesteryl ester formation (44). Thus, the enhanced synthesis of apo B in hyperapoB may be related either to an increased flux of FFA to the liver or to an abnormal effect of basic protein II (Figure 3).

It will also be important to determine what effect the basic proteins may have on oleate incorporation into cholesteryl ester and triglyceride in other types of cultured cells. In normal human monocyte-derived macrophages, basic protein III significantly stimulated oleate incorporation into cholesteryl esters, an effect that was time dependent (Figure 10). No such effect was seen on triglyceride synthesis in these cells. However, basic proteins I and II showed no effect on oleate incorporation into either triglyceride or cholesteryl esters. These data suggest that the effect of basic proteins may be cell specific (Figure 3).

Binding of ASP to Normal and HyperapoB Cells

The possible mechanisms of action of the basic proteins include (a) an interaction at the surface of the cell, facilitating the binding or uptake (or both) of FFA or the induction of a second message; (b) transport of FFA across the cell membrane; (c) intracellular movement of FFA; and (d) a direct or indirect effect on enzymes involved in triglyceride and phospholipid synthesis and in cholesterol esterification or hydrolysis.

Cianflone et al. (45) recently examined the possibility that the binding of ASP to hyperapoB fibroblasts may be defective. They found that in normal cells the amount of cell-associated 125I-labeled ASP was concentration dependent and the ASP was rapidly degraded. Although both the hyperapoB and normal cells showed ASP concentration-dependent differences in cell-associated ASP, the difference was substantially less in the hyperapoB cells, but only at the highest concentration of ASP in the medium (45). The data suggested that ASP exhibited the properties of specific and saturable binding to the normal cells. Analysis of Scatchard plots indicated the presence of a single class of binding sites with a binding constant ($K_d$) of $1.05 \times 10^{-8}$ mol/L (45). In the hyperapoB cells, the apparent $K_d$ was normal but the specific binding of ASP was decreased about 50%, indicating that the mutant cells manifest half the normal number of ASP-binding sites (45). Thus, the number of ASP receptors, on average, appeared to be reduced by half in the hyperapoB cells. However, only five of the 10 hyperapoB cell lines had a 50% or less defect in binding of ASP. Thus, a specific defect in ASP binding appeared to be more characteristic of some hyperapoB cell lines than others. Moreover, this did not appear to be related to the degree of hyperlipidemia. It is interesting to speculate that those individuals that did not manifest a decrease in ASP receptors may have some other defect, such as the defect in lipoprotein lipase activity described by Babirak et al. (28).

When Cianflone et al. (45) examined the relationship between the percent of normal binding and the stimulation of triglyceride synthesis (as judged by oleate incorporation), they found that the decrease in binding of ASP to the cell surface was directly related to the deficiency in the stimulation of triglyceride formation in the cells (Figure 11).

Using a competitive enzyme-linked immunosorbent assay (ELISA), Cianflone et al. (46) measured ASP in normal human plasma. In seven normal subjects, the average concentration of ASP was 4.71 $\mu$mol/L (range 1.64–7.42). Previously, these workers found that 0.36 $\mu$mol of ASP could be isolated from 50 mL of plasma.

![Fig. 10. Effect of basic protein III on incorporation of [14C]oleate into cholesteryl esters and triglyceride in cultured normal human monocyte-derived macrophages.](image-url)
purification was 436-fold and the total yield of acylation stimulatory activity was 14% (41). After correcting for losses during purification, the plasma concentration of ASP would have been 5.00 μmol/L, a value that agrees well with the results of the ELISA (46). After an oral fat load, normal subjects had a significant (twofold) increase in the plasma concentrations of ASP (46). The concentration of either fasting ASP or peak ASP (after the fat load) was significantly inversely related to the degree of postprandial lipemia. These data suggest a role of ASP in the processing of dietary fat. The precise role of ASP (or basic protein 1) is not known. Whether ASP facilitates the entry of FFA at the surface of the cell, or the intracellular transport or the esterification of FFA, the process should result in an increased rate of fatty acid uptake by the cell. Because FFA may inhibit the activity of lipoprotein lipase (Figure 3), the expedient uptake of FFA may allow more efficient hydrolysis of postprandial triglyceride by lipoprotein lipase and improve the clearance of triglyceride-rich lipoproteins (Figure 3). Perhaps the increase in FFA observed in hyperapoB patients after a normal fat load may be related to deficient binding of ASP to the surface of the cell. Further experiments in vivo will be necessary to confirm this speculation.

Summary and Future Research Directions

The identification of three basic proteins in normal human serum and the demonstration of their acylation-stimulatory activity provide new insights into normal lipid, lipoprotein, and FFA metabolism and the pathophysiology of hyperapoB. The future development of specific and highly sensitive ELISAs should permit measurement of the concentrations in serum of these basic proteins in normal and hyperapoB subjects, as well as their response to various metabolic perturbations such as intake of dietary fat. Immunoassay of the basic proteins should also permit us to assess their intracellular distribution in various cell types and better understand their sites of synthesis and the regulation of their production and degradation. The primary structure of the proteins and their possible role in binding to FFA, apolipoproteins, or lipoproteins remain to be determined. The precise biochemical mechanism(s) of the effect of the three basic proteins remains to be elucidated, but might include an interaction with the albumin:FFA complex facilitating FFA uptake; alternatively, the basic proteins may bind directly to cell surface receptor(s), promoting either the uptake or transmembrane transport of FFA. The basic proteins might also facilitate the intracellular transport of FFA, facilitate delivery to sites of esterification, or participate as co-factors in the synthesis of triglyceride, cholesteryl esters, and (or) phospholipids. Finally, it remains to be determined whether the effect of the different basic proteins is tissue-specific. The clarification of these questions should help resolve the significant genetic heterogeneity that underlies hyperapoB (and FCH).

The work described here was presented in part at the annual meeting of the AACC, San Francisco, July 1990, and was supported in part by the following U.S. Public Health Service Grants from the National Institutes of Health: HL-31497 DK-31722; General Clinical Research Center Program, RR-52, RR-35, and CLINFLO. We thank N. K. Ghoob for help with the immunoblot, P. Gugliotta for preparing the manuscript, and H. Smith for assisting with the family studies.

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