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Acylation-stimulatory activity in hyperapobetalipoproteinemic fibroblasts: Enhanced cholesterol esterification with another serum basic protein, BP II

(fatty acids/coronary artery disease/apolipoprotein B/triglyceride/cholesteryl ester)

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ABSTRACT **Cultured fibroblasts from patients with familial hyperapobetalipoproteinemia (hyperapoB) were used to determine if a defect in lipid metabolism was present. Three basic proteins (BP I, BP II, and BP III) were isolated from normal human serum by preparative isoelectric focusing, preparative SDS/PAGE, and reversed-phase HPLC. The M_r and pI values of these proteins were 14,000 and 9.10 for BP I, 27,500 and 8.48 for BP II, and 55,000 and 8.73 for BP III. These proteins differed significantly in their content of arginine, cysteine, proline, histidine, serine, and methionine. BP I appears to be the same protein as acylation-stimulating protein, but BP II and BP III appeared different from acylation-stimulating protein and other lipid carrier proteins. BP I, BP II, and BP III stimulated the incorporation of [^{14}C]oleate into lipid esters in normal fibroblasts, an effect that was time and concentration dependent. In hyperapoB cells, BP II markedly increased (up to 9-fold) the incorporation of [^{14}C]oleate into cholesteryl ester compared with that in normal cells; in addition, there was a 50% decrease in the stimulation of triglyceride acylation and cholesterol esterification with BP I. No difference between normal and hyperapoB cells was observed with BP III. In summary, the identification of another serum basic protein, BP II, led to the elucidation of another cellular defect in hyperapoB fibroblasts, enhanced cholesterol esterification, which may be related to the precocious atherosclerosis and abnormal lipoprotein metabolism seen in hyperapoB.**

Hyperapobetalipoproteinemia (hyperapoB) is a familial lipoprotein disorder commonly found in patients with coronary artery disease (1–6). In hyperapoB, apolipoprotein B (apoB), the major apolipoprotein of low density lipoprotein (LDL), is elevated in the presence of normal or moderately elevated levels of total and LDL cholesterol (1–3). Two metabolic defects have been described. In one, the hepatic synthesis of very low density lipoprotein is increased, resulting in the overproduction of small, dense LDL particles (7, 8). In the other, there is a decreased clearance of postprandial triglyceride that is accompanied by an abnormal increase in free fatty acids (FFA) (8, 9). A significant decrease in the incorporation of FFA into triglyceride of adipocytes from hyperapoB patients has been observed (10). Those studies led to the partial purification of a protein fraction from human plasma that stimulated the incorporation of FFA into triglyceride of both normal adipose tissue and cultured fibroblasts (11). This stimulatory activity appeared to reside in a small molecular weight basic protein that was recently isolated and characterized and is known as the acylation-stimulating protein (ASP) (12).

In the process of isolating an acylation-stimulatory factor from human serum for use in studies of lipid metabolism in hyperapoB fibroblasts, we discovered that there were three basic proteins that had acylation-stimulatory activity. One of these was ASP, while the other two appeared to be different basic serum proteins. We report here the isolation and partial characterization of these proteins and their effect on the incorporation of [^{14}C]oleate into lipid esters in normal human and hyperapoB fibroblasts.

MATERIALS AND METHODS

Subjects. Six normolipidemic subjects (three males: D.B., J.O., and M.E.; three females: K.C., D.R., and M.M.) and six hyperapoB subjects (C.L., B.O., C.H., W.Y., W.B., and G.G.) were studied (Table 1). HyperapoB was defined as an elevated plasma level of LDL apoB (>120 mg/dl) and a low ratio (<1.27) of LDL cholesterol to LDL apoB (5). Each hyperapoB subject was a proband from a family that had at least one other similarly affected member. All but one (B.O.) of the probands had premature coronary artery disease (before 50 years of age) and were ascertained through the Johns Hopkins Coronary Artery Disease Study (13).

Blood. For lipid and lipoprotein measurements, blood was collected into tubes containing EDTA (1.5 mg per ml of blood) after a 12-hr fast, and the plasma was separated from erythrocytes by centrifugation. None of the subjects was on lipid-lowering medication. For the isolation of basic proteins, citrated plasma (250 ml) was obtained by plasmaphoresis from two normolipidemic male donors (D.B. and J.O.), and serum was prepared from plasma by treating it with thrombin (14).

Lipid, Lipoprotein, and LDL apoB Levels. Plasma levels of lipids, LDL, very low density lipoprotein cholesterol, and high density lipoprotein (HDL) cholesterol were determined by using methods of the Lipid Research Clinics Program as described before (6). LDL apoB levels were measured by using a radial immunodiffusion assay (6).

Fibroblasts. Skin biopsies were obtained from the forearms of the normal (n = 6) and hyperapoB (n = 6) subjects after informed consent. Fibroblasts were grown from skin biopsies as described (15).

Preparative Flat-Bed Isoelectrofocusing. A mixture of basic proteins was isolated from human serum (500 mg of serum proteins) on a Multiphor II system (Pharmacia LKB) using Ampholine, pH 3.5–10.0. The gel bed was then cut into 30 equal fractions, and the proteins were eluted with 0.02 M phosphate buffer at pH 7.1. Every three fractions (1–3, 4–6, etc.) were combined and then subjected to analytical isoelectric focusing. The major fractions containing proteins with a basic pI (>8.15) were pooled and referred to as the mixture of basic proteins. To remove Ampholine, a salt solution was

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Abbreviations: BP I, II, and III, basic proteins I, II, and III; LDL, low density lipoprotein; HDL, high density lipoprotein; FFA, free fatty acids; ASP, acylation-stimulating protein; SCP_2, sterol carrier protein 2; hyperapoB, hyperapobetalipoproteinemia; apoB, apolipoprotein B.

Table 1. Plasma lipid, lipoprotein, and LDL apoB levels

Subject	Age	Sex	Total chol	Total tri	LDL chol	LDL apoB	HDL chol
			HyperapoB				
C.L.	43	F	229	139	128	176	51
B.O.	28	M	261	145	169	174	63
C.H.	50	M	237	152	158	183	44
W.Y.	51	M	255	272	145	178	55
W.B.	43	M	194	70	120	127	50
G.G.	44	M	232	330	151	196	38
			Normal				
D.B.	23	M	129	69	43	77	72
J.O.	22	M	113	41	28	48	77
M.E.	22	M	163	36	83	61	73
K.C.	23	F	132	31	49	77	77
D.R.	28	F	154	34	79	83	68
M.M.	27	F	159	46	79	79	71

chol, cholesterol; tri, triglyceride.

added to a final concentration of 0.5 M NaCl in 0.02 M phosphate buffer at pH 7.1. The mixture of basic proteins was dialyzed extensively at 8°C against either 0.02 M phosphate buffer or acetic acid [10%, 25%, and then 50% (vol/vol)], lyophilized, and stored at −20°C until used.

Preparative SDS/PAGE. The lyophilized basic protein mixture was reconstituted with 0.02 M phosphate buffer at pH 7.1. Preparative SDS/PAGE was performed with a Protean II slab cell (Bio-Rad) as described by Laemmli (16). The sample was heated for 4 min at 100°C under reducing conditions (10% 2-mercaptoethanol), and 100-μg samples were applied to wells cut into the slab. After SDS/PAGE, the proteins were eluted from the cut-out gels with 0.02 M phosphate buffer, centrifuged to remove residues of the gel, dialyzed extensively against 0.02 M phosphate buffer, lyophilized, and stored at −20°C until used.

Analytical SDS/PAGE. The mixture of basic proteins and the isolated major basic proteins were subjected to analytical SDS/PAGE using 12.5% and 17.5% acrylamide (16). The gels were run under reducing and nonreducing conditions. M_r determinations were made under reducing conditions using 12.5% acrylamide and standards of known M_r (16).

Analytical Isoelectric Focusing. Serum proteins were characterized by using LKB analytical isoelectric focusing PAGE plates (broad pH range, 3.5–10.5) and pH markers (3.5–10.5), following the procedure of the manufacturers (Pharmacia LKB).

Reversed-Phase HPLC. A Hi-Pore RP-304 column (Bio-Rad) and a gradient of 0–75% acetonitrile in 0.1% trifluoroacetic acid were used.

Amino Acid Analysis. The Pico-Tag amino acid analysis system of Waters-Millipore was used. The basic proteins isolated by HPLC (about 15 μg) were hydrolyzed with 6 M HCl containing 1% (vol/vol) phenol at 110°C for 24 hr. The hydrolysate was dried, and the amino acids were derivatized with phenylisothiocyanate to yield the corresponding phenylthiocarbamyl derivatives following standard procedures (17). These derivatives were analyzed on the Pico-Tag amino acid analysis system, which had been previously calibrated with a standard mixture of amino acids.

N-Terminal Amino Acid Analysis. The basic proteins were subjected to microsequencing on an Applied Biosystems model 470A sequencer equipped with on-line phenylthiohydantoin analysis.

Western Immunoblot Assay. The basic proteins (3–4 μg) and standard proteins were subjected to Western immunoblot analysis as described by Chatterjee and Ghosh (18). The following previously described basic proteins and antisera against them were used: ASP (12), sterol carrier protein 2 (SCP_2) (19), and protein 422 (29).

Protocol for the Cell Experiments. Cells (1×10^5) were seeded in Petri dishes and grown in minimal essential medium containing 10% (vol/vol) fetal calf serum, 1% amino acids, 100 units of penicillin per ml, and 100 μg of streptomycin per ml for 6 days (15). The medium was then changed to a supplemented serum-free medium for 24 hr (11). [^{14}C]Oleate/albumin (10 mM oleate, specific activity = 10,000 dpm/nmol; ratio of oleate to albumin = 4.6:1) and the indicated amounts of basic proteins were then added to duplicate or triplicate dishes and incubated for the specified time. The medium was then removed, and the amount of [^{14}C]oleate incorporated into individual lipid esters per milligram of cell protein was determined using the method of Goldstein *et al.* (20).

Other Methods. The protein content was determined by the method of Lowry *et al.* (21) with serum albumin as a standard. Tests of significance were performed by using Student's *t* test or standard regression analysis.

RESULTS AND DISCUSSION

Isolation and Characterization of Basic Serum Proteins. After SDS/PAGE of the mixture of basic proteins (see *Materials and Methods*), three major protein bands were seen: basic protein I (BP I), basic protein II (BP II), and basic protein III (BP III) (Fig. 1*A*). BP I, BP II, and BP III were separated from each other and other minor contaminants by using preparative SDS/PAGE (see *Materials and Methods*). The apparent M_r of each isolated basic protein was 14,000 for BP I, 27,500 for BP II, and 55,000 for BP III (Fig. 1*A*). After SDS/PAGE under reducing conditions, BP III did not generate BP II or BP I, and BP II did not give rise to BP I (Fig. 1*A*). When the isolated proteins were also subjected to analytical isoelectric focusing, a single band was found for each (Fig. 1*B*). The pI of each was 9.10 for BP I, 8.48 for BP II, and 8.73 for BP III.

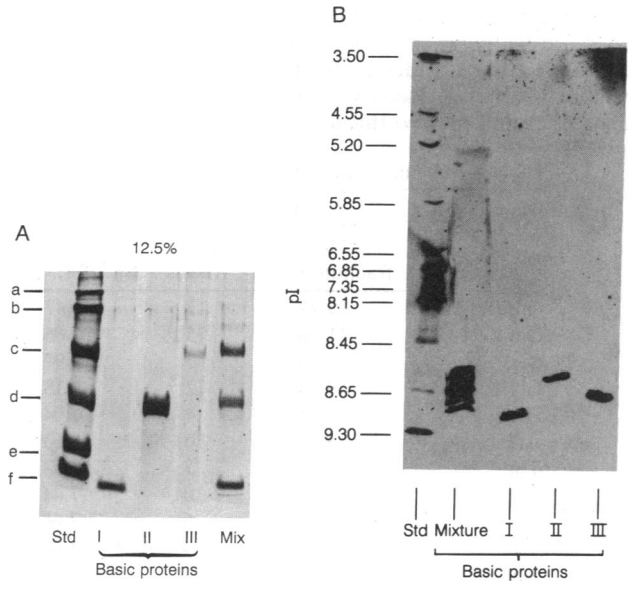

FIG. 1. (*A*) SDS/PAGE of serum basic proteins. Lyophilized basic proteins were dissolved in sample buffer and an aliquot (15 μg) was applied. M_r standards (Std) used were a, phosphorylase b (92,500); b, bovine serum albumin (66,200); c, ovalbumin (45,000); d, carbonic anhydrase (31,000); e, soybean trypsin inhibitor (21,000); and f, lysozyme (14,400). Mix, mixture. (*B*) Analytical isoelectric focusing. Serum basic proteins (15 μg) were applied and focused at 1500 V for 1.5 hr. Calibration markers (Std) were trypsinogen (pI = 9.30); lentil lectin, basic (pI = 8.65); lentil lectin, middle (pI = 8.45); lentil lectin, acidic (pI = 8.15); myoglobin, basic (pI = 7.35); myoglobin, acidic (pI = 6.85); bovine carbonic anhydrase b (pI = 5.85); β-lactoglobin A (pI = 5.20); soybean trypsin inhibitor (pI = 4.55); and amyloglucosidase (pI = 3.50).

When the mixture of basic proteins was subjected to reversed-phase HPLC, three peaks were observed (data not shown). By using 12.5% SDS/PAGE, the first peak was found to contain BP III; the second peak, BP II; and the third peak, BP I. The basic proteins isolated by this method were used for N-terminal amino acid analysis and amino acid composition.

The N-terminal amino acid was blocked in each basic protein. The use of preparative isoelectric focusing may have artifactually blocked these N-terminal amino acids (22). However, it has been estimated that about 80% of the soluble proteins in mammalian cells have acetylated N-terminal amino acids, and additional studies will be required to elucidate the nature of the N-terminal amino acids of these proteins (23). The amino acid compositions of BP I, BP II, and BP III are summarized in Table 2. The estimated minimum M_r differed for the three proteins. Significant differences were also found in their amino acid compositions. BP I had twice as much arginine and at least 3 times as much cysteine as BP II and BP III. BP II differed from BP III in that it had twice as much proline and 2.41 times as much histidine; BP III had twice as much serine, and 3.44 times as much methionine as BP II. To account for their high pI, most of the aspartic or asparagine residues and glutamic or glutamine residues of these basic proteins are probably in their amidated form.

These data do not exclude the possibility that there is some homology between the three proteins or that they may be derived from a larger M_r parent protein. However, in one experiment, we added proteolytic inhibitors [sodium azide at 0.05%, gentamycin sulfate at 0.5 mg/ml, and 1,5'-dithiobis(2-nitrobenzoic acid) at 1.4 mM] to plasma and at each isolation step before SDS/PAGE. Three basic proteins were again observed, indicating that they were not artifactually generated by proteolysis after the blood was drawn. A similar pattern was seen on SDS/PAGE under nonreducing conditions, indicating that the three basic proteins were not generated *in vitro* from a parent protein by cleavage of sulfhydryl groups. Indeed, BP II had no detectable cysteine residues (Table 2). However, the proteins might still have been generated *in vivo* through proteolysis. Finally, we do not believe that preparative isoelectric focusing artifactually caused these proteins to manifest a basic pI (24) since BP I, BP II, and BP III were all detected by analytic isoelectric focusing of fractions obtained after chromatography of serum on an Affi-Gel blue column.

By use of Western immunoblot assays, none of the three basic proteins reacted with antisera to two other basic proteins, SCP_2 [also called nonspecific lipid transfer protein (ns-LTP) (19, 23)] and protein 422, a fatty acid binding protein from adipose tissue (29). The immune serum to ASP (12), but not the preimmune serum, reacted with BP I, indicating that they are likely the same protein (data not shown). Both the preimmune and immune serum against ASP reacted with BP II and BP III, so no conclusions could be drawn about any possible homology between ASP and BP II and BP III. Fatty acid binding protein, a low M_r acidic protein, had previously been shown to be a protein different from ASP (12). Significant differences between the amino acid compositions of BP I, BP II, and BP III and those of both SCP_2 and fatty acid binding protein (25) also indicate that our basic proteins are different from these two proteins.

Effect of the Three Basic Proteins on [^{14}C]Oleate Incorporation into Lipid Esters in Normal Cells. Each of the three basic proteins stimulated the incorporation of [^{14}C]oleate into lipid esters in normal fibroblasts. The effect was both time and concentration dependent (data not shown). For triglyceride and phospholipid, esterification reached a plateau by 6 hr, whereas for cholesteryl ester, [^{14}C]oleate incorporation was linear for at least 6 hr. The stimulatory effect was the greatest for BP I, which at 6 hr was 2.7-fold greater for both triglyceride and cholesteryl ester and 4- to 5-fold greater for phospholipid than that seen in the control medium without basic protein (data not shown).

The concentration curves for the effect of each of the three basic proteins on oleate incorporation into triglyceride were saturable; BP I again had the greatest effect, (about a 3-fold stimulation) and BP II and BP III had a lesser effect (about a 2-fold stimulation) (data not shown). The concentration curves for the incorporation of [^{14}C]oleate into cholesteryl esters were also saturable. When present in equimolar

Table 2. Amino acid composition of basic serum proteins

	BP I		BP II			BP III		
Residue	Mole fraction × 100	Integer (no.)	Mole fraction × 100	Integer (no.)	Residues,* moles	Mole fraction × 100	Integer (no.)	Residues,* moles
Asp/Asn	9.47	12	12.47	13	31	8.35	10	41
Glu/Gln	11.76	15	10.66	11	27	12.95	16	66
Ser	5.75	7	5.97	6	15	9.66	12	50
Gly	9.14	12	8.18	8	20	5.42	7	29
His	2.95	4	4.04	4	10	1.67	2	8
Arg	8.25	10	3.63	4	10	4.05	5	21
Thr	5.16	7	5.53	6	14	4.15	5	21
Ala	7.54	10	7.44	8	19	9.47	11	45
Pro	5.02	6	7.68	8	20	3.80	5	21
Tyr	3.11	4	3.08	3	7	2.65	3	12
Val	5.23	7	6.51	7	15	7.56	9	37
Met	1.59	2	1.07	1	2	3.69	4	17
Cys	3.01	4	0.02	0	0	0.85	1	4
Ile	3.61	5	2.04	2	5	5.06	7	29
Leu	8.03	10	10.69	11	27	8.86	11	45
Phe	3.61	5	4.33	4	10	5.40	6	25
Lys	6.75	9	6.73	7	17	5.82	7	29
Trp	ND		ND			ND		
Number of residues	129		103			121		
Minimum M_r	14,335		11,243			13,317		

ND, not determined. Values for each protein were determined on two different samples and averaged.
*Based on molecular weight estimated by SDS/PAGE.

amounts, BP I (6 μg/ml, 42.86 nmol/liter) stimulated 2.8-fold, BP II (12 μg/ml, 43.64 nmol/liter) stimulated 2.2-fold, and BP III (24 μg/ml, 43 nmol/liter) stimulated 1.6-fold compared with the control. The effects of the basic proteins on the incorporation of [^{14}C]oleate into phospholipid were of a lower magnitude (about 1.5- to 2.0-fold) than those for triglyceride.

We do not believe that the effect of these proteins was simply related to their basic pI. When cytochrome *c* (M_r = 12,327, pI = 10.25) and trypsinogen (M_r = 24,000, pI = 9.30) were subjected to the same isolation procedures as BP I, BP II, and BP III, neither stimulated the incorporation of [^{14}C]oleate (in units of nmol per mg of cell protein per hr) into triglyceride (10.29 and 11.67) or cholesteryl ester (0.154 and 0.18), respectively, compared with F-12 control medium (13.92 and 0.40; averages from three normal cell lines). SCP_2 (a gift from J. Billheimer, E. I. DuPont de Nemours, Wilmington, DE) also did not stimulate [^{14}C]oleate (in units of nmol per mg of cell protein per hr) into triglyceride (13.24) or cholesteryl ester (0.30), compared with F-12 control medium (12.03 and 0.28; averages from two normal cell lines).

Effect of the Basic Proteins on [^{14}C]Oleate Incorporation into Cellular Lipid Esters in HyperapoB Cells. When the effect of the basic proteins with time was studied in the hyperapoB cells, a 50% decrease in the effect of BP I on [^{14}C]oleate incorporation into both cellular triglyceride and cholesteryl ester compared with the normal cell line was found (Fig. 2). There was no decrease in the incorporation of [^{14}C]oleate into triglyceride with BP II in the hyperapoB cells, but a markedly (3-fold) enhanced incorporation of [^{14}C]oleate into cholesteryl esters was observed (Fig. 2). No differences between normal and hyperapoB cells were found with BP III for either triglyceride or cholesteryl esters (Fig. 2). For oleate incorporation into phospholipid, no differences were observed between normal and hyperapoB cells.

When all six normal and six hyperapoB lines were studied, no significant differences were seen between the normal and hyperapoB cells when control medium without basic proteins was used (Table 3). This suggested that the differences observed after the addition of the basic proteins to the cells were not simply related to differences in cellular lipid pools. Stimulation of [^{14}C]oleate incorporation into both cellular triglyceride and cholesteryl ester with BP I was significantly less in the hyperapoB than in the normal cells, whereas there was a markedly *enhanced* stimulation (9-fold) of [^{14}C]oleate into cholesteryl ester with BP II in the hyperapoB cells (Table 3). There were no differences between normal and hyperapoB cells when BP III was used (Table 3).

In another experiment, lipid esters of normal and hyperapoB fibroblasts were pulse-labeled for 6 hr, using [^{14}C]oleate/albumin (27,636 dpm/μl), followed by a chase (0, 0.5, 1, 2, 4, 8, and 24 hr) in either F-12 medium alone or F-12 medium containing BP I, BP II, or BP III (6 μg per ml of medium). After the pulse, a similar amount of [^{14}C]oleate had been incorporated into the lipid classes in normal and hyperapoB cells. During the chase, no significant differences in the rate of disappearance of the label from triglyceride, cholesteryl ester, or phospholipid were found between normal and hyperapoB cells. These results indicated, first, that in normal cells the basic proteins did not inhibit lipid hydrolysis (and therefore act by stimulating lipid synthesis and, second, that the defects in hyperapoB cells were not due to abnormal hydrolysis of lipid esters.

Cianflone *et al.* did not report on the effect of ASP on cholesterol esterification in normal (12) or hyperapoB (26) fibroblasts. We found that BP I (or ASP) significantly stimulated the incorporation of oleate into cholesteryl esters in normal cells and that there was about a 50–60% deficiency in this effect in hyperapoB cells. Such an effect of BP I might also result from a defect of FFA uptake at the cell surface. However, we did not find such a deficiency with BP I in the

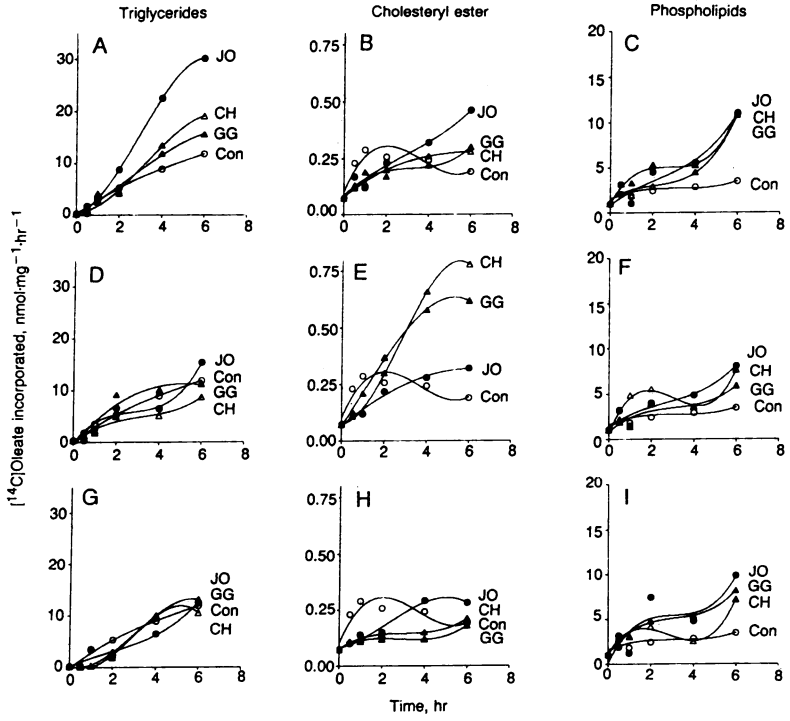

FIG. 2. Effect of BP I, BP II, and BP III on [^{14}C]oleate incorporation into cellular lipids with time in hyperapoB and normal cells. One normal (J.O.) and two hyperapoB (C.H. and G.G.) cell lines were studied. Con, normal cell line without basic proteins. Cells were grown as described in *Materials and Methods*. At zero time, cells were changed to F-12 medium containing [^{14}C]oleate/albumin. BP I (*A–C*), BP II (*D–F*), or BP III (*G–I*) (6 μg/ml of medium) was added to J.O., G.G., and C.H. cell lines or omitted (Con), and the cells were incubated for up to 6 hr. Cells were then harvested, and the incorporation of [^{14}C]oleate into triglyceride (*A*, *D*, and *G*), cholesteryl ester (*B*, *E*, and H), and phospholipid (*C*, *F*, and *I*) was determined.

Table 3. Effect of basic serum proteins on the incorporation of [^{14}C]oleate into cellular triglyceride, cholesteryl ester, and phospholipid in normal and hyperapoB cultured fibroblasts

	[^{14}C]Oleate incorporation, nmol·mg^{-1}·hr^{-1}					
	Triglyceride		Cholesteryl ester		Phospholipid	
Protein added	Normal	HyperapoB	Normal	HyperapoB	Normal	HyperapoB
BP I	23.0 ± 5.4	12.7 ± 4.2*	0.24 ± .09	0.09 ± .06*	1.3 ± 5.1	0.5 ± 4.0
BP II	3.8 ± 2.3	3.5 ± 2.8	0.01 ± .06	0.09 ± .09†	‡	‡
BP III	1.8 ± 2.5	2.3 ± 3.8	0.01 ± .09	0.01 ± .05	‡	1.1 ± 6.7
None	10.6 ± 2.6	11.8 ± 2.9	0.27 ± .07	0.22 ± .04	12.4 ± 3.5	13.8 ± 4.2

Data represent the mean ± 1 SD. Normal, $n = 6$; hyperapoB, $n = 6$. Cells were grown as described in *Materials and Methods* and then incubated in F-12 medium with BP I, BP II, or BP III (6 μg/ml of medium) or without protein for 6 hr. Stimulation in the absence of basic serum proteins was subtracted from that found in the presence of basic proteins.
*$P = 0.0001$.
†$P = 0.02$.
‡No stimulation above F-12 control medium was observed.

incorporation of FFA into phospholipid. Cianflone and coworkers (26) recently presented evidence for a decreased number of receptors for ASP on the surface of hyperapoB fibroblasts. Further studies are necessary to determine whether patients with hyperapoB have significantly elevated ASP levels (as well as elevated FFA levels) after a fatty meal (27), consistent with a defect in the uptake of FFA at the cell surface.

In contrast to BP I, BP II markedly *stimulated* the incorporation of oleate into cholesteryl ester in hyperapoB cells. Further, the defect in triglyceride metabolism with BP I was not seen with BP II, further indicating that the physiological role of BP II differs from that of BP I. Although the basis for the enhanced cholesterol ester synthesis in hyperapoB cells with BP II is not known, if such an abnormality were found in other cell types, such as hepatocytes, it might indicate a role of BP II in the pathophysiology of hyperapoB. For example, changing the concentration of cholesterol within some regulatory pool in liver might contribute to the overproduction of apoB seen in hyperapoB (28).

We also looked at the correlation between the results of the cellular assays and the plasma lipid, lipoprotein, and LDL apoB levels (Table 1). The effect of BP I on oleate incorporation into cell triglyceride (Table 3) was significantly and inversely correlated with plasma levels of LDL apoB ($r = -0.68, P = 0.0003$), LDL cholesterol ($r = -0.64, P = 0.0008$), and triglyceride ($r = -0.57, P = 0.001$) but positively correlated with HDL cholesterol ($r = 0.59, P = 0.002$). Similar findings were observed for the effect of BP I on oleate incorporation into cholesteryl esters. These findings indicate that the pleiotrophic phenotype of hyperapoB (elevated LDL apoB, with higher total and LDL cholesterol and triglyceride levels but a lower HDL cholesterol level) is related to the deficiency of acylation with BP I in hyperapoB cells. When similar analyses were performed for BP II, interestingly, only LDL apoB ($r = 0.52, P = 0.009$) and total ($r = 0.52, P = 0.008$) and LDL cholesterol ($r = 0.50, P = 0.01$) levels were significantly (and positively) correlated with the BP II-mediated effect of oleate incorporation into cholesteryl esters, perhaps suggesting that BP II may influence LDL levels by a different mechanism.

The abnormalities reported here were consistently seen in cells from six unrelated families with familial hyperapoB, several of which also fit the picture of familial combined hyperlipidemia (6). Further studies are needed to determine the effect of these basic proteins on other cell types, their *in vivo* metabolism in normal and hyperapoB patients, and their precise role in the pathophysiology of hyperapoB.

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