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Studies of Plasma Lipids, Lipoproteins and Apolipoproteins in Patients with Small Bowel Resection

by

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To Michael,

my loving husband,

for his constant support

and to my

Mom and Dad,

for their encouragement and belief in my

ability to achieve this goal.

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Studies of Plasma Lipids, Lipoproteins and Apolipoproteins in Patients with Small Bowel Resection

by

Kathleen Jo La Sala

Both liver and intestine contribute to the circulating mass of lipoproteins in plasma. To determine whether the contribution of the small intestine leads to formation of discrete species of lipoproteins other than chylomicrons, a detailed analysis of the plasma lipoproteins of individuals with near complete resection of the small intestine was performed in comparison with the lipoproteins of control plasma. The patients had lower total plasma cholesterol levels reflecting decreased concentrations of low density lipoproteins (LDL) and high density lipoproteins (HDL). The ratio of surface to core components of very low density lipoproteins (VLDL) suggested that VLDL particle diameters are smaller in patients with small bowel resection. Low LDL concentrations correlated with a fifty percent reduction in levels of apoprotein B-100. Patient LDL had significantly smaller particle diameters than those of controls, 227 ± 26 Å versus 238 ± 27 Å, $p<0.0001$. Low HDL levels in patients were associated with a thirty three percent reduction in plasma apoprotein A-I concentrations. Plasma apoprotein E concentrations were similar in patients and controls indicating that a full complement of apo E can be generated by tissues other than the intestine. None of the major apolipoproteins other than apoB-48 was absent from plasma of

patients. Levels of HDL with pre-beta electropheretic mobility were fifty percent lower in the patients, (5.4 versus 10.5%, $p<0.001$). Electrophoresis of apoA-I-containing lipoproteins isolated by immunosorption revealed similar speciation in patients and controls. These data indicated that LDL of small particle diameter were generated in the absence of hypertriglyceridemia in patients with small bowel resection, perhaps due to a lack of absorption of exogenous cholesterol or failure to reabsorb bile acids. The low levels of pre-beta HDL may reflect either the failure to generate this subspecies because of the absence of chylomicrons or the lack of a direct secretion by the intestine. The presence of most of the remaining discrete species of HDL suggested that they are not secreted as discrete products by the intestine.

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INTRODUCTION

The principal lipoproteins of plasma are large, macromolecular complexes that transport lipids (primarily triglycerides and cholesteryl esters) through the bloodstream. Each lipoprotein particle contains a nonpolar core surrounded by a polar monolayer of phospholipids and free cholestserol. Each lipoprotein particle also contains specific proteins (apoproteins) that are at least partly exposed at the particles' surface. Some of these apoproteins bind to specific enzymes or to receptors on cell membranes, thus mediating the metabolism of the lipoproteins.

Although plasma lipoproteins were described as early as 1929 (Macheboeuf), it was not until the early 1940's that these complexes were to be isolated and studied in detail. It was the pioneering work of Gofman and Havel, independently, which yielded a comprehensive view of the lipoprotein particles of plasma. Employing the preparative ultracentrifuge, Havel determined the distribution and chemical composition of major human serum lipoproteins. Gofman, using the analytical ultracentrifuge, studied their hydrodynamic properties and their association with risk of atherosclerotic heart disease.

Listed in Table 1 are the five major classes of plasma lipoproteins and some of their characteristics. These lipoprotein classes are characterized by differences in density, particle size, electrophoretic mobility, and content of lipids and apoproteins. The major lipoproteins

are known as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL).

Though each is within a specific density interval, the particles in each class show some heterogeneity with respect to hydronamic properties. For example, the HDL class can be divided into two or more subclasses by a variety of techniques. Traditionally, when isolated with a preparative ultracentrifuge, HDL are separated into two subclasses, HDL_2 and HDL_3, based on flotation at densities 1.063 to 1.125 g/ml and 1.125 to 1.21 g/ml, respectively (Havel et al. 1955). However, particle heterogeneity within the HDL class has been demonstrated using a variety of other techniques. Further fractionation of HDL_2 into HDL_{2b} (d=1.063-1.100 g/ml) and HDL_{2a} (d=1.100-1.125 g/ml) by equilibrium density gradient ultracentrifiguation results in three major components of HDL (Anderson et al.). Other centrifugation techniques, such as rate zonal centrifugation, have also allowed further separation of HDL (Ewing et al., Kostner et al., Chung et al.). Moreover, confirmation of a varied and heterogeneous population of HDL particles has been achieved by electrophoretic and chromatographic techniques (Utermann, Janecki et al., Bittolo et al., Blanche et al., Okazaki et al., Rudel et al., Mitamura). Most recently, selected-affinity immunosorption has been used to demonstrate as many as seven subspecies of HDL (McVicar et al., Cheung et al.).

Table 1. Properties of Normal Human Plasma Lipoproteins

Class	Diameter (nm)	Density (g/ml)	Electrophoretic mobility	Chemical Composition (% dry mass)				
				TG	CE	FC	PL	Protein
Chylomicrons	75-1200	0.93	Remains at origin	86	3	2	7	2
VLDL	30-80	0.93-1.006	Pre-beta	55	12	7	18	8
IDL	25-35	1.006-1.019	Slow pre-beta	23	29	9	19	19
LDL	18-25	1.019-1.063	Beta	6	42	8	22	22
HDL_2	9-12	1.063-1.125	Alpha	5	17	5	33	40
HDL_3	5-9	1.125-1.210	Alpha	3	13	4	25	55

Chylomicrons

Within the intestinal epithelial cells, triglycerides synthesized from beta-monoglycerides and free fatty acids of dietary origin are incorporated with cholesterol and phospholipids into large lipoprotein particles called chylomicrons. Chylomicrons contain a central core containing mainly triglycerides with a small amount of cholesteryl esters, surrounded by a monolayer of phospholipids, free cholesterol and apoproteins B-48, A-I, A-II, and possibly E (Green et al. 1979, Imaizumi et al.). The chylomicrons are secreted into the intestinal lymph and pass later, via the thoracic duct into the general circulation where they acquire additional apoproteins (C proteins) from HDL (Havel et al. 1973, Glickman et al. 1974, Mattock et al.). The chylomicrons are transported to the capillaries of adipose tissue, skeletal muscle, and other tissues where they adhere to binding sites on the capillary endothelium. While bound to the endothelial surface, the chylomicrons are exposed to the enzyme lipoprotein lipase. Apoprotein C-II activates the lipase, liberating free fatty acids and monoglycerides which can be further hydrolyzed by monoglyceridases. The fatty acids pass through the endothelial cells and enter the underlying adipocytes or muscle cells, where they are either re-esterified to triglycerides or oxidized.

After the bulk of core triglycerides have been removed, perhaps in several such steps of binding and hydrolysis, the remainder of the chylomicron dissociates from the capillary endothelium and re-enters the circulation. It has now been transformed into a particle that is relatively poor in triglyceride and enriched in cholesteryl esters via

the centripital transport system. It has also undergone an exchange of apoproteins with other plasma lipoproteins, in particular apoA-I is transferred to HDL as are surface phospholipids (Schaefer et al. 1978a, Imaizumi et al.). The net result is the conversion of the chylomicron to a remnant particle, enriched in cholesteryl esters and containing apoproteins B-48 and E. This remnant particle appears to be taken up by hepatocytes through a high affinity receptor pathway which recognizes apoprotein E on the remnant particle (Havel 1986). The surface-bound remnants are taken into the cell and degraded within lysosomes. The overall result of the chylomicron transport process is to deliver dietary triglyceride to peripheral tissues and cholesterol to the liver.

Very Low Density Lipoproteins

The liver converts carbohydrates to fatty acids, esterifies the fatty acids with glycerol and forms triglycerides. The liver also forms triglycerides from free fatty acids abstracted from plasma. These triglycerides are secreted into the bloodstream in the cores of very low density lipoproteins. As is the case with chylomicrons, the central core contains mainly triglycerides with a small amount of cholesteryl ester and is surrounded by phospholipids, free cholesterol and apoproteins. The major protein components of VLDL are apoB-100, apoC-I, apoC-II, apoC-III, and apoE.

The VLDL are secreted by the parenchymal cells into the portal bloodstream and are transported to tissues. Here they interact with lipoprotein lipase and are catabolized in the same manner as

chylomicrons. Remnants are formed by the degradation of VLDL analogous to those formed from chylomicrons. It has recently been shown that VLDL vary dramatically in size and that the size of VLDL seems to determine it's fate (Stalenhoef et al.). Remnants of larger VLDL are removed from the circulation by the liver, just like the chylomicron remnants. Some of the smaller VLDL remnants remain in the plasma and undergo further degradation to particles which have had nearly all of the triglycerides removed and replaced with cholesteryl esters. During this conversion, all the apoproteins are removed from the remnant except apoprotein B-100. The final products of VLDL catabolism are cholesteryl ester rich lipoproteins, LDL.

Low Density Lipoproteins

The cores of LDL are composed almost entirely of cholesteryl esters. The lipoprotein contains only one apoprotein, apoprotein B-100. Approximately seventy percent of the total cholesterol in normal human plasma is contained within LDL particles. The function of LDL is to deliver cholesterol to a variety of extrahepatic tissues.

LDL range in size from twenty to twenty-five nanometers. This small diameter allows the LDL to pass through the capillary endothelium and reach cell membranes. Many cells, such as adrenal cortical cells, lymphocytes, muscle cells, renal cells, and smooth muscle cells, have high affinity receptors on their membranes which bind LDL by interacting with apoB-100. The pioneering work of Brown and Goldstein (1973) has elucidated this LDL-receptor pathway. The LDL-receptor complex

invaginates into the cell, forming an endocytic vesicle. This vesicle delivers the LDL to secondary lysosomes where apoB is hydrolyzed to amino acids and the cholesteryl esters are hydrolyzed by a lysosomal esterase to free cholesterol. The liberated cholesterol is used for membrane synthesis. When sufficient cholesterol has accumulated to saturate the requirement for membrane synthesis, the excess cholesterol is re-esterified by a microsomal enzyme, acyl CoA:cholesterol acetyl transferase (ACAT); the activity of which appears to be regulated by the intracellular cholesterol levels. In addition, the cell is protected from an overaccumulation of cholesterol by suppression of its own cholesterol synthesis. Cells can also regulate the number of LDL receptors on the plasma membrane in response to intracellular levels of cholesterol or some metabolite thereof. This LDL receptor pathway serves as the major route for the degradation of at least half of the LDL circulating in plasma.

In addition to its degradation by the LDL pathway in extrahepatic parenchymal cells, some of the LDL is degraded by a scavenger cell system that consists of phagocytic cells in the reticuloendothelial system (Goldstein et al. 1979, Basu et al., Pitas et al., Nagelkerke et al.). However, the LDL has to be chemically modified in some way before being taken up by the macrophages. It is likely that peroxidative modification is the principal reaction which targets LDL to this pathway in vivo.

High Density Lipoproteins

HDL are derived from the catabolism of triglyceride-rich lipoproteins when apoA-I, and apoA-II and phospholipids from the surface monolayer of these lipoproteins are released into the plasma during the hydrolysis of their core triglycerides (Imaizumi et al., Green et al. 1979, Tall et al.). These elements apparently organize into new HDL pseudo-micelles. While it is known that chylomicrons are involved in the formation of HDL, Eisenberg (1980) has also postulated the degradation of the other triglyceride-rich lipoproteins, i. e. VLDL, contribute to the formation of HDL. It has been shown that interchange of apoC-II and apoC-III occurs between HDL and both chylomicrons (Havel et al. 1973) and VLDL (Eisenberg et al. 1973).

Nascent HDL are also secreted from the liver in the form of disc micelles containing phospholipid, free cholesterol, and the apoproteins E and A-I (Hamilton et al. 1976, Havel 1978). These discs are changed into spherical HDL when newly formed esterified cholesterol--formed by the action of lecithin:cholesterol acyl transferase (LCAT)--seeks the hydrophobic region between the monolayers. LCAT forms a cholesteryl ester by coupling a fatty acid derived from the second position of lecithin to free cholesterol aquired from tissues.

High density lipoproteins are a heterogeneous group of lipoproteins and are known to perform a wide variety of functions. A postulated major function of HDL is to remove cholesterol from peripheral tissues and deliver it to the liver for excretion from the body (Gordon et al.

1977). Indeed, biliary excretion or the conversion to bile acids are essentially the only ways in which cholesterol is removed from the body. During this process, free cholesterol aquired from tissues is again esterified by the action of LCAT. The cholesteryl esters that are formed on the surface of HDL are transferred to the cores of VLDL, VLDL remnants, and LDL. This establishes a cycle by which LDL delivers cholesterol to extrahepatic cells and by which cholesterol is returned to LDL from extrahepatic cells via HDL. Centripetal transport may counter the development of coronary artery disease (Fielding et al. 1977). Other major roles are those of carrier for C-apoproteins for transfer to nascent chylomicrons and VLDL and the acceptance of monolayer derived lipids during intravascular lipolysis. Other functions attributed to HDL include delivering cholesterol to steroidogenic tissues (Chen et al. 1980, Ohashi et al.); inhibiting infective agents (Kane et al. 1979, Levy et al. 1982); regulating coagulation (Carson et al.); and stimulating vascular endothelial cell growth (Tauber et al.). Presumably only selected HDL subspecies can perform each of the functions.

Previous techniques for isolating HDL have not proven useful for recovering HDL from serum in native form. Two of these techniques, ultracentrifugation and polyanion precipitation, disrupt the HDL particles by causing dissociation of apoproteins, particularly apoA-I. Kunitake and Kane (1982) have reported that repeated ultracentrifugation of high density lipoproteins results in a loss of as much of 35% of the apoprotein A-I present in serum. Other isolation techniques, such as

electropheresis and chromatography, require preliminary ultracentrifugation to remove many plasma proteins which contaminate the HDL preparations.

Recently, McVicar et al. developed the technique of selected-affinity immunosorption for isolation of HDL from plasma. This isolating technique has many advantages over other procedures used to isolate HDL. The native structure of HDL is minimally disturbed because monospecific antibodies directed against apoA-I are selected for their ability to dissociate from the apoA-I-containing particles (HDL) under gentle elution conditions. These investigators have shown as many as seven subspecies of HDL in the immunosorbates.

Using this new technique to isolate HDL, I participated in the isolation and characterization of a new subspecies of HDL (Kunitake et al. 1985). This subpopulation of HDL has pre-beta electrophoretic mobility, unlike the bulk of high density lipoproteins which have alpha electrophoretic mobility. Pre-beta HDL contain phospholipid and free and esterified cholesterol but no triglycerides. Protein constitutes ninety percent of its mass and apolipoprotein A-I is the only apoprotein detected in the particle. The protein moiety of this subpopulation exhibits markedly lower helicity than that of high density lipoproteins isolated by ultracentrifugation or than HDL of alpha mobility isolated by immunosorption.

The differences in lipid composition and in the secondary structure of the protein of the pre-beta HDL probably reflect important differences

between the metabolic functions of this subpopulation and the bulk of HDL. This relatively lipid poor particle might acquire and stablize surface lipid components derived from hydrolysis of triglyceride-rich lipoproteins or it might acquire cholesterol from peripheral cells.

Disorders of lipid metabolism

The most common life threatening disease involving plasma lipids and lipoproteins is atherosclerosis. Each year in the United States more adults die from coronary artery disease due to atherosclerosis than from any other single disease (Mahley 1979, Levy et al 1980.). There are a variety of genetic and environmental "risk factors" that are thought to contribute to the development of the disease. These risk factors include hyperlipidemia, hypertension, cigarette smoking, and diabetes mellitus. It is thought that the effect of the risk factors is at least additive and that individuals who are diagnosed as having more than one risk factor develop atherosclerosis in higher incidence than those who only exhibit one of these factors (Levy et al. 1980, Kannel et al.).

Pathologically, the atherosclerotic lesion consists of a plaquelike thickening of the intima of the artery wall. Although the sequence of events which cause this arterial thickening has not been fully elucidated, most researchers agree that the following sequence is pathologically correct. First there is a focal injury to the arterial wall. This increases permeability to circulating macromolecules and causes thickening of the subendothelial space and secondary cell proliferation (Ross et al.). Next, proliferation of the intimal smooth

muscle cells and synthesis and deposition by these cells of connective tissue matrix proteins including collagen, elastin, glycosaminoglycans and fibrin (Wissler, Geer et al.). Finally, lipids accumulate within the proliferating smooth muscle cells and macrophages, as well as in the newly formed connective tissue matrix (Geer et al.). Both lipid accumulation and continued smooth muscle proliferation, may occur after recurrent sequences of proliferation and regression, and may eventually lead to the development of complicated lesions that may go on to calcify. This sequence of events may lead to the clinical presentation of thrombosis and infarction.

Most of the cholesterol that accumulates within the atherosclerotic arterial intima is in the form of cholesteryl ester. It has been shown that this esterified cholesterol originates principally from LDL (Goldstein et al. 1977b, Smith). Moreover, LDL particles have actually been found in the arterial wall (Hoff et al.). This incriminating data has led to the belief that LDL play a central role in the atherogenic process and that these lipoproteins are themselves, atherogenic (Ross et al.).

Proliferating smooth muscle cells of the injured arterial intima are known to remove foreign plasma constituents by endocytosis and to synthesize collagen, glycosaminoglycans, and elastin. It is possible that the uptake of LDL by these smooth muscle cells occurs by the LDL binding to glycosaminoglycans of the interstitial space and are consequently internalized by a scavenger pathway (Small et al.).

Epidemiologic studies, such as the Framingham study, have shown there is a direct correlation between high levels of plasma LDL and the development of coronary artery disease (Gofman et al., Avogaro et al., Carlson et al. 1972). It is also known that the amount of circulating LDL required to supply sufficient cholesterol to cells to support cell growth is five-fold lower than the mean 120 mg/dl LDL-cholesterol level present in the average American man (Goldstein et al. 1977a). LDL receptor activity is suppressed and the number of LDL receptors is decreased when LDL reaches a critical concentration in plasma. Therefore the rate of LDL uptake is decreased and the "excess" plasma LDL is removed slowly by other pathways, such as the scavenger cells of the liver or it may find its way to the smooth muscle cells or macrophages of an injured arterial endothelium where it is endocytosed and incorporated into a developing atherosclerotic lesion. It is believed that lowering the concentration of cholesterol in plasma, the bulk of which is carried by LDL, will ultimately lower the risk of developing coronary artery disease.

Contrary to the detrimental effects of high plasma concentrations of LDL, increased concentrations of HDL are thought to confer protection against developing atherosclerosis. Epidemiological data have shown that low levels of plasma HDL are associated with an increased incidence of premature atherosclerosis (Gordon et al. 1977, Berg et al., Miller et al.).

The involvement of lipoproteins in the pathogenesis of atherosclerosis has yet to be elucidated. However, we have enough information

implicating some lipoproteins as atherogenic and others as mitigating elements to justify intense study of disease states where these lipoproteins may be altered in order to identify further risk factors and strategies for potential intervention.

Role of the intestine in lipoprotein synthesis, secretion and metabolism

The absorptive surface of the mucosa of the small intestine varies along the length of the intestine. Circular mucosal folds that project into the lumen are taller and more numerous in the proximal jejunum that in the distal ileum. Tiny villi less than one millimeter in height line the surface of these mucosal folds. Villi are in turn covered by columnar epithelial cells that have a brush border consisting of microvilli one micrometer in height. The outer surface of the microvillus membrane is covered by a continuous glycoprotein coat. The microvillus membrane also contains specific hydrolytic enzymes, specific transport proteins, and has more cholesterol and glycolipids than other portions of the intestinal cell plasma membrane (Brasitus et al.) The total absorptive area of the small intestine is 200-500 meters squared.

Columnar cells, mucous cells, Paneth cells, and enteroendocrine cells are the four major cell types in the epithelium of the small intestine. Columnar cells, which are responsible for absorption, arise from mitotically active cells comprising the base of the crypts of Lieberkuhn. The cells at the base of the crypts are functionally immature lacking a well developed microvillus membrane and

disaccharidase enzyme activity. These continually proliferating undifferentiated cells migrate to the tips of the villi over the course of four to seven days. Their life span is five to six days. Mucous cells also originate in crypts and migrate to the tips of villi. Mature mucous cells are known as goblet cells. Paneth cells are found only in the crypts and their function is unknown. Enteroendocrine cells are formed in the crypts and migrate to the villi. They have cytoplasmic granules which contain polypeptides such as motilin. These polypeptides have been implicated in lipoprotein secretion (Glickman et al. 1973).

The cells involved in intestinal lipoprotein formation are the columnar epithelial cells. Pancreatic lipase and colipase work together to hydrolyze dietary fat (mainly triglycerides) to fatty acids and beta-monoglycerides. When the concentration of bile acids in the intestine reaches a specific level, the bile acids spontaeously aggregate to form micelles. These micelles have a hydrophobic core and a water soluble surface layer. Hydrophobic molecules such as fatty acids and cholesterol enter the core of the micelles and are transported across the layer of water that coats the mucosa. Monoglycerides and fatty acids are released when the micelles reach the microvilli. The fatty acids and monoglycerides enter the cell by passively crossing the lipid soluble surface membrane of the epithelial cell.

Fatty-acid-binding protein in the cell cytosol avidly binds fatty acids and appears to function as an intracellular transport protein for long-chain fatty acids (Ockner et al. 1976) When fatty acid binding to this protein is inhibited, less fatty acid is available for triglyceride

resynthesis. The fatty-acid-protein complex travels to the smooth endoplasmic reticulum where triglyceride resynthesis occurs (Strauss, and Sabesin et al.). The movement of these lipid droplets can be followed morphologically through the endoplasmic reticulum to the Golgi apparatus in the apical portion of the cell (Glickman et al. 1976b). Chylomicrons can be seen in the Golgi vesicles. These vesicles migrate to the base of the cell where they fuse with the basolateral cell membrane and discharge the chylomicrons into the intercellular space (Cardell et al.).

While it is well documented that the intestine synthesizes and secretes chylomicrons (Glickman et al. 1974, Sabesin et al., Mahley et al. 1971), there are differing opinions on the intestine's capability to synthesize and secrete other lipoproteins. In 1971, Tytgat et al. demonstrated that fasting and postabsorptive human jejunal mucosa contain VLDL size particles. The lipid and apoprotein content of intestinal VLDL resembles that of chylomicrons rather than plasma VLDL (Green et al. 1981, Ockner et al. 1969), and for this reason have been regarded as small chylomicrons. However, some experimental evidence shows that some of the intestinal VLDL may be produced by a different process or in a different location from that involved in chylomicron formation (Mahley et al. 1971).

The intestine has also been implicated as a source of high density lipoproteins. HDL have been isolated from rat mesenteric lymph (Green et al. 1978, Windmueller et al. 1973). Lymph HDL contained both spherical HDL, similar to serum HDL, and discoidal particles which

resembled hepatic nascent HDL secreted by isolated perfused rat liver (Hamilton et al. 1976). HDL have been isolated from human thoracic duct lymph (Alaupovic et al.), from chylous urine (Green et al. 1979), and have been visualized in human mesenteric lymph (Jones et al.). These HDL were spherical and except for having a slightly higher triglyceride content, resembled serum HDL.

Experimental studies in the rat suggest that there are chemically and morphologically distinct forms of HDL in mesenteric lymph, however, secretion of these particles has not been demonstrated. Moreover, discoidal HDL have not been demonstrated in intestinal epithelial cells in rat or man. Nor has discoidal HDL been identified in human intestinal lymph.

It is well documented that lipoproteins, once they are secreted, are in a constant state of metabolism and degradation. Surface components are exchanged and core lipids are transferred to other lipoproteins or tissues. So while there is exchange and filtration between the plasma compartment and lymph system, there is inconclusive evidence that the intestine secretes any lipoproteins except chylomicrons.

Although the intestine may not be a primary synthetic source of lipoproteins, it is a major synthetic source of apolipoproteins. ApoB synthesis has been demonstrated in microsomes from rat intestine (Kessler et al. 1970). Further studies by Kessler et al. (1975) revealed apoprotein synthesis in the microsomes and carbohydrate addition to the apoproteins in the Golgi. Other studies have shown that

mucosal apoB concentrations increase after triglyceride feeding (Schonfield et al. 1978a, Glickman et al. 1976a). It should be interjected here that there are two major forms of apoB. ApoB-100, a higher molecular weight form, is the principal apoprotein of LDL. ApoB-48 is the major apoB species of chylomicrons and therefore is presumably of intestinal origin (Kane et al. 1980).

ApoB synthesis has also been demonstrated in human intestine (Rachmilewitz et al. 1978). This study also revealed de novo synthesis of apoA-I and apoA-II. ApoA-I has also been demonstrated in human intestinal biopsies using immunochemical techniques (Schwartz et al., Hopf et al.). Early studies on human intestinal biopsies seem to indicate that the concentration of apoA-I in jejunal cells is the same as that in distal ileal cells. The intestine has also been implicated in the synthesis of apoA-IV (Green et al. 1980).

The short bowel syndrome

Having established the importance of the small intestine in lipoprotein and apolipoprotein synthesis, secretion, and metabolism, it follows that small bowel dysfunction would cause disturbances in lipoprotein homeostasis. While temporary interruptions in bowel function may cause temporary changes in plasma lipid and lipoprotein profiles, more severe interruptions, such as massive resection of the small bowel, could cause permanent alterations in lipid and lipoprotein patterns. It is my intent to study the plasma lipids, lipoproteins, and apolipoproteins in

patients who have had such massive bowel resections. These patients are suffering from what has come to be known as the short bowel syndrome.

Short bowel syndrome develops after extensive resection of the small intestine. Resection of small amounts of the small intestine usually causes no clinical symptoms as the remaining absorptive and digestive surface of the small bowel is sufficient to maintain adequate nutrition. The severity of symptoms encountered after extensive resection of the small intestine is related to the amount of functional absorptive and digestive surface remaining, and to the specific level of the resected bowel. Normally, most of the dietary fat, carbohydrate, and protein are absorbed in the jejunum. If the jejunum is resected, the ileum is able to take over most of this absorptive function. However, extensive loss of the proximal bowel may result in decreased hormonal stimulation of pancreatic enzyme and bile secretion since cholecystokinin and secretin are released from the duodenum and jejunum. Moreover, iron, folate, and calcium are mainly absorbed in the proximal bowel. Conversely, bile salts, vitamin B_{12}, and cholesterol transport occurs in the distal portion of the small intestine. Resection of this region causes diarrhea, steatorrhea and malabsorption. Resection of up to 40% of the total length of the small bowel is usually well tolerated, provided the duodenum, the proximal jejunum, the distal half of the ileum, and the ileocecal valve are spared. Resection of more than 50% of the small intestine usually results in significant malabsorption, and resection of more than 70% causes severe malabsorption and profound nutritional consequences.

The most common clinical causes for massive resection of the small bowel are thrombosis or emboli of the superior mesenteric artery, low flow ischemia of the superior mesenteric arterial bed, thrombosis of the superior mesenteric vein and its branches, volvulus of the small intestine, and strangulated internal or external hernias. Other indications for resection include radiation enteropathy, Crohn's disease, neoplasm, and trauma.

The minimal amount of small intestinal absorptive surface required to sustain life varies from patient to patient. If the duodenum remains intact, but less than two feet of jejunum or ileum remain, long-term total parental nutrition is required for patient survival.

Extensive small bowel resection results in reduced absorption of virtually all nutrients, including water, electrolytes, fat, protein, carbohydrate, vitamins, and trace elements. However, if the patient survives the first few weeks after massive resection, there is evidence that adaptive changes occur which facilitate absorption and digestion by the remaining intestine (Weser 1979, Williamson). The exposure of the remaining small intestine to dietary nutrients, bile and pancreatic secretions, and the effect of trophic gut peptides have all been implicated in the adaptative ability of the remaining intestine (Porus, Dowling et al., Weser 1981, Levine). In rats, adaptation of the remaining bowel does not occur if they receive conventional parenteral alimentation. However, if secretin and cholecystokinin octapeptide are added to the parenteral infusions, or if the rats are fed

intraluminally, there is partial adaptation of the remaining bowel (Levine).

In man, it has not been established whether the absorptive surface of the remaining bowel increases in every patient, but there is evidence that absorptive function increases with time after massive resection. Epithelial cell hyperplasia seems to be consistent in these patients (Levine, Porus).

Another detrimental physiologic response occurring in these patients is an oversecretion of gastric acid (Windsor et al.). This may cause peptic ulcer disease which may decrease absorption further by damaging the mucosa, and inhibit fat digestion and micelle formation. Though gastric hypersecretion may persist for years in some patients, it seems to be a transient response in most, and gastric secretion returns to normal if the patients survive the acute problems of extensive resection.

Two of the most serious problems for patients who have had the distal ileum resected are diarrhea and steatorrhea. Because conjugated bile salts are absorbed in the ileum, removal of this segment disrupts the enterohepatic circulation. If too much of the ileum has been resected, and maximum hepatic synthesis of bile is not enough to maintain an adequate intraluminal bile salt pool, the malabsorbed fatty acids as well as bile acids enter the colon and impair colonic water and ion absorption and stimulate colonic fluid secretion (Bright-Asare et al.). Bile acid binding resins have been useful in treating cholerrheic

diarrhea, but this further depletes the bile acid pool thereby aggravating steatorrheic diarrhea.

Depletion of the bile salt pool results in lithogenic bile, and hence, these patients have three times as many gallstones as control populations (Heaton et al., Hill et al.). Extensive resection of the small bowel results in an accelerated loss of bile acids from the body. In normal individuals, the return of bile salts to the liver serves as a negative feedback mechanism to suppress 7-alpha-hydroxylase, the enzyme responible for initiating the conversion of cholesterol in the liver to bile acids. Removal of this suppresive effect results in an increased rate of conversion of hepatic cholesterol to bile acids creating a metabolic drain on the total body pool of cholesterol. It also results in a depletion of the bile salt pool, leading to an imbalance in the critical ratio of cholesterol, bile acids, and phospholipid. This leads to a supersaturation of the bile with cholesterol--theoretically the first stage of gallstone formation (Bennion et al.).

Lastly, massive small bowel resection results in initial fluid and electrolyte imbalances. Potassium, sodium, calcium and magnesium serum levels may be low. Serum prothrombin, carotene, and cholesterol levels decrease. Hypoalbuminemia may arise if a negative nitrogen balance persists.

However, all of these derangements can be alleviated with parenteral nutrition. Early implementation of parenteral alimentaion is essential for patients with an extensive small bowel resection. Glucose, amino

acids, electrolytes, vitamins, trace minerals, and essential fatty acids are required for balanced nutrition. Oral feedings should be started as soon as possible to promote adaptation of the remaining bowel. Yet, initial feedings should be small and require minimal digestion. More complex meals should be added slowly, and TPN therapy should be used to maintain adequate nutrition until patients can maintain an acceptable nutritional status on their own.

Still, not all patients can achieve independent nutritional status and must remain on home parenteral nutrition for the rest of their lives. It has been shown that these patients can establish nutritional and electrolyte balance and lead active lives (Gouttebel et al.). Some of these patients rely totally on TPN for their nutritional needs, while others only supplement their diet a few times a week with TPN to achieve appropriate nutrition.

While there have been many studies on patients receiving total parenteral nutrition, few have focused on possible derangements in lipoprotein structure and metabolism. Because patients on long-term TPN must receive a portion of their calories as fat, there have been a certain number of investigations concerning the role of fat emulsions and lipoprotein metabolism. Yet, few of these studies compare lipoproteins before and after an infusion of fat, and even fewer investigate lipoprotein disturbances in the absence of the small bowel. Rather, most of these studies involve patients at bowel rest or normal volunteers whose lipoproteins are studied subsequent to a bolus fat infusion.

The most common fat emulsion administered to patients on long-term parenteral nutrition is Intralipid 10%. Intralipid 10% is made from soybean oil and is composed of 97% fatty acids, including linoleic, oleic, palmitic, and linolenic, 1% egg yolk phospholipids, and 2% glycerol. One milliliter of an Intralipid 10% solution delivers 1.1 calories. Intralipid particles are larger than naturally occurring chylomicrons, but have a similar lipid composition and triglyceride clearance kinetics (Hansen et al., Wretlind). In contrast to chylomicrons, which contain intestinally derived protein components when they enter the circulation (Green et al. 1981), Intralipid particles have no apoprotein components when infused. Upon entering the bloodstream, Intralipid particles acquire C apoproteins from normal, circulating lipoproteins (Sata et al., Havel et al. 1973, Carlson 1980). The C-II apoprotein serves to activate lipoprotein lipase and thus are essential for Intralipid triglyceride hydrolysis. There are no reported studies of the metabolic fate of Intralipid particles following the removal of triglycerides, i.e. "Intralipid remnants." However, the following hypothetical metabolic sequence for "Intralipid remnant" removal can be proposed based on information regarding chylomicron metabolism and cholestasis (Seidel et al., Hamilton et al. 1971, Felker et al.), and LCAT deficiency (Gjone et al.).

After the removal of Intralipid triglycerides at the surface of capillary endothelial cells, the Intralipid remnants containing phospholipids and cholesterol are released back into plasma. A deficiency of specific apoprotein components on the remnant particles

prevents normal mechansims of cholesterol esterification by LCAT from occurring. Deficiency of certain apoproteins also prohibits the subsequent removal from plasma via cell surface receptors. The Intralipid remnants then form abnormal phospholipid-cholesterol particles, like Lp-X, as a stable physiochemical arrangement. Some particles, however, may attract apolipoprotein E and become capable of binding to apoB-100-E receptors in liver and thus be removed through this receptor pathway. Alternatively, these particles may removed by mechanisms other than receptor-mediated uptake.

Indeed, it has been reported that adminsitration of total parenteral nutrition containing Intralipid alters plasma lipids and cholesterol balance. Continuous Intralipid administration tends to increase plasma free cholesterol and phospholipid concentrations, and if Intralipid is infused at a particular rate which exceeds removal mechanisms in plasma, the accumulation of an abnormal low density lipoprotein, Lp-X may occur.

Animal Studies

Steiger et al. studied serum lipids in rats given TPN without fat and TPN with 40% of the calories as Intralipid. Compared to the group given TPN without fat, the rats which received Intralipid had significantly higher levels of serum cholesterol (81 ± 12 vs. 276 ± 47 mg/dl) and serum phospholipids (125 ± 19 vs. 644 ± 142 mg/dl) at the end of the study period. There was no significant difference between groups in the levels of serum triglycerides.

Breckenridge et al. infused fasting rats with Intralipid 10% for twenty-four hours. Plasma lipids and lipoproteins were isolated and compared to those of fed animals and animals with bile duct ligatures. Compared to the fed animals, the animals infused with Intralipid and those with ligated bile ducts had six to ten fold increases in the plasma concentration of free cholesterol and phospholipids which were largely restricted to an abnormal low density lipoprotein identified as Lp-X.

Dogs which had daily infusions of TPN containing Intralipid 10% for periods of four to eight weeks showed marked elevations in the levels of plasma cholesterol, triglycerides, phospholipids after two weeks of treatment. High density and low density lipoproteins were also increased. These elevations were sustained during the study period, but returned to pretreatment levels shortly after cessation of TPN (Koga et al. 1974).

Clinical Studies

Studies in adult humans also show serum lipid abnormalities. In a study by Broviac et al., patients received Intralipid 10% for seven days as forty percent of nonprotein calories in their TPN solutions. The mean serum cholesterol concentrations rose dramatically (156 vs. 315 mg/dl) and the mean serum triglyderide levels fell (164 vs. 74 mg/dl) during the course of Intralipid administration.

Mean serum cholesterol levels were also increased in a study by Zohrab et al. in patients receiving TPN containing Intralipid 10% for thirty

one days. However, these authors report that mean serum triglyceride levels rose (from 111 to 213 mg/dl).

Changes in serum lipid levels have also been reported by Singh et al. Twelve patients with various gastro-intestinal disorders were given daily infusions of TPN containing Intralipid 10% for twenty-one days. Serum lipids were measured before the TPN was initiated and at weekly intervals thereafter for the duration of the study period. Blood samples were obtained eight to twelve hours after the infusion of Intralipid. At the end of the three week study period, mean total serum cholesterol increased (from 150 to 198 mg/dl, $p<0.05$) as did serum phospholipid concentrations (from 200 to 283 mg/dl, $p<0.05$). Mean serum triglyceride levels did not change. However, eight out of twelve patients showed a decrease in triglycerides, while two out of twelve patients showed an increase in triglyceride concentrations. HDL cholesterol levels also decreased. The authors report that one week after TPN therapy was initiated, lipoprotein-X was identified in the plasma of six out of seven patients by density gradient ultracentrifiugation. However, there is no chemical compositional or electron micrographic data presented to substantiate this statement.

Tashiro et al. studied seven patients at bowel rest over a period of three to seven weeks. Four patients received TPN containing 1000ml/day Intralipid 10%, while three patients received TPN without fat. Blood sampling was performed weeky, twelve hours after the end of the Intralipid infusion. VLDL, LDL and HDL were isolated by density gradient ultracentrifugation.

Fat-free TPN lowered plasma lipids, especially low density lipoproteins and high density lipoproteins. Conversely, TPN containing Intralipid caused a marked increase of mean serum cholesterol, especially free cholesterol, and phospholipids. Further analysis of these data showed that the majority of the increase in total lipid occurs in the LDL fraction. There was a slight increase in cholesterol content in the VLDL fraction. HDL remain unchanged. It is important to remember that separation of lipoproteins from blood obtained after an Intralipid infusion may contain Intralipid particles, or "Intralipid remnants" in certain density intervals. This is especially true in the VLDL fraction. Daily infusions of Intralipid may overload clearance mechanisms in plasma and lead to decreased removal of these particles from plasma. Hence, lipid compositional data from lipoproteins at the density interval of <1.006 g/ml may be skewed.

Another study involving patients at bowel rest during TPN with and without Intralipid 10%, seems to give conflicting results with the above report (Taskinen et al.). Thirteen patients with postoperative hypercatabolism were randomly assigned either to a TPN program consisting only of glucose and amino acids or a program containing 50% of the calories supplied by Intralipid. The Intralipid solution was given as a continuous infusion for 18 hours and the glucose and amino acid mixture was infused continuously for 24 hours. Baseline blood samples were taken after 12 hours of a saline infusion and treatment samples were taken after four days of infusion with either glucose or Intralipid plus glucose. VLDL and LDL were isolated by stepwise

flotation at the solvent densities of 1.006 g/ml and 1.063 g/ml, respectively. The 1.063 g/ml infranatant material was used to determine lipids and proteins of HDL.

TPN without fat caused mean total serum cholesterol levels to fall, pretreatment concentration was 3.8 mmol/l while post-treatment values were 3.2 mmol/l, $p<0.05$. Intralipid infusion was associated with a slight rise in total serum cholesterol, from 4.0 to 5.0 mmol/l, $p<0.05$. This cholesterol increase occured mainly in the LDL while HDL cholesterol levels fell. The authors did not determine free or esterified cholesterol concentrations. This would have been informative, as free cholesterol has been shown to increase after Intralipid infusions.

Both infusion programs caused an increase in mean total serum triglyceride concentrations. The glucose IV caused a marked elevation in VLDL triglicerides, from 0.51 to 0.79 mmol/l, $p<0.01$. LDL triglycerides increased slightly. Infusion of glucose plus Intralipid did not change VLDL triglycerides, but increased LDL and HDL triglycerides, from 0.39 to 0.52 mmol/l and 0.19 to 0.31 mmol/l, $p<0.01$, respectively.

Serum apolipoprotein A-I levels were approximately fifty percent lower than normal before the iniation of TPN and did not change after the four day infusion of either TPN solution. Serum apoA-I concentrations of patients receiving TPN containing glucose and amino acids was 77 mg/dl before TPN was initiated and 72 mg/dl after treatment. Similarly, the

patients who received Intralipid in their TPN solutions had a baseline serum apoA-I concentration of 81 mg/dl and a post-treatment level of 86 mg/dl. Serum apoA-II levels remained the same during the glucose infusion, 30 mg/dl pretreatment and 31 mg/dl after the infusion period, and increased during Intralipid infusion, from 30 to 34 mg/dl, $p<0.05$.

Judd et al. measured plasma lipoprotein composition in seven adult surgical patients prior to parenteral alimentation, seven days after infusion of a glucose and amino acid solution, and five days after 500 ml/day of Intralipid were included in the TPN regimen. Intralipid was infused over a twelve hour period and the final blood sample was obtained twelve hours after the final Intralipid infusion. VLDL, LDL, and HDL were isolated by discontinuous gradient ultracentrifugation.

There were no significant changes reported for the percent composition of VLDL, LDL, or HDL after the Intralipid infusion. Total plasma cholesterol levels rose by forty percent ($p<0.02$) during the Intralipid infusion period. Analysis of the available data indicate the rise in serum cholesterol is reflected in the LDL fraction. There was no increase in the post-Intralipid LDL phospholipid concentration, which would have suggested the presence of lipoprotein-X. There is a 15% decrease in total plasma HDL and a 29% decrease ($p<0.01$) in total plasma VLDL after the Intralipid infusion.

By the authors own admission, these patients were in a poor nutritional state when this study was undertaken and the data should be evaluated with this fact in mind.

Untracht measured serum lipids, HDL composition and LCAT activity in three patients at bowel rest and in two healthy volunteers. The three patients received daily infusions of 500-1000 ml of Intralipid 10% over a twelve hour infusion period for three to five weeks. After an overnight fast, the two normal volunteers received a bolus intravenous infusion of 500 ml of Intralipid 10% over the course of four hours. HDL were obtained by precipitation with dextran sulfate.

There was a marked elevation in total serum cholesterol and phospholipid concentrations in two patients after the last infusion of Intralipid. The total serum cholesterol of subject #1 increased from 189 mg/dl to 493 mg/dl, and the serum cholesterol of subject #2 increased from 168 mg/dl to 276 mg/dl. The first two subjects also had marked elevations of serum phospholipids, 550 mg/dl and 450 mg/dl, after the infusion of fat. Serum triglycerides of these subjects remained unchanged. In each subject, the increased cholesterol concentration was primarily due to an increase in free cholesterol. The abnormal free cholesterol concentrations were 213 mg/dl and 180 mg/dl. This data coupled with the increased phospholipid concentrations are indicative of the presence of lipoprotein-X. The serum lipid profile of the third subject remained unchanged during the study.

Lipoprotein-X was isolated and characterized from the plasma of the first two subjects. These particles were isolated by isopycnic density-gradient ultracentrifugation in the density range 1.02-1.034 g/ml. Electron micrographic studies revealed vesicular particles which

were 300-600 angstroms in diameter. Chemically, the vesicles were comprised of 66% phospholipids, 28% free cholesterol, and 5% protein.

In all subjects, total HDL and LCAT concentrations decreased by 40% after three weeks of daily Intralipid infusions. Upon cessation of the lipid infusions, all abnormal lipid and enzyme levels returned to normal within four days.

A final study on fat emulsions and lipoprotein alterations, by Hailer and Wolfram, employed six healthy volunteers rather than a patient population. Five hundred milliliters of Intralipid 10% was intraveneously infused over the course of four hours. In contrast to the results by Untracht, serum cholesterol levels did not change. The VLDL cholesterol level increased, the LDL and HDL_3 cholesterol levels decreased, and the HDL_2 cholesterol level remained the same. Total serum triglyceride concentrations rose markedly. All of the lipoprotein fractions have an increased triglyceride concentration. Total serum phospholipid levels also increased after Intralipid infusion. Phospholipids increased in all lipoprotein fractions, with the largest increase in the LDL, however these researchers did not attempt to isolate LP-X lipoproteins.

Because the liver and the intestine are the major sites of apoprotein synthesis (Schaefer et al. 1978a), it would be expected that small bowel dysfunction and or extensive small bowel resection would effect plasma concentrations of circulating apolipoproteins. It has been postulated that the intestine may synthesize approximately 50% of the total plasma

apoA-I (Wu et al., Glickman et al. 1981). ApoA-II has also been shown to be synthesized and secreted by the intestine (Rachmilewitz et al. 1978). Theoretically, patients with intestinal failure from either extensive resections or disease should have reduced plasma levels of intestinal apoA-I and, perhaps, apoA-II.

To date, only a few studies have addressed this issue. Two recent investigations demonstrated the effect of intraveneous fat infusion on the serum levels of apoproteins A-I, A-II, C-II and C-III (Koga et al. 1982, Hailer et al.). Both studies used normolipemic, healthy volunteers who had been given an intravenous bolus of Intralipid 10%. In these normal subjects, serum apoA-I concentrations ranged between 130 mg/dl and 150 mg/dl and apoA-II levels between 45 mg/dl and 55 mg/dl. Both the apoA-I and apoA-II concentrations remained the same or decreased by less than ten percent when the fat emulsion was infused. Both the apoC-II and apoC-III levels increased after Intralipid infusion.

The only data available on apolipoprotein concentrations in patients with short bowel syndrome was recently reported by Badimon et al. Fourteen patients in this study had less than thirty percent of their small bowel remaining. All patients were receiving long-term parenteri nutrition. Blood was also obtained from thirty-five normolipemic volunteers to determine normal apoplipoprotein concentrations.

Patients with massive small bowel resection had mean serum apoA-I concentrations of 98 mg/dl. This is thirty percent lower than the 145

mg/dl reported for controls. Likewise, there is a fifty percent reduction in serum apoA-II levels when compared to controls, 23 vs 46 mg/dl, respectively. The average apolipoprotein B concentration of the patients was 88 mg/dl, similar to the control's 83 mg/dl. However, further analysis of the available data indicate that there are three abnormally low apoB concentrations in the patient group, i.e. 19, 27 and 38 mg/dl.

To this point, a variety of conflicting results have been presented. In most subjects, Intralipid infusion caused an increase in total serum cholesterol concentrations, however, the distribution of free and esterified cholesterol was rarely determined. Phospholipids increased in these individuals as well. However, triglyceride concentrations were shown to increase in some subjects and decrease in others. Lipoprotein profiles were assessed in only a few studies, and generally only the cholesterol and phospholipid content was studied. Again, free and esterified cholesterol was rarely determined. As I stated earlier, most of the subjects in these studies were normal volunteers or surgical patients.

It is the purpose of this thesis effort to investigate in detail the effect of near complete surgical resection of the small intestine on plasma lipids and lipoproteins. It will be determined whether the contribution of the small intestine leads to the formation of discrete species of lipoproteins other than chylomicrons. In the following pages, the analyses of alterations of plasma lipids, apolipoproteins, lipoproteins in patients who have had greater than ninety percent of

their small bowel resected are compared to data obtained from age and sex matched control subjects. All of these parameters will be determined with and without the infusion of Intralipid. These subjects were all in good health and caloric balance at the time of this study and therefore the results from these investigations will show the homeostasis of lipoproteins in the absence of a small bowel.

MATERIALS AND METHODS

Experimental Design

The purpose of this work was to examine the effect of extensive small bowel resection on several parameters of lipoprotein metabolism. These included analyses of serum lipids and apolipoproteins, chemical compositions of the major lipoprotein fractions, and subspeciation of HDL. The subjects included eight patients who had less than fifteen percent of small bowel remaining and had been receiving total parenteral nutrition for at least 1.5 years (Table 2). Plasma from eight age, sex, and race matched controls was also studied. Blood samples were obtained from fasting subjects. Two of the patients with small bowel resection received a four hour infusion of 500 ml of Intralipid 10% and a second blood sample was obtained from each of these subjects ninety minutes after the infusion period. During the course of this investigation I had the unique opportunity to study the serum lipoproteins of a patient who had been at bowel rest for a year and again after this patient had ninety percent of her small bowel resected. While the patient was at bowel rest, blood samples were obtained prior to and ninety minutes after a four hour infusion of 500 ml of Intralipid 10%.

The analyses performed on either serum or plasma included determination of total cholesterol, free, and esterified cholesterol, triglycerides, and total phospholipids. Plasma concentrations of apolipoproteins A-I,

Table 2. Patients with Extensive Small Bowel Resection

Subject	Sex	Age (yrs.)	Weight (kgs.)	Height (ft.)	Years on TPN	Length of Remaining Bowel*	Reasons for Resection
P1	F	62	52	5'4"	1.5	22 in.	Received radiation therapy for cervical CA: developed radia-enteritis
P2	F	67	46	5'1"	5	30 in.	Received radiation therapy for pelvic CA: developed radiation enteritis
P3	F	53	49	5'2"	4	24 in.	Mesenteric vein thrombosis
P4	F	48	64	5'7"	2	24 in.	Mesenteric vein thrombosis
P5	F	33	58	5'5"	3	8 in.	Mesenteric vein thrombosis
P6	M	52	65	5'7"	3	30 in.	Embolus to mesenteric vascular bed
P7	M	40	77	6'0"	4	18 in.	Crohns disease since age 17
P8	M	55	75	5'10"	3	24 in.	Mesenteric vein thrombosis

*measured at time of surgery.

Table 3. Controls

Subject	Sex	Age (yrs.)	Weight (kgs.)	Height (ft.)
C1	F	63	59	5'6"
C2	F	67	58	5'5"
C3	F	53	60	5'7"
C4	F	50	67	5'8"
C5	F	31	56	5'6"
C6	M	49	78	6'1"
C7	M	41	72	6'2"
C8	M	56	76	5'11"

B, and E were determined. HDL subspecies were analyzed directly from serum.

The major lipoprotein classes were separated from serum by sequential preparative ultracentrifugation. Very low density lipoproteins (VLDL) were recovered in the density interval d<1.006 g/ml. Intermediate density (IDL) and low density lipoproteins (LDL) were recovered at the density intervals d=1.006 to 1.109 g/ml and d=1.019 to 1.063 g/ml, respectively. High density lipoproteins (HDL_2 and HDL_3) were recovered at the density intervals d=1.063 to 1.125 g/ml and 1.125 to 1.21 g/ml, respectively. Total lipid and protein analyses were determined for each class.

Preparation of Human Serum or Plasma

Plasma was obtained from eight patients who had undergone near complete resection of the small intestine. During the course of this study three patients received a four hour infusion of five hundred milliliters of Intralipid and a second blood sample was obtained ninety minutes after the end of the infusion.
Plasma was also obtained from eight age, sex, and weight matched controls.

Blood donors had fasted for at least 14 hours. Plasma was prepared by drawing venous blood into vacuum tubes containing EDTA. The blood was centrifuged immediately at 4^{o} C, 1000g, for 15 minutes. The plasma was recovered and disodium EDTA, sodium azide, and gentamycin were added as preservatives in final concentrations of 0.001 M, 0.002%, and 5.0 ug/ml,

respectively. A sample of the plasma was removed and to it was added DTNB in a final concentration of 0.14mM to inhibit the action of LCAT.

Serum was isolated after venous blood was allowed to clot for thirty minutes at 4^{o} C and was recovered in the same manner as plasma. Both serum and plasma were either analyzed immediately or stored at 4^{o} C until all analyses were performed.

Preparation of Lipoproteins

Lipoproteins were separated from plasma by sequential preparative ultracentrifugation (Havel et al. 1955). Intermediate density lipoproteins, low density lipoproteins, and high density lipoproteins were dialyzed at 7^{o} C against 0.15 M NaCl containing 0.001 M disodium EDTA and 0.002% NaN_3, pH 7.4. The lipids recovered in the centrifuged lipoprotein fractions averaged eighty-five percent of the initial serum concentrations.

Isolation of ApoA-I-containing Lipoproteins

The apoA-I-containing lipoproteins were isolated from plasma or serum by selected-affinity immunosorption (McVicar et al.). This isolation procedure was selected to minimize alteration of the fine structure of the HDL particles. Monospecific antibodies directed against apoA-I were selected for their ability to dissociate from apoA-I under gentle elution conditions. The paucicional antibodies were then bound to CNBr-activated sepharose beads and packed into a column. Human serum or

plasma was washed over the column for two and a half hours with saline, pH 7.4, the apoA-I containing particles were eluted with 0.2M acetic acid, pH 3.0, then they were neutralized immediately with 2M Tris buffer. Columns containing 70 mg of antibody have been found to bind all of the HDL in 2.5 milliliters of human serum.

Chemical Analysis of Isolated Samples

Chemical compositions of plasma and ultracentrifugally isolated VLDL, IDL, LDL, HDL_2, and HDL_3 were determined as follows: the protein content was determined by a modification of the Lowry method which corrects for differences in chromogenicity between apo LDL and apo HDL and the bovine serum albumin standard based on the aminoacyl mass of purified apo LDL and apo HDL and the standard. The triglyceride content was measured using a quantitative, enzymatic procedure which is modeled after that described by Bucolo and David. The lipid phosphorus content was measured as described by Stewart and Hendry. Free and esterified cholesterol were measured by an enzymatic method employing fluorometry (Huang, Kuan, and Guilbault).

Analysis of Apoproteins

Tago immunoassay systems were used to determine plasma concentrations of apoproteins A-I and B-100 in all subjects (Tago Inc., Burlingame, CA). These assay systems allow the equivalent quantitation of apoB-100 or apoA-I in any lipoprotein form because of the presence of two

amphipathic substances which cause these apolipoproteins to be presented to antibodies in a uniform state.

Plasma apoprotein E content was measured by radioimmunoassay (Havel et al. 1980).

Analytical Gel Electrophoresis

SDS Gradient Electrophoresis:
Apolipoprotein components of the various lipoprotein subfractions were analyzed by SDS gradient (2.7-27%) polyacrylamide gels using a discontinuous buffer system (Laemmli).

Polyacrylamide Electrophoresis under Non-denaturing Conditions:
Qualitative visualization of the distribution of the immunosorbed HDL was achieved by employing a modification of the techniques of Utermann and Janecki et al. The techniques of these researchers used whole serum pre-stained for lipid. Our modification of these methods allows post-staining of the proteins from immunosorbed HDL with Coomassie Blue and allows a clear visualization of the HDL subspecies present. We explored a variety of conditions to obtain the maximum separation and highest resolution of the HDL subspecies and found this was best achieved with an 8.5% polyacrylamide gel, pH 8.9. One hundred micrograms of sample was layered on top of a discontinuous gel composed of a 1-cm 2.5% stacking gel and an 8-cm resolving gel. Constant current was applied at 1.25 ma/gel until the sample is stacked, at which time the current was increased to 2.5 ma/gel. Samples were electrophoresed

for three hours. Gels were stained with Coomassie Blue R-25, and then destained and stored in 7% methanol, 5% acetic acid, and 0.01mM Coomassie Blue.

Immunoelectrophoresis:

The electrophoretic mobilities of plasma lipoproteins were determined by immunoelectrophoresis in agarose gel. Immunoelectrophoresis, modified from Grabar and Williams, was performed by placing five microliters of sample in a well cut in a 1% agarose gel containing 0.05M barbital buffer, pH 8.6. A potential of 165 volts was placed across the plate until the front had migrated 3.5 cm. After electrophoresis, troughs were cut between the sample wells, parallel to the direction of migration. One hundred fifty microliters of goat anti-apoA-I antiserum was placed in each trough. After 18 hours of incubation at 25 C, immunoprecipitin lines formed. The gel was placed in saline for 48 hours and then dried and stained with Coomassie Blue R-250.

Determination of Particle Size

Low density lipoproteins were negatively stained with 2% potassium phosphotungstate (Hamilton et al.) and electron photomicrographs were graciously obtained by R. L. Hamilton, Department of Anatomy, University of California at San Francisco. The distribution of particle diameters was determined from the electron photomicrographs at a magnification of 60,000 by a semiautomated method, utilizing a digitizer (Chen et al.). In order to obtain the most accurate determination of the particle diameters, four points were marked on the circumference of each of two

hundred particles. To avoid selection bias, all contiguous particles were measured moving concentrically from a randomly selected particle until a total of 200 sets of points was accumulated. While only three points on the circumference of a circle are necessary to estimate its diameter, we wanted to know the uncertainty of accurately determining the diameter due to two sources. The inaccuracy of data collection and the noncircularity of particles were assessed when four points on the circumference of the particles were used to determine the diameter of the particles. Four sets of three points were used to determine the mean particle diameters. The data were analyed and all particles with a standard error greater then ten percent were discarded.

The algorithm employed for computation is as follows: Let X_0 and Y_0 be the linear coordinates of the center of a particle, and let X_1, Y_1, X_2, Y_2, and X_3, Y_3 be the known coordinates of three nonidentical points of the periphery. By use of the Pythagorean theorem, the radii to the perimeter points may be equated to one another, for example

$$\sqrt{(X_1 - X_0)^2 + (Y_1 - Y_0)^2} = \sqrt{(X_2 - X_0)^2 + (Y_2 - Y_0)^2}$$

etc. to generate a set of simultaneous equations which yield the coordinates of the center of the circle. From the coordinates, radii (r) were calculated. The diameter (d) of the image was calculated as follows:

d = 2r(EM magnification factor)(photographic magnification factor)(calibration factor for digitizer)

Isolation of ApoA-I-containing Lipoproteins with Pre-Beta Electrophoretic Mobility

ApoA-I-containing lipoproteins with pre-beta mobility were isolated from plasma by a modification of the starch block electrophoresis technique of Kunkel and Slater (Kane et al.). Potato starch was washed with distilled water and then suspended in 0.076M barbital buffer, pH 8.6. The starch was poured into a 1 x 40 x 10 centimeter block. One milliliter of plasma was applied in a zone 5 centimeters from the anode end of the block and electrophoresed for 18 hours at a constant current of 80 milliamperes. The block was divided into 1-cm segments along the direction of migration and the lipoproteins in each segment were recovered by two 3-ml washes of the starch with 0.15 M NaCl that contained preservatives. Apolipoprotein distributions in the fractions were determined by a modification of the immunonephelometric technique of Wieland, Cremer, and Seidel with a Beckman ICS analyzer II. The apoA-I content of each fraction determined by immunonephelometry allowed the quantitative determination of the proportion of apoA-I found in the lipoproteins with pre-beta and alpha mobility.

Statistical Methods

Signifance of differences between means was determined by Student's t test (unpaired) and by analysis of variance.

RESULTS

Presented here are lipid and protein data of serum and lipoproteins isolated from patients who were on long-term total parenteral nutrition (TPN) therapy due to extensive small bowel resection (see Methods, Table 2). The medical indications for surgical removal of the small intestine included ischemia of the mesenteric vascular supply, i.e. thrombosis to either the superior mesenteric artery or vein, Crohns disease, radiation enteritis, and cancer. All of the patients had been on TPN for at least a year and some for as long as four or five years. All of the patients were in a state of nutritional balance, within ten percent of their ideal body weight, and had no other medical problems at the time of this study. The patients included five women, one of whom was premenopausal, and three men. The mean age of the patients was 51 years.

Also presented are data obtained from age, race and sex matched controls. The individuals in the control group were all healthy, and within ten percent of their ideal body weights.

Serum Lipid Composition

The lipid compositions of fasting serum from the control and patient groups are presented in Tables 4 and 5, respectively. The control data are consistent with results reported for a healthy, normolipidemic population. The mean total serum cholesterol level was 177 mg/dl with the free cholesterol comprising twenty-seven percent (49 mg/dl) and

esterified cholesterol comprising seventy-three percent (128 mg/dl) of the total. The mean total serum triglyceride and phospholipid concentrations were 130 mg/dl and 214 mg/dl, respectively.

In the patient group, the mean total serum cholesterol concentration was 135 mg/dl. The patients' total serum cholesterol was twenty-five percent lower than that of the control group. Their free cholesterol level was 44 mg/dl and was similar to the control's free cholesterol level. However, the patients' esterified cholesterol concentration was significantly lower than the control's, 92 mg/dl vs. 128 mg/dl. The mean serum triglyceride value of the patients was 124 mg/dl (similar to that of the control group) and the mean serum phospholipid concentration was 182 mg/dl (slightly lower than the control group).

Table 4. Lipid Composition of Serum from Control Subjects

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
C1	172*	121	51	170	200
C2	180	128	52	171	215
C3	200	145	55	113	210
C4	171	126	45	133	235
C5	177	129	48	92	206
C6	176	133	43	194	248
C7	168	123	45	88	170
C8	174	120	54	79	228
Mean	177	128	49	130	214
±SD	±9.9	±8.1	±4.5	±43	±24

*All values reported in mg/dl.

Table 5. **Lipid Composition of Serum from Patients with Small Bowel Resection**

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
P1	100*	52	48	122	174
P2	173	113	60	191	210
P3	159	105	54	140	240
P4	92	58	34	84	116
P5	165	122	43	126	240
P6	109	71	38	84	88
P7	165	120	45	170	250
P8	120	90	30	71	141
Mean ±SD	135 ±33	92 ±28	44 ±10	124 ±42	182 ±62

*All values reported in mg/dl.

Table 6. Lipid Composition of Serum: Controls vs. Patients with Small Bowel Resection

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	177±9.9*	128±8.1	49±4.5	130±43	214±24
Patients n=8	135±33**	92±28#	44±10	124±42	182±62

*All values reported in mg/dl. **$p<0.001$. #$p<0.02$.

Serum Apoprotein Determinations

Concentrations of serum apoproteins A-I, B, and E were also determined. As can be seen in Table 7, the average serum apoA-I concentration for controls was 159 mg/dl. The average serum apoA-I concentration of the patients was 108 mg/dl (Table 8), and is thirty-three percent below that of the control population.

The mean serum apoE concentrations were the same in both the control and the patient groups, 4.6 mg/dl and 4.8 mg/dl, respectively.

The average serum apoB levels were surprisingly lower in the patient group. Their mean apoB concentration was determined to be 46 mg/dl, forty percent lower than the average value of the control group (80 mg/dl).

Serum Pre-beta and Alpha HDL Determinations

In addition to classical HDL of alpha mobility, we have isolated and characterized a subspecies of apoA-I-containing lipoproteins with pre-beta electrophoretic mobility (Kunitake et al. 1985). ApoA-I-containing lipoproteins of both alpha and pre-beta mobilities were found in plasma and among immunosorbed HDL as seen by the presence of two immunoprecipitin arcs developed by antiserum directed against apoA-I. However, immunoelectrophoresis is only a semiqualitative technique and the relative size of the arcs cannot be directly compared.

Qualitative representation of the relative percentages of pre-beta and alpha HDL isolated from controls and patients with small bowel resection

Table 7. Apoprotein A-I, B, and E Concentrations in Serum of Control Subjects

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
C1	136*	79	4.7
C2	142	85	4.8
C3	192	85	5.9
C4	177	84	5.2
C5	177	68	3.0
C6	152	95	5.3
C7	147	66	4.6
C8	152	74	3.6
Mean	159	80	4.6
±SD	±20	±9.6	±0.94

*All values reported in mg/dl.

Table 8. Apoprotein A-I, B, and E Concentrations in Serum of Patients with Small Bowel Resection

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
P1	72*	24	3.3
P2	130	56	6.4
P3	155	60	9.1
P4	96	21	4.6
P5	147	79	3.1
P6	72	32	5.0
P7	100	51	4.6
P8	84	46	2.0
Mean ±SD	108 ±34	46 ±19	4.8 ±2.2

*All values reported in mg/dl.

Table 9. Serum Apoprotein Concentrations: Controls vs. Patients with Small Bowel Resection

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
Controls n=8	159±20*	80±9.6	4.6±0.94
Patients n=8	108±34**	46±19#	4.8±2.2

*All values reported in mg/dl. **$p<0.02$. #$p<0.001$.

are shown in Figures 1-7. It can be seen that the electrophoretic migration of plasma is the same in both groups. That is to say, the immunoprecipitin arcs of the pre-beta and alpha HDL of the patients have contours exactly like those of the controls. The arcs of all subjects showed a tapering at the beginning and end of the migration zone. The presence of pre-beta HDL has been verified in the plasma of patients with small bowel resection by this technique, however, quantification of the relative amount of pre-beta HDL present requires other methods.

Quantitatively, the relative percents of pre-beta HDL and alpha HDL were determined by immunonephelometery after starch block electrophoresis of serum. The apoA-I distributed into two peaks upon starch block electrophoresis, a pre-beta (slow) and an alpha (fast) subpopulation. The block was divided into 1-cm segments along the direction of migration and the lipoproteins in each segment were eluted in saline. The apolipoprotein distributions in the fractions were determined by immunonephelometry. The apoA-I content of each fraction determined by immunonephelometry allowed the quantitative determination of the proportoin of apoA-I found in the lipoproteins with pre-beta mobility.

These data are presented in Tables 10-12 and in Figures 8-23. The peak contours and electrophoretic mobilities of the two subpopulations were similar in controls and patients. However, on the average, the control pre-beta HDL accounted for 10.5%, and alpha HDL for 89.5% of the apoA-I-containing lipoproteins (HDL) in serum. The average contribution of the pre-beta HDL in serum of patients with small bowel resection was approximately 50% lower at 5.4%.

Figure 1. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from control subjects #1 (A&B), #2 (C&D), and #3 (E&F) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

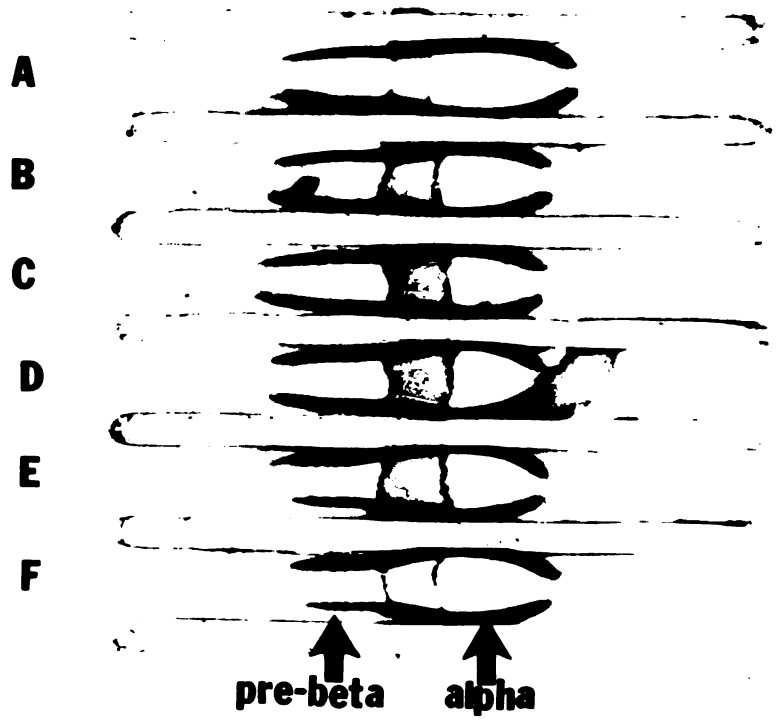

A
B
C
D
E
F
pre-beta
alpha

Figure 2. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from control subjects #4 (A), #5 (B), #6 (C&D) and #7 (E) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

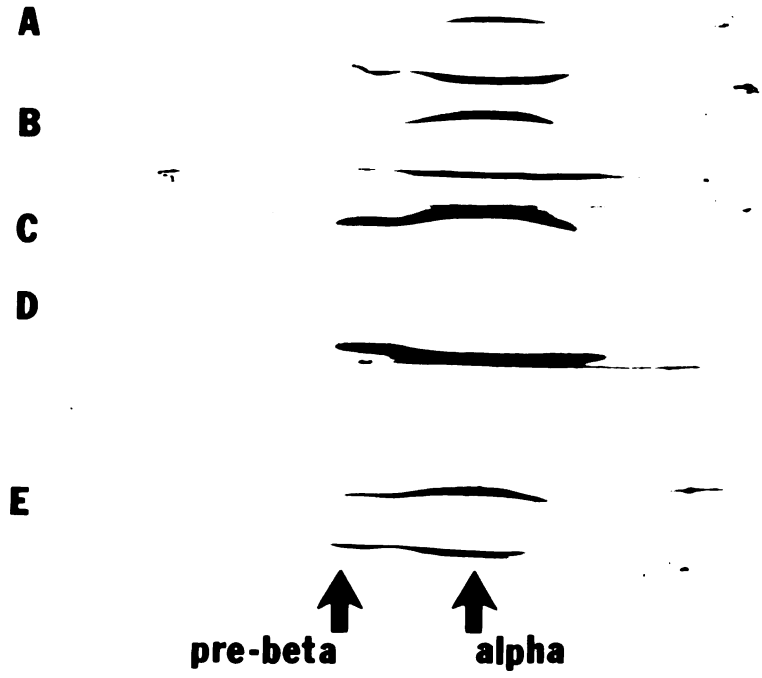

A
B
C
D
E
pre-beta
alpha

Figure 3. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from control subject #8 (A&B) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

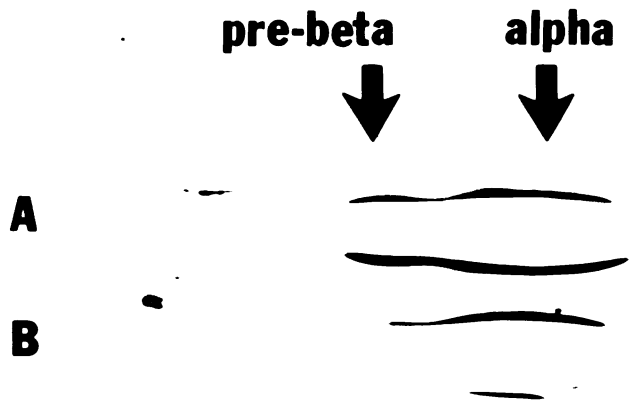

pre-beta
alpha
A
B

Figure 4. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 (A) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

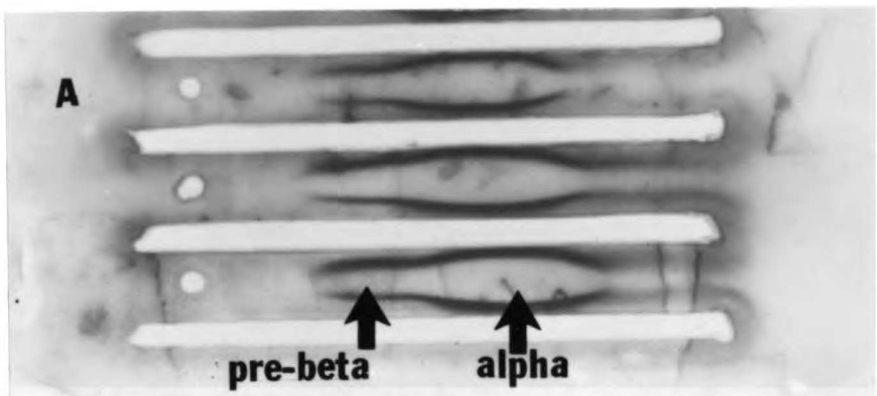

A
pre-beta
alpha

Figure 5. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #2 (A&B) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

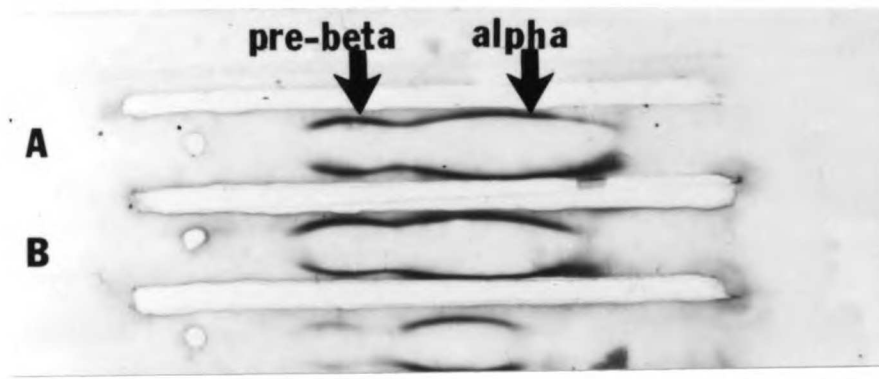

pre-beta
alpha
A
B

Figure 6. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patients #3 (A), #4 (B), #5 (C), and #6 (D) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

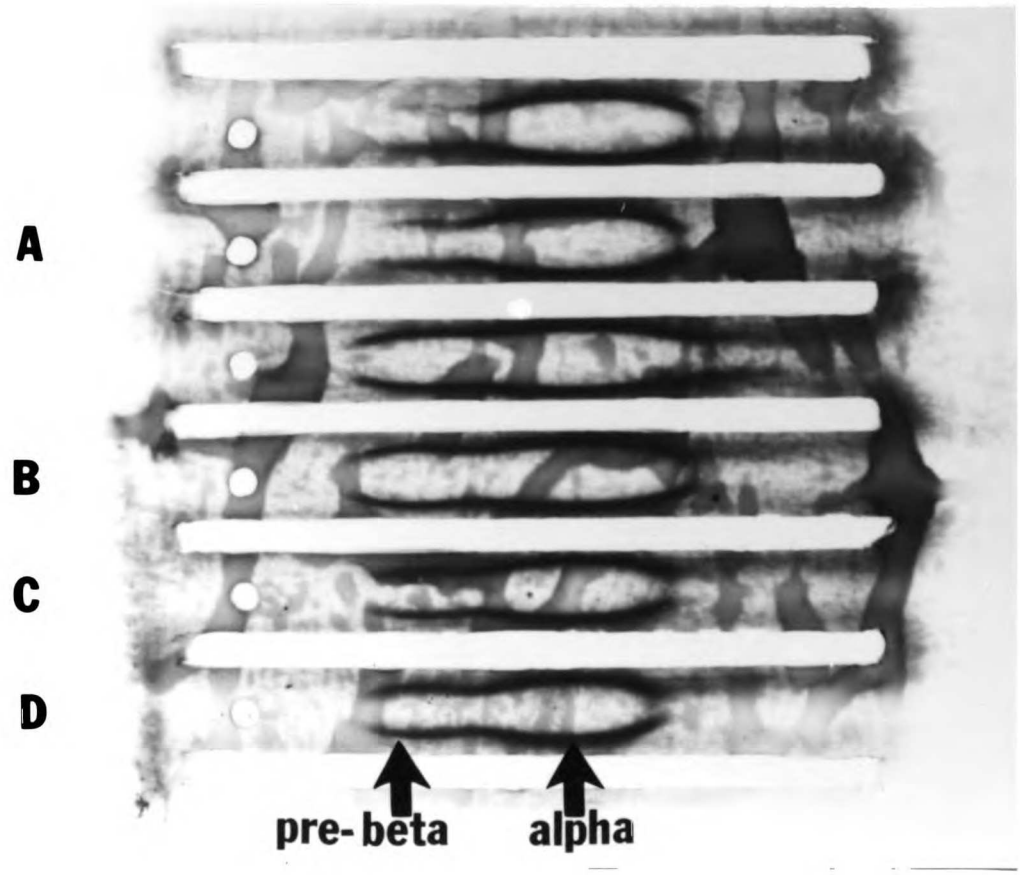

A
B
C
D
pre-beta
alpha

Figure 7. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patients #7 (A) and #8 (B) was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verifies that the apoA-I distributed into two components, a pre-beta (slow) and an alpha (fast) subpopulation.

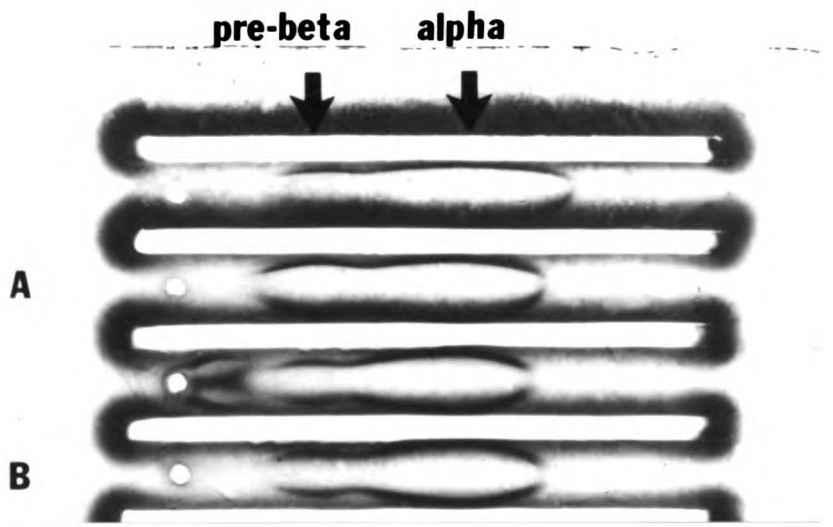

pre-beta
alpha
A
B

Table 10. Relative Percentages of Pre-Beta HDL and Alpha HDL in Serum of Control Subjects

Subjects	% Pre-Beta HDL	% Alpha HDL
C1	9.1	90.9
C2	9.0	91.0
C3	10.0	90.0
C4	13.2	86.8
C5	10.4	89.6
C6	11.1	88.9
C7	11.6	88.4
C8	9.6	90.4
Mean ±SD	10.5 ±1.4	89.5 ±1.4

Table 11. Relative Percentages of Pre-Beta HDL and Alpha HDL in Serum of Patients with Small Bowel Resection

Subject	% Pre-Beta HDL	% Alpha HDL
P1	5.4	94.6
P2	3.4	96.6
P3	6.9	93.1
P4	7.1	92.9
P5	4.5	95.5
P6	6.7	93.3
P7	5.2	94.8
P8	4.6	95.4
Mean ±SD	5.4 ±1.3	94.6 ±1.3

Table 12. Pre-Beta and Alpha HDL in Serum: Controls vs. Patients with Small Bowel Resection

Subjects	% Pre-Beta HDL	% Alpha HDL
Controls n=8	10.5	89.5
Patients n=8	5.4*	94.6

*$p<0.001$.

Figure 8. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #1 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

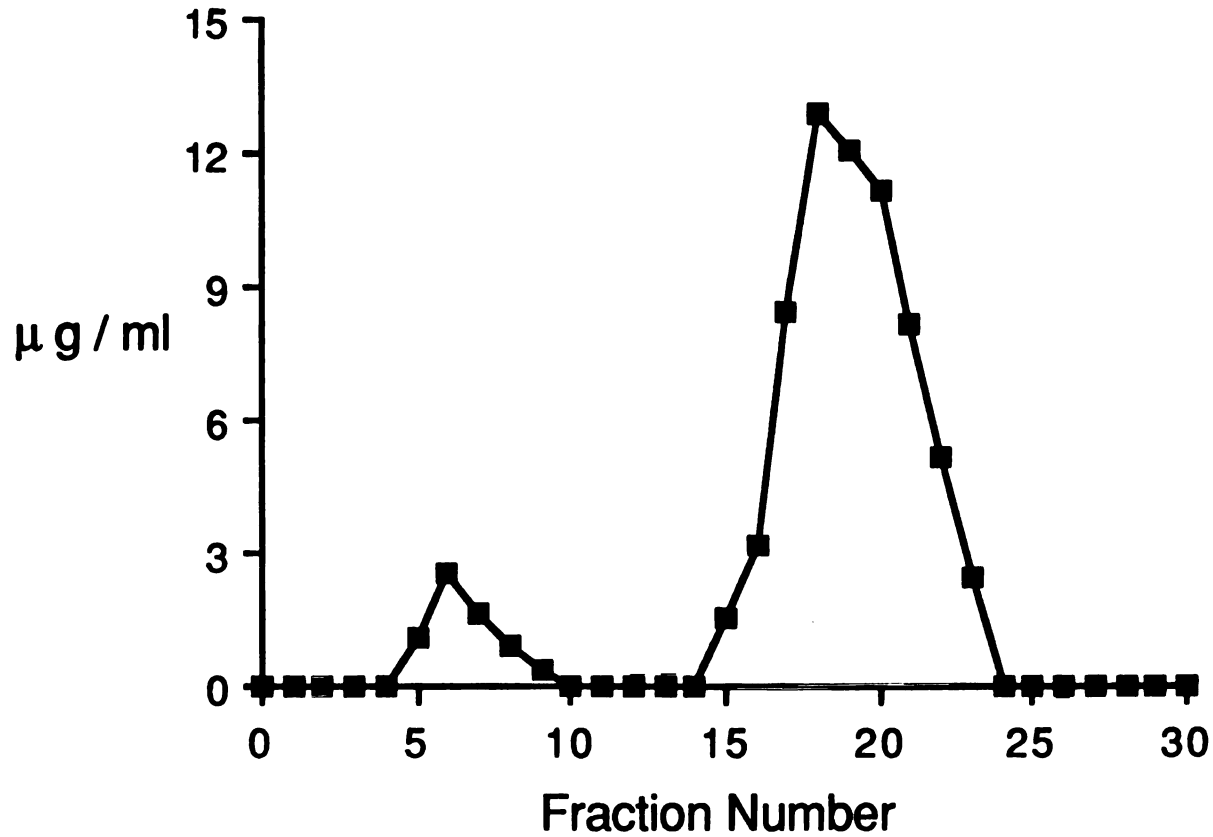

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 9. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #2 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

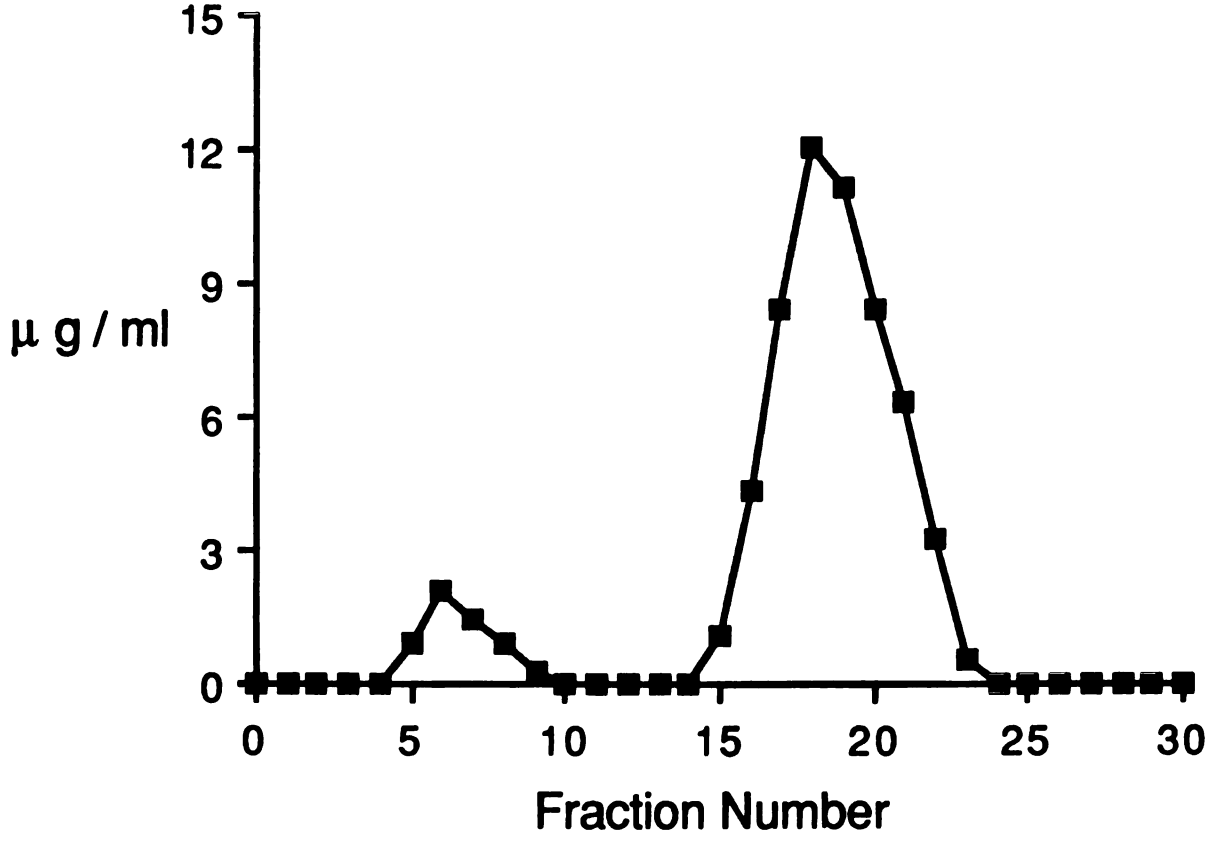

15
12
9
6
3
0
µ g / ml
0
5
10
15
20
25
30
Fraction Number

Figure 10. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #3 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

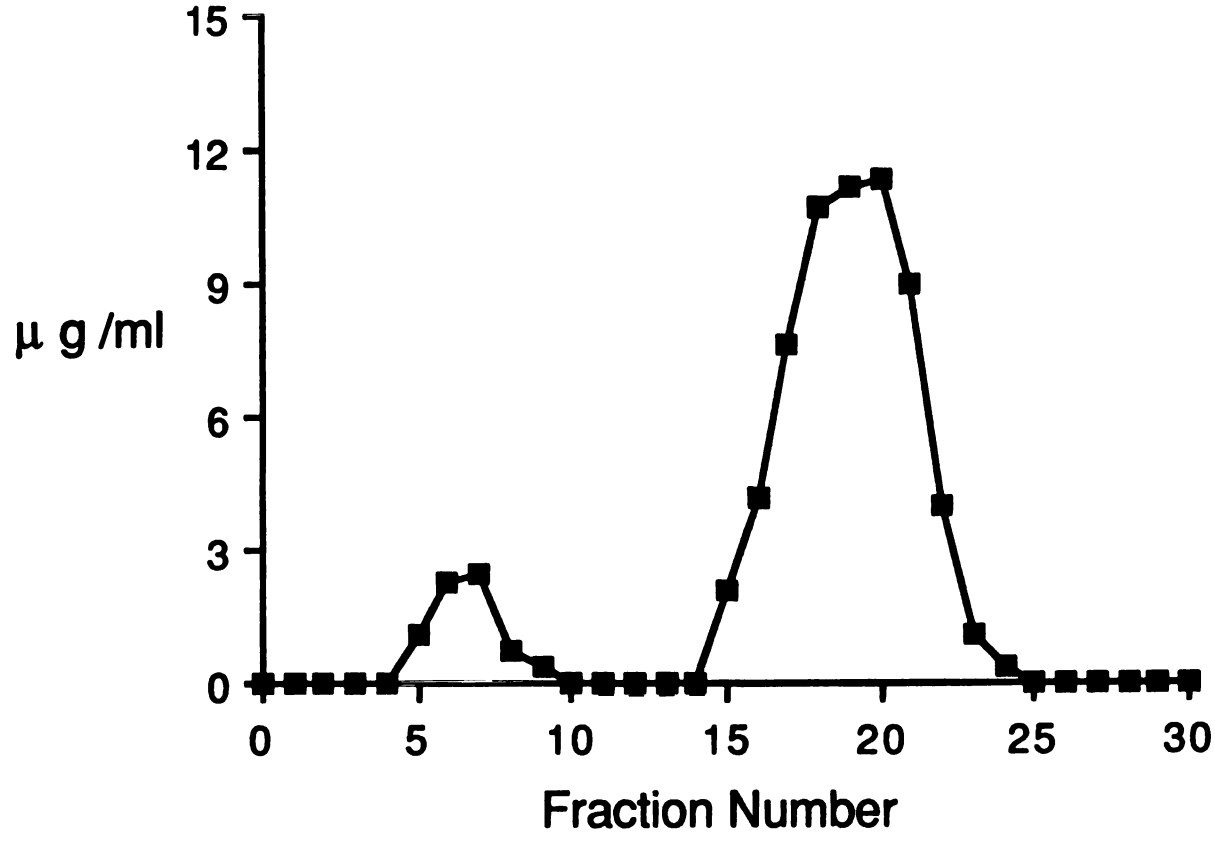

15
12
9
6
3
0
μ g /ml
0
5
10
15
20
25
30
Fraction Number

Figure 11. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #4 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

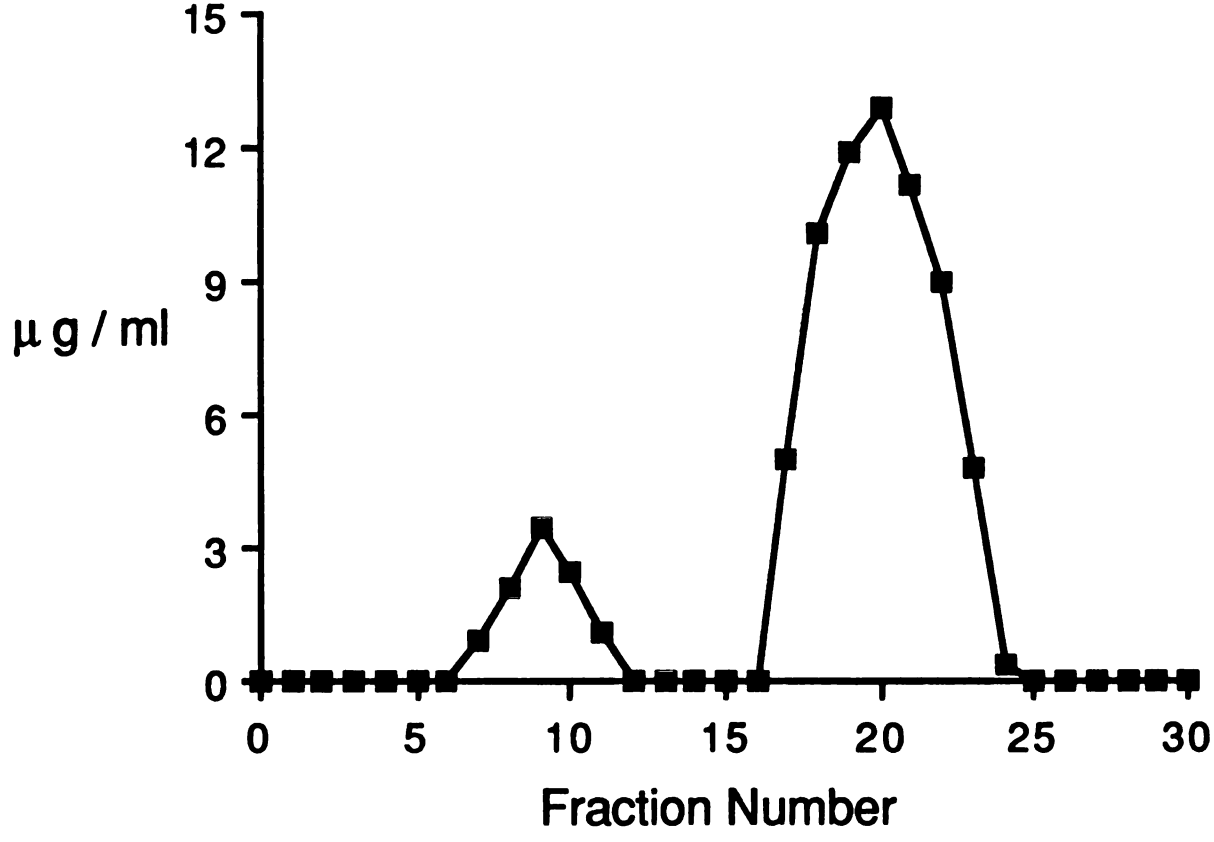

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 12. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #5 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

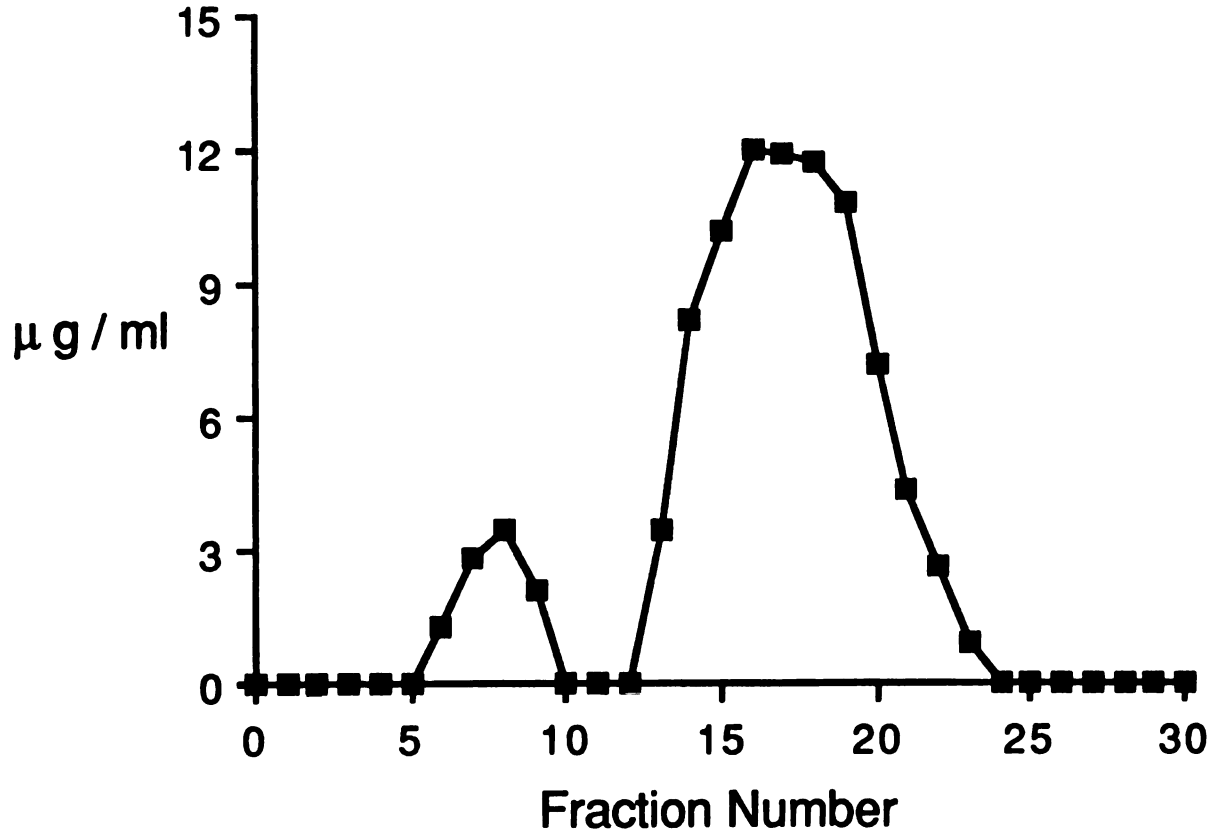

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 13. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #6 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

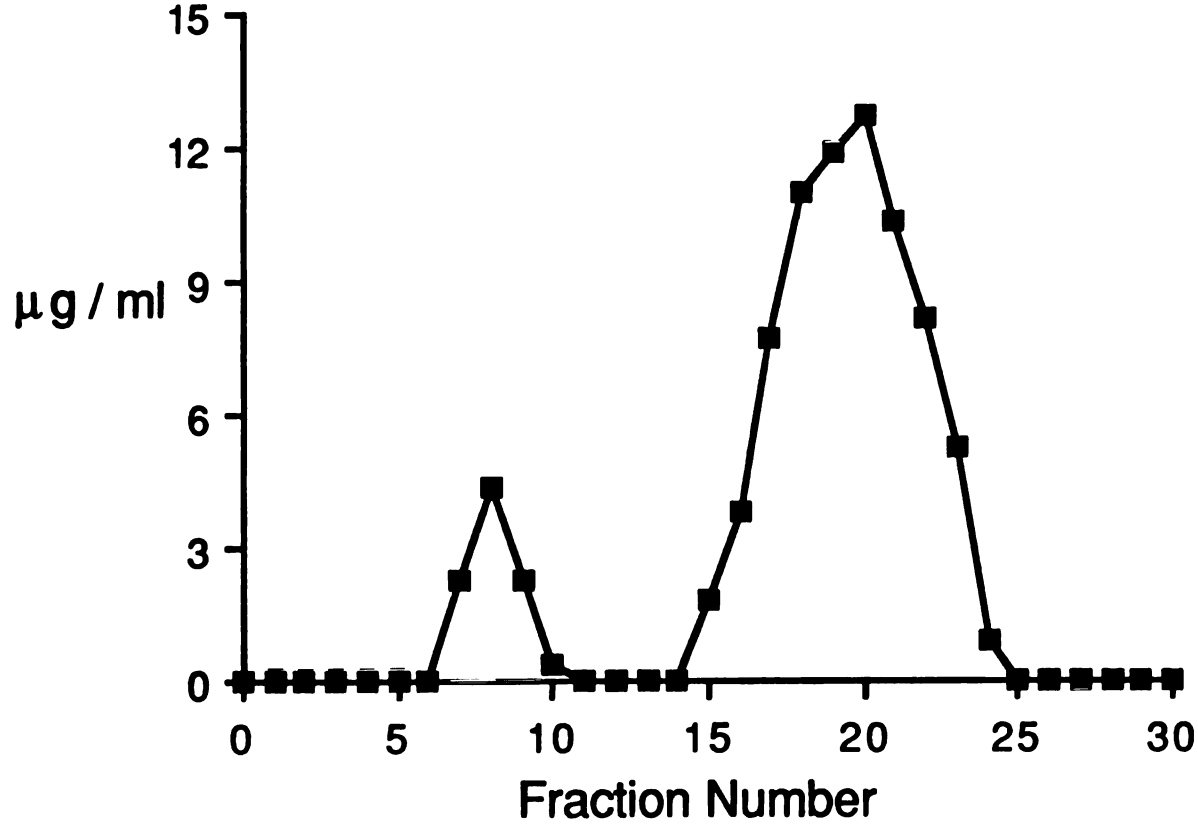

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 14. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #7 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

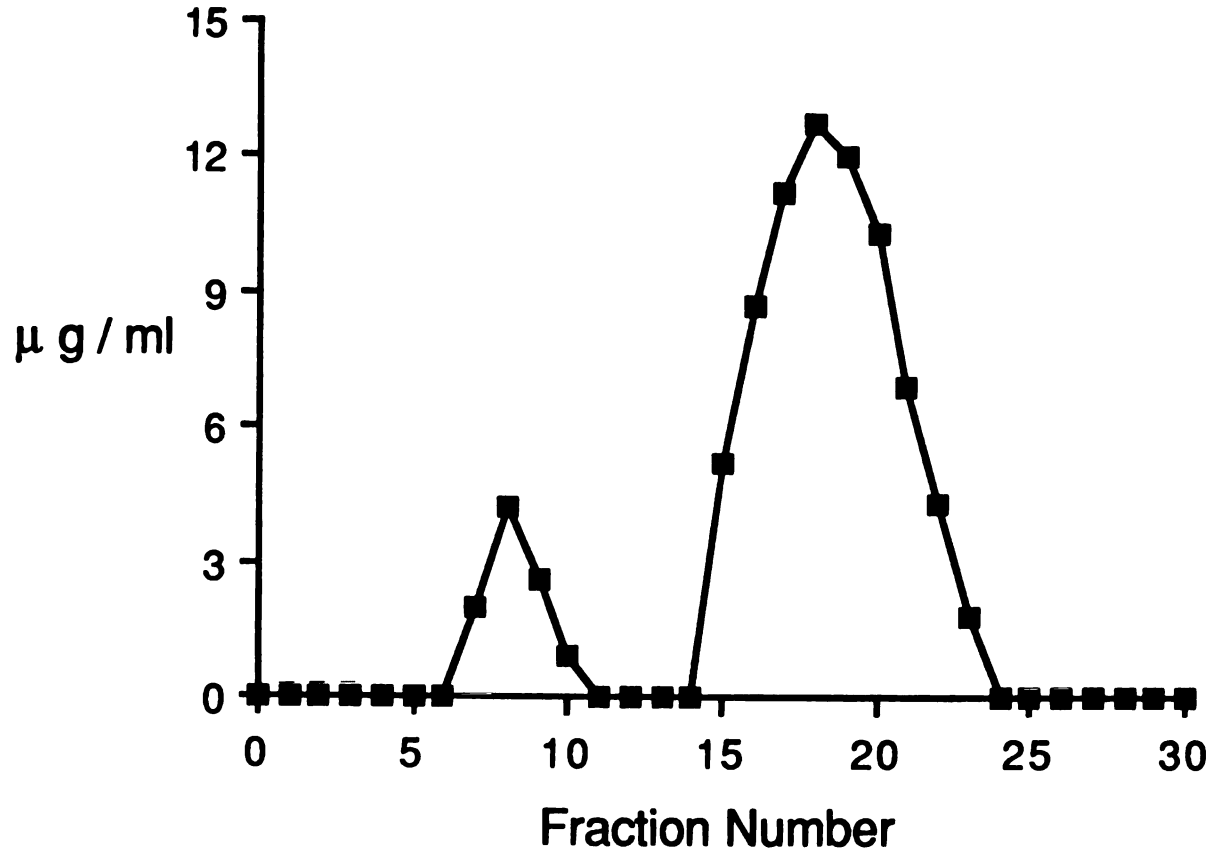

15
12
9
6
3
0
μ g / ml
0
5
10
15
20
25
30
Fraction Number

Figure 15. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from control #8 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

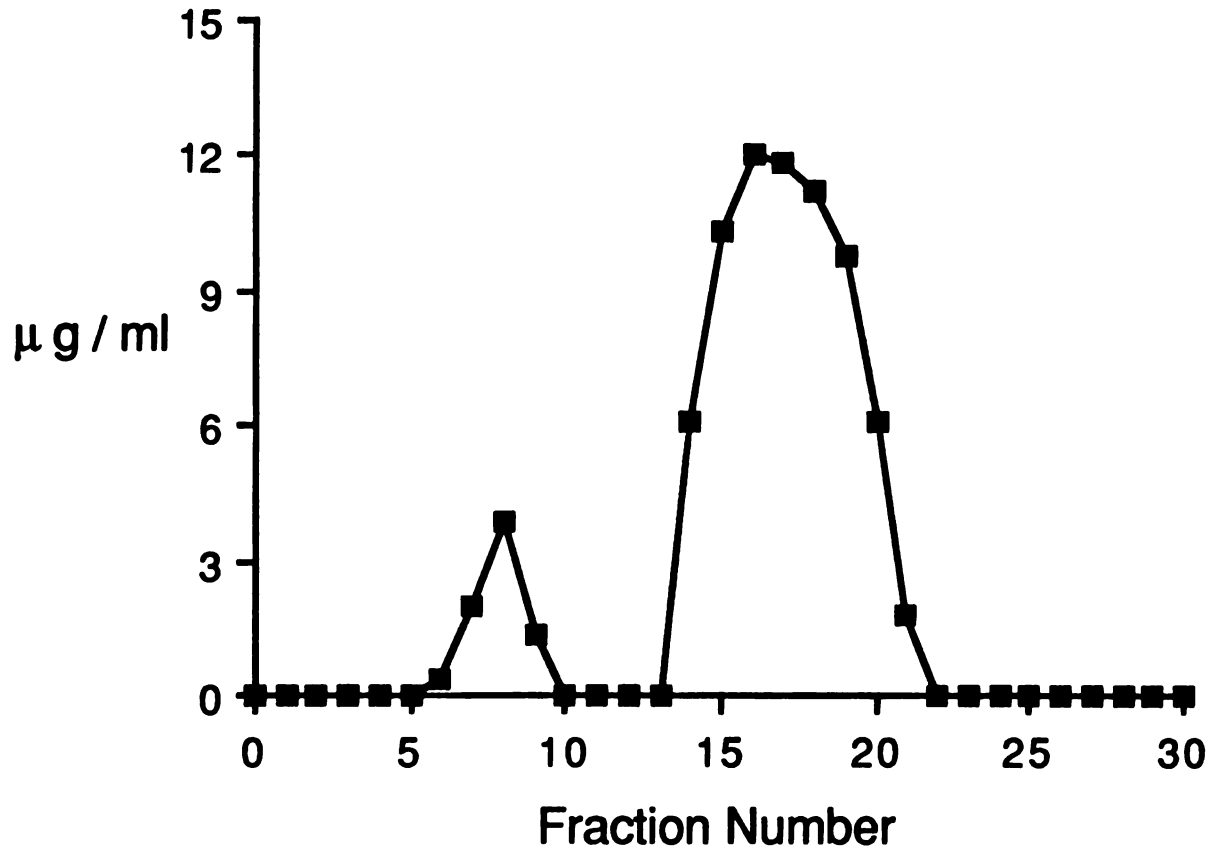

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 16. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

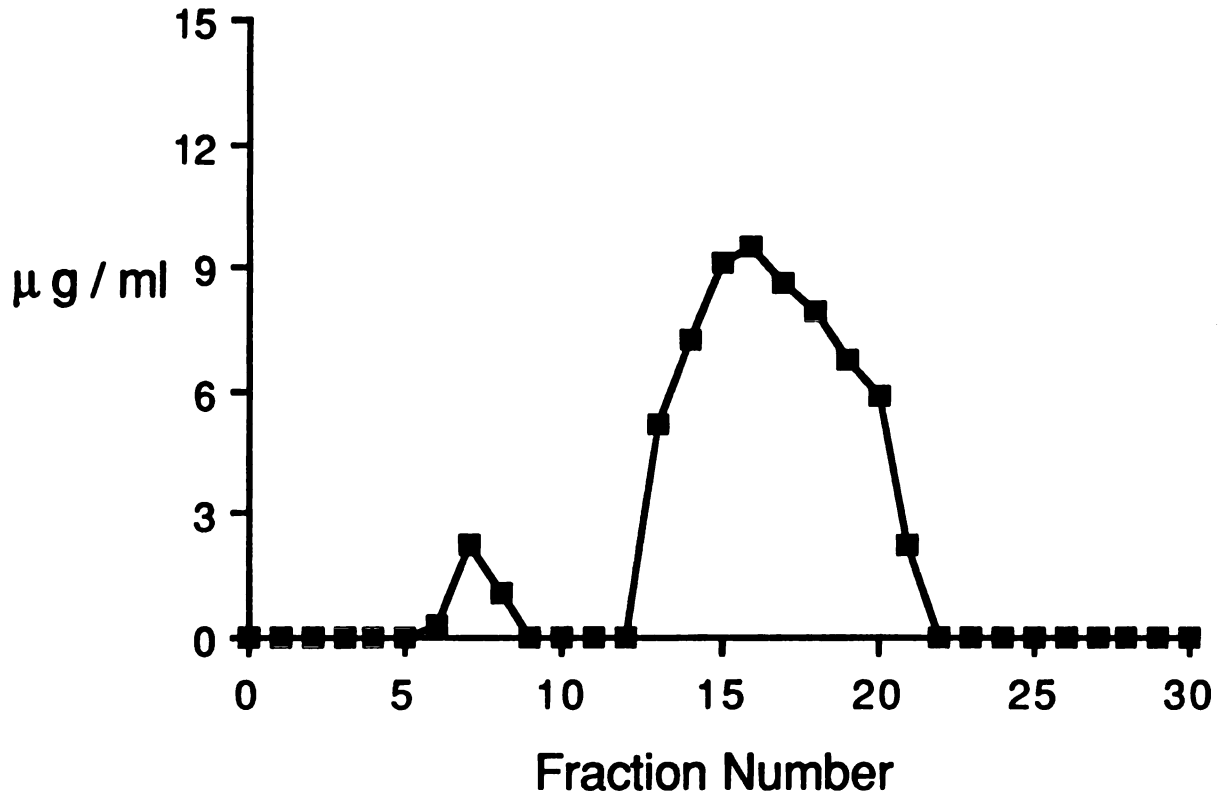

15
12
µg/ml 9
6
3
0
0
5
10
15
20
25
30
Fraction Number

Figure 17. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #2 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

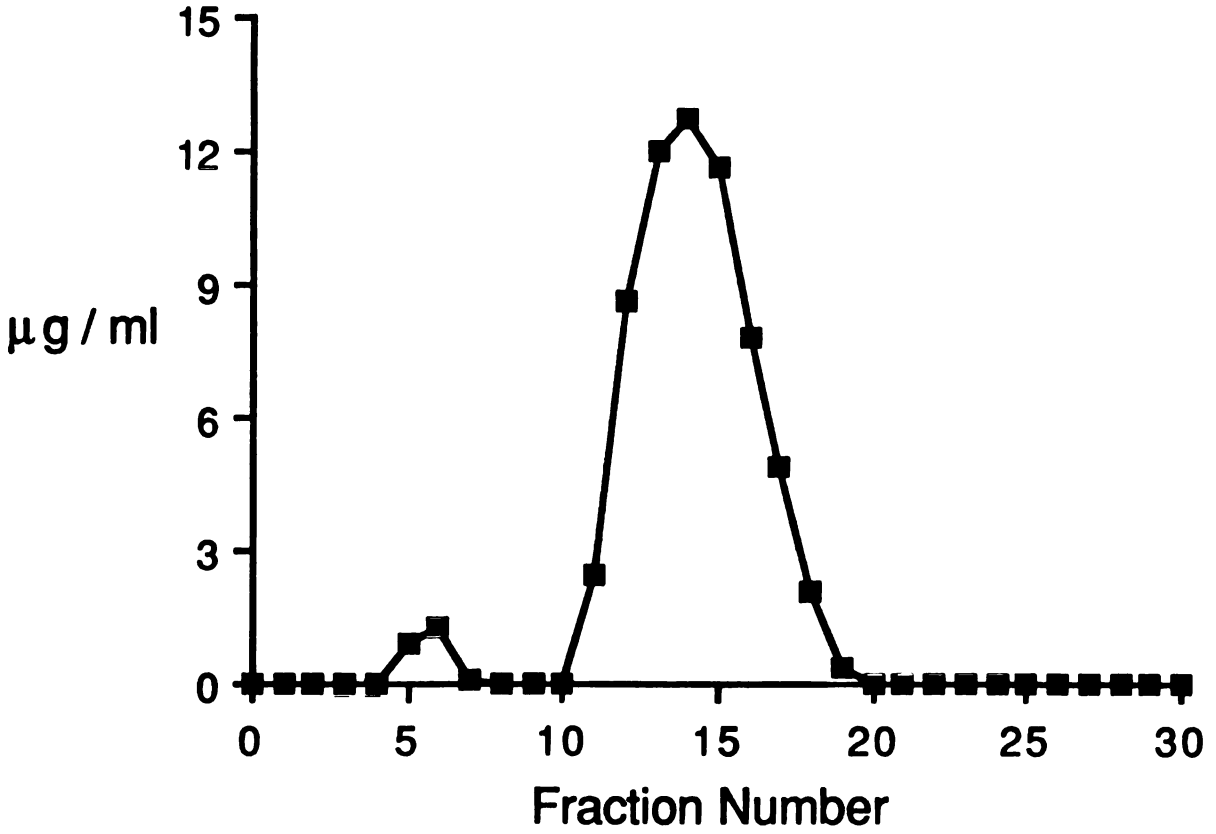
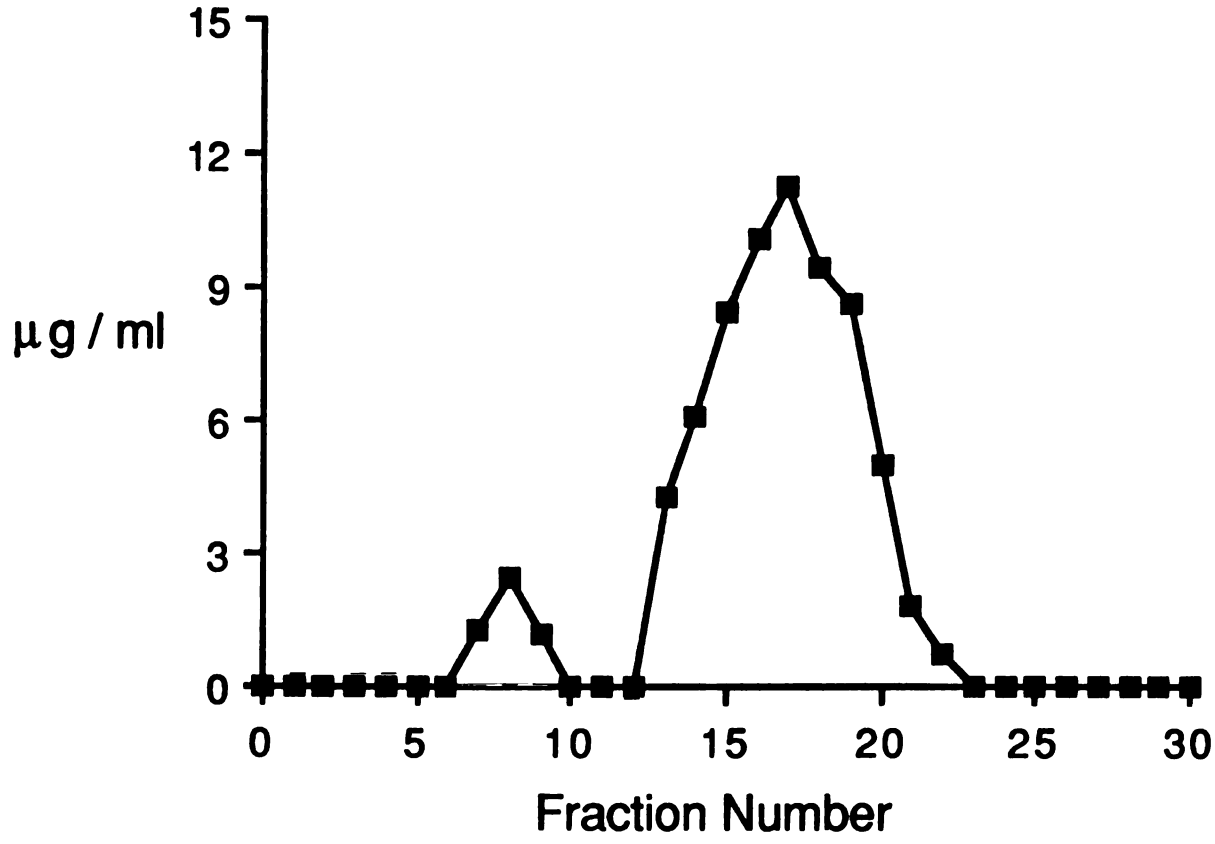

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 18. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #3 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.


15
12
9
6
3
0
µg / ml
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5
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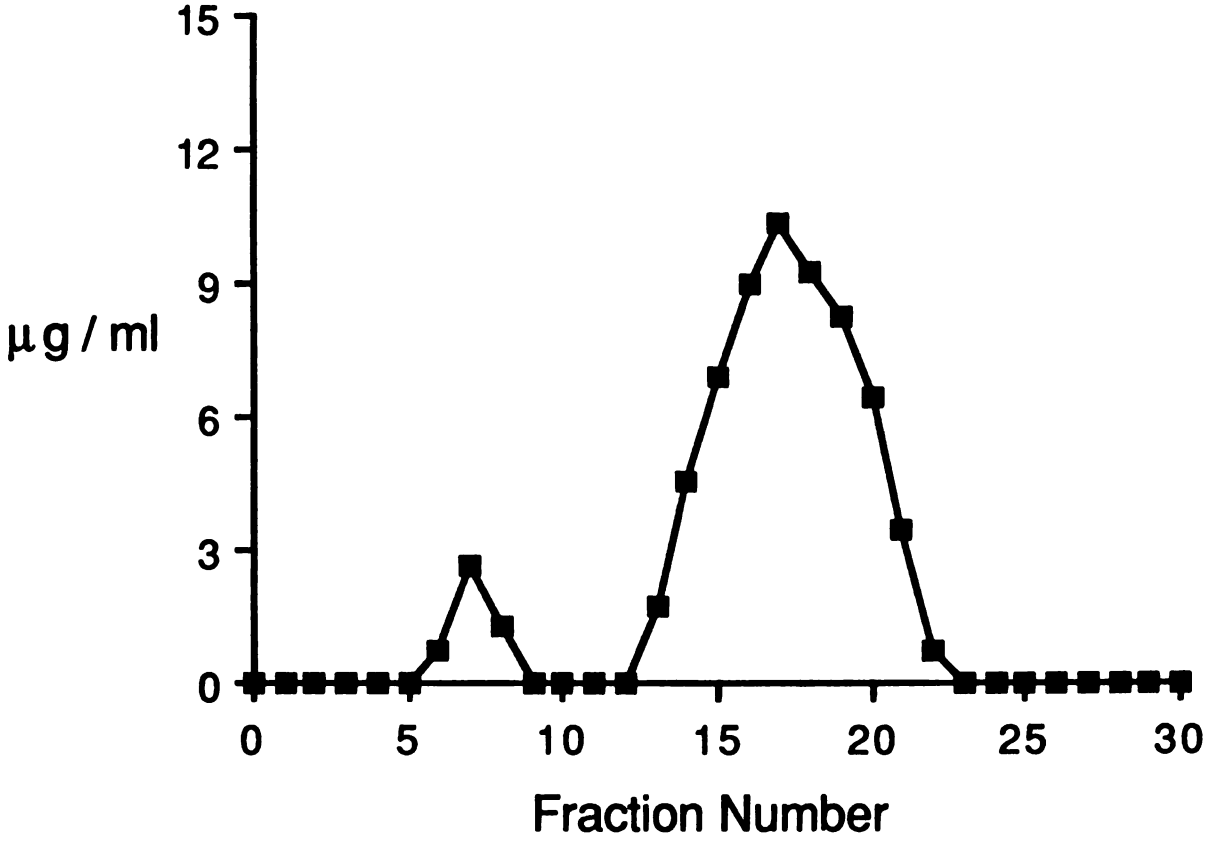
15
20
25
30
Fraction Number

Figure 19. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #4 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.


15
12
9
6
3
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µg / ml
0
5
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15
20
25
30
Fraction Number

Figure 20. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #5 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

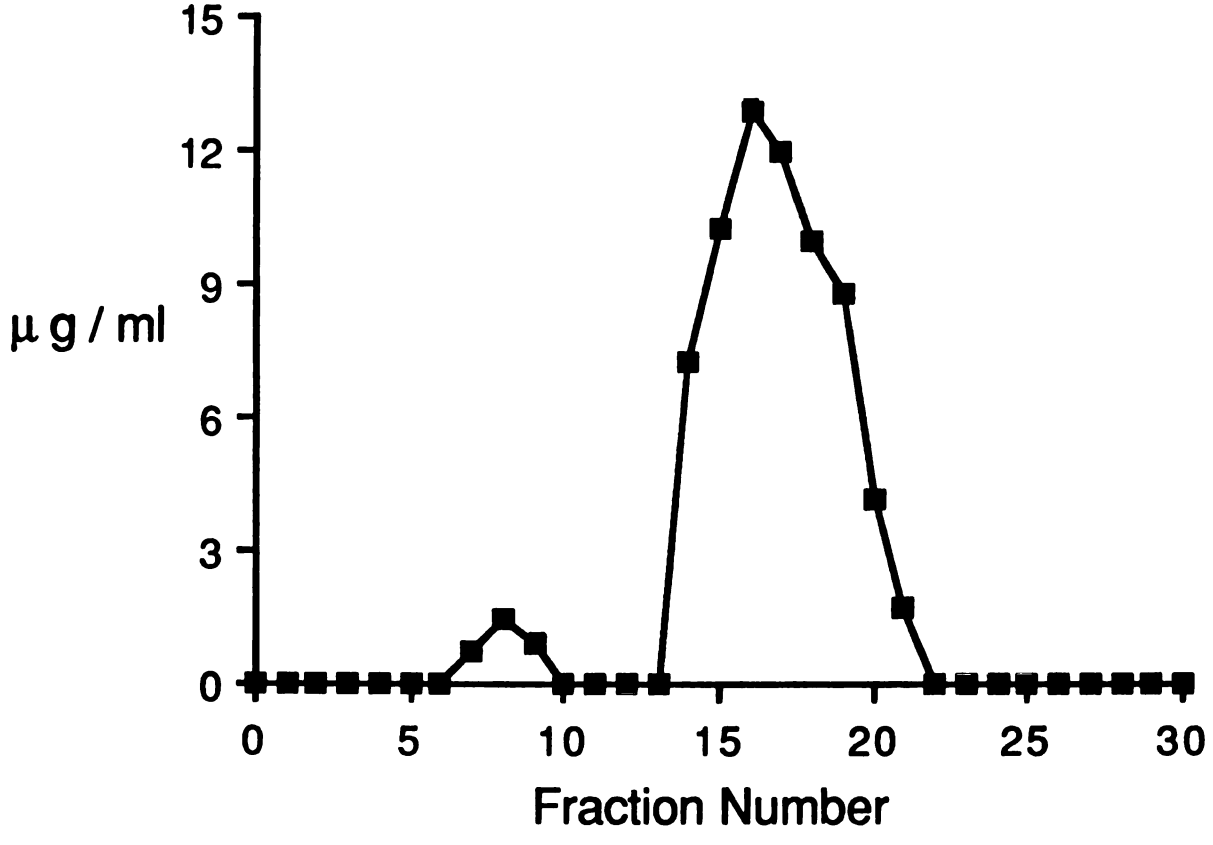

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 21. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #6 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

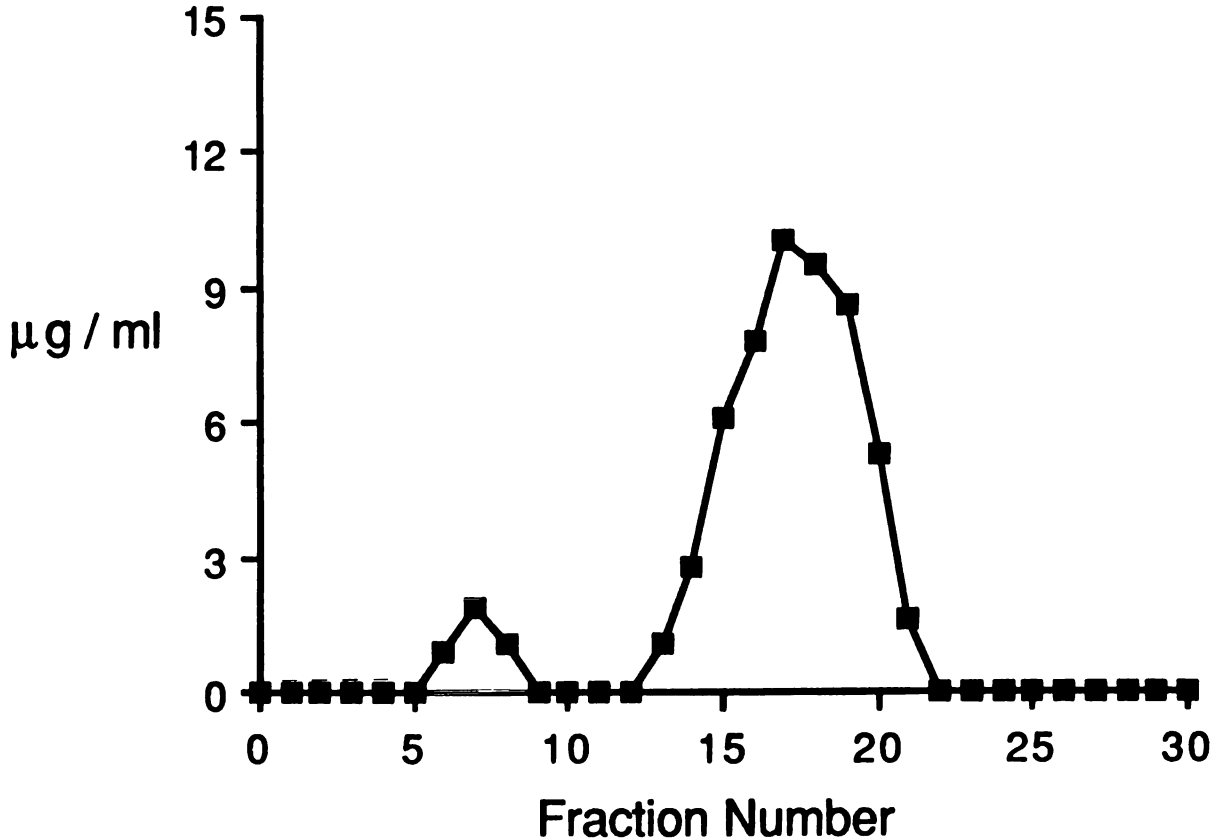

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 22. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #7 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.

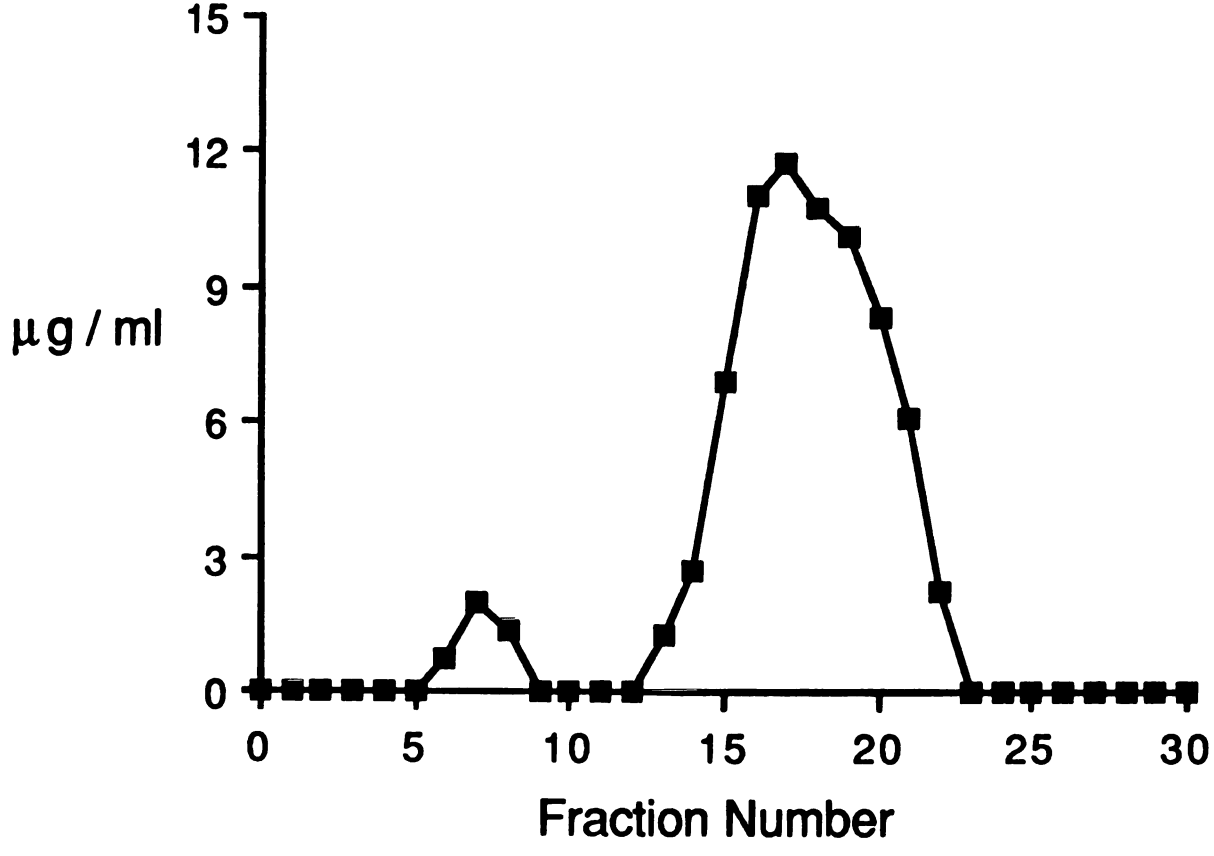
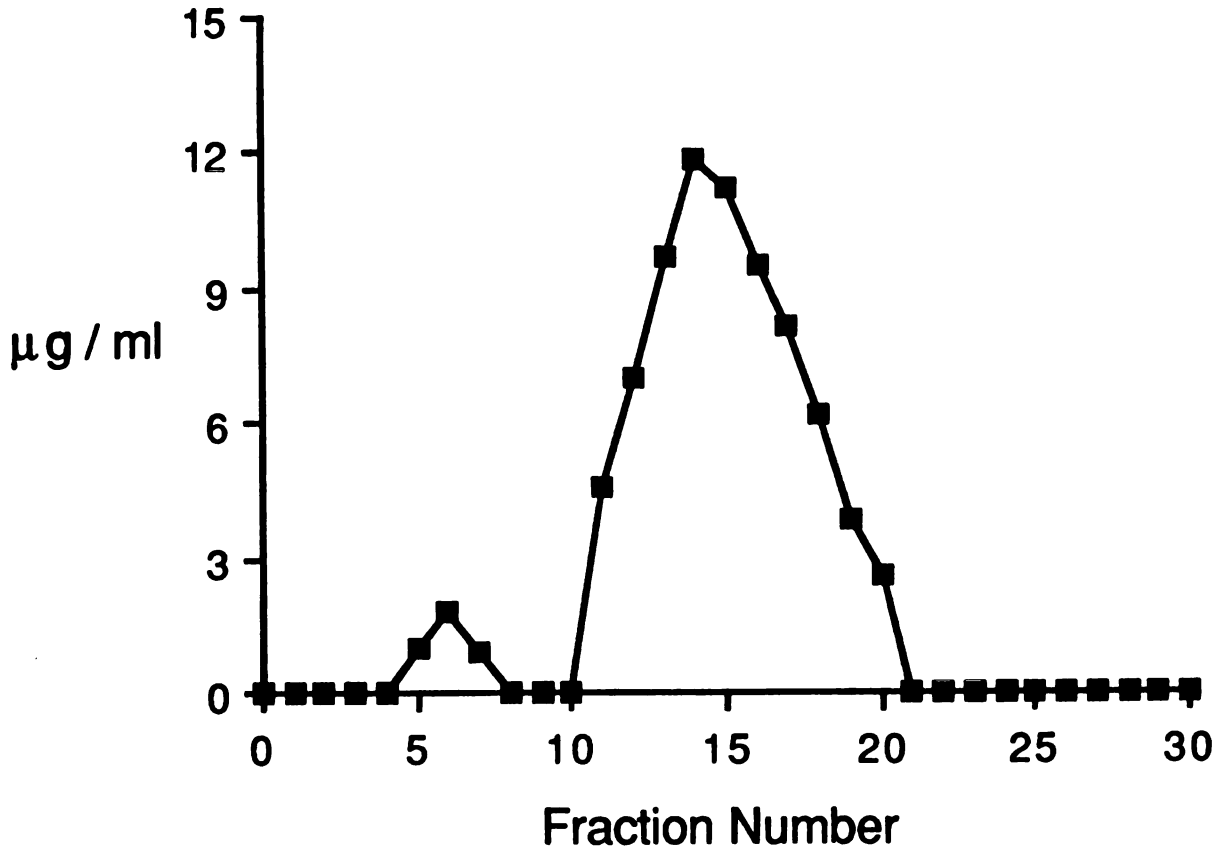

15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Figure 23. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #8 was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation.


15
12
9
6
3
0
µg / ml
0
5
10
15
20
25
30
Fraction Number

Chemical Composition of Serum Lipoproteins

Very Low Density Lipoproteins (VLDL)

Serum lipoproteins were isolated using the preparative ultracentrifuge and the chemical composition was determined for each fraction. VLDL were isolated at a density interval less than 1.006 g/ml. The chemical compositions for control and patient VLDL are given in Tables 13 and 14, respectively.

Mean protein levels in control VLDL were 8.9%. The cholesteryl ester and free cholesterol concentrations were 10.3% and 5.4%, respectively. Triglycerides comprised 55.8% of the particle and 19.6% were phospholipids.

Patient VLDL contained 10.2% protein and 22.3% phospholipid. Free cholesterol accounted for 7.5% of the particles' mass, while the core lipids, triglycerides and cholesteryl esters comprised the remaining 47.3% and 12.7%, respectively.

Patient VLDL contained more protein, more cholesteryl esters ($p<0.05$), more free cholesterol ($p<0.001$), and more phospholipids than control VLDL. However, patient VLDL had less triglyceride than control VLDL ($p<0.005$).

Intermediate Density Lipoproteins (IDL)

Intermediate density lipoproteins were isolated at a density between 1.006 and 1.019 g/ml. Chemical compositions of control and patient IDL

Table 13. Percent Chemical Composition of VLDL from Control Subjects

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
C1	8.9*	10.0	5.4	59.4	16.3
C2	6.9	12.9	6.4	49.7	24.1
C3	11.2	11.9	5.8	49.8	21.1
C4	8.5	11.3	6.5	58.7	15.0
C5	8.0	9.3	5.4	54.0	23.3
C6	6.3	9.0	4.1	60.0	20.6
C7	11.7	8.7	4.3	55.8	19.5
C8	9.7	9.6	5.5	58.7	16.5
Mean	8.9	10.3	5.4	55.8	19.6
±SD	±1.9	±1.5	±0.87	±4.2	±3.3

*All values are expressed as percent dry mass.

Table 14. Percent Chemical Composition of VLDL from Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
P1	10.6*	13.9	7.3	44.1	24.1
P2	10.7	9.1	6.4	54.1	19.1
P3	8.7	16.8	7.4	41.3	25.9
P4	12.1	15.2	8.7	39.9	24.1
P5	9.1	11.0	8.3	48.7	22.8
P6	11.9	12.6	5.9	48.2	21.4
P7	10.5	9.5	6.9	53.8	19.3
P8	7.9	13.5	9.3	47.9	21.4
Mean	10.2	12.7	7.5	47.3	22.3
±SD	±1.5	±2.7	±1.1	±5.3	±2.4

*All values expressed as percent dry mass.

Table 15. Percent Chemical Composition of VLDL: Controls vs. Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	8.9*	10.3	5.4	55.8	19.6
Patients n=8	10.2	12.7**	7.5#	47.3##	22.3

*All values expressed as percent dry mass.
**p<0.05. #p<0.001. ##p<0.005.

Table 16. Percent Chemical Composition of IDL from Control Subjects

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
C1	14.6*	29.6	6.8	27.4	21.6
C2	14.1	30.1	9.4	22.9	23.5
C3	17.8	25.9	9.0	23.2	24.1
C4	20.0	29.8	8.4	23.0	18.8
C5	15.8	30.9	8.0	29.9	15.4
C6	18.9	27.2	8.7	24.1	21.1
C7	16.9	32.4	12.6	22.1	16.0
C8	17.6	32.9	13.1	16.4	20.0
Mean	17.0	29.9	9.4	23.6	20.1
±SD	±2.0	±2.4	±2.2	±4.0	±3.2

*All values expressed as percent dry mass.

Table 17. Percent Chemical Composition of IDL from Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
P1	23.7*	14.2	9.2	30.8	22.1
P2	16.0	22.7	9.1	28.6	23.6
P3	14.8	34.5	9.4	12.7	28.6
P4	22.4	28.1	8.3	26.0	15.0
P5	18.1	15.2	7.3	30.7	28.7
P6	19.8	10.8	7.6	36.7	25.1
P7	17.6	29.1	10.9	18.9	23.5
P8	20.2	27.7	9.1	23.4	19.6
Mean	19.1	22.8	8.7	26.0	23.4
±SD	±3.0	±8.4	±1.2	±7.6	±4.5

*All values expressed as percent dry mass.

Table 18. Percent Chemical Composition of IDL: Controls vs. Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	17.0*	29.9	9.4	27.4	21.6
Patients n=8	19.1	22.8**	8.7	26.0	23.4

*All values expressed as percent dry mass.
**$p<0.05$.

are listed in Tables 16 and 17, respectively. Control IDL were determined to contain 17% protein, 30% cholesteryl ester, 9% free cholesterol, 24% triglyceride, and 20% phospholipid.

Patient IDL contained slightly more protein, triglyceride and phospholipids than control IDL. The percent of free cholesterol was the same in both groups while the percent of cholesteyl esters in the patient IDL was lower than in the controls', 23% vs. 30% ($p<0.05$).

Low Density Lipoproteins (LDL)

Low density lipoproteins were isolated at a density interval between 1.019 and 1.063 g/ml. There were striking differences between the chemical compositions of LDL of controls and LDL of patients (Tables 19 and 20). LDL of both groups contain approximately 21% protein and 23% phospholipid. However, pateint LDL contain appreciably less esterified cholesterol, 31% vs. 40% ($p<0.005$). Moreover, LDL of patients with small bowel resection is comprised of more triglyceride, 8% vs. 12% ($p<0.001$), and more free cholesterol, 8% vs. 12% ($p<0.002$).
The chemical composition data of the patient group imply that patients with extensive small bowel resection have smaller LDL than the control population. This implication was further investigated by negatively staining LDL of three patients and three controls with 2% potassium phosphotungstate (Hamilton et al. 1971). The distribution of particle diameters was determined from electron photomicrographs at a magnification of 60,000 (Figures 24-29) by a semiautomated method,

utilizing a digitizer. Four points were marked on the circumference of each of two hundred particles.

As can be seen from the data in Table 22, all three mean LDL particle diameters of patients were lower than the three mean LDL particle diameters of controls. Skewness values for all subjects were close to zero and kurtosis values close to three which indicated a near gaussian distribution of the diameters of all the measured particles for a given subject. Also, a kurtosis value of 3 indicated a normal, unskewed distribution of elements within a given population. Therefore, from the available data it can be said that for each subject there is a single population of particles present which showed a near gaussian distribution about the mean particle diameter.

For statistical analysis, the mean particle diameters of LDL of patients (n=600) were averaged and compared to those of controls (n=600). Patients with small bowel resection had significantly smaller LDL than controls (Table 23). The mean diameter of patient LDL was 227 ± 26 Å versus a mean diameter of 238 ± 27 Å of control LDL, $p<0.001$. Moreover, by analysis of variance there is a difference in LDL size between the control and patient groups of much less than $p<0.001$. Comparison of the two groups by analysis of variance resulted in an f value of 0.5, indicating a significant difference between LDL particle diameters of controls and LDL particle diameters of patients with small bowel resection. However, when analysis of variance of particle diameters was performed within a given group, the f value was 5.3 and indicated that

no statistical differences existed between the particle diameters within a given group of subjects.

Table 19. Percent Chemical Composition of LDL from Control Subjects

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
C1	21.8*	39.2	8.3	8.5	22.2
C2	22.9	41.0	8.4	7.0	20.7
C3	22.5	37.7	8.1	8.4	23.3
C4	22.2	40.2	8.1	7.7	21.8
C5	21.8	41.5	6.2	9.2	21.3
C6	21.1	40.9	7.5	7.6	22.9
C7	25.1	39.0	6.9	6.6	22.4
C8	23.0	39.6	9.7	6.3	21.4
Mean	22.6	39.9	7.9	7.7	22.0
±SD	±1.2	±1.3	±1.1	±1.0	±0.89

*All values expressed as percent dry mass.

Table 20. Percent Chemical Composition of LDL from Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
P1	22.2	28.8	10.1	12.8	26.1
P2	27.1	26.1	8.8	13.1	24.9
P3	23.7	27.4	9.8	11.8	27.3
P4	18.7	29.8	10.3	14.8	26.4
P5	19.7	44.0	12.0	7.4	16.9
P6	16.1	45.2	14.0	10.0	14.7
P7	24.4	24.2	8.8	12.1	30.5
P8	19.4	38.8	11.8	10.0	19.9
Mean	20.6	31.7	11.3	11.8	24.6
±SD	±3.9	±9.8	±2.3	±2.5	±7.1

*All values expressed as percent dry mass.

Table 21. Percent Chemical Composition of LDL: Controls vs. Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	22.6*	39.9	7.9	7.7	22.0
Patients n=8	20.6	31.7**	11.3#	11.8##	24.6

*All values expressed as percent dry mass.
**$p<0.05$. #$p<0.002$. ##$p<0.001$.

Table 22. Mean LDL particle Diameters of Controls and Patients

Subjects	n	Diameter (Å)	SD	SE	Skew	Kurtosis
C3	200	239	25	2.7	-0.19	3.0
C5	200	244	27	2.9	-0.12	3.4
C8	200	232	27	2.8	-0.56	3.3
P3	200	228	27	2.8	-0.36	2.9
P5	200	225	25	2.7	0.30	3.1
P8	200	227	25	2.8	-0.28	3.6

*n is equal to the number of LDL particles measured on each electron photomicrograph.

Table 23. Mean LDL Particle Diameters in Controls and Patients

Subjects	n	Diameter (Å)	SD	SE	Skew	Kurtosis
Controls	600	238	27	1.7	-0.27	3.4
Patients	600	227*	26	1.6	-0.11	3.1

*$p<0.0001$.

Figure 24. Electron photomicrograph of LDL from control #3, visualized by negative staining with 2% potassium phosphotungstate.

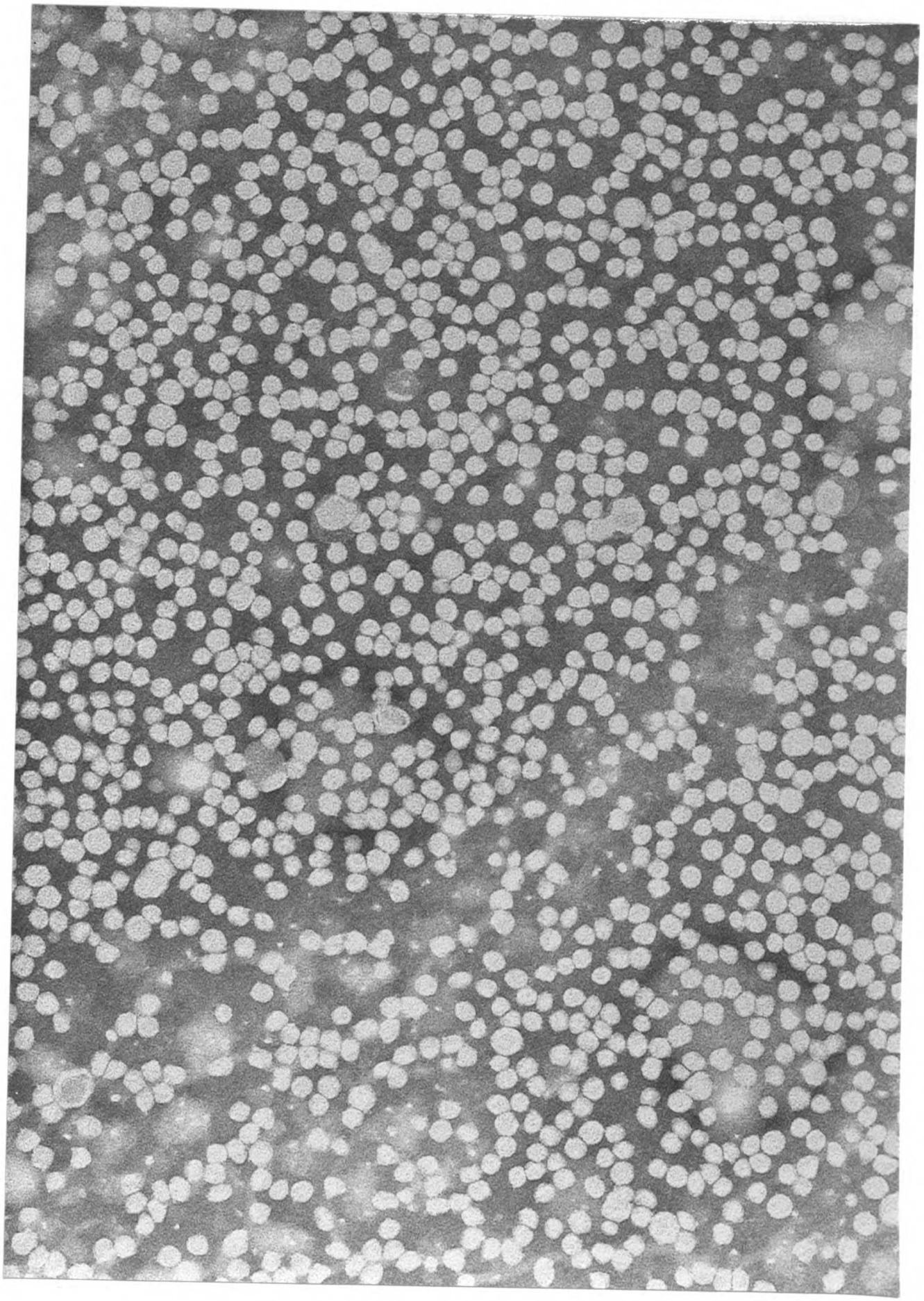


Figure 25. Electron photomicrograph of LDL from control #5, visualized by negative staining with 2% potassium phosphotungstate.

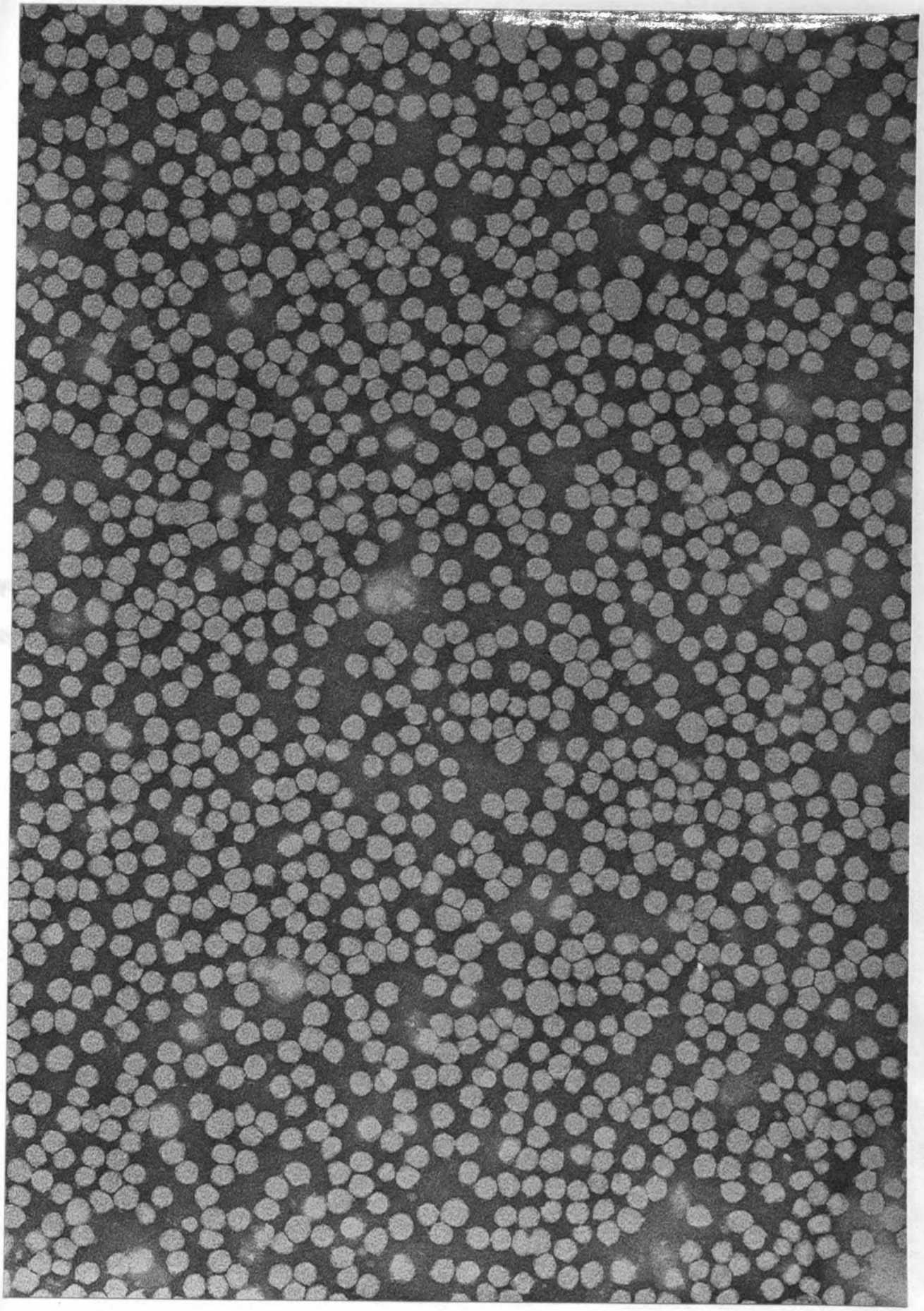


Figure 26. Electron photomicrograph of LDL from control #8, visualized by negative staining with 2% potassium phosphotungstate.

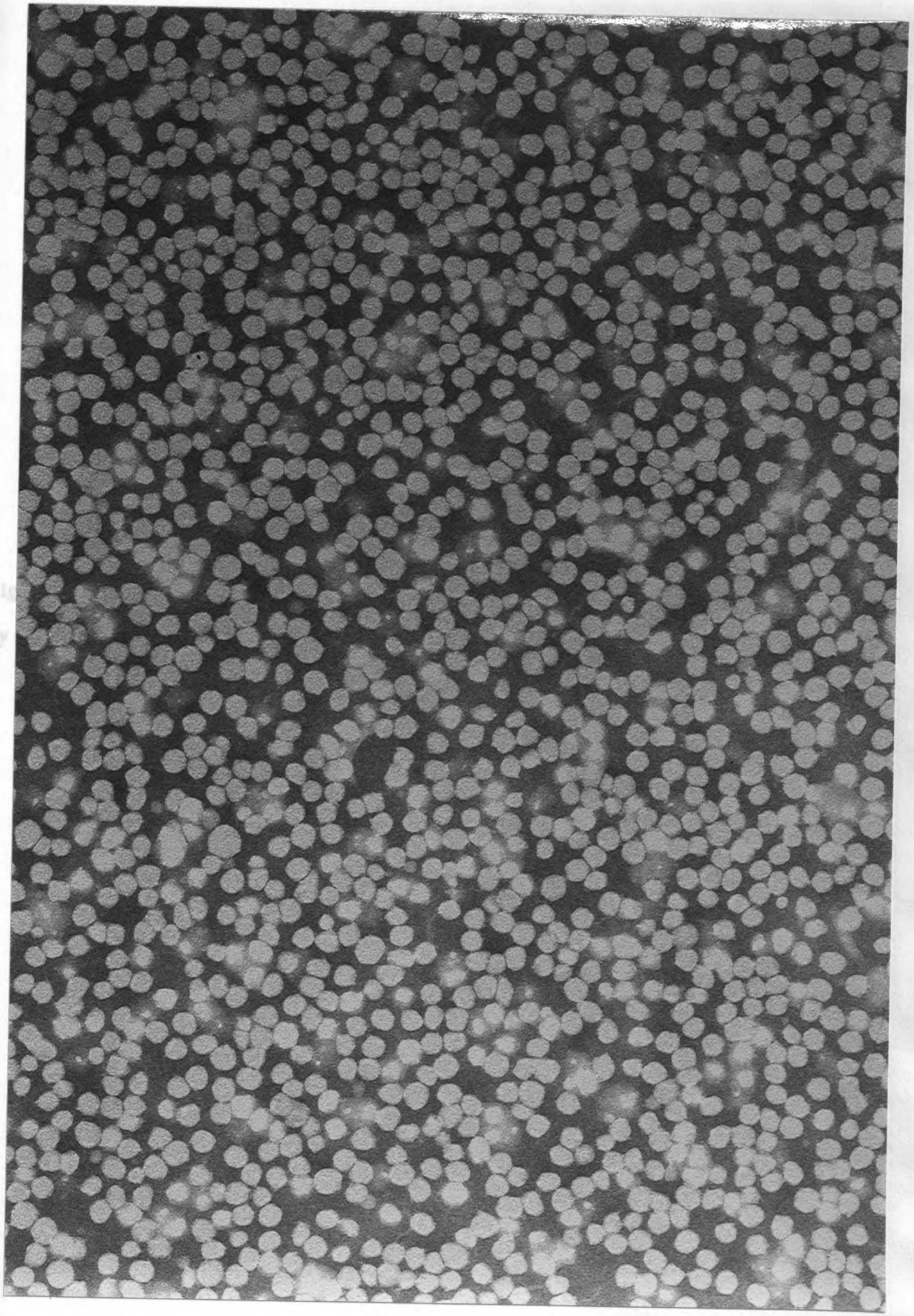


Figure 27. Electron photomicrograph of LDL from patient #3, visualized by negative staining with 2% potassium phosphotungstate.

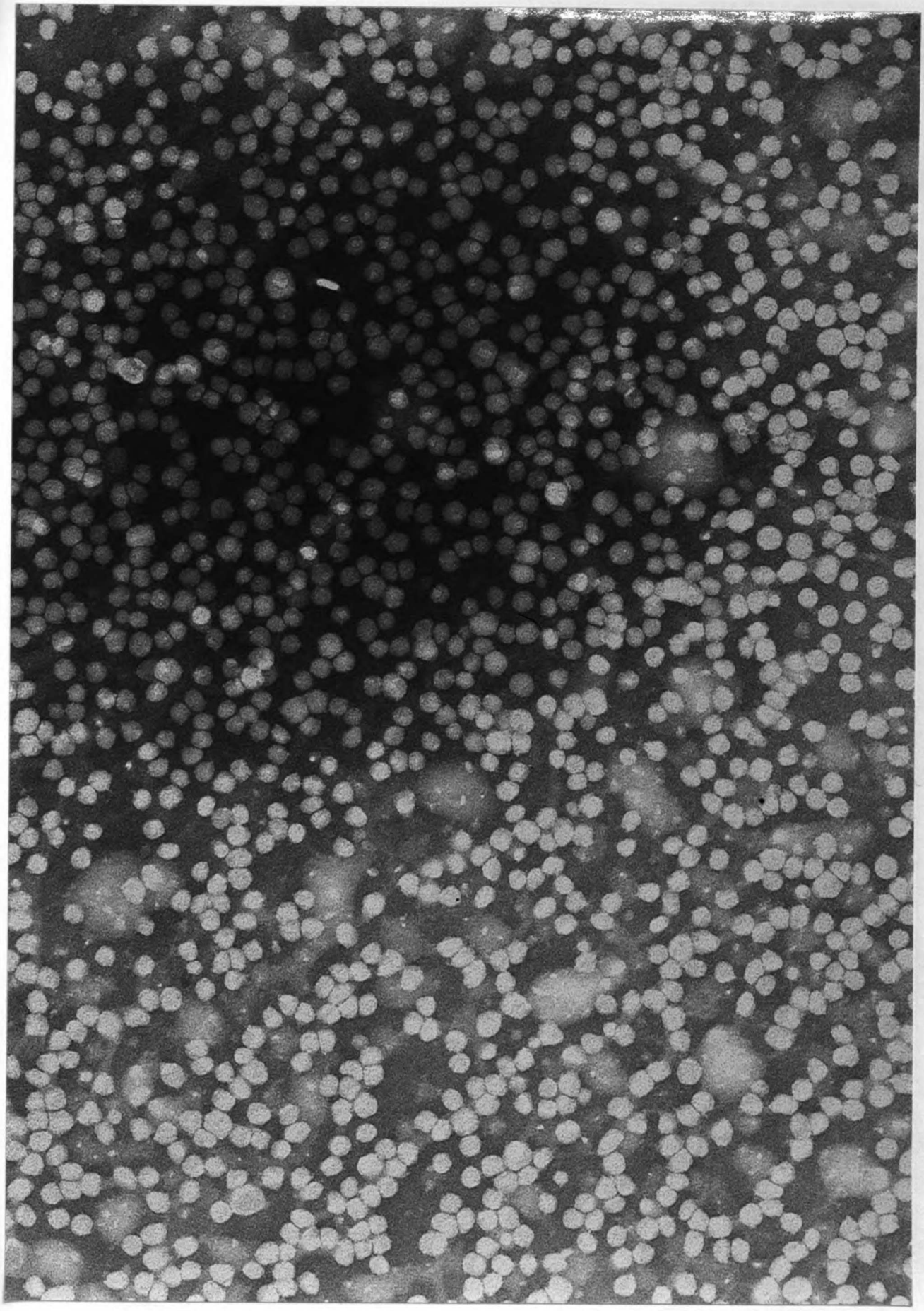


Figure 28. Electron photomicrograph of LDL from patient #5, visualized by negative staining with 2% potassium phosphotungstate.

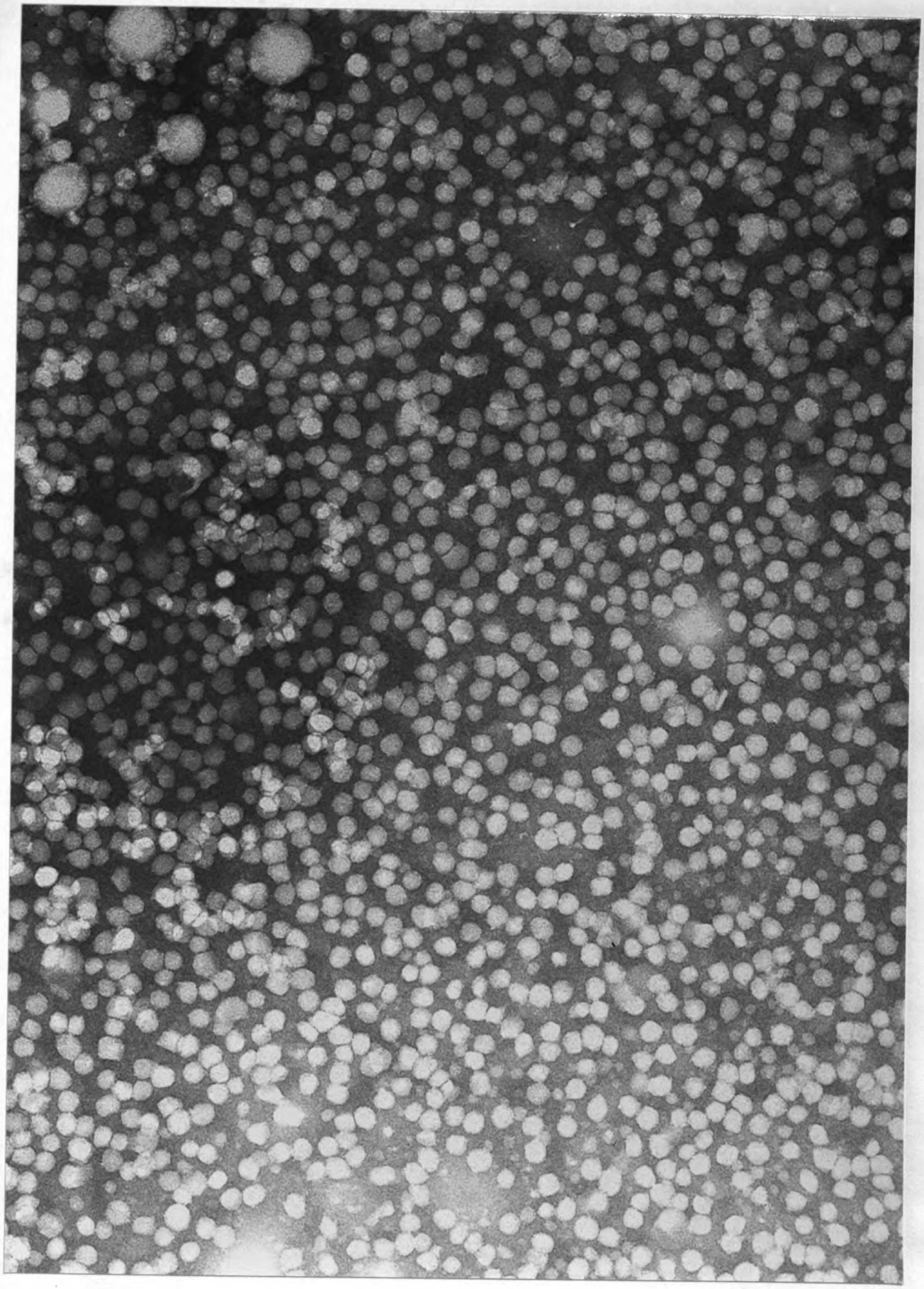
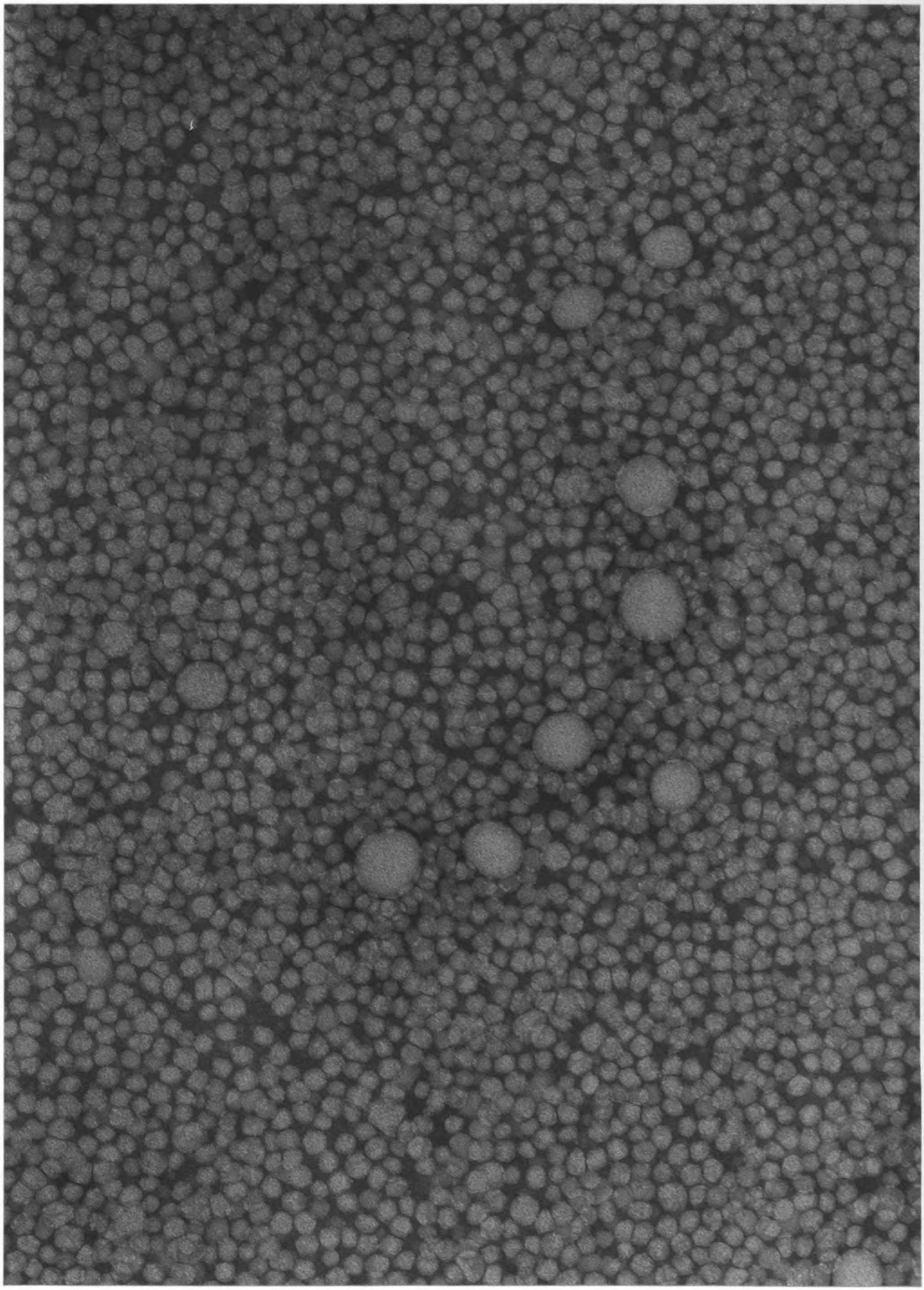


Figure 29. Electron photomicrograph of LDL from patient #8, visualized by negative staining with 2% potassium phosphotungstate.



High Density Lipoproteins

HDL_2

As is illustrated in Tables 24 and 25, the percent chemical compositions of control HDL_2 were almost identical to patient HDL_2. The only difference is a slight increase in the triglyceride of patient HDL_2, 5% vs 9%. Otherwise HDL_2 are composed of 40% protein, 30% phospholipid, 4% free cholesterol, and 17% cholesteryl esters.

HDL_3

The chemical compositions of control and patient HDL_3 were also the same (Tables 27 and 28). The particles were comprised of 56% protein, 23% phospholipids, 5% triglycerides, 13% cholesteryl esters, and 3% free cholesterol.

Table 24. Percent Chemical Composition of HDL_2 from Control Subjects

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
C1	42.7*	20.4	4.2	5.4	27.3
C2	37.2	19.9	4.8	5.3	32.8
C3	41.2	21.3	4.6	4.7	28.2
C4	40.9	16.3	3.4	6.5	32.9
C5	41.5	15.1	3.6	5.7	34.1
C6	42.0	15.4	3.4	6.7	32.5
C7	42.9	14.4	2.9	6.0	33.8
C8	39.3	20.0	4.3	3.4	33.0
Mean	40.9	17.9	3.9	5.5	31.8
±SD	±1.8	±2.8	±0.67	±1.1	±2.6

*All values expressed as percent dry mass.

Table 25. Percent Chemical Composition of HDL_2 from Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
P1	40.9*	19.4	6.1	7.9	25.7
P2	42.4	17.9	5.2	5.1	29.4
P3	41.6	13.5	2.3	8.9	33.7
P4	39.9	14.9	4.4	8.7	32.2
P5	37.2	21.1	4.3	5.9	31.5
P6	41.2	15.3	4.6	13.4	25.5
P7	42.2	14.1	3.9	12.2	27.6
P8	37.8	18.5	6.7	5.1	31.9
Mean	40.4	16.6	4.7	8.9	29.4
±SD	±1.9	±2.6	±1.4	±3.3	±3.5

*All values expressed as percent dry mass.

Table 26. Percent Chemical Composition of HDL_2: Controls vs. Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	40.9*	17.9	3.9	5.5	31.8
Patients n=8	40.4	16.6	4.7	8.9**	29.4

*All values expressed as percent dry mass.
**$p<0.02$.

Table 27. Percent Chemical Composition of HDL_3 from Control Subjects

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
C1	56.5*	12.7	1.9	5.2	23.7
C2	55.2	14.9	2.7	4.4	22.8
C3	58.2	11.3	2.0	3.8	24.7
C4	55.7	11.0	2.4	5.0	25.9
C5	57.7	12.0	2.5	5.3	22.5
C6	55.8	11.6	2.4	6.3	23.9
C7	56.8	14.1	2.5	5.9	20.7
C8	57.8	15.0	2.8	3.2	21.2
Mean	56.7	12.8	2.4	4.9	23.2
±SD	±1.1	±1.6	±0.31	±1.0	±1.7

*All values expressed as percent dry mass.

Table 28. Percent Chemical Composition of HDL_3 from Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
P1	53.1*	14.5	3.1	7.2	22.1
P2	53.1	15.9	5.0	3.3	22.7
P3	55.2	12.6	3.3	7.8	21.1
P4	58.2	14.8	2.7	4.0	20.3
P5	57.7	15.3	2.2	2.5	22.3
P6	53.9	12.1	2.2	9.0	22.8
P7	55.5	11.7	2.6	6.0	24.2
P8	56.9	14.2	2.8	3.9	22.2
Mean	55.5	13.8	3.0	5.5	22.2
±SD	±2.0	±1.5	±0.90	±2.3	±1.2

*All values expressed as percent dry mass.

Table 29. Percent Chemical Composition of HDL_3: Controls vs. Patients with Small Bowel Resection

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
Controls n=8	56.7*	12.8	2.4	4.9	23.2
Patients n=8	55.5	13.8	3.0	5.5	22.2

*All values expressed as percent dry mass.

SDS Gradient Electrophoresis

The apoproteins of VLDL, IDL, LDL, HDL_2, and HDL_3 from controls and patients were resolved on SDS gradient gels (2.7-27%). These lipoprotein fractions were not double spun and there was a slight contamination by albumin in some of the samples. All samples were loaded onto the gels in order of increasing density beginning with VLDL to the far left and ending with HDL_3 to the right, followed by a lane of marker proteins for comparison of molecular weights.

The apolipoproteins of controls displayed a normal distribution in polyacrylamide gels. Figure 30 exemplifies the resolution of apoproteins seen in all the control gels. VLDL contain apoB-100 at the top of the gel, apoE is present between the 42,700 and 31,000 marker proteins and the C proteins are seen near the bottom of the gel below the 14,000 molecular weight marker. ApoB-100 and apoE are present in the IDL while LDL contains only apoB-100. The HDL subfractions contain apoA-I at a molecular weight of 28,000 while apoA-II and the C proteins are located at the bottom of the gels below 14,000.

None of the major apolipoproteins other than apoB-48 were absent from the plasma of patients with small bowel resection and the apolipoproteins of patients displayed a similar distribution as controls (Figure 31).

Figure 30. SDS gradient electrophoresis. The apoproteins of the lipoprotein fractions from control #8 were resolved on SDS gradient gels. The lipoproteins from left to right are: VLDL (A); IDL (B); LDL (C); HDL_2 (D); and HDL_3 (E). Marker proteins are displayed for comparison (F)--molecular weights equal the number shown multiplied by 1000.

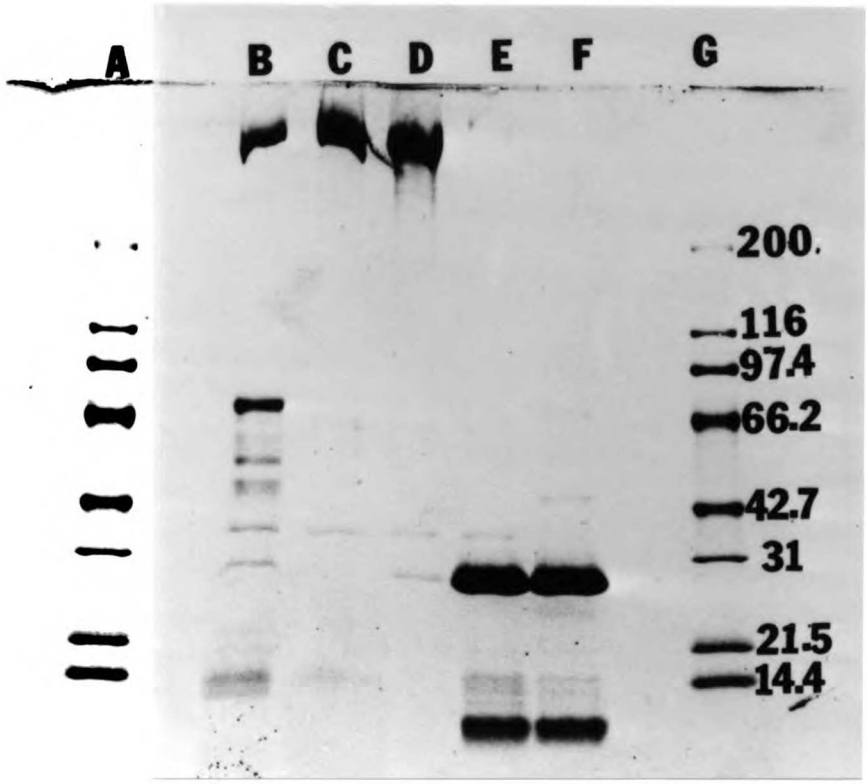
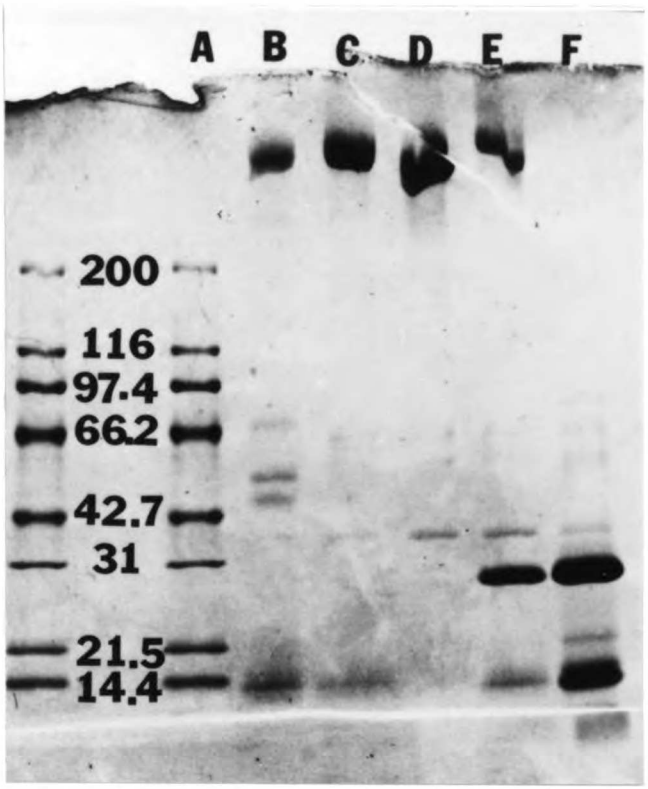

A
B
C
D
E
F
G
200.
116
97.4
66.2
42.7
31
21.5
14.4

Figure 31. SDS gradient electrophoresis. The apoproteins of the lipoprotein fractions from patient #1 were resolved on SDS gradient gels. The lipoproteins from left to right are: VLDL (B); IDL (C); LDL (D); HDL_2 (E); and HDL_3 (F). Marker proteins are displayed for comparison (A)--molecular weights equal the number shown multiplied by 1000.


A
B
C
D
E
F
200
116
97.4
66.2
42.7
31
21.5
14.4

Analytical Electrophoresis In Non-denaturing Gels

The apoA-I-containing lipoproteins isolated from plasma by immunosorption were subjected to electrophoresis in polyacrylamide gels under non-denaturing conditions (Figures 32 and 33). Post-staining of the gels with Coomassie Blue revealed discrete subspecies of apoA-I-containing lipoproteins. The four major species seen in the lower portion of the gels were present in both patients and controls. There appeared to be some slight differences in speciation among the lipoproteins seen in the upper portion of the gels, however, the differences can be seen within groups in addition to between groups. It can therefore be concluded that except for reductions in pre-beta HDL the absence of the small intestine does not affect the speciation of apoA-I-containing lipoproteins.

Figure 32. Electrophoresis of apoA-I-containing particles under nondenaturing conditions. The lipoproteins from controls 1-8 (left to right) were isolated by immunosorption. The gels were stained with Coomassie Blue after electrophoresis.

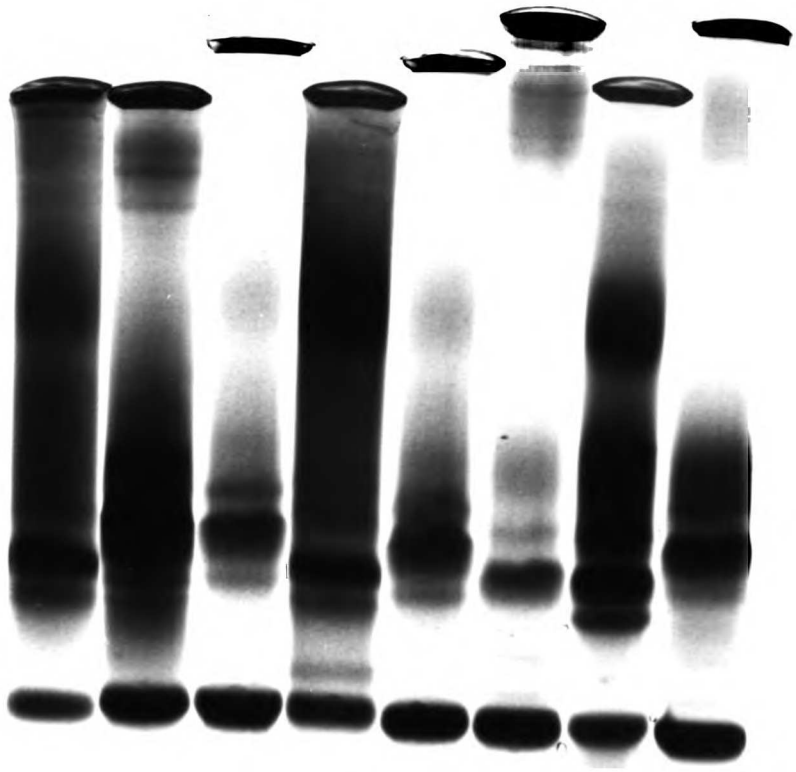


Figure 33. Electrophoresis of apoA-I-containing particles under nondenaturing conditions. The lipoproteins from patients 1-8 (left to right) were isolated by immunosorption. The gels were stained with Coomassie Blue after electrophoresis.

Effect of Infusion of Intralipid 10% on Serum Lipid, Apoprotein and Lipoprotein Profiles in Two Patients With Extensive Small Bowel Resection

Serum was also obtained from patients #3 and #8 ninety minutes after completion of a four hour infusion of Intralipid. Serum lipid and apoprotein compositions were determined as well as the concentrations of pre-beta and alpha HDL. Chemical compositions of the major lipoprotein classes were also determined.

Serum Lipid Composition

Table 30 indicates a dramatic shift in serum lipid profiles after the infusion of Intralipid 10%. In each patient, the triglyceride concentration was 6-7 times greater after the fat infusion than before the fat infusion. In patient #3, the triglyceride content rose from 140 to 994 mg/dl, and in patient #8 the concentration increased from 71 to 392 mg/dl. The phospholipid levels increased by 45%; from 240 to 442 mg/dl in patient #3 and from 141 to 228 mg/dl in patient #8. The total cholesterol for both patients remained the same. However, the free cholesterol concentration increased while the esterified cholesterol concentration decreased. This effect was observed in both patients, but was more pronounced in patient #3 where the free cholesterol rose from 54 to 59 mg/dl and the esterified cholesterol dropped from 105 to 91 mg/dl.

Table 30. Lipid Composition of Serum from Patients #3 and #8 Before and After Intravenous Infusion of Intralipid

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
$P3_{-IL}$	159*	105	54	140	240
$P3_{+IL}$	150	91	59	992	442
$P8_{-IL}$	120	90	30	71	141
$P8_{+IL}$	125	85	40	392	228

*All values reported in mg/dl.

Serum Apoprotein Determinations

The serum apoprotein A-I, B, and E concentrations before and after infusion of Intralipid 10% are reported in Table 31. There was no change in the apoA-I or apoE concentrations of either patient. The apoA-I of patient #3 was 155 mg/dl before Intralipid 10% and 160 mg/dl after the infusion of fat. Similarly, patient #8 had an apoA-I level of 84 mg/dl before and 87 mg/dl after the fat emulsion. In patient #3, the apoE content was 9.1 before and 8.6 after Intralipid 10%. In patient #8, the apoE concentration was 2.0 mg/dl before and 2.2 mg/dl after the fat infusion. However, by radial immunodiffusion there was an apparent decrease of almost 50% in the already depressed level of apoB; from 60 to 32 mg/dl in patient #3, and from 46 to 25 mg/dl in patient #8. It was possible that Intralipid infusion caused a masking of the antigenic sites of the protein and not allowed them to interact with the antiserum, leading to an apparent decrease in apoB concentration. This hypothesis was investigated by employing another technique to measure the apoB-100. Immunonephelometric determination of apoB-100 in serum of both patients was the same before and after Intralipid infusion. In patient #3 the levels of apoB were 58 mg/dl before and 56 mg/dl after the infusion. In patient #8 the apoB concentrations were 46 mg/dl before and 48 mg/dl after infusion of Intralipid.

Table 31. Apoprotein A-I, B, and E Concentrations in Serum of Patients #3 and #8 Before and After Intravenous Infusion of Intralipid

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
P3 Before Intralipid	155*	60	9.1
P3 After Intralipid	160	58	8.6
P8 Before Intralipid	84	46	2.0
P8 After Intralipid	87	48	2.2

*All values reported in mg/dl.

Serum Pre-beta and Alpha HDL Determinations

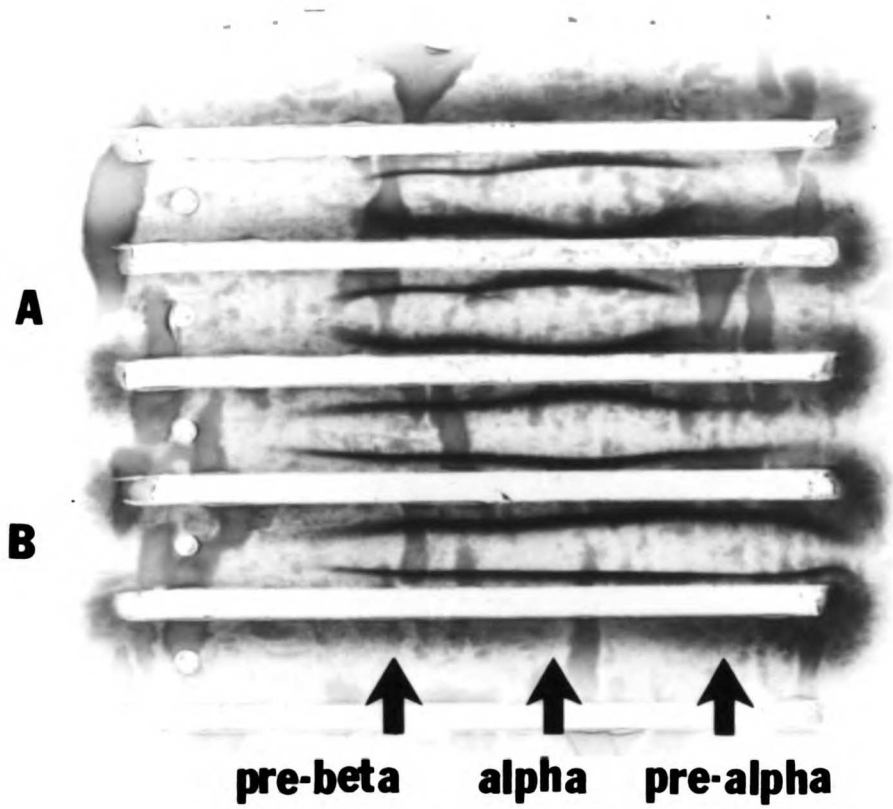
The levels of pre-beta and alpha HDL were also determined before and after Intralipid administration. As can be seen in Table 32, and is illustrated in Figures 34 and 35, the amount of apoA-I-containing lipoproteins (HDL) contributed by pre-beta HDL did not change in either patient after infusion of Intralipid 10%. In the serum of patient #3, there was 6.9% pre-beta HDL before and 7.0% after lipid infusion. In patient #8, there was 4.6% pre-beta HDL before and 4.8% after Intralipid infusion.

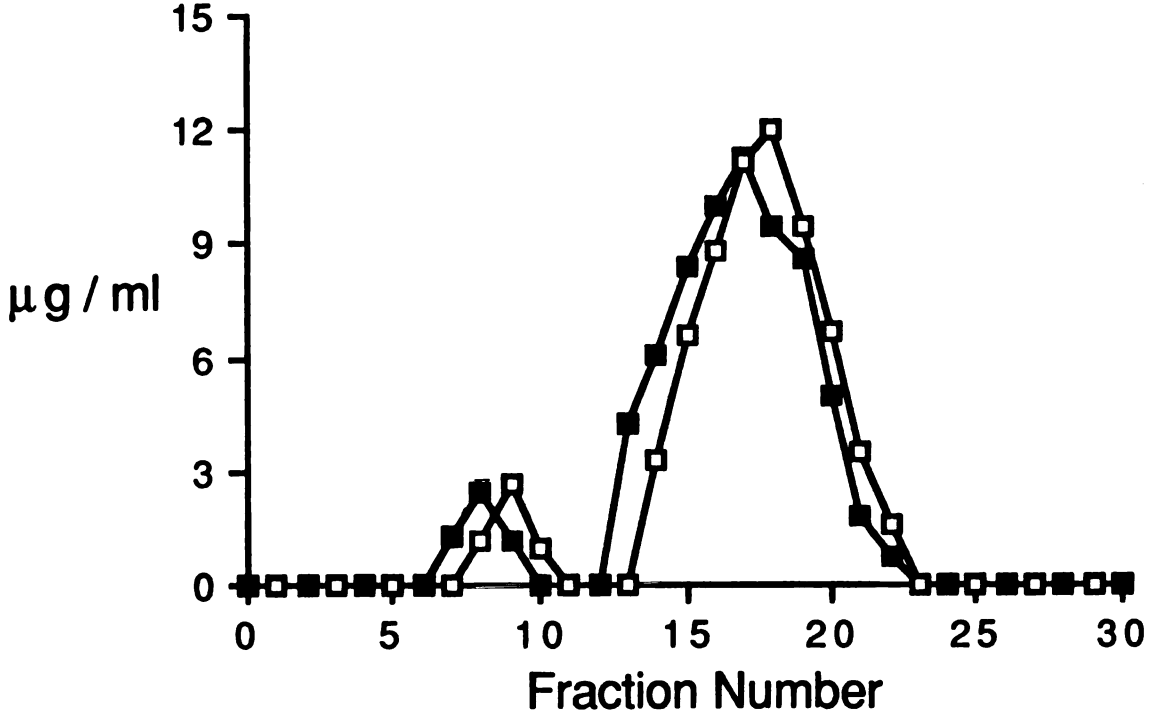
Immunoelectrophoresis of serum shows a difference in the distribution of apoA-I-containing lipoproteins before versus after the infusion of Intralipid (Figures 36 and 37). Before the Intralipid infusion, both patients have a normal separation of pre-beta and alpha HDL (sample A in both figures). After the infusion of fat, serum reacted with a monospecific antibody directed against apoA-I displays a pre-alpha immunoprecipitin arc in addition to the pre-beta and alpha immunoprecipitin arcs (sample B in both figures). This increase in migration is attributed to an increase in negative charge on the HDL. This may arise from the attachment of excess free fatty acids circulating in the plasma of patients receiving a bolus infusion of Intralipid (Wolfe et al.).

Table 32. Relative Percentages of Pre-Beta HDL and Alpha HDL in Serum of Patients #3 and #8 Berfore and After Intravenous Infusion of Intralipid

Subject	% Pre-Beta HDL	% Alpha HDL
P3 Before Intralipid	6.9	93.1
P3 After Intralipid	7.0	93.0
P8 Before Intralipid	4.6	95.4
P8 After Intralipid	4.8	95.2

Figure 34. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patients #3 before (closed squares) and after (open squares) an infusion of Intralipid was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation. The relative percent of pre-beta HDL did not change after the fat infusion.


A
B
pre-beta
alpha
pre-alpha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µg / ml
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20
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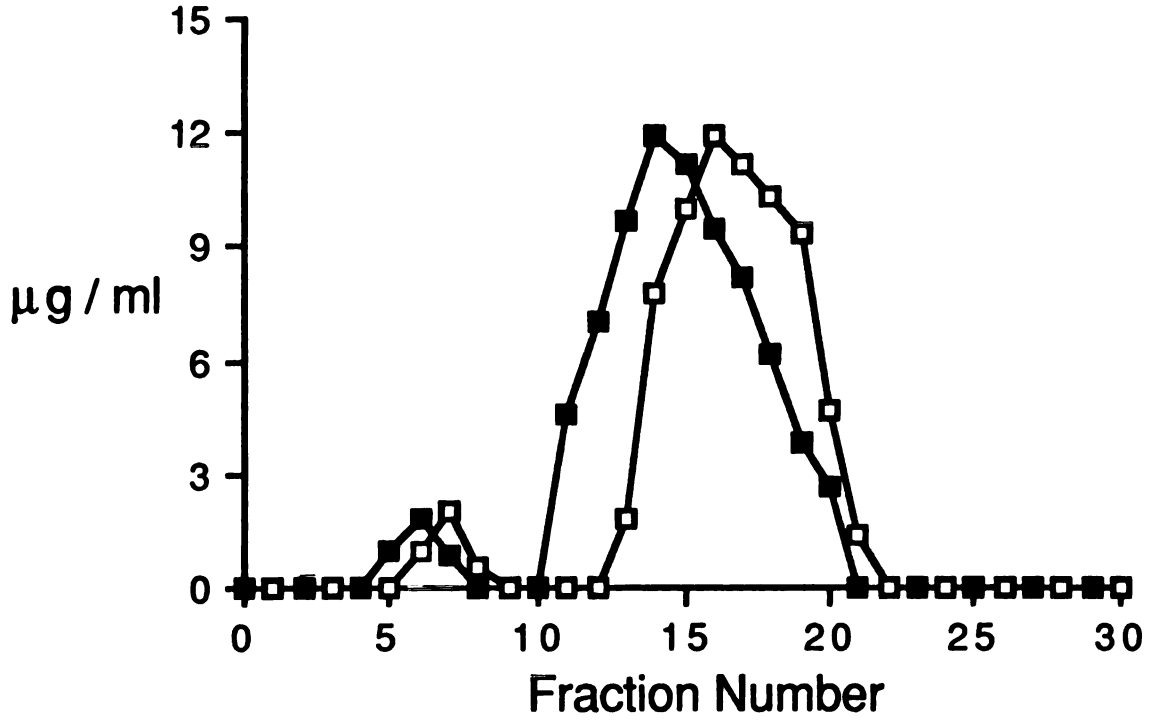
Fraction Number

Figure 35. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patients #8 before (closed squares) and after (open squares) an infusion of Intralipid was subject to electrophoresis on a starch block. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation. The relative percent of pre-beta HDL did not change after the fat infusion.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µg / ml
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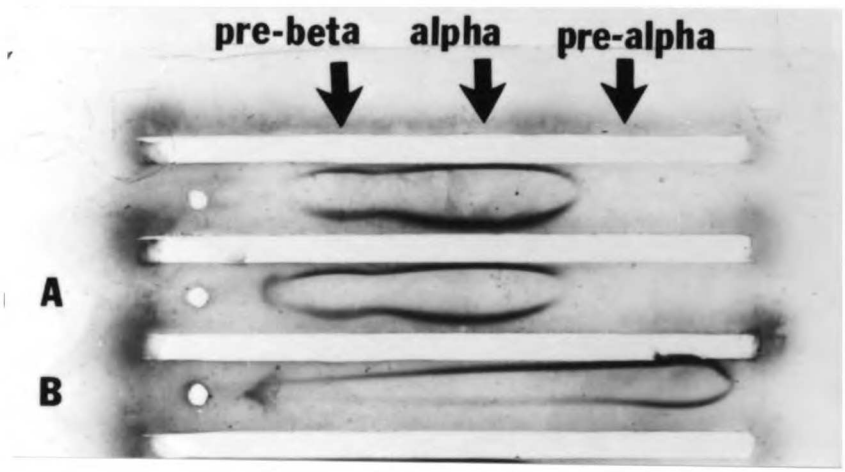
Fraction Number

Figure 36. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #3 before (A) and after (B) an infusion of Intralipid was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The immunoprecipitin arcs reveal an abnormal apoA-I distribution of a pre-beta (slow), and an alpha (fast) subpopulation which extends to a pre-alpha zone (faster).

Figure 37. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #8 before (A) and after (B) an infusion of Intralipid was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The immunoprecipitin arcs reveal an abnormal apoA-I distribution of a pre-beta (slow), and an alpha (fast) subpopulation which extends to a pre-alpha zone (faster).


pre-beta
alpha
pre-alpha
A
B

Chemical Composition of Serum Lipoproteins

The chemical compositions of the lipids of the five major density intervals before and after administration of Intralipid are reported in Tables 33 and 34. Similar changes occurred in each density interval of both patients.

Lipoproteins of the d<1.006 supernatant

In the Intralipid and lipoprotein mixture, the protein content remained the same in patients #3 and #8. The triglyceride concetration rose dramatically in each patient, from 41.3% to 53.4% in patient #3 and from 47.9% to 58.3% in patient #8. The phospholipid, free cholesterol and cholesteryl ester levels fell in both samples after the Intralipid infusion. In patient #3, the cholesteryl esters dropped from 16.8% to 11.4%, and the free cholesterol fell from 7.4% to 5.5%. In patient #8, the cholesteryl esters dropped from 13.5% to 9.4% and the free cholesterol fell from 9.3% to 6.7%. The phospholipid decreased from 25.9% to 22.2% in patient #3, and from 21.4% to 17.2% in patient #8.

IDL

There do not appear to be any significant changes in the IDL of either patient. The protein, phospholipid, and free and esterified cholesterol content remained the same in both patients. The triglyceride content was unchanged in patient #8, but increased slightly in patient #3.

LDL

There are two consistent changes in the LDL of each patient after the infusion of Intralipid 10%. First, the cholesteryl esters fell from 27.4% to 23.9% in patient #3 and from 38.8% to 32.5% in patient #8. Second, the triglycerides increased from 11.8% to 16.8%. In patient #3 and from 10.0% to 15.6% in patient #8. The percent of protein, free cholesterol and phospholipid remain unchanged in both patients after the Intralipid infusion. Infusion of a fat emulsion alters the core components of LDL so they become more triglyceride rich.

HDL_2

In the HDL_2 fraction, triglycerides increased after Intralipid in both patients, while cholesteryl esters decreased. Protein, free cholesterol, and phospholipids remained unchanged.

HDL_3

The changes which occurred in the HDL_2 fraction also occured in the HDL_3 fraction. Triglycerides increased after the Intralipid infusion and cholesteryl esters decreased. Again, protein, phospholipids and free cholesterol remained the same.

Table 33. Percent Chemical Composition of the Major Lipoproteins of Patient #3 Before and After Intravenous Infusion of Intralipid

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
$VLDL_{-IL}$	8.7*	16.8	7.4	41.3	25.9
$VLDL_{+IL}$	7.5	11.4	5.5	53.4	22.2
IDL_{-IL}	14.8	34.5	9.4	12.7	28.6
IDL_{+IL}	15.4	33.1	8.1	17.2	26.2
LDL_{-IL}	23.7	27.4	9.8	11.8	27.3
LDL_{+IL}	25.6	23.9	9.3	16.8	27.4
HDL_{2-IL}	41.6	13.5	2.3	8.9	33.7
HDL_{2+IL}	42.9	11.2	2.5	11.4	32.7
HDL_{3-IL}	55.2	12.6	3.3	7.8	21.1
HDL_{3+IL}	56.8	9.4	2.0	12.7	19.1

*All values expressed as percent dry mass.

Table 34. Percent Chemical Composition of the Major Lipoproteins of Patient #8 Before and After Intravenous Infusion of Intralipid

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
$VLDL_{-IL}$	7.9*	13.5	9.3	47.9	21.4
$VLDL_{+IL}$	8.4	9.4	6.7	58.3	17.2
IDL_{-IL}	20.2	27.7	9.1	23.4	19.6
IDL_{+IL}	19.5	28.2	7.0	24.9	20.4
LDL_{-IL}	19.4	38.8	11.8	10.0	19.9
LDL_{+IL}	21.6	32.5	10.9	15.6	19.4
HDL_{2-IL}	37.8	18.5	6.7	5.1	31.9
HDL_{2+IL}	40.0	15.9	6.6	7.7	29.4
HDL_{3-IL}	56.9	14.2	2.8	3.9	22.2
HDL_{3+IL}	54.2	12.8	2.6	6.6	23.8

*All values expressed as percent dry mass.

Effect of Infusion of Intralipid 10% on Serum Lipids, Apoproteins, and Lipoprotein Profiles in a Patient at Bowel Rest

During the course of this study I had the oppurtunity to study the serum lipids, apoproteins and lipoprotein profiles of a patient before and after surgical removal of all but 22 inches of her small intestine. At the beginning of the study, patient #1 had been at bowel rest for a year and relied soley on TPN for nutritional support. During the course of the study, patient #1 underwent surgery for massive ileal resection.

While the patient was at bowel rest, serum was obtained before and ninety minutes after a four hour infusion of Intralipid 10%. The results from this patient are expressed in Tables 35-38 and Figures 38 and 39.

Serum Lipid Composition

There was quite a difference in the serum lipid profile before versus after the Intralipid infusion (Table 35). Before the infusion, the total serum cholesterol concentration was 152 mg/dl, with esterified cholesterol contributing 82 mg/dl and free cholesterol 70 mg/dl. Serum triglycerides were 143 mg/dl and phospholipids were 280 mg/dl.

One and a half hours after the infusion of Intralipid, the total serum cholesterol was slightly higher at 162 mg/dl, but the esterified cholesterol decreased to 87 mg/dl and the free cholesterol increased to 75 mg/dl. Phospholipid content increased almost two-fold and triglyceride content more than doubled.

Table 35. Lipid Composition of Serum from Patient #1 at Bowel Rest Before and After Intravenous Infusion of Intralipid

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
Pl_{-IL}	152*	82	70	143	280
Pl_{+IL}	162	87	75	318	472

*All values reported in mg/dl.

Serum Apoprotein Determinations

Changes in serum apoprotein concentrations in the patient at bowel rest before and after the administration of Intralipid 10% are expressed in Table 36. The apparent apoB values decreased by fifty percent (56 mg/dl vs. 27 mg/dl) after the fat infusion, just as it did in the patients with extensive small bowel resection. However, immunonephelometric determination revealed that the concentration of apoB did not change after Intralipid infusion. The apoA-I and apoE levels increased; apoA-I rose from 78 mg/dl to 105 mg/dl and the apoE rose from 4.5 mg/dl to 10.4 mg/dl. These levels did not change in the patients with small bowel resection after the Intralipid infusion.

Table 36. **Apoprotein A-I, B, and E Concentrations in Serum of Patient #1 at Bowel Rest Before and After Intravenous Infusion of Intralipid**

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
P1 at Bowel Rest Before Intralipid	78*	56	4.5
P1 at Bowel Rest After Intralipid	105	54	10.4

*All values reported in mg/dl.

Serum Pre-Beta and Alpha HDL Determinations

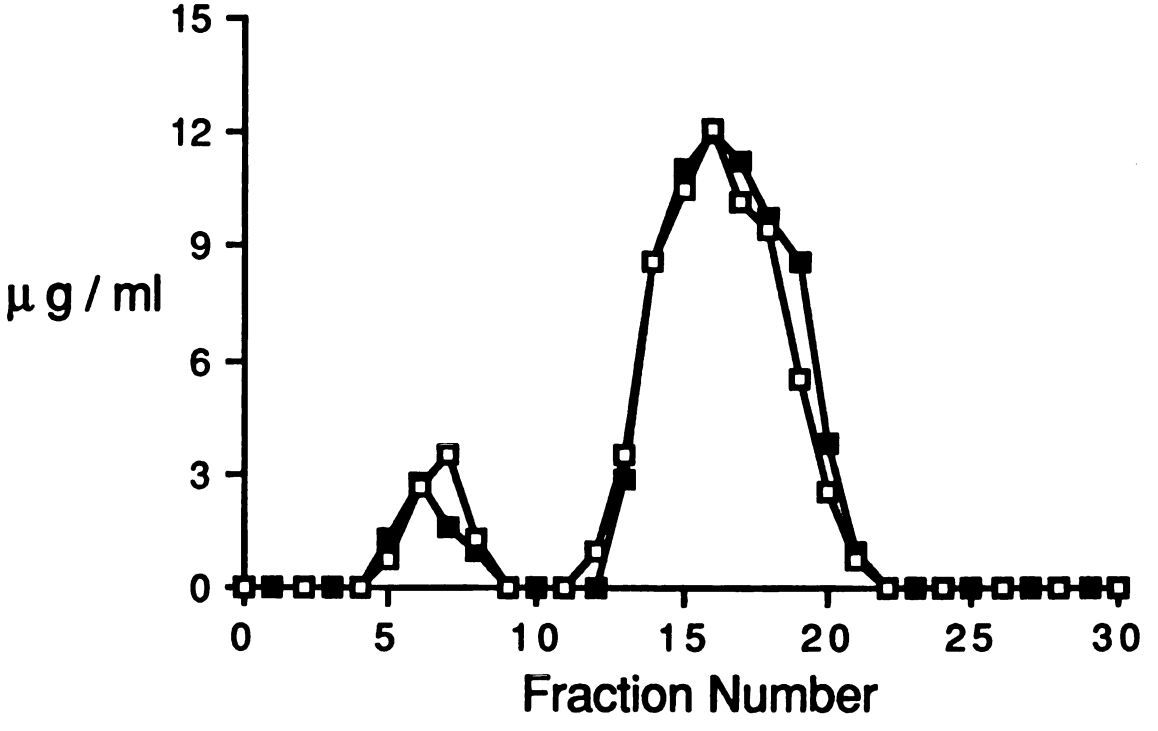
The relative percent of pre-beta HDL increased slightly after the administration of the fat emulsion in the patient at bowel rest (Table 37 and Figure 38). Before Intralipid 10% was infused, the pre-beta HDL accounted for 8.7% of the total HDL and alpha HDL for 91.3%. After the fat infusion, the pre-beta increased to 11.2% of the total HDL. These pre-beta values are more consistent with values determined for fasting controls rather than patients who have had surgical resection of their small intestine.

Immunoelectropheresis of serum of patient #1 at bowel rest showed the same migration pattern of apoA-I-containing lipoprotiens after the Intralipid infusion as the serum of patients with small bowel resection. Before the fat infusion, a normal pre-beta and alpha HDL distribution is shown in sample B, Figure 39. After the Intralipid infusion, reactivity of the monospecific antibody directed against apoA-I revealed a pre-alpha immunoprecipitin arc in addition to the pre-beta and alpha immunoprecipitin arcs.

Table 37. Relative Percentages of Pre-Beta HDL and Alpha HDL in Serum of Patient #1 at Bowel Rest Before and After Intravenous Infusion of Intralipid

Subject	% Pre-Beta HDL	% Alpha HDL
Pl_{-IL}	8.7	91.3
Pl_{+IL}	11.2	88.8

Figure 38. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 at bowel rest was subject to electrophoresis on a starch block before (closed squares) and after (open squares) an infusion of Intralipid. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation. The relative percent of pre-beta HDL increased with the administration of the fat emulsion.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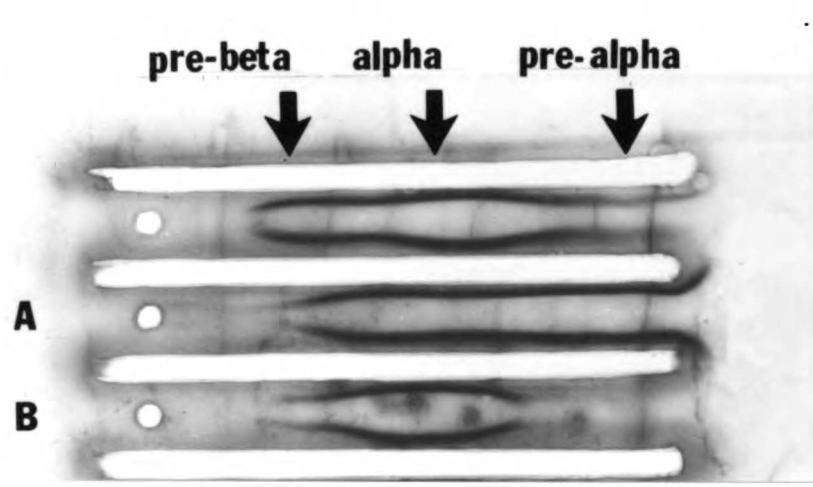
Fraction Number

Figure 39. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 at bowel rest before (A) and after (B) an infusion of Intralipid was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The immunoprecipitin arcs reveal an abnormal apoA-I distribution of a pre-beta (slow), and an alpha (fast) subpopulation which extends to a pre-alpha zone (faster).


pre-beta
alpha
pre-alpha
A
B

Chemical Composition of Serum Lipoproteins

The chemical composition of the major lipoproteins before and after fat infusion in patient #1, at bowel rest, are reported in Table 38. In the VLDL fraction the protein remained the same, while the cholesteryl ester, free cholesterol, and phospholipid decreased. The triglyceride content increased. There were no significant changes in the IDL fraction. The LDL protein remained constant but cholesteryl ester levels fell as free cholesterol levels rose after the Intralipid infusion. Triglycerides increased and phospholipids remained unchanged. The only changes in the HDL fractions were an increase in triglyceride content.

SDS Gradient Electrophoresis

The distribution of the apolipoproteins of this patient did not change with infusion of Intralipid. A unique characteristic of this patient is the presence of a protein of molecular weight appropriate for apoB-100 in the HDL_2. This apoB is present before and after the fat infusion. The presence of apoB in the HDL subfraction has been termed a "sinking" beta protein and is sometimes seen in patients with hyperthyroidism and in patients with high levels of Lp(a) lipoprotein.

Table 38. Percent Chemical Composition of the Major Lipoproteins of Patient #1 at Bowel Rest Before and After Intravenous Infusion of Intralipid

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phsopholipid
$VLDL_{-IL}$	12.0*	11.8	9.2	46.1	20.9
$VLDL_{+IL}$	11.6	8.3	5.0	57.5	17.6
IDL_{-IL}	25.1	14.6	10.3	30.8	18.6
IDL_{+IL}	25.4	12.6	9.1	32.5	20.4
LDL_{-IL}	21.4	26.7	11.4	14.5	26.0
LDL_{+IL}	20.6	20.0	12.4	22.1	24.9
HDL_{2-IL}	42.4	20.7	5.8	4.2	26.9
HDL_{2+IL}	41.1	19.1	5.0	7.2	27.6
HDL_{3-IL}	56.3	15.2	3.2	2.8	22.5
HDL_{3+IL}	55.1	13.4	2.8	5.2	23.5

*All values expressed as percent dry mass.

Changes in Serum Lipids, Apoproteins, and Lipoprotein Profiles Before and After Surgical Resection of the Small Bowel

The data obtained from patient #1 before and after surgical resection of all but 22 inches of her small bowel are reported in Tables 39-42 and Figures 40 and 41.

Serum Lipid Composition

Changes in serum lipid compositions before and after bowel resection are presented in Table 39. Total serum cholesterol concentration at bowel rest was 152 mg/dl, esterified cholesterol contributing 82 mg/dl and free cholesterol contributing 70 mg/dl. After bowel resection, total cholesterol fell to 100 mg/dl, with the contribution of esterified and free cholesterol becoming almost equal; 52 mg/dl and 48 mg/dl, respectively. Triglyceride and phospholipid values fell slightly, from 143 mg/dl to 122 mg/dl and 283 mg/dl to 174 mg/dl, respectively.

Table 39. **Lipid Composition of Serum from Patient #1 Before and After Surgical Resection of the Small Bowel**

Subject	Total Cholesterol	Esterified Cholesterol	Free Cholesterol	Triglyceride	Phospholipid
P1 Before Surgery	152*	82	70	143	280
P1 After Surgery	100	52	48	122	174

*All values reported in mg/dl.

Serum Apoprotein Determinations

The apoprotein data obtained after the bowel was removed was consistent with that reported for the other patients with small bowel resection (Table 40). The serum apoA-I and apoE concentrations fell slightly after resection of the bowel. At bowel rest, the apoA-I was determined to be 78 mg/dl and the apoE was 4.5 mg/dl. After surgery, the apoA-I was 72 mg/dl and the apoE was 3.3 mg/dl. The apparent apoB concentration decreased by more than fifty percent after surgery, from 56 mg/dl to 24 mg/dl.

Table 40. Apoprotein A-I, B, and E Concentrations in Serum of Patient #1 Before and After Surgical Resection of the Small Bowel

Subject	Apoprotein A-I	Apoprotein B	Apoprotein E
P1 Before Surgery	78*	56	4.5
P1 After Surgery	72	24	3.3

*All values reported in mg/dl.

Serum Pre-Beta and Alpha HDL Determinations

The relative percentages of pre-beta HDL determined for patient #1 before and after resection of the small bowel showed striking differences (Table 41 and Figure 40). At bowel rest, the pre-beta HDL contribution was 8.7%. However after surgery, this value dropped dramatically to 5.4%. While a pre-beta HDL value of 8.7% is consistent with average pre-beta values found in fasting controls, 5.4% pre-beta HDL is consistent with other patients who had extensive small bowel resection.

Immunoelectrophoresis of the serum of this patient before and after resection of the small bowel displays a normal pre-beta and alpha HDL distribution (Figure 41).

Table 41. **Relative Percentages of Pre-Beta HDL and Alpha HDL in Serum of Patient #1 Before and After Surgical Resection of the Small Bowel**

Subject	% Pre-Beta HDL	% Alpha HDL
P1 Before Surgery	8.7	81.3
P1 After Surgery	5.4	94.6

Figure 40. Starch block electrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 was subject to electrophoresis on a starch block before (closed squares) and after (open squares) extensive ileal resection. The block was sliced into 1-cm fractions and analyzed for apoA-I content by immunonephelometry. The apoA-I distributed into two peaks, a pre-beta (slow) and an alpha (fast) subpopulation. The relative percent of pre-beta HDL decreased with the resection of the small bowel.

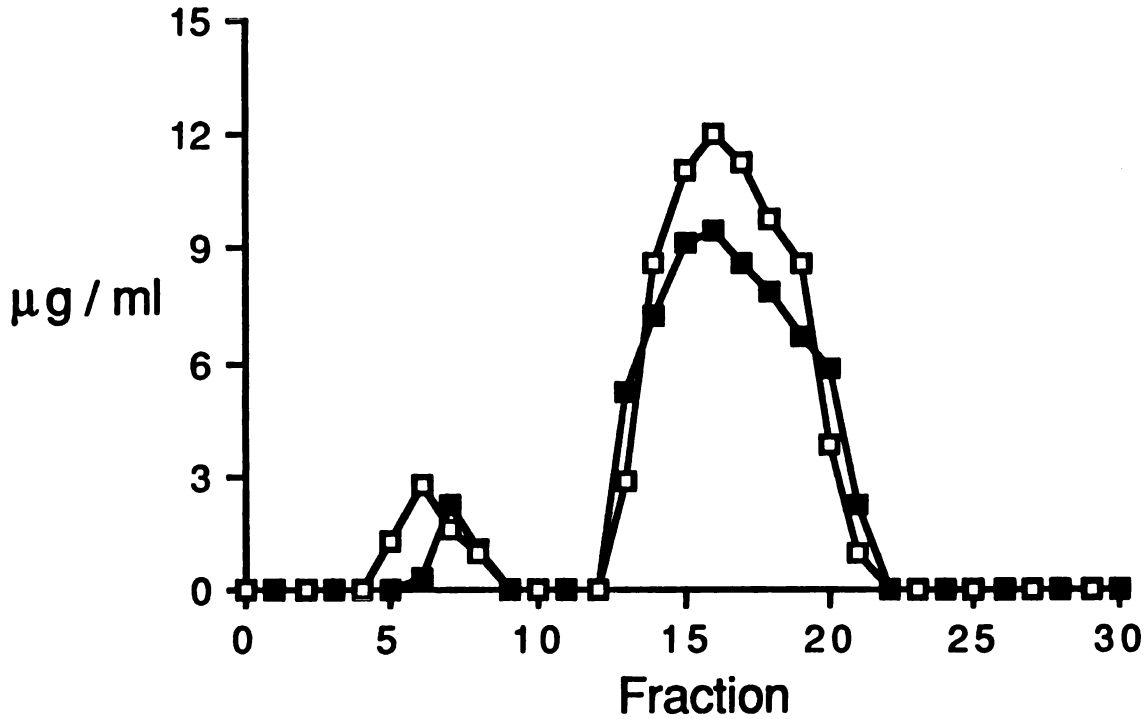

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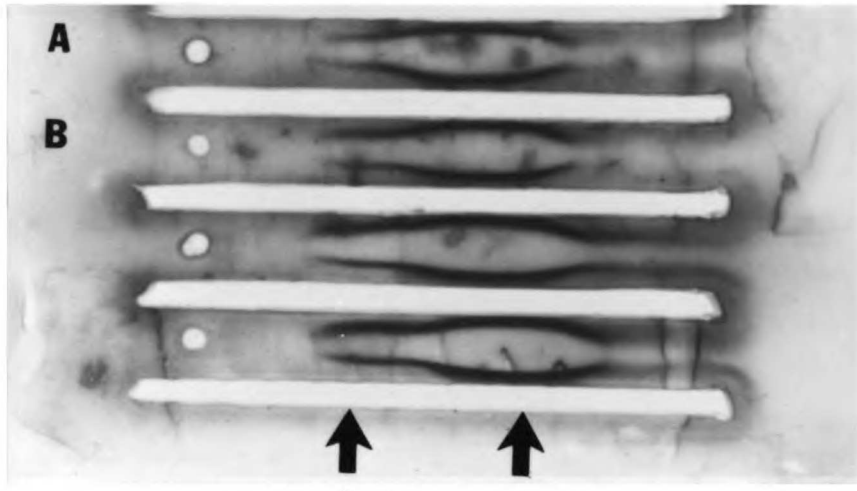
Figure 41. Immunoelectrophoresis of apoA-I-containing lipoproteins. Plasma from patient #1 before (A) and after (B) extensive ileal resection was electrophoresed in agarose then reacted with a monospecific anti-apoA-I antiserum. The presence of two immunoprecipitin arcs verify a normal apoA-I distribution of a pre-beta (slow), and an alpha (fast) subpopulation.


A
B
pre-beta
alpha

Chemical Composition of Serum Lipoproteins

The chemical composition of the major lipoproteins isolated before and after bowel resection are presented in Table 42. The VLDL and IDL remained unchanged with the exception of a slight increase in the phospholipid content. The only significant change in the LDL was a decrease in cholesteryl ester, from 27% to 23%. Protein, free cholesterol, phospholipids, and triglycerides remained the same. In HDL there was a decrease in protein and an increase in triglyceride. Cholesteryl ester, free cholesterol, and phospholipid contents remained unchanged.

Table 42. Percent Chemical Composition of the Major Lipoproteins of Patient #1 Before and After Surgical Resection of the Small Bowel

Subject	Protein	Cholesteryl Esters	Free Cholesterol	Triglyceride	Phospholipid
$VLDL_{bs}$*	12.0#	11.8	9.2	46.1	20.9
$VLDL_{as}$**	10.6	13.9	7.3	44.1	24.1
IDL_{bs}	25.1	14.6	10.3	30.8	18.6
IDL_{as}	23.7	14.2	9.2	30.8	22.1
LDL_{bs}	21.4	26.7	11.4	14.5	26.0
LDL_{as}	22.2	28.8	10.1	12.8	26.1
HDL_{2bs}	42.4	20.7	5.8	4.2	26.9
HDL_{2as}	40.9	19.4	6.1	7.9	25.7
HDL_{3bs}	56.3	15.2	3.2	2.8	22.5
HDL_{3as}	53.1	14.5	3.1	7.2	22.1

#All values expressed as percent dry mass.
*bs--before surgery.
**as--after surgery.

DISCUSSION

Investigation of the effects of extensive surgical resection of the small bowel on serum lipids and proteins

Extensive resection of the small intestine causes a reduction in total serum cholesterol levels. Patients' total cholesterol concentrations were twenty-five percent lower than controls'. The patients' free cholesterol concentrations remain similar to controls', however, their esterified cholesterol concentrations were thirty percent lower than control values. Depressed total serum cholesterol levels have recently been reported in patients at bowel rest by Badimon et al., but free and esterified cholesterol concentrations were not determined. Serum triglyceride and phospholipid levels do not appear to be affected by small bowel resection as these concentrations are the same in both patients and controls. Most of the triglyceride present in plasma is carried within VLDL. The normal fasting plasma triglyceride levels observed in patients in the present study who were in caloric balance indicate normal VLDL production.

While patients' total cholesterol concentration is lower than controls', it is not abnormally low. The decrease may be related to the fact that these individuals take little if any food by mouth and hence have no exogenous sterol input. It is well known that one's total serum cholesterol concentration can be lowered by limiting the amount of saturated fat in one's diet. Patients on long-term total parenteral

nutrition require minimal fat intake to insure that essential fatty acids are obtained.

Other mechanisms relating to decreased total serum cholesterol levels may be associated with decreased concentrations of VLDL. Indeed, concentrations of VLDL have been shown to decrease in patients receiving long-term TPN. Because VLDL is the precursor of LDL, the lipoprotein responsible for transporting the majority of cholesterol in plasma, it therefore follows that decreased concentrations of precursor may translate to decreased concentrations of end product. VLDL formed in the absence of food intake may be metabolized differently from "normal" VLDL and contribute another mechanism that would lead to decreased concentrations of LDL. Stalenhof et al. have shown not all VLDL are catabolized in the same manner. Larger size VLDL appear to removed by the liver, in a manner similar to chylomicron remnants, while only the smaller VLDL are further degraded to LDL.

Apoprotein Data

The major sites of synthesis of the apolipoproteins A-I, B, and E are liver and small intestine (Glickman et al. 1976a, Schaefer et al. 1978a, Windmueller et al. 1973, Wu et al., and Rachmilewitz et al. 1978). The mechanisms responsible for regulation of synthesis and secretion of these apoproteins have not yet been fully explored. However, it is known that in the small intestine, synthesis and secretion of apolipoprotein A-I can be stimulated by lipid absorption (Glickman et al. 1978, Hopf et al., and Schwartz et al.).

Patients with extensive small bowel resection who take litle, if any, food by mouth should theoretically have decreased plasma apoA-I levels. There is no stimulation of epithelial cells for apoprotein synthesis by an oral fat load and little surface area remaining with biosynthetic capacity.

Plasma apoA-I concentrations of patients in the present study with extensive small bowel resection were thirty percent lower than plasma apoA-I levels of controls. These results are in agreement with a recent study of individuals with intestinal dysfunction and were at bowel rest whose apoA-I levels were also thirty percent below values reported for a normolipemic control population (Badimon et al.). This decrease in total apoA-I levels may be directly related to the loss of use or resection of the small intestine. Because patients with intestinal failure as well as patients with small bowel resection have depressed plasma apoA-I levels, it could be the absence of lumenal stimulation, as well as the lack of the intestine which is responsible for the decline in apoA-I.

This hypothesis was tested in a study in which oral and intravenous fat were given to normal volunteers. Fat ingestion by healthy subjects caused serum apoA-I levels to rise (Koga). This increase in serum apoA-I levels in normal volunteers is not unexpected as increases in serum apoA-I concentrations after fat feeding has been reported by others (Tall et al., Alpers et al., and Schonfeld et al. 1978a). When the volunteers received the fat emulsions intravenously, serum apoA-I levels did not change. In these normal volunteers at bowel rest, the

apoA-I concentrations neither increased or decreased. In the present study, infusion of fat in patients with small bowel resection did not change their serum apoA-I levels. Therefore, in patients at bowel rest it appears that intestinal dysfunction is responsible for decreases in serum apoA-I concentrations rather than a lack of intralumenal stimulation. In patients with extensive small bowel resection, the actual loss of the bowel appears to cause reductions in serum apoA-I concentrations.

It is interesting that there is only a thirty percent reduction in plasma apoA-I levels in patients with small bowel resection because it has been reported that the intestine may produce up to fifty percent of the total apoA-I in plasma (Glickman et al. 1981, Rachmilewitz et al. 1979). The reasons for this may be two-fold. First, Windmueller and Wu (1981) have suggested that apo A-I synthesis occurs despite active triglyceride absorption. As these patients have eight to thirty inches of small bowel remaining, this portion of bowel may be responsible for synthesizing a small percentage of the total serum apoA-I. Second, the liver may compensate for the loss of input by the intestine and produce more apoA-I when intestinal function is impaired. Further studies are needed to determine whether this is the case.

Because the patient serum apoE concentrations were the same as the controls, it is evident that the loss of the small intestine does not affect the capacity of other tissues to synthesize and secrete this apoprotein. Tissues other than the intestine can thus supply the entire complement of apoprotein E.

The serum apoB-100 concentrations of patients with small bowel resection were significantly lower than controls. Although liver function tests were not performed for this study, it has been reported by others that long-term TPN does not impair liver function. However, removal of the small bowel prohibits the absorption of bile salts and consequently the rate of conversion of hepatic cholesterol to bile acids increases and a metabolic drain on the total body pool of cholesterol is established. This enhances receptor-mediated catabolism of LDL. Indeed, these patients have lower total serum cholesterol levels than controls. The depressed serum apoB-100 levels may be a reflection of decreased VLDL synthesis and secretion, or increased catabolism of the VLDL remnants by the liver. Increased uptake of the remnants by the liver prevents further degradation to LDL. Lower than normal apoB-100 concentrations in patients with extensive small bowel resection may also be a consequence of increased clearance of the LDL particles.

In the present study, there was a change in immunoreactivity in serum apoB-100 after infusion of Intralipid. Because the apoB in this study was measured by radial immunodiffusion, we cannot assume that there is less of this apoprotein in circulation after Intralipid infusion. In fact, this is not the case as the concentrations of LDL are the same before and after Intralipid. Moreover, when the serum apoB content was determined by immunonephelometry, the apoprotein concentrations did not change after the infusion of Intralipid. The apparent decrease in apoB may result from a masking of the antigenic determinant sites on the protein and render it unrecognizable by the antiserum.

After Intralipid infusion in patients with extensive small bowel resection, serum apoA-I and apoE concentrations did not change. These results contrast with reports of increases in serum apoA-I during alimentary lipemia (Tall et al., Schonfeld et al. 1978a).

Investigation of the effects of extensive surgical resection of the small bowel on serum lipoproteins

There are subtle changes in the chemical composition of lipoprotein fractions in which triglycerides are more abundant in the cores of these particles isolated from patients with extensive small bowel resection than in lipoproteins isolated from controls. The most dramatic changes occur in the LDL fraction, where it was determined that patients with extensive small bowel resection have smaller particle diameters than control subjects. This was first suggested by composition data, and was subsequently substantiated by particle sizing of LDL in electron photomicrographs.

The biological significance of a smaller LDL particle has yet to be elucidated, however it has recently been reported that small diameter LDL may be implicated in the atherogenesis of some inherited diseases. Heterogenity of LDL particle size has been reported in normal individuals as well as in patients with certain genetic diseases (Austin et al., Krauss et al., Shen et al., Teng et al., Fisher). Familial combined hyperlipidemia and hyperapobetalipoproteinemia are two diseases accompanied by elevations in plasma lipids (Goldstein et al. 1973), VLDL and/or LDL (Brunzell et al.), and an increased production of apoprotein

B (Chait et al. 1981, Kissebah et al.). Familial combined hyperlipidemia appears to be transmitted as a mendilian dominance with high penetrance (Goldstein et al., Brunzell et al.). Homozygosity for the trait may result in severe hypertriglyceridemia (Chait et al. 1980).

The marked elevation of apolipoprotein B-100 content of plasma is due to an appreciably increased rate of production of the apoprotein (Chait et al. 1981, Kissebah et al.). Though VLDL composition is normal, the particles tend to be smaller than normal. Some patients with hyperapobetalipoproteinemia and familial combined hyperlipidemia have more dense LDL than normal subjects (Teng et al.). These dense LDL were depleted of cholesteryl esters and enriched in apoB-100 and triglycerides. Denser LDL particles have been associated with hypertriglyceridemia raising the question as to whether hypertriglyceridemia per ce influences LDL diameters. The abnormalities in LDL composition, size and density may be the result of transfer and exchange of core constituents between VLDL and LDL.

While increased production rates of apoB may signal the underlying abnormality in these disorders, increased production rates of VLDL may also be involved (Chait et al. 1980, Janus et al). The hypertriglyceridemia observed in some patients may be due to increased plasma pools of VLDL. Normal catabolism of this larger VLDL population could lead to elevated levels of LDL observed in others. Regulation of secretion of VLDL, or apoB-100, or a metabolic pathway involved in the triglyceride production of the liver may be responsible for the primary

defect of these disorders. Whatever the cause, there is a significant predilection toward coronary artery disease among these patients.

In the present study, patients with extensive small bowel resection have been found to have smaller, more dense LDL particles. However, in these patients LDL of smaller size are present in the absence of hypertriglyceridemia.

The low total serum cholesterol levels, normal fasting triglyceride levels, and decreased apoprotein B and apoprotein A-I concentrations found in the patients with extensive small bowel resection are also characteristics which are found in patients with Anderson's disease (Levy et al. 1987, Roy et al., and Bouma et al.). Anderson's disease, also known as chylomicron retention disease, is further characterized by fat malabsorption and the inability to secrete chylomicrons after a fat meal. In patients with small bowel resection and patients with chylomicron retention disease, there are no circulating chylomicrons, and hence, no contribution by dietary fat to lipoporotein metabolism and homeostasis. In chylomicron retention disease there are low levels of LDL which are characterized by a decreased content of cholesteryl ester and an increased content of triglyceride (Levy et al. 1987). This is the same compositional change found in the LDL of patients in the present study.

Moreover, the LDL of patients with chylomicron retention disease are smaller particles than normal LDL. As is the case with patients with small bowel resection, the small LDL observed in chylomicron retention

disease are present in patients with normotriglyceridemia rather than hypertriglyceridemia. Thus smaller, more dense LDL are not necessarily a consequence of hypertriglyceridemia.

The lack of contribution of dietary cholesterol to the plasma lipids in patients with either disease may possibly lead to a decrease in cholesteryl esters available as core material for the lipoproteins. This may lead directly to enrichment of the core region by triglycerides and hence create a smaller, denser LDL particle.

Furthermore, because the total plasma cholesterol pool is reduced in patients with small bowel resection, there is a reduction in cholesterol flux with less LDL being delivered to the peripheral tissues. Connor et al. (1961) have shown that reductions of cholesterol in the diet leads to a decrease in total LDL cholesterol in plasma. It follows that patients with extensive small bowel resection, who receive no dietary cholesterol, should have decreased levels of LDL cholesterol. This could result from either a lower content of cholesterol per LDL particle or fewer particles. Perhaps a decreased flux of cholesterol via the LCAT pathway could result in a lower cholesterol content of VLDL which would lead to cholesterol-poor LDL.

The interruption of the return of bile acids to the liver may also play a role in determining the smaller size of LDL particles observed in patients with small bowel resection. In normal individuals and patients with hypercholesterolemia, administration of bile acid binding resins interrupts the biliary cycle and prevents return of the bile acids to

the liver. Witztum et al. (1985) determined that guinea pigs treated with cholestyramine, a bile acid sequestrant resin, have smaller LDL which are depleted of cholesteryl ester and phospholipid. Moreover, the compositional changes induced by the cholestyramine altered the metabolism of the smaller LDL such that its fractional catabolic rate was slower than normal LDL. Marked alterations in the composition of LDL also occur in man when bile acid binding resins are administered (Witztum et al. 1976 and Witztum et al. 1979) The LDL are smaller, more dense and have a decreased cholesterol:protein ratio.

The decreased diameter of the LDL in patients with extensive small bowel resection, coupled with the severely depressed concentrations of pre-beta HDL, which may play a role in reverse cholesterol transport, may possibly result in an increased risk for coronary heart disease.

Pre-beta and Alpha HDL

Recently we have identified a subpopulation of apoA-I-containing lipoproteins which possess pre-beta electrophoretic mobility in agarose gels (Kunitake et al. 1985). This pre-beta subpopulation of apoA-I-containing lipoproteins is protein-rich. The method used to visualize this subpopulation is immunoelectrophoresis of plasma which is subsequently reacted with an antiserum against apoA-I. Indeed, nearly 20 years ago Levy and Frederickson detected immunopricipitin arcs with pre-beta mobility in ultracentrifuged fractions using anti-HDL and anti-apo-A-I serum, respectively. Other researchers have noted HDL of

small particle size that contain only apoA-I (Schonfeld et al. 1978b, Albers et al.) but have not associated these HDL to the pre-beta HDL.

In addition to the unique composition of pre-beta HDL, the apoA-I has apparently assumed a conformation that is quite different from the apoA-I in the bulk of HDL. When analyzed by circular dichroism, the protein in pre-beta HDL contained 52% helix compared to 62% helix of alpha mobility HDL, implying that the apoA-I in pre-beta HDL is in a different conformation.

Pre-beta HDL also react differently from alpha migrating HDL with monoclonal antibodies against apoA-I. In liquid phase competition assays, pre-beta HDL were less capable of displacing ^{125}I labeled HDL from binding to selected monoclonal antibodies (Kunitake, La Sala, and Kane unpublished results), implying that some apoA-I epitopes are not exposed to the same extent on pre-beta HDL as they are on alpha HDL. In addition, we have found that the apoA-I in pre-beta HDL is more sensitive to proteolytic degradation than the apoA-I in alpha HDL. In fact, the proteolytic fragments of apoA-I are found predominantly in pre-beta HDL. The implication is that the apoA-I in pre-beta HDL is in a different conformation from the apoA-I in alpha HDL and is more susceptible to proteolysis. This finding is of interest because recently, Gregg et al. reported that proteolytically cleaved apoA-I is more rapidly removed from circulation than undegraded apoA-I, inferring that the more pre-beta HDL in circulation the greater the turnover of apoA-I. This could also explain the selective degradation of apoA-I

relative to HDL cholesterol in circulation observed by other investigators (Glass et al.).

The observed structural differences between pre-beta and alpha HDL apparently have led to differences in their biological activities. We have found that pre-beta and alpha HDL interact differently with liver membrane surfaces. The measured dissociation constant for the binding of pre-beta HDL to membranes is two orders of magnitude greater than that for alpha HDL, as determined by competition with labeled E-free HDL_3 for membrane binding, indicating that recognition sites on the ligand are masked or absent on pre-beta HDL, and that there probably is no interaction between pre-beta HDL and liver membranes on a physiological level (Kunitake et al. 1987).

Of further interest is our finding that pre-beta HDL are converted to alpha migrating HDL upon incubation at 37^{o} C (Kunitake, La Sala, and Kane unpublished results). This conversion appears to be dependent on the activity of lecithin-cholesterol acyltransferase (LCAT) because the addition of 1.4mM DTNB or 50mM menthol, inhibitors of LCAT, block the conversion. This finding, combined with reports of a similar HDL subspecies found in LCAT-deficient patients (Chen et al. 1984) and that HDL containing only apoA-I are necessary for cholesterol efflux (Fielding et al. 1981), suggests that pre-beta HDL provide a key function in the centripetal transport system.

With this in mind, the fact that patients with small bowel resection have fifty percent less pre-beta HDL than controls may be of

significance. There may be several factors influencing this dramatic decrease in pre-beta HDL. First, there is no exogenous fat entering the plasma of these patients and hence, no chylomicrons are formed. The average individual consumes 40-50% of his daily calories as fat, most of which circulates at least briefly in the bloodstream in chylomicrons. The amount of fat carried by chylomicrons in the plasma of typical Americans is three to four times greater than that carried by VLDL (Connor et al. 1982). If the formation of pre-beta HDL is directly related to the degradation of triglyceride-rich lipoproteins, the absence of chylomicrons removes the greatest portion of "precursor" particles available for the formation of pre-beta HDL. Thus, if pre-beta HDL are among the metabolic products of the catabolism of triglyceride-rich lipoproteins, the total plasma pool of pre-beta HDL would be expected to decrease in the absence of chylomicrons. Second, pre-beta HDL may similarly be formed from VLDL degradation. Though VLDL do not contain apoA-I, they may contribute phospholipid and free cholesterol to HDL. These hypotheses are substantiated by data obtained from patients with abetalipoproteinemia (Malloy, La Sala, Kunitake, and Kane unpublished results). These patients do not have any circulating chylomicrons, VLDL, or LDL. The pre-beta HDL concentrations in patients with abetalipoproteinemia were also fifty percent lower than concentrations found in control subjects. Lastly, the intestine may directly secrete pre-beta HDL. Hence resection of the bowel might result directly in diminished concentrations of this lipoprotein in plasma.

If it is true that pre-beta HDL play an important role in reverse cholesterol transport and its removal from circulation, then diminished quantities of this particle may put these patients at risk for atherosclerosis. Recent improvements in total parenteral nutrition have extended the lifespan of these patients such that this possible increased risk coronary artery disease may become significant.

There were other changes observed in the apoA-I-containing lipoproteins in plasma of patients who had received an infusion of Intralipid. In addition to depressed levels of pre-beta HDL, patients receiving Intralipid exhibited increased electrophoretic mobility of the apoA-I-containing lipoproteins. After Intralipid infusion, lipoproteins electrophoresed in agarose gels and reacted with a monospecific anti-serum directed against apoA-I exhibited different mobility than the lipoproteins of plasma obtained before Intralipid infusion. In addition to a pre-beta and alpha migrating immunoprecipitin arc (as seen in the pre-Intralipid plasma), a pre-alpha immunoprecipitin arc appeared.

This increase in electrophoretic mobility is apparently due to an increase in serum free fatty acids during the infusion of Intralipid. Plasma free fatty acid levels, from four normal human subjects infused with a soybean fat emulsion, have been shown to increase from 500 meq/l to over 2500 meq/l (Wolfe et al.). Abbott et al. have shown that free fatty acid levels also increase when fat emulsions are infused over a twenty-four hour period, but the electrophoretic mobility of lipoproteins was not addressed in this study. Increases in serum free fatty acids levels after infusion of a fat emulsion have also been

reported by others (Andrew et al., Friedman et al.). Increases in plasma triglycerides and free fatty acids associated with the infusion of fat emulsions may be the result of kinetic saturation of lipoprotein lipase and peripheral uptake of fatty acids.

It has been shown by Gordon (1955) and others that free fatty acids increase the electrophoretic mobility of serum lipoproteins when they are added directly to serum in vitro and in vivo by heparin-released lipoprotein lipase (Herbst et al., Laurell). The influence of free fatty acids on the mirgration rates of lipoproteins may be due to an interaction of positively charged groups on the lipoprotein surface or to an intercalation of the fatty acids into the surface monolayer, leaving their carboxylic groups partly dissociated. It has also been shown that lipoprotein lipase, when incubated with artificially produced lipemic serum, caused an increase in the electrophoretic migration of serum lipoproteins (Laurell). Moreover, when human albumin was added to the serum incubated with both lipoprotein lipase and serum incubated with free fatty acids, the migration rates of the lipoproteins returned to normal. Albumin binds free fatty acids in plasma and binds them with higher affinity than lipoproteins. However, it has been demonstrated that there is a critical concentration of albumin required to prevent excess free fatty acids from circulating in plasma. Once the free fatty acids to albumin ratio exceeds this crucial molar ratio, excess free fatty acids in the circulation may bind to lipoproteins and cause an increase in their electrophoretic mobility (Shafrir).

Further evidence for free fatty acids being responsible for the increased migration of the apoA-I-containing lipoproteins comes from data obtained from an experiment involving serum of a lipoprotein lipase deficient patient (Kunitake, La Sala, and Kane unpublished results). Serum from this patient was incubated with bovine milk lipase and electrophoresed in agarose then reacted with a monospecific antiserum against apoA-I. Unincubated serum without bovine milk lipase was run as a control. The unincubated serum of this patient exhibited a pre-beta and alpha HDL distribution seen in controls. The serum incubated with lipase showed the pre-beta and alpha migrating bands, as well as an immunoprecipitin arc with pre-alpha mobility. This increased migration was undoubtedly caused by free fatty acids liberated by the action of bovine milk lipase.

Speciation of apoA-I-containing lipoproteins

The speciation of apoA-I-containing lipoproteins does not appear to be affected by resection of the small intestine. As can be seen in native polyacrylamide gels, the distribution of the major species present in the lower portion of the gels (Figures 32 and 33) is the same in patients and controls. There are some slight variations present in the species found in the upper portion of the gels, but these variations are found within each of the two groups as well as between them. The presence of nearly all of the apoA-I-containing subspecies in patients with extensive small bowel resection indicates that these lipoproteins are not directly secreted as discrete species from the intestine but rather, speciation of HDL depends on secretion by other tissues or by

metabolic processing in plasma. There is evidence that the speciation of HDL is not a process which reaches equilibrium state and remains there, but rather is the consequence of thermodynamic determinants and kinetic transformations in plasma. Hence, other than decreased concentrations of pre-beta HDL, the speciation of HDL cannot be attributed to direct secretion by the intestine.

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