Title
Gene-Diet Interactions in Lipoproteins Metabolism

Authors
Dreon, D.M.
Krauss, R.M.

Publication Date
1992

LBL-32067
UC-000

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

To be published as a chapter in *Molecular Genetics of Coronary Heart Disease and Stroke*, A. Lusis, J. Rotter, and R. Sparkes, Eds., Karger Medical and Scientific Publishers, Basel, Switzerland, 1992

Gene-Diet Interactions in Lipoprotein Metabolism

D.M. Dreon and R.M. Krauss

March 1992

Donner Laboratory

Biology & Medicine Division

Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

DISCLAIMER

LBL 32067

GENE-DIET INTERACTIONS IN LIPOPROTEIN METABOLISM

Darlene M. Dreon, M.S., M.P.H., R.D. and Ronald M. Krauss, M.D.

Donner Laboratory
University of California

and

Life Sciences Division
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

Address Correspondence and Reprint Requests to:
Ronald M. Krauss, M.D.
Lawrence Berkeley Laboratory
University of California
Donner Laboratory, Room 465
One Cyclotron Road
Berkeley, CA 94720
(415) 486-4277

This work was supported by a grant from the National Dairy Promotion and Research Board, and was conducted at the Lawrence Berkeley Laboratory (Department of Energy contract DE-AC03-76SF00098 to the University of California).

OUTLINE

Gene-Diet Interactions in Lipoprotein Metabolism

I. Introduction

II. LDL Receptor in Cholesterol Homeostasis

- A. Plasma Cholesterol Level and Dietary Fat and Cholesterol Responsiveness

III. Apolipoprotein B

- A. Hypertriglyceridemia and Diet Responsiveness
- B. Apolipoprotein B and Diet Responsiveness

IV. Apolipoprotein E

- A. Apolipoprotein E and Diet Responsiveness

V. Defective HDL Metabolism

- A. Apolipoprotein A and C Polymorphism in Relation to Diet Responsiveness

VI. LDL Subclass Patterns

- A. Hyperinsulinemia
- B. LDL Heterogeneity and Diet Responsiveness

VII. Conclusion

GENE-DIET INTERACTIONS IN LIPOPROTEIN METABOLISM

I. Introduction

The study of genetic variations affecting lipoprotein metabolism has added to our understanding of genetic contributions to atherosclerosis. Although genetics plays an important role in CAD [1], single gene defects affecting lipoprotein metabolism which cause early CAD account for only a small percentage (5%) of coronary disease in the population [2, 3]. These include Type III hyperlipoproteinemia (apolipoprotein E mutation), familial defective apoB-100 (elevated apoB), and familial hypercholesterolemia (elevated LDL) [4]. These disorders are more important for elucidating mechanisms for lipoprotein metabolism and CAD rather than for public health intervention. However, genetic defects affecting proteins do relate to the variability of lipoprotein concentrations in the general population [5] and alter the lipoprotein transport pathways for fat and cholesterol metabolism [6, 7]. Diet also affects the levels of lipoproteins [8, 9], yet it has become apparent that the wide variation of lipid and lipoprotein values of individuals on the "average American diet"[10-20] is strongly influenced by genetic differences. For the population at large, current preventive efforts for improving lipoprotein profiles are aimed at reducing total fat intake (particularly saturated fat) and cholesterol and increasing the carbohydrate content of the diet. Yet on average, these efforts decrease total cholesterol levels 10-15% with initial reductions in dietary fat [21].

Hypo- or hyper-responsiveness to dietary cholesterol and saturated fat have been difficult to document because of intraindividual variability in the range of dietary modification employed [22]. Yet, individual metabolic characteristics in cholesterol metabolism do contribute to the regulation of total cholesterol levels. Some of these known mechanisms underlying

differences in dietary responsiveness have been reviewed by Grundy and Denke [9] and include factors associated with the production, transport, and secretion of lipoproteins. Individual genetic susceptibility to diet-induced changes in lipid and lipoprotein levels have also recently been considered. A threshold for cholesterol absorption and metabolism forms the evidence for genetic as well as environmental factors that are involved in cholesterol metabolism.

Following is apo brief summary of the genetic lipoprotein disorders that account for both premature CAD in families and lipoprotein heterogeneity in the general population. Accompanying the description of each lipoprotein disorder is a summary of the current evidence in humans for genetic-mediated responsiveness to dietary fat and cholesterol.

II. LDL Receptor in Cholesterol Homeostasis. The LDL-cholesterol level in the blood is controlled, in part, by LDL receptors located in cell membranes [23]. These LDL receptors recognize and bind to two protein-components of lipoproteins, apoB-100 and apoE [24].

At least thirty-four different mutations in the LDL-receptor gene have been identified [25]. A genetic mutation that affects the gene which encodes the receptor (occurring in 1/500 persons) impairs LDL receptor function and may result in elevated blood LDL levels [familial hypercholesterolemia (FH)] and increased risk of CAD [23]. However, FH patients show marked interindividual variation in clinical symptoms [26], indicating that factors other than this genetic mutation must influence the pathogenesis of CAD [27, 28]. One possible mechanism for this observed variation may be the existence of a second LDL receptor-related protein (LRP) which functions as a receptor for apoE-enriched lipoproteins [29], and which has been shown to prevent the accumulation of atherogenic chylomicron remnants and beta

VLDL [30, 31] in patients with homozygous FH (possessing two defective alleles) [32]. Normolipidemic FH heterozygotes (possessing one defective allele) have higher than usual fractional catabolic rates for LDL and low input of LDL apoB [33], which may explain normal concentrations of LDL-C. Also, the possible existence of a suppressor gene, which when inherited with the mutant LDL receptor gene, may explain the observation of normal LDL-cholesterol levels in FH heterozygotes [34]. Other candidate genes currently under investigation for LDL lowering in FH include apoC-III, hepatic lipase, and HMG-CoA reductase (controls sterol synthesis) [25]. Other factors thought to influence genetic expression of the LDL receptor defect include variation at the gene loci for apoB, Lp(a), and apoE as discussed below.

Genetic variation at the LDL-receptor gene locus has also been associated with differing plasma lipid levels in the normal population [35-37], explaining 9.6% of the population variance in LDL-C levels [37].

A. Plasma Cholesterol Level and Dietary Fat and Cholesterol Responsiveness. The relationship between changes in nutrient intake and changes in blood cholesterol is shown to be strongly influenced by the initial concentration of total cholesterol [38]. Figure 1 [38] shows that the usefulness of serum cholesterol as a marker of change in dietary fat intake in women depends, in part, on the distribution of cholesterol values in the population studied. The difference between predicted and observed change in serum cholesterol is greatest at the extremes of the cholesterol distribution. Thus, variation in study results of dietary influences on lipoprotein levels may be a result of the choice of study subjects.

Recent studies [39-42] have suggested that there may be a correlation between an individual's sensitivity to changes in dietary saturated fat intake and sensitivity to dietary cholesterol. From these studies it appears that

dietary sensitivity might be defined more precisely if individuals are first identified as responsive or unresponsive to dietary fat manipulation as well as normo- or hypercholesterolemic (Tables 1,2) [40]. The cholesterolemic response to dietary change is consistently individually determined and suggests fundamental differences in cholesterol metabolism. For instance, responsiveness or unresponsiveness to dietary cholesterol in humans is shown to be stable for at least 6 years [43]. This phenomenon may be important for studying genetically determined hypercholesterolemia for the majority of the population without single gene defects such as FH.

Miettinen and Kesaniemi [44] suggest that cholesterol absorption efficiency and absorbed dietary cholesterol regulate cholesterol synthesis and elimination in normal populations and are important determinants of within-population variation in the levels of total, LDL, and HDL cholesterol. In normal subjects, efficient intestinal cholesterol absorption down-regulates LDL-receptor mediated catabolism of LDL and enhances LDL synthesis [45]. The percentage decrease in LDL receptor activity correlates inversely with the percentage increase in LDL cholesterol; implying that high levels of dietary cholesterol down-regulate the LDL receptor. These changes in LDL reflect changes in the degree of saturation of the receptor-dependent component of LDL degradation [46]. In FH patients, however, cholesterol absorption and synthesis are inversely related and no association is observed between the efficiency of cholesterol absorption and total cholesterol levels [47] due to defective LDL-ApoB receptor.

McNamara et al. [48] conducted 75 studies in 50 normolipidemic men to examine the effects of dietary fat and cholesterol on lipid and lipoprotein levels, cholesterol absorption, and sterol synthesis. The range of intakes of dietary cholesterol tested were 159-905 mg/day and variations in

polyunsaturated to saturated fat ratio (P:S) were 0.20-2.37 within a diet supplying 35% of calories from fat. The results indicate that the change in dietary cholesterol has a marginal influence on lipid levels; while changes in dietary fat quality have a small but consistent effect on plasma cholesterol levels. About 20% of the subjects exhibited a decrease in total cholesterol on a high P:S diet, regardless of cholesterol intake. The lack of a significant dietary cholesterol effect on total cholesterol levels in most subjects was attributed to a reduction in the absorption of exogenous cholesterol and the feedback suppression of endogenous cholesterol synthesis. The results also demonstrate that the degree of saturation of dietary fat has no effect on the feedback response to dietary cholesterol. Additionally, the response to dietary fat and cholesterol were highly individualized; the thirty-one percent of the subjects who lacked feedback control of endogenous cholesterol synthesis may benefit from dietary modification.

It has been well documented that hepatic LDL receptors regulate cholesterol synthesis in the liver in accordance with the influx of dietary cholesterol, yet it has not been clear if there is also an effect on intestinal cholesterol synthesis. Current available data, however, do support a role of dietary cholesterol in the regulation of cholesterol synthesis in the intestine (reviewed by Field [49]). Cholesterol synthesis (and/or esterification) in the intestine is shown to be regulated by the cholesterol requirements of the cell [49]. For instance, during influx of cholesterol and certain fats into the intestine, cholesterol synthesis decreases and esterification increases. Dietary fats are known to alter membrane fatty acid composition and promote lipoprotein secretion and cholesterol absorption. The exact mechanisms for regulation of cholesterol synthesis in the intestine, however, are unknown.

Other investigators have demonstrated metabolic compensation for changes in dietary fat and cholesterol [50, 51]. Craig et al. [50] demonstrated in vitro that the supply of lipid is a determinant of apolipoprotein synthesis and secretion by the liver, and that cholesterol may be of particular importance in the initiation of apolipoprotein synthesis. For example, cholesterol alone stimulates synthesis and secretion of Apos E and A-I as well as HDL formation. Fatty acids and cholesterol are both necessary, however, for the cell to synthesize apoB-containing lipoproteins. Additionally, Patsch et al. [51] demonstrated that dietary cholesterol increases the size of HDL, possibly reflecting an increase in reverse cholesterol transport.

III. Apolipoprotein B. Genetic variation of the principal ligand (recognition site) for the LDL receptor, apoB, is shown to predict atherosclerosis [52], and may contribute to serum cholesterol regulation in FH [53]. ApoB is also essential for the synthesis and secretion of chylomicrons and VLDL, in addition to serving as the ligand for the interaction of LDL with the LDL receptor [24, 54]. ApoB exists in two major forms: apoB-100 derived from the liver, and apoB-48 from the intestine [55]. Genetic disorders affecting apoB include: familial combined hyperlipoproteinemia and familial defective apoB-100.

Familial combined hyperlipidemia (elevated cholesterol and triglycerides) is another condition in which increased CAD risk may be related to distribution of IDL [30, 31, 56] and LDL [57] subsclasses, although its association with mutations in the apoB gene is still under question [58]. These patients have relatively more VLDL, LDL-III, and IDL than do unaffected controls [57]. In contrast, patients with familial hypertriglyeridemia (FHTG) have elevated VLDL, due to genetically low VLDL triglyceride removal, but normal or low LDL levels [59]. Again, the

basic genetic defects have not been identified in either of these two disorders, although recent findings have identified associations of DNA variation at the apoB gene locus with VLDL levels and CAD [60], and an association between FCHL and variation within the apolipoprotein AI-CIII-AIV gene cluster [61].

Elevated serum apoB levels in **familial defective apoB-100** are associated with elevated LDL levels and an increased risk of premature atherosclerosis [62, 63]. This altered protein, with only 32% of normal LDL-receptor binding activity, occurs at a frequency of about 1/500 in populations in the United States [64] where elevated LDL levels are common. Familial defective apoB-100 is apparently not, however, a major contributor to high LDL cholesterol levels in Finland, another population characterized by both elevated LDL levels and a high incidence of CAD [65]. Thus, elevated LDL in different populations may, in part, be due to different genetic defects.

Hyperapobetalipoproteinemia (hyperapoB) is also a familial lipoprotein disorder characterized by an increase in small dense LDL particles, and a delayed clearance of post-prandial triglycerides and free fatty acids. To date, defects in the apoB gene do not appear to explain the hyperapoB phenotype. The activity of three proteins involved in triglyceride acylation and cholesterol esterification are being investigated in relation to the pathophysiology of hyperapoB [66].

Recent data suggests that variation in the apoB gene also influences the metabolism of LDL in the normal population, and that the variation in LDL metabolism differs by ethnic background [67]. For example, variation in the apoB gene in subjects from the United Kingdom and Finland was shown to affect the rate of LDL catabolism, which is not observed in subjects from Italy and Spain. The major observed effect of apoB variation in a South African

population is on the rate of LDL synthesis. These findings may help to explain different population responses to dietary manipulation.

A. Hypertriglyceridemia and Diet Responsiveness.

Hypertriglyceridemic patients treated with fish oil [n-3 fatty acids (FA)] show consistent reductions in total and VLDL-triglyceride levels [68] caused primarily by an inhibition of VLDL-triglyceride synthesis [69]. However, LDL response during n-3 FA supplementation is much more variable [70] and seems to vary depending on differences in LDL and VLDL composition and metabolic behavior [71-73]. For example in FH treated with n-3 FA, LDL is decreased [74] while LDL is increased in FHTG (Figure 2)[71], FCHL, mild hypercholesterolemia [75], and in patients with non-insulin dependent diabetes mellitus. This variability in response may be a result of the range of dose administered, but is most likely due to the heterogeneity of lipoprotein disturbances [72]. For instance, FHTG is characterized by an overproduction of VLDL triglyceride relative to LDL apoB and cholesterol levels [76]. FCHL, on the other hand, is associated with increased LDL apoB in the presence of small, dense LDL subsclasses [57].

Hypertriglyceridemic patients treated with high carbohydrate diets (60% energy) show consistent increases in plasma glucose, insulin, and triglyceride concentrations and reductions in HDL cholesterol levels [77]. Although high carbohydrate-low fat diets have been universally recommended to reduce CAD risk [21], these metabolic events in hypertriglyceridemic patients in response to carbohydrate feeding have been associated with enhanced risk of CAD [78]. These results point to the importance of learning more about the interaction of metabolic lipoprotein abnormalities with dietary intervention before uniform dietary recommendations are made.

B. Apolipoprotein B and Diet Responsiveness. Lipoprotein responses to an oral fat test were studied in hypertriglyceridemic patients with and without hyperapobetalipoproteinemia (HyperapoB) and in normal triglyceridemic controls with and without HyperapoB [79]. After the fat load, elevations in triglyceride and decreases in HDL_2 cholesterol levels were greater for all patients with HyperapoB. This study suggests that the measurement of LDL apoB may lead to the recognition of metabolic relationships previously unrecognized and provide a clue to interactions of diet with VLDL chylomicron and HDL_2 metabolism.

Genetic variation at the apoB gene locus was found to explain 6.3% of the phenotypic variance in apoA-I change in response to a low-fat diet (24.1% fat) [80]. One common apoB allele (M+) was associated with a reduction in apoA-I levels, whereas a less common apoB allele (M-) was associated with an increase in apoA-I levels in response to change in dietary fat intake.

IV. <u>Apolipoprotein E</u>. The role of genetic variation at the apoE locus in determining the interindividual variation in lipid and lipoprotein levels in populations is well documented [81-83] and also seems to be involved in influencing the genetic risk of atherosclerosis [84]. ApoE is a protein constituent of chylomicrons, chylomicron remnants, VLDL, IDL, and HDL. Its major physiologic role is to mediate the interaction of these lipoproteins with the LDL receptor and the chylomicron remnant receptor [85]. The three major allelic forms of apoE (apo E2, E3, and E4) create three homozygous phenotypes (apo E2/2, E3/3, and E4/4) and three heterozygous phenotypes (apo E3/2, E4/3, and E4/2). ApoE3 is the most common form of apoE and apo E4 and E2 are variants. Sing and Davignon [86] have reported that as much as 16% of the genetic variation of LDL cholesterol levels may be associated with these allelic differences in the apoE gene which have been shown to

effect LDL-apoB variability [87]. The average effects of the apoE alleles on apoB levels, however, vary in different populations [88].

The apoE4 allele predisposes to lower triglyceride and apoE concentrations, higher LDL cholesterol, and elevated LDL apoB [81, 82, 89-93] and occurs at a higher frequency in FH patients [94] and in patients with CAD [91, 95]. The apoE4 phenotype effect, however, is shown to be independent of LDL receptor status, although the magnitude of the effect is greatest in FH [96]. U.S. blacks [97] and Finns, [98, 99] with high rates of CAD, have relatively lower frequencies of the apoE3 allele and higher frequencies of apoE4 than U.S. whites. Patients with Type V hyperlipoproteinemia (elevated chylomicrons and VLDL) also have a high apoE4 gene frequency which is 2.6-fold higher than the Finnish population [100].

The apoE2 allele is defective as a ligand for the receptor mediated clearance of chylomicron remnants, VLDL and IDL. Homozygote (apoE2/2) hypolipidemia population frequencies are about 1% and heterozygotes (apo E4/2 and E3/2) about 14%. Individuals who inherit the apoE2/2 genotype have reduced levels of plasma cholesterol due in part to impaired LDL formation. Approximately 5% of patients with apoE2/2 have an accumulation of chylomicron remnants and IDL and increased frequency of atherosclerotic cardiovascular disease (type III hyperlipoproteinemia). This disorder may be due to interaction of apoE2 with other genetic or environmental factors that act to increase plasma triglyceride levels. This clinical syndrome is similar to familial apoE deficiency [101].

The majority of people with the apoE2/2 phenotype, however, are normolipidemic and do not show atherosclerosis susceptibility. Thus, expression of type III hyperlipoproteinemia requires the interaction of a

known apolipoprotein genotype with additional genetic or environmental factors [102]. One such focus of gene-gene interactions on cholesterol levels is the interaction between genetic variation at the LDL receptor locus and the apoE allele [35, 103]. Environmental factors that could alter the composition of lipoproteins and modulate the binding of apoE with the LDL receptor, thus interfering with hepatic lipoprotein clearance, include age, gender, diet, and hormonal status [103] (see below).

The occurrence of dominant and recessive forms of apoE2/2 may also explain the variability of binding that occurs [103]. A recessive mode of inheritance caused by one apoE2 variant shows reduced expression of FD (no type III hyperlipoproteinemia), while a dominant mode of inheritance with increased expression of FD (as type III hyperlipoproteinemia) occurs in the case of another apoE2 mutant [103].

Individuals who are heterozygous for the apoE2 phenotype (apoE4/2, or apoE3/2) generally show a milder increase in chylomicrons and IDL, low LDL cholesterol, and no increased risk for CAD, unless an individual inherits the aforementioned apoE2 mutant which predisposes to FD [104]. Additionally, the combination of endogenous hypertriglyceridemia and apoE3/2 phenotype is associated with increased VLDL-C levels [96]. Two other genetic apoE variants (apoE1 and apoE5) have also been described [105-107] which lead to FD, again with variable expression of type III hyperlipoproteinemia.

A. Apolipoprotein E and Diet Responsiveness. Most of the studies conducted on genetic-diet interactions and lipoprotein response have evaluated the influence of apoE isoforms as a genetic determinant of lipoprotein levels. The lipoprotein response to diet-induced weight gain is shown to be related to apoE isoform. Gueguen [108] demonstrated in 158

families in France that in conjunction with longitudinal weight gain, individuals with an apoE4 allele show a larger increase in triglyceride and beta lipoprotein levels compared with individuals with no apoE4 allele. It is also shown cross-sectionally, that obese subjects with apoE2 and/or apoE4 also have a higher frequency of hyperlipoproteinemia than non-obese or than obese subjects with the apoE3 phenotype [109]. However, in a different analysis of 63 premenopausal women, Pouliot et al. [110] demonstrated that the associations of body fatness and plasma lipoproteins usually seen cross-sectionally in the population are mainly accounted for by the high frequency of the apoE3 allele, as these associations are altered in the presence of apoE2 and E4 isoforms (Tables 3-5)[110]. Thus, the interaction between the apoE locus and weight gain on change in lipoprotein levels needs further clarification.

Cobb et al. [111] analyzed the association of apoE polymorphism, gender, and dietary fat composition on plasma lipid levels in 67 subjects fed both a "Western Diet" and a "Therapeutic Diet." Dietary polyunsaturated to saturated fatty acid ratio (P:S) was a stronger predictor of lipid concentrations than apoE phenotype, gender, or the latter two combined, although all these factors independently influenced lipoprotein levels. However, in combination with a "Therapeutic Diet," apoE polymorphism has a stronger influence on lipid levels than gender. Finally, an apoE4 isoform was associated with an increase in LDL cholesterol following both the low and high P:S diets.

Tikkanen et al. [112] also demonstrated that apoE isoform-related differences and LDL cholesterol were due to differential responses to dietary lipids. Lipid response to high- and low-fat diets according to apoE phenotypes was analyzed in 110 individuals (56 men and 54 women). The changes in

total cholesterol concentrations were significantly influenced by the apoE phenotypes during exchange from high- to low-fat diet, the magnitude of which again appeared to be greater in subjects with the apoE4 phenotype (Table 6) [112]. Interestingly, it was noted that the correlation between cholesterol levels and apoE phenotype was not significant on the low-fat diet suggesting that the metabolic influence of the apoE phenotype may be dependent on the quantity of fat in the diet. The lack of correlation of apoE phenotype to total serum cholesterol levels has also been demonstrated in human umbilical cord blood where the newborn state provides a model to analyze the influence of the apoE polymorphism on lipoproteins independent of intestinal nutrition in the relatively constant *in utero* environment [113].

In 230 male subjects with hypercholesterolemia of the Helsinki Heart Study [114], apolipoprotein E polymorphism also influenced the serum cholesterol response to dietary intervention. Subjects with the E4 allele exhibited a greater reduction in serum total cholesterol and LDL-cholesterol than those without the E4 allele, while the changes in HDL-C and triglycerides were not influenced by E4 status. Both baseline serum total cholesterol and E4 phenotype independently predicted the reduction in cholesterol level. However, in contrast with other studies [44, 46] which suggest that the E4 allele is associated with a greater sensitivity to dietary manipulations, there were no differences in baseline levels of total cholesterol and LDL-cholesterol in subjects with and without the E4 allele.

In a recent study [80], Xu et al. also demonstrated that on a basal high fat diet (38.9% fat) the major effect of genotype on lipid traits was due to variation at the apoE gene locus, explaining 14.6% of the phenotypic variance in LDL-C levels and 12.7% of the variance in total cholesterol levels.

However, when 107 individuals switched to a low fat diet (24.1% fat) these effects of variation at the apoE gene locus on the phenotypic variation of LDL and total cholesterol levels disappeared.

Savolainen et al. [115] also showed that the absolute and percentage lipid changes on low- and high-fat diets were equal in 44 subjects (22 men and 22 women) with the common apoE phenotype E3/3 and in those homozygous and heterozygous for the E4 allele (E4/4 and E4/3). Significant gender differences were noted, however; the men responded to dietary change with greater changes in total cholesterol and LDL-C levels than women.

The significance of the association between a particular apoE allele and total cholesterol level in different populations may especially depend on the amount of fat consumed. For example, Finns who have a high frequency of the apoE4 allele, along with high fat diets, have high cholesterol levels, and high rates of CAD [98]. U.S. blacks with similar CAD risk factors as U.S. whites have some of the highest rates of CAD; they also have a high frequency of apoE4 allele compared to U.S. whites [97]. In contrast, Nigerian blacks, with the highest observed frequencies of apoE4 allele among world populations, have the lowest mean cholesterol level reported among studies of the cholesterol/apoE relationship [116]. Nigerians consume a diet that is lower in animal fat than that of U.S. blacks, and in addition, have marked variation in the alleles observed at the genetic loci of apoA-IV, C-II, and H compared to Caucasians [117]. Although the average effect of the apoE4 allele is to increase the cholesterol level in populations, the magnitude of this effect may be modulated by diet or other genetic-environmental interactions.

The mechanisms by which different isoforms of apoE regulate cholesterol concentrations have been explored [82, 118, 119]. Genetically,

intestinal cholesterol absorption efficiency has been related to apoE phenotype in both normal and FH subjects [120, 121], which may contribute to the variation in total cholesterol concentration. Subjects with the E2 allele absorb less cholesterol and those with the E4 allele absorb more cholesterol than individuals with the E3 allele (Figure 3) [120]. Apo E3 and E4 have a different lipoprotein particle distribution (lower density lipoproteins are relatively enriched with apoE4 and high density lipoproteins relatively depleted of apoE4 as compared to apoE3) which may account for the elevated total and LDL cholesterol levels seen in carriers of the E4 allele [119].

Hepatic lipoprotein clearance has also been related to apoE phenotype. Triglyceride-rich particles containing apoE2 are removed more slowly by the liver and apoE4 particles are removed more efficiently than the apoE3-containing particles, an observation not confounded by age, gender, or baseline diet (Figure 4) [118]. This rate of clearance of dietary fat among the apoE phenotypes is related to LDL receptor regulation and could therefore account for the allelic effect on LDL cholesterol levels in the population [118].

Despite the well established effect of different apoE phenotypes on total and LDL cholesterol levels in normal populations, the apoE polymorphism does not contribute to the variation in these levels in FH patients cross-sectionally or when treated with a low-fat diet, and may be masked by the deficiency of the LDL receptor in FH.

V. Defective HDL Metabolism. Defective HDL production (*familial hypoalphalipoproteinemia*), due to an inability to synthesize its major apolipoprotein, apoA-I, leads to premature CAD [122]. This homozygous apoA-I deficiency has a frequency of < 1/1,000,000 in the population [123]. Eleven variants in the apoA-I gene have been characterized [124]. For

instance, low HDL mass and HDL_2 subfraction due to a structural mutation in the apoA-I gene occurs in the disorders apoA-I_{Milano} [125], apoA-I Giessen, A-$I_{Marburg}$, and A-$I_{Munster2}$ [126] in which subjects have a partial LCAT deficiency [126-128](see below).

Heterozygotes for gene mutations of the apo A-I,-C-III, and -A-IV gene complex (disrupting apoA-I, C-III expression) have HDL levels 62% of normal [129] and also have a higher risk of CAD [130]. Variations in the apoA-IV gene have been shown to account for 11% of the total variability in HDL-cholesterol levels in studies from Austria [131]. In general, however, apoA-IV genetic variability contributes minimally to normal variation in lipids, lipoproteins, and apolipoproteins in the population [132]. Although these particular defects are rare (reviewed by Schaefer [133]), it is still not certain how common other types of apoA-I defects might be present in the general population. However, the apoA-I:apoB ratio has been shown to be the most consistent discriminator of CAD severity [62, 124, 134-137].

Plasma HDL can be separated into two subpopulations of apoA-I containing particles: those that contain apoA-II and those that do not. The proportion of apoA-I with apoA-II to apoA-I without apoA-II in CAD patients is comparable to that in healthy normolipidemics [138]. However, the particle size distribution of these two HDL subpopulations in CAD patients tend to differ significantly from those in healthy normolipidemics. A preponderance of small HDL particles is associated with CAD in a study of 17 patients with CAD and 27 normolipidemic controls [138]. Earlier studies have suggested, however, that both small and large HDL particles are inversely correlated with CAD risk [139, 140].

These human traits in ApoA-I variability phenotypically resemble a newly described *Ath-1* gene in the mouse which forms the genetic basis for

the differences in atherosclerotic lesion formation and HDL cholesterol levels among different strains [141]. The *Ath-1* gene appears to act by altering the catabolism of HDL in response to a high-fat diet, resulting in low HDL levels in susceptible mice strains.

A. Apolipoprotein A and C Polymorphism in Relation to Diet Responsiveness. In experiments by Schonfeld et al. [14], levels of apoA-I and B in subjects respond to variations in dietary cholesterol or P/S ratio, whereas levels of apo A-II, C-II, C-III or E do not. ApoA-I levels are highest in cholesterol-enriched diets. Zanni [18] also demonstrated an increase in apoA-I levels in test diets highest in saturated fat and cholesterol. In these studies, apoB levels varied consistently with changes in LDL-cholesterol concentrations.

The mechanism by which apoA-I regulates lipoprotein concentration has been explored. Within normal subjects, upon changing from a high to low intake of saturated fat and cholesterol, HDL-C and apoA-I levels decrease and are correlated with a decrease in apoA-I synthetic rate. In contrast, the differences in HDL concentrations between subjects on a high fat diet correlate with apoA-I fractional catabolic rate (FCR) defined as the rate at which labeled apoA-I disappears from plasma [142] (Figure 5) [142]. Differences in hepatic lipase levels between subjects appear to account for a large share of this intersubject variability in apoA-I FCR. This finding suggests that the intrasubject diet-induced decreases in HDL may not be metabolically equivalent to low HDL levels between people on a standard diet. Brinton et al. [143] further hypothesized that lipase activity and plasma triglycerides affect HDL composition which modulates FCR, in turn regulating intersubject HDL-C levels.

Others have explored the relationship of interindividual differences in lipoprotein response to diet as a function of apoC-II levels. In normolipidemic subjects, the fasting level of apoC-II is not associated with clearance of triglyceride after a fat load [144]. ApoC-II seems to be mainly involved in cholesterol metabolism through its association with HDL rather than VLDL.

VI. LDL Subclass Patterns. In most healthy people, the major LDL subclass is either LDL-I or LDL-II, while the smaller, denser LDL subclasses, LDL-III and LDL-IV, are present in very small amounts [145]. These LDL subclass patterns follow a dominant mode of inheritance [146] and are related to risk of myocardial infarction [147]. A recently identified genetic trait, termed atherogenic lipoprotein phenotype *(alp)* found to be present in up to 40% of the population, predisposes to production of small, dense LDL subspecies III and IV, and is associated with increased levels of triglycerides and reduced levels of HDL [148]. This phenotype, however, is not fully expressed until after age 20 in men and after menopause in women [146], which suggests that other genetic or environmental factors may interact to influence expression of *alp*. *Alp* may, however, be a marker for one or more genes which influence lipoprotein profiles that predispose an individual to increased risk of atherosclerosis.

A. Hyperinsulinemia. High fasting plasma insulin (hyperinsulinemia), an indicator of CAD [149], is another metabolic feature associated with an atherogenic lipoprotein profile (high triglycerides and low HDL-C). Hyperinsulinemia may reflect insulin resistence and be the cause or result of a constellation of associated metabolic factors, such as a high body mass index and/or abdominal adipose tissue (a high waist to hip girth ratio), hypertension, high triglycerides and LDL-III, or reduced HDL-C levels [150].

The possibility that the fatty acid composition of plasma and membrane lipids has a role in insulin resistence was investigated by Salomaa et al. [151]. In a cross-sectional study, increased intake of dietary saturated fats and subsequent higher proportion of saturated fatty acids in serum and membrane lipids was higher in subjects with impaired glucose tolerance and NIDDM than controls.

The possible enhanced lipolytic activity of abdominal adipose tissue may lead to higher plasma free fatty acids which in turn decrease both hepatic removal of insulin and insulin-stimulated glucose uptake by peripheral tissues [152]. Obesity has also been associated with increased activity of HTGL and decreased activity of LPL which in turn may also contribute to increases in triglycerides and reductions in HDL cholesterol levels. Hypertriglyceridemic subjects with normal glucose tolerance often have peripheral insulin resistence, but hepatic insulin sensitivity is preserved [153].

Individuals with the *alp* phenotype (predominance of LDL-III subclass) have a similar clinical profile as patients with hyperinsulinemia, namely, higher fasting triglycerides and lower HDL-C [154]. These lipid and lipoprotein associations with hyperinsulinemia have also been shown in a cross-sectional epidemiological study of 542 persons, independent of glucose intolerance, obesity, and hypertension [155].

B. LDL Heterogeneity and Diet Responsiveness. Small, dense LDL subclasses may be a genetic marker, tied to a gene whose expression affects lipoprotein response to dietary fat and carbohydrate. A low fat-high carbohydrate diet has been shown to induce expression of the *alp* trait in those who do not express it on a high-fat diet (Dreon et al. unpublished observations, 1991). Additionally, reduction of fat intake and substitution of carbohydrate in diets of individuals with the *alp* phenotype results in

aggravation of some of their metabolic abnormalities, namely increases in triglyceride and reductions in HDL cholesterol (Table 7; Dreon et al., unpublished observations). These responses are not observed in individuals who have more of the large, less dense LDL subsclasses I and II.

It is known that, in general, consumption of a high carbohydrate diet is associated with increases in plasma triglycerides and decreases in HDL-C [156, 157], which parallels the diet response seen in subjects with the *alp* trait. Many other studies have also demonstrated relationships between LDL particle size and triglyceride metabolism [158-160]. Additionally, studies of post-prandial lipidemia in normal individuals have suggested that HDL-C is also influenced by triglyceride metabolism through lipolytic enzymes [51]. For example, a possible mechanism for this response was observed in a diet study of thirteen normal male and female subjects where changes in plasma triglycerides and HDL-C seen with low fat-high carbohydrate feeding were associated with reductions in the activity of LPL [161]. Reductions in LPL acitivity may be the consequence of an increased insulin secretion associated with a high carbohydrate diet [161]. A decline in LPL activity with a low fat-high carbohydrate diet was also observed in subjects with *alp* (Campos et al. unpublished observations, 1991), which suggests that expression of the *alp* trait may be linked to reductions in LPL activity which accompanies dietary change.

The influence on insulin sensitivity of a high carbohydrate (55% carbohydrate, 20% fat) versus a high fat (31% carbohydrate, 50% fat) diet was tested in a group of eight normal subjects (three males and five females) [162]. Fasting blood glucose and serum insulin concentrations were unchanged on the two diets, as was the mean whole body glucose uptake (assessed by glucose clamps). A high carbohydrate diet did not enhance

insulin sensitivity yet affected lipoprotein metabolism significantly by reducing LDL-C and HDL-C, and increasing triglycerides.
However, in patients with insulin resistance [157, 163, 164], the hyperinsulinemia is accentuated when dietary carbohydrate is increased by 16% of total calories (from 40-56%) and dietary fat is decreased [163].

Although substitution of carbohydrate for saturated fat in the diet is the proposed remedy for high LDL-C levels, there is an associated negative influence resulting in reduced HDL-C and increased triglyceride levels. Substitution of monounsaturated fat for saturated fat has been reported to have a more favorable effect on lipoprotein risk profiles than exchanging carbohydrate for saturated fat, as the former increases the ratio of apoA-I to apoB and HDL to LDL [165, 166].

Recently, others have reported that substitution of dietary protein for carbohydrate significantly lowers plasma total cholesterol, LDL-C, and triglycerides while increasing HDL-C in hypercholesterolemic subjects which may enhance cardiovascular risk reduction obtained by restriction of dietary fat and cholesterol [167].

VII. Conclusion

As evidenced by this review, little is known regarding genetic-diet interactions and plasma lipoproteins. Relatively few genes and their phenotypic expression in response to diet have as yet been examined. Examples of other factors that need to be studied in lipoprotein transport pathways for fat and cholesterol metabolism include the role of lipoprotein and triglyceride lipases, cholesterol ester transfer protein, and polymorphisms in apolipoproteins. Additionally, the genes that control responsiveness to diet need to be identified. As more genetic information about lipoprotein

traits becomes available, it can play a role in more accurately assigning risk to those individuals who do or do not respond to diet therapy.

Successful dietary intervention for lipoprotein modification may in the future be based on identifying genetic markers whose effects we are attempting to modify. We now need to focus our attention on gene identification when looking at lipoprotein responses to diet, as an extension of the studies in diet and lipoproteins performed over the past several decades. The studies of the future will need to involve larger numbers of people. They will also need to include family units to identify the gene(s) and demonstrate genetic heterogeneity. Future research may lead to the development of diets that are tailored to an individual's need. Appropriate prescriptions can then be made with primary focus on prevention. Diet therapy for the high risk individual in preventive medicine may be different than the conventional public health approach to dietary modification. The optimal dietary strategy that will improve lipoprotein levels and reduce heart disease risk may not be the same for each type of genetic variant.

REFERENCES

1. Sing, C.F.; Moll, P.P.: Genetics of atherosclerosis. Annu. Rev. Genet. 24: 171-187 (1990).

2. Lusis, A.J.: Genetic factors affecting blood lipoproteins: The candidate gene approach. J. Lipid Res. 29: 397-429 (1988).

3. Goldstein, J.L.; Brown, M.S.: Familial hypercholesterolemia; in Scriver, C.R.;Beaudet, A.L.;Sly, W.S.; Valle, D., The Metabolic Basis of Inherited Disease; 6th edition ed. No. pp. 1215-1250 (McGraw-Hill Book Co., New York 1989).

4. Mahley, R.W.; Weisgraber, K.H.; Innerarity, T.L.; Rall, S., Jr.: Genetic defects in lipoprotein metabolism. Elevation of atherogenic lipoproteins caused by impaired catabolism. J.A.M.A. 265: 78-83 (1991).

5. Utermann, G.: Apolipoprotein polymorphism and multifactorial hyperlipidaemia. J. Inherited Metab. Dis. 11: 74-86 (1988).

6. Breslow, J.L.: Genetic basis of lipoprotein disorders. J. Clin. Invest. 84: 373-380 (1989).

7. Breslow, J.L.: Lipoprotein transport gene abnormalities underlying coronary heart disease susceptibility. Annu Rev Med 42: 357-371 (1991).

8. Kris-Etherton, P.M.; Krummel, D.; Russell, M.E.; Dreon, D.; Mackey, S.; Borchers, J.; Wood, P.D.: The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. J Am Diet Assoc 88: 1373-1400 (1988).

9. Grundy, S.M.; Denke, M.A.: Dietary influences on serum lipids in lipoproteins. J. Lipid Res. 31: 1149-1172 (1990).

10. Keys, A.; Anderson, J.T.; Grande, F.: Prediction of serum cholesterol responses of man to changes in fats in the diet. Lancet 2: 959-966 (1957).

11. Hegsted, D.M.; McGrandy, R.B.; Myers, M.L.; Stare, F.J.: Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17: 281-290 (1965).

12. Anderson, J.T.; Grande, F.; Keys, A.: Independence of the effect of cholesterol and degree of saturation of the fat on serum cholesterol in man. Am. J. Clin. Nutr. 25: 589-594 (1978).

13. Mistry, F.; Miller, N.E.; Laker, M.; Hazzard, W.B.; Lewis, B.: Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular homeostasis in man. J. Clin. Invest. 67: 493-502 (1981).

14. Schonfeld, G.; Patsch, W.; Rudel, L.L.; Nelson, C.; Epstein, W.: Effects of dietary cholesterol and fatty acids on plasma lipoproteins. J. Clin. Invest. 69: 1072-1080 (1982).

15. Jacobs, D.R.; Anderson, J.T.; Hannan, P.; Keys, A.; Blacktown, H.: Variability in individual serum cholesterol response to change in diet. Atherosclerosis 48: 1161-1165 (1983).

16. Katan, M.B.; Beynen, A.C.; De Vries, J.H.; Nobels, A.: Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol 123: 221-234 (1986).

17. Beynen, A.C.; Katan, M.B.: Hypo and hyperresponders to dietary cholesterol. An J Clin Nutr 43: 974-976 (1986).

18. Zanni, E.E.; Zannis, V.I.; Blum, C.B.; Herbert, P.N.; Breslow, J.L.: Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins, and apoproteins of normal women consuming natural diets. J. Lipid Res. 28: 518-527 (1987).

19. Bowman, M.P.; van Doren, J.; Taper, J.T.; Thye, F.W.; Ritchey, S.J.: Effect of dietary fat and cholesterol in plasma lipids and lipoprotein fractions in normolipidemic men. J Nutr 118: 555-560 (1988).

20. Grundy, S.M.; Vega, G.L.: Plasma cholesterol responsiveness to saturated fatty acids. Am. J. Clin. Nutr. 47: 822-824 (1988).

21. National Cholesterol Education Program Expert Panel: National Heart, Lung, and Blood Institute. Report of the National Cholesterol Education Program expert panel on detection, evaluation, and

treatment of high blood cholesterol in adults. Arch Intern Med 148: 36-69 (1988).

22. Beynen, A.C.; Katan, M.B.; van Zutphen, L.F.M.: Hypo- and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. Adv Lipid Res 22: 115-171 (1987).

23. Goldstein, J.L.; Brown, M.S.: The LDL receptor defect in familial hypercholesterolemia. Implications for pathogenesis and therapy. Med. Clin. North Am. 66: 335-362 (1982).

24. Mahley, R.W.; Innerarity, T.L.: Lipoprotein receptors and cholesterol homeostasis. Biochim. Biophys. Acta 737: 197-222 (1983).

25. Grundy, S.M.; Getz, G.: Aspen hepatic cholesterol and lipoprotein conference 1989. Report of a conference. J. Lipid Res. 31: 1327-1333 (1990).

26. Berg, K.: Role of genetic factors in atherosclerotic disease. Am. J. Clin. Nutr. 49: 1025-1029 (1989).

27. Scriver, C.R.: Nutrient-gene interactions:the gene is not the disease. Am. J. Clin. Nutr. 48: 1505-1509 (1988).

28. Hill, J.S.; Hayden, M.R.; Frohlich, J.; Pritchard, P.H.: Genetic and environmental factors affecting the incidence of coronary artery disease

in heterozygous familial hypercholesterolemia. Arteriosclerosis 11: 290-297 (1991).

29. Beisiegel, U.; Weber, W.; Ihrke, G.; Herz, J.; Stanley, K.K.: The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. Nature 341: 162-164 (1989).

30. Musliner, T.A.; Krauss, R.M.: Lipoprotein subspecies and risk of coronary disease. Clin. Chem. 34: B78-B83 (1988).

31. Krauss, R.M.: Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. Am. Heart J. 113: 578-582 (1987).

32. Kowal, R.C.; Herz, J.; Goldstein, J.L.; Esser, V.; Brown, M.S.: Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. Proc. Natl. Acad. Sci. USA 86: 5810-5814 (1989).

33. Vega, G.L.; Hobbs, H.H.; Grundy, S.M.: Low density lipoprotein kinetics in a family having defective low density lipoprotein receptors in which hypercholesterolemia is suppressed. Arteriosclerosis 11: 578-585 (1991).

34. Hobbs, H.H.; Leitersdorf, E.; Leffert, C.C.; Cryer, D.R.; Brown, M.S.; Goldstein, J.L.: Evidence for a dominant gene that suppresses

hypercholesterolemia in a family with defective low density lipoprotein receptors. J. Clin. Invest. 84: 656-664 (1989).

35. Pedersen, J.C.; Berg, K.: Interaction between low density lipoprotein receptor (LDLR) and apolipoprotein E (apoE) alleles contributes to normal variation in lipid level. Clin Genet 35: 331-337 (1989).

36. Schuster, H.; Rauh, G.; Kormann, B.; Hepp, T.; Humphries, S.; Keller, C.; Wolfram, G.; Zöllner, N.: Familial defective apolipoprotein B-100. Comparison with familial hypercholesterolemia in 18 cases detected in Munich. Arteriosclerosis 10: 577-581 (1990).

37. Humphries, S.; Coviello, D.A.; Masturzo, P.; Balestreri, R.; Orecchini, G.; Bertolini, S.: Variation in the low density lipoprotein receptor gene is associated with differences in plasma low density lipoprotein cholesterol levels in young and old normal individuals from Italy. Arteriosclerosis 11: 509-516 (1991).

38. Boyd, N.F.; Cousins, M.; Beaton, M.; Kriukov, V.; Lockwood, G.; Tritchler, D.: Quantitative changes in dietary fat intake and serum cholesterol in women: results from a randomized trial. Am. J. Clin. Nutr. 52: 470-476 (1990).

39. Katan, M.B.; Berns, M.A.M.; Glatz, J.F.C.; Knuiman, J.T.; Nobels, A.; deVries, J.H.M.: Congruence of individual responsiveness to dietery cholesterol and to saturated fat in humans. J. Lipid Res. 29: 883-892 (1988).

40. Clifton, P.M.; Kestin, M.; Abbey, M.; Drysdale, M.; Nestel, P.J.: Relationship between sensitivity to dietary fat and dietary cholesterol. Arteriosclerosis 10: 394-401 (1990).

41. Keys, A.: Diet and blood cholesterol in population surveys--lessons from analysis of the data from a major survey in Israel. Am. J. Clin. Nutr. 48: 1161-1165 (1988).

42. Kestin, M.; Clifton, P.M.; Rouse, I.L.; Nestel, P.J.: Effect of dietary cholesterol in normolipidemic subjects is not modified by nature and amount of dietary fat. Am. J. Clin. Nutr. 50: 528-532 (1989).

43. Beynen, A.C.; Katan, M.B.: Reproducibility of the variations between humans in the response of serum cholesterol to cessation of egg consumption. Atherosclerosis 57: 19-31 (1985).

44. Miettinen, T.A.; Kesäniemi, Y.A.: Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. Am. J. Clin. Nutr. 49: 629-635 (1989).

45. Applebaum-Bowden, D.; Haffner, S.M.; Hartsook, E.; Luk, K.H.; Albers, J.J.; Hazzard, W.R.: Down-regulation of the low-density lipoprotein receptor by dietary cholesterol. Am. J. Clin. Nutr. 39: 360-367 (1984).

46. Meddings, J.B.; Dietschy, J.M.: Regulation of plasma levels of low-density lipoprotein cholesterol: interpretation of data on low-density lipoprotein turnover in man. Circulation 74: 805-814 (1986).

47. Gylling, H.; Miettinen, T.A.: Absorption and metabolism of cholesterol in familial hypercholesterolaemia. Clin Sci 76: 297-301 (1989).

48. McNamara, D.J.; Kolb, R.; Parker, T.S.; Batwin, H.; Samuel, P.; Brown, C.D.; Ahrens, E.H., Jr.: Heterogeneity of cholesterol homeostasis in man. J. Clin. Invest. 79: 1729-1739 (1987).

49. Field, F.J.; Kam, N.; Mathur, S.N.: Regulation of cholesterol metabolism in the intestine. Gastroenterology 99: 539-551 (1990).

50. Craig, W.Y.; Nutik, R.; Cooper, A.D.: Regulation of apoprotein synthesis and secretion in the human hepatoma Hep G2. The effect of exogenous lipoprotein. J. Biol. Chem. 263: 13880-13890 (1988).

51. Patsch, J.R.; Prasad, S.; Gotto, A.M., Jr.; Patsch, W.: High density lipoprotein2: Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. J. Clin. Invest. 80: 341-347 (1987).

52. Tybjærg-Hansen, A.; Nordestgaard, B.G.: Apolipoprotein B genotypes predict atherosclerosis and hyperlipidemia. Circulation 82 Supp.III: III-621 (1990).

53. Aalto-Setala, K.; Tikkanen, M.J.; Taskinen, M.R.; Nieminen, M.; Holmberg, P.; Kontula, K.: XbaI and c/g polymorphisms of the apolipoprotein B gene locus are associated with serum cholesterol and LDL-cholesterol levels in Finland. Atherosclerosis 74: 47-54 (1988).

54. Brown, M.S.; Goldstein, J.L.: A receptor-mediated pathway for cholesterol homeostatis. Science 232: 34-47 (1986).

55. Lee, D.M.; Dashti, N.; Mok, T.: Apolipoprotein B-100 is the major form of this apolipoprotein secreted by human intestinal caco-2 cells. Biochem. Biophys. Res. Comm. 156: 581-587 (1988).

56. Krauss, R.M.; Lindgren, F.T.; Williams, P.T.; Kelsey, S.F.; Brensike, J.; Vranizan, K.; Detre, K.M.; Levy, R.I.: Intermediate-density lipoproteins and progression of coronary artery disease in hypercholesterolaemic men. Lancet 2: 62-66 (1987).

57. Krauss, R.M.; Albers, J.J.; Brunzell, J.D.: An apolipoprotein B-enriched low density lipoprotein subspecies in familial combined hyperlipidemia. Clin. Res. 31: 503A (1983).

58. Rauh, G.; Schuster, H.; Muller, B.; Schewe, S.; Keller, C.; Wolfram, G.; Zollner, N.: Genetic evidence from 7 families that the apolipoprotein

B gene is not involved in familial combined hyperlipidemia. Atherosclerosis 83: 81-87 (1990).

59. Sane, T.; Nikkila, E.A.: Very low density lipoprotein triglyceride metabolism in relatives of hypertriglyceridemia probands. Evidence for genetic control of triglyceride removal. Arteriosclerosis 8: 217-226 (1988).

60. Paulweber, B.; Friedl, W.; Krempler, F.; Humphries, S.E.; Sandhofer, F.: Association of DNA polymorphism at the apolipoprotein B gene locus with coronary heart disease and serum very low density lipoprotein levels. Arteriosclerosis 10: 17-24 (1990).

61. Wojciechowski, A.P.; Farrall, M.; Cullen, P.; Wilson, T.; Bayliss, J.D.; Farren, B.; Griffin, B.A.; Caslake, M.J.; Packard, C.J.; Shepherd, J.; Thakker, R.; Scott, J.: Familial combined hyperlipidaemia linked to the apolipoprotein AI-CIII-AIV gene cluster on chromosome 11q23-q24. Nature 349: 161-164 (1991).

62. Brunzell, J.D.; Sniderman, A.D.; Albers, J.J.; Kwiterovich, P.O., Jr.: Apoproteins B and A-I and coronary heart disease in humans. Arteriosclerosis 4: 79-83 (1984).

63. Ladias, J.; Kwiterovich, P.J.; Smith, H.H.; Miller, M.; Bachorik, P.S.; Forte, T.; Lusis, A.J.; Antonarakis, S.E.: Apolipoprotein B-100 Hopkins (arginine4019-->tryptophan). A new apolipoprotein B-100 variant in a

family with premature atherosclerosis and hyperapobetalipoproteinemia. J.A.M.A. 262: 1980-1988 (1989).

64. Innerarity, T.L.: Familial hypobetalipoproteinemia and familial defective apolipoprotein B100: Genetic disorders associated with apolipoprotein B. Curr Opin Lipidology 1: 104-109 (1990).

65. Hämäläinen, T.; Palotie, A.; Aalto, S.K.; Kontula, K.; Tikkanen, M.J.: Absence of familial defective apolipoprotein B-100 in Finnish patients with elevated serum cholesterol. Atherosclerosis 82: 177-183 (1990).

66. Kwiterovich, P.O., Jr.; Motevalli, M.; Miller, M.; Bachorik, P.S.; Kafonek, S.D.; Chatterjee, S.; Beaty, T.; Virgil, D.: Further insights into the pathophysiology of hyperapobetalipoproteinemia:. Clin. Chem. 37: 317-326 (1991).

67. Houlston, R.S.; Turner, P.R.; Lewis, B.; Humphries, S.E.: Genetic epidemiology of differences in low-density lipoprotein (LDL) cholesterol concentration: possible involvement of variation at the apolipoprotein B gene locus in LDL kinetics. Genet Epidemiol 7: 199-210 (1990).

68. Harris, W.S.: Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J. Lipid Res. 30: 785-807 (1989).

69. Harris, W.S.; Connor, W.E.; Illingworth, D.R.; Rothrock, D.W.; Foster, D.M.: Effects of fish oil on VLDL triglyceride kinetics in humans. J. Lipid Res. 31: 1549-1558 (1990).

70. Kinsella, J.E.; Lokesh, B.; Stone, R.A.: Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr. 52: 1-28 (1990).

71. Inagaki, M.; Harris, W.S.: Changes in lipoprotein composition in hypertriglyceridemic patients taking cholesterol-free fish oil supplements. Atherosclerosis 82: 237-246 (1990).

72. Schectman, G.; Kaul, S.; Kissebah, A.H.: Heterogeneity of low density lipoprotein responses to fish-oil supplementation in hypertriglyceridemic subjects. Arteriosclerosis 9: 345-354 (1989).

73. Mölgaard, J.; Von, S.H.; Lassvik, C.; Kuusi, T.; Olsson, A.G.: Effect of fish oil treatment on plasma lipoproteins in type III hyperlipoproteinaemia. Atherosclerosis 81: 1-9 (1990).

74. Friday, K.E.; Failor, R.A.; Childs, M.T.; Bierman, E.L.: Effects of n-3 and n-6 fatty acid-enriched diets on plasma lipoproteins and apolipoproteins in heterozygous familial hypercholesterolemia. Arteriosclerosis 11: 47-54 (1991).

75. Abbey, M.; Clifton, P.; Kestin, M.; Belling, B.; Nestel, P.: Effect of fish oil on lipoproteins, lecithin:cholesterol acyltransferase, and lipid transfer protein activity in humans. Arteriosclerosis 10: 85-94 (1990).

76. Chait, A.; Albers, J.J.; Brunzell, J.D.: Very low density lipoprotein overproduction in genetic forms of hypertriglyceridemias. Eur. J. Clin. Invest. 710: 17-22 (1980).

77. Liu, G.; Coulston, A.; Hollenbeck, C.; Reaven, G.: The effect of sucrose content in high and low carbohydrate diets on plasma glucose, insulin, and lipid responses in hypertriglyceridemic humans. J. Clin. Endocrinol. Metab. 59: 636-642 (1984).

78. Castelli, W.P.; Doyle, J.T.; Gordon, T.; Hames, C.B.; Hjortland, M.C.; Hulley, S.B.; Kagan, A.; Zukel, W.J.: HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation 55: 767-772 (1977).

79. Genest, J.; Sniderman, A.; Cianflone, K.; Teng, B.; Wacholder, S.; Marcel, Y.; Kwiterovich, P.J.: Hyperapobetalipoproteinemia. Plasma lipoprotein responses to oral fat load. Arteriosclerosis 6: 297-304 (1986).

80. Xu, C.F.; Boerwinkle, E.; Tikkanen, M.J.; Huttunen, J.K.; Humphries, S.E.; Talmud, P.J.: Genetic variation at the apolipoprotein gene loci contribute to response of plasma lipids to dietary change. Genet Epidemiol 7: 261-275 (1990).

81. Utermann, G.: Apolipoprotein E polymorphism in health and disease. Am. Heart J. 113: 433-440 (1987).

82. Davignon, J.; Gregg, R.E.; Sing, C.F.: Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8: 1-21 (1988).

83. Shriver, M.D.; Boerwinkle, E.; Hewett- Emmett, D.; Hanis, C.L.: Frequency and effects of apolipoprotein E polymorphism in Mexican-American NIDDM subjects. Diabetes 40: 334-337 (1991).

84. Miller, N.E.: Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am. Heart J. 113: 589-597 (1987).

85. Mahley, R.W.: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240: 622-630 (1988).

86. Sing, C.F.; Davignon, J.: Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. Am. J. Hum. Genet. 37: 268-285 (1985).

87. Pairitz, G.; Davignon, J.; Mailloux, H.; Sing, C.F.: Sources of interindividual variation in the quantitative levels of apolipoprotein B in pedigrees ascertained through a lipid clinic. Am. J. Hum. Genet. 43: 311-321 (1988).

88. Smit, M.; de Knijff, P.; Rosseneu, M.; Bury, J.; Klasen, E.; Frants, R.; Havekes, L.: Apolipoprotein E polymorphism in the Netherlands and its effect on plasma lipid and apolipoprotein levels. Hum. Genet. 80: 287-292 (1988).

89. Kobori, S.; Nakamura, N.; Uzawa, H.; Shichiri, M.: Influence of apolipoprotein E polymorphism on plasma lipid and apolipoprotein levels, and clinical characteristics of type III hyperlipoproteinemia due to apolipoprotein E phenotype E2/2 in Japan. Atherosclerosis 69: 81-88 (1988).

90. Boerwinkle, E.; Visvikis, S.; Welsh, D.; Steinmetz, J.; Hanash, S.M.; Sing, C.F.: The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglycerides in a sample of unrelated individuals. Am J Med Genet 27: 567-582 (1987).

91. Eto, E.; Watanabe, K.; Ishii, K.: Reciprocal effects and apolipoprotein E alleles (E2 and E4) on plasma lipid levels in normolipidemic subjects. Clin Genet 29: 477-484 (1986).

92. Eichner, J.E.; Kuller, L.H.; Ferrell, R.E.; Meilahn, E.N.; Kamboh, M.I.: Phenotypic effects of apolipoprotein structural variation on lipid profiles. III. Contribution of apolipoprotein E phenotype to prediction of total cholesterol, apolipoprotein B, and low density lipoprotein

cholesterol in the healthy women study. Arteriosclerosis 10: 379-385 (1990).

93. Eichner, J.E.; Kuller, L.H.; Ferrell, R.E.; Kamboh, M.I.: Phenotypic effects of apolipoprotein structural variation on lipid profiles. IV. Apolipoprotein polymorphism in a small group of black women from healthy women study. Genet Epidemiol 6: 681-689 (1989).

94. Eto, M.; Watanabe, K.; Chonan, N.; Ishii, K.: Familial hypercholesterolemia and apolipoprotein E4. Atherosclerosis 72: 123-128 (1988).

95. Kuusi, T.; Nieminen, M.S.; Ehnholm, C.; Yki, J.H.; Valle, M.; Nikkilä, E.A.; Taskinen, M.R.: Apoprotein E polymorphism and coronary artery disease. Increased prevalence of apolipoprotein E-4 in angiographically verified coronary patients. Arteriosclerosis 9: 237-241 (1989).

96. Dallongeville, J.; Roy, M.; Leboeuf, N.; Xhignesse, M.; Davignon, J.; Lussier-Cacan, S.: Apolipoprotein E polymorphism association with lipoprotein profile in endogenous hypertriglyceridemia and familial hypercholesterolemia. Arteriosclerosis 11: 272-278 (1991).

97. Kamboh, M.I.; Sepehrnia, B.; Ferrell, R.E.: Genetic studies of human apolipoproteins VI. Common polymorphism of apolipoprotein E in blacks. 7: 49-55 (1989).

98. Ehnholm, C.; Lukka, M.; Kuusi, T.; Nikkila, E.; Utermann, G.: Apolipoprotein E polymorphism in the Finnish population: gene frequencies and relation to lipoprotein concentrations. J. Lipid Res. 27: 227-235 (1986).

99. Lehtimaki, T.; Moilanen, T.; Viikari, J.; Åkerblom, H.K.; Ehnholm, C.; Rönnemaa, T.; Marniemi, J.; Dahlen, G.; Nikkari, T.: Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study. J. Lipid Res. 31: 487-495 (1990).

100. Kuusi, T.; Taskinen, M.-R.; Solakivi, T.; Kauppinen-Makelin, R.: Role of lipoproteins E and C in type V hyperlipoproteinemia. J. Lipid Res. 29: 293-298 (1988).

101. Schaefer, E.J.; Gregg, R.E.; Ghiselli, G.; Forte, T.M.; Ordovas, J.M.; Zech, L.A.; Brewer, H.B., Jr.: Familial apolipoprotein E deficiency. J. Clin. Invest. 78: 1206-1219 (1986).

102. Rall, S.C., Jr.; Newhouse, Y.M.; Clarke, H.R.; Weisgraber, K.H.; McCarthy, B.J.; Mahley, R.W.; Bersot, T.P.: Type III hyperlipoproteinemia associated with apolipoprotein E phenotype E3/3. Structure and genetics of an apolipoprotein E3 variant. J. Clin. Invest. 83: 1095-1101 (1989).

103. Innerarity, T.L.; Hui, D.Y.; Bersot, T.P.; Mahley, R.W.: Type III hyperlipoproteinemia: a focus on lipoprotein receptor-apolipoprotein E2 interactions. Adv Exp Med Bio 201: 273-288 (1986).

104. Smit, M.; de Knijff, P.; van der Kooij-Meijs, E.; Groenendijk, C.; van den Maagdenberg, A.M.J.M.; Gevers Leuven, J.A.; Stalenhoef, A.F.H.; Stuyt, P.M.J.; Frants, R.R.; Havekes, L.M.: Genetic heterogeneity in familial dysbetalipoproteinemia. The E2(lys146-->gln) variant results in a dominant mode of inheritance. J. Lipid Res. 31: 45-53 (1990).

105. Steinmetz, A.; Assefbarkhi, N.; Eltze, C.; Ehlenz, K.; Funke, H.; Pies, A.; Assmann, G.; Kaffarnik, H.: Normolipemic dysbetalipoproteinemia and hyperlipoproteinemia type III in subjects homozygous for a rare genetic apolipoprotein E variant (apoE1). J. Lipid Res. 31: 1005-1013 (1990).

106. Mann, W.A.; Gregg, R.E.; Sprecher, D.L.; Brewer, H.J.: Apolipoprotein E-1Harrisburg: A new variant of apolipoprotein E dominantly associated with type III hyperlipoproteinemia. Biochim. Biophys. Acta 1005: 239-244 (1989).

107. Ordovas, J.M.; Litwack-Klein, L.; Wilson, P.W.F.; Schaefer, M.M.; Schaefer, E.J.: Apolipoprotein E isoform phenotyping methodology and population frequency with identification of apoE1 and apoE5 isoforms. J. Lipid Res. 28: 371-380 (1987).

108. Gueguen, R.; Visvikis, S.; Steinmetz, J.; Siest, G.; Boerwinkle, E.: An analysis of genotype effects and their interactions by using the apolipoprotein E polymorphism and longitudinal data. Am. J. Hum. Genet. 45: 793-802 (1989).

109. Eto, M.; Watanabe, K.; Ishii, K.: Apolipoprotein E polymorphism and hyperlipoproteinemia in obesity. Int J Obes 13: 433-440 (1989).

110. Pouliot, M.C.; Després, J.P.; Moorjani, S.; Lupien, P.J.; Tremblay, A.; Bouchard, C.: Apolipoprotein E polymorphism alters the association between body fatness and plasma lipoproteins in women. J. Lipid Res. 31: 1023-1029 (1990).

111. Cobb, M.M.; Teitelbaum, H.S.; Jekel, J.J.; Ostfield, A.M.: Apolipoprotein E polymorphism and gender compounds a "Western Diet" as determinants of plasma lipid levels. Circulation 82 Supp.III: III-348 (1990).

112. Tikkanen, M.J.; Huttunen, J.K.; Ehnholm, C.; Pietinen, P.: Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. Arteriosclerosis 10: 285-288 (1990).

113. Steinmetz, A.; Thiemann, E.; Czekelius, P.; Kaffarnik, H.: Polymorphism of apolipoprotein E influences levels of serum apolipoproteins E and B in the human neonate. Eur J Clin Invest 19: 390-394 (1989).

114. Mänttäri, M.; Koskinen, P.; Ehnholm, C.; Huttunen, J.K.; Manninen, V.: Apolipoprotein E polymorphism influences the serum cholesterol response to dietary intervention. Metabolism 40: 217-221 (1991).

115. Savolainen, M.J.; Rantala, M.; Kercinen, K.; Jarvi, L.; Suvanto, K.; Rantala, T.; Kesaniemi, Y.A.: Magnitude of dietary effects on plasma cholesterol concentration: Role of sex and apolipoprotein E phenotype. Atherosclerosis 86: 145-152 (1991).

116. Sepehrnia, B.; Kamboh, M.I.; Adams, C.L.; Bunker, C.H.; Nwankwo, M.; Majumder, P.P.; Ferrell, R.E.: Genetic studies of human apolipoproteins. X. The effect of the apolipoprotein E polymorphism on quantitative levels of lipoproteins in Nigerian blacks. Am. J. Hum. Genet. 45: 586-591 (1989).

117. Sepehrnia, B.; Kamboh, M.I.; Adams-Campbell, L.L.; Nwankwo, M.; Ferrell, R.E.: Genetic studies of human apolipoproteins. VII. Population distribution of polymorphism of apolipoproteins A-I, A-II, A-IV, C-II, E, and H in Nigeria. Am. J. Hum. Genet. 43: 847-853 (1988).

118. Weintraub, M.S.; Eisenberg, S.; Breslow, J.: Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J. Clin. Invest. 80: 1571-1577 (1987).

119. Steinmetz, A.; Jakobs, C.; Motzny, S.; Kaffarnik, H.: Differential distribution of apolipoprotein E isoforms in human plasma lipoproteins. Arteriosclerosis 9: 405-411 (1989).

120. Kesäniemi, A.Y.; Ehnholm, C.; Miettinen, T.A.: Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. J. Clin. Invest. 80: 578-581 (1987).

121. Gylling, H.; Kuusi, T.; Vanhanen, H.; Miettinen, T.A.: Apolipoprotein E phenotype and cholesterol metabolism in familial hypercholesterolemia. Atherosclerosis 80: 27-32 (1989).

122. Schaefer, E.J.; Heaton, W.H.; Wetzel, M.G.; Brewer, H.B.J.: Plasma apolipoprotein A-I absence associated with a marked reduction of high density lipoproteins and premature coronary artery disease. Arteriosclerosis 2: 16-26 (1982).

123. Breslow, J.L.: Familial disorders of HDL metabolism; in Shriver, C.R.;Beaudet, A.L.;Sly, W.S.; Valle, D., The Metabolic Basis of Inherited Disease. McGraw-Hill Book Co., New York; 6th ed. No. pp. 1251-1266 (McGraw-Hill Book Co., New York 1988).

124. Hegele, R.A.; Breslow, J.L.: Apolipoprotein genetic variation in the assessment of atherosclerosis susceptibility. Genet. Epidemiol. 4: 163-184 (1987).

125. Franceschini, G.; Calabresi, L.; Tosi, C.; Sirtori, C.R.; Fragiacomo, C.; Noseda, G.; Gong, E.; Blanche, P.; Nichols, A.V.: Apolipoprotein A-I_{Milano}. Correlation between high density lipoprotein subclass distribution and triglyceridemia. Arteriosclerosis 7: 426-435 (1987).

126. Rall, S.C.J.; Weisgraber, K.H.; Mahley, R.W.; Ogawa, Y.; Fielding, C.J.; Utermann, G.; haas, J.; Steinmetz, A.; Menzel, H.J.; Assmann, G.: Abnormal lecithin: cholesterol aclytransferase activation by a human

apolipoprotein A-I variant in which a single lysine residue is deleted. J. Biol. Chem. 259: 10063-10070 (1984).

127. Franceschini, G.; Baio, M.; Calabresi, L.; Sirtori, C.R.; Cheung, M.C.: Apolipoprotein AI_{Milano}. Partial lecithin:cholesterol acyltransferase deficiency due to low levels of a functional enzyme. Biochim. Biophys. Acta 1043: 1-6 (1990).

128. Utermann, G.; Steinmetz, A.; Paetzold, R.; Wilk, J.; Feussner, G.; Kaffarnik, H.; Mueller-Eckhardt, C.; Seidel, D.; Vogelberg, K.H.; Zimmer, F.: Studies on two kindreds with a mutant of human apolipoprotein AI. Hum. Genet. 61: 329-337 (1982).

129. Ordovas, J.M.; Cassidy, D.K.; Civeira, F.; Bisgaier, C.L.; Schaefer, E.J.: Familial apolipoprotein A-I, C-III, and A-IV deficiency and premature atherosclerosis due to deletion of a gene complex on chromosome 11. J. Biol. Chem. 264: 16339-16342 (1989).

130. Wile, D.B.; Barbir, M.; Gallagher, J.; Myant, N.B.; Ritchie, C.D.; Thompson, G.R.; Humphries, S.E.: Apolipoprotein A-I gene polymorphisms: Frequency in patients with coronary artery disease and healthy controls and association with serum apo A-I and HDL-cholesterol concentration. Atherosclerosis 78: 9-18 (1989).

131. Menzel, H.-J.; Boerwinkle, E.; Schrangl-Will, S.; Utermann, G.: Human apolipoprotein A-IV polymorphism: frequency and effect on lipid and lipoprotein levels. Hum. Genet. 79: 368-372 (1988).

132. Hanis, C.L.; Douglas, T.C.; Hewett- Emmett, D.: Apolipoprotein A-IV protein polymorphism: Frequency and effects on lipids, lipoproteins, and apolipoproteins among Mexican-Americans in Starr County, Texas. Hum. Genet. 86: 323-325 (1991).

133. Schaefer, E.J.: Clinical, biochemical, and genetic features in familial disorders of high density lipoprotein. Arteriosclerosis 4: 303-322 (1984).

134. Schwartzkopff, W.; Schleicher, J.; Pottins, I.; Yu, S.B.; Han, C.Z.; Du, D.Y.: Lipids, lipoproteins, apolipoproteins, and other risk factors in Chinese men and women with and without myocardial infarction. Atherosclerosis 82: 253-259 (1990).

135. Reinhart, R.A.; Gani, K.; Arndt, M.R.; Broste, S.K.: Apolipoproteins A-I and B as predictors of angiographically defined coronary artery disease. Arch Intern Med 150: 1629-1633 (1990).

136. Sniderman, A.D.; Shapiro, S.; Marpole, D.; Skinner, B.; Teng, B.; Kwiterovich, P.O., Jr.: Association of coronary atherosclerosis with hyperapobetalipoproteinemia (increased protein but normal cholesterol levels) in human plasma low density (β) lipoproteins. Proc. Natl. Acad. Sci. USA 77: 604-608 (1980).

137. Pan, Q.X.; Liu, P.; Wang, S.C.; Pan, J.T.; Sun, B.Y.; Wu, X.Y.; Saltups, A.: The study of serum apoprotein levels as indicators for the severity of

angiographically assessed coronary artery disease. Am J Clin Pathol 95: 597-600 (1991).

138. Cheung, M.C.; Brown, B.G.; Wolf, A.C.; Albers, J.J.: Altered particle size distribution of apolipoprotein A-I-containing lipoproteins in subjects with coronary artery disease. J. Lipid Res. 32: 383-394 (1991).

139. Miller, N.E.; Hammett, F.; Saltissis, S.; Rao, S.; Van Zeller, H.; Coltart, J.; Lewis, B.: Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. Br. Med. J. 282: 1741-1744 (1981).

140. Gofman, J.W.; Young, W.; Tandy, R.: Ischemic heart disease, atherosclerosis, and longevity. Circulation 34: 679-697 (1966).

141. Paigen, B.; Mitchell, D.; Reue, K.; Morrow, A.; Lusis, A.; LeBoeuf, R.: Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc. Natl. Acad. Sci. USA 84: 3763-3767 (1987).

142. Brinton, E.A.; Eisenberg, S.; Breslow, J.L.: A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J. Clin. Invest. 85: 144-151 (1990).

143. Brinton, E.A.; Eisenberg, S.; Breslow, J.L.: Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein-

cholesterol levels with or without hypertriglyceridemia. J. Clin. Invest. 87: 536-544 (1991).

144. Annuzzi, G.; Holmquist, L.; Carlson, L.A.: Concentrations of apolipoproteins B, C-I, C-II, C-III, E and lipids in serum and serum lipoproteins of normal subjects during alimentary lipaemia. Scand J Clin Lab Invest 49: 73-81 (1989).

145. Krauss, R.M.; Burke, D.J.: Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J. Lipid Res. 23: 97-104 (1982).

146. Austin, M.A.; King, M.C.; Vranizan, K.M.; Newman, B.; Krauss, R.M.: Inheritance of low-density lipoprotein subclass patterns: results of complex segregation analysis. Am. J. Hum. Genet. 43: 838-46 (1988).

147. Austin, M.A.; Breslow, J.L.; Hennekens, C.H.; Buring, J.E.; Willett, W.C.; Krauss, R.M.: Low-density lipoprotein subclass patterns and risk of myocardial infarction. J.A.M.A. 260: 1917-1921 (1988).

148. Austin, M.A.; King, M.C.; Vranizan, K.M.; Krauss, R.M.: Atherogenic lipoprotein phenotype: A proposed genetic marker for coronary heart disease risk. Circulation 82: 495-506 (1990).

149. Rönnemaa, T.; Laakso, M.; Pyörälä, K.; Kallio, V.; Puukka, P.: High fasting plasma insulin is an indicator of coronary heart disease in non-

insulin-dependent diabetic patients and nondiabetic subjects. Arteriosclerosis 11: 80-90 (1991).

150. Reaven, G.M.: Insulin resistance and compensatory hyperinsulinemia: Role in hypertension, dyslipidemia, and coronary heart disease. Am. Heart J. 2: 1283-1288 (1991).

151. Salomaa, V.; Ahola, I.; Tuomilehto, J.; Aro, A.; Pietinen, P.; Korhonen, H.J.; Penttilä, I.: Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: A population-based study. Metabolism 39: 1285-1291 (1990).

152. Golay, A.; Chen, N.; Chen, Y.; Hollenbeck, C.; Reaven, G.M.: Effect of central obesity on regulation of carbohydrate metabolism in obese patients with varying degrees of glucose tolerance. J. Clin. Endocrinol. Metab. 71: 1299-1304 (1990).

153. McKane, W.R.; Stevens, A.B.; Woods, R.; Andrews, W.J.; Henry, R.W.; Bell, P.M.: The assessment of hepatic and peripheral insulin sensitivity in hypertriglycerid emia. Metabolism 39: 1240-1245 (1990).

154. Zavaroni, I.; Bonora, E.; Pagliara, M.; Dall'Aglio, E.; Luchetti, L.; Buonanno, G.; Bonati, P.A.; Bergonzani, M.; Gnudi, L.; Passeri, M.; Reaven, G.: Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N. Engl. J. Med. 320: 702-706 (1989).

155. Modan, M.; Halkin, H.; Lusky, A.; Segal, P.; Fuchs, Z.; Chetrit, A. .: Hyperinsulinemia is characterized by jointly disturbed plasma VLDL, LDL, and HDL levels: A population-based study. Arteriosclerosis 8: 227-236 (1988).

156. Kashyap, M.L.; Barnhart, R.L.; Srivastava, L.S.; Perisuth, G.; Vink, P.; Allen, C.; Hogg, E.; Brady, D.; Glueck, C.J.; Jackson, R.L.: Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-IIIin healthy men. J. Lipid Res. 23: 877-886 (1982).

157. Garg, A.; Bonanome, A.; Grundy, S.M.; Zhang, Z.-J.; Unger, R.H.: Comparison of a high-carbohydrate diet with a high-monounsaturated fat diet in patients with non-insulin-dependent diabetes mellitus. N. Engl. J. Med. 319: 829-834 (1988).

158. Deckelbaum, R.J.; Granot, E.; Oschry, Y.; Rose, L.; Eisenberg, S.: Plasma triglyceride determines structure-composition in low and high density lipoproteins. Arteriosclerosis 4: 225-231 (1984).

159. Fisher, W.R.: Heterogeneity of plasma low density lipoprotein: Manifestations of the physiologic phenomenon in man. Metabolism 32: 283-291 (1983).

160. Vega, G.L.; Grundy, S.M.: Kinetic heterogeneity of low density lipoproteins in primary hypertriglyceridemia. Arteriosclerosis 4: 395-406 (1986).

161. Jackson, R.L.; Yates, M.T.; McNerney, C.A.; Kashyap, M.L.: Relationship between post-heparin plasma lipases, triglycerides and high density lipoproteins in normal subjects. Horm Metab Res 22: 289-294 (1990).

162. Borkman, M.; Campbell, L.V.; Chisholm, D.J.; Storlien, L.H.: Comparison of the effects on insulin sensitivity of high carbohydrate and high fat diets in normal subjects. J. Clin. Endocrinol. Metab. 72: 432-437 (1991).

163. Parillo, M.; Coulston, A.; Hollenbeck, C.; Reaven, G.: Effect of a low fat diet on carbohydrate metabolism in patients with hypertension. Hypertension 11: 244-248 (1988).

164. Reiser, S.; Powell, A.S.; Scholfield, D.J.; Panda, P.; Fields, M.; Canary, J.J.: Day-long glucose, insulin, and fructose responses of hyperinsulinemic and nonhyperinsulinemic men adapted to diets containing either fructose or high-amylose cornstarch. Am. J. Clin. Nutr. 50: 1008-1014 (1989).

165. Mensink, R.P.; deGroot, M.J.M.; van den Broeke, L.T.; Severijnen-Nobles, A.P.; Demacker, P.N.M.; Katan, M.B.: Effects of monosaturated fatty acids v complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. Metabolism 38: 172-178 (1989).

166. Grundy, S.M.: Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N. Eng. J. Med. 314: 745-748 (1986).

167. Wolfe, B.M.; Giovannetti, P.M.: Short-term effects of substituting protein for carbohydrate in the diets of moderately hypercholesterolemic human subjects. Metabolism 40: 338-343 (1991).

TABLE 1. Effect of Dietary Fat Supplementation (5 g Saturated Fat) in Diet-Sensitive and Diet-Insensitive Hypercholesterolemic and Normocholesterolemic Subjects.

Source: Clifton, P.M., et al. Arteriosclerosis 10: 394-401 (1990).

Reference Number: [40]

	Hypercholesterolemic group			
	Diet-sensitive (n=23)	Diet-insensitive (n=22)	Normocholesterolemic group (n=11)	Combined (n=56)
Plasma cholesterol	0.32±0.41	0.13±0.34	0.03±0.31	0.19±0.38*
LDL cholesterol	0.20±0.40	0.06±0.31	−0.02±0.28	0.10±0.35†
HDL cholesterol	0.12±0.15	0.09±0.18	0.01±0.14	0.08±0.17‡
VLDL cholesterol	0.01±0.13	−0.02±0.12	0.05±0.03	0.00±0.13
Plasma triglyceride	−0.02±0.34	−0.05±0.32	0±0.45	−0.03±0.35

Values of changes in concentration are mmol/l±SD.
Comparisons with baseline values: *$p<0.001$, †$p<0.01$, ‡$p<0.05$.

LDL = low density lipoprotein

HDL = high density lipoprotein

VLDL = very low density lipoprotein

TABLE 2. Effect of Dietary Cholesterol Supplementation (700 mg) in Diet-Sensitive and Diet-Insensitive Hypercholesterolemic and Normocholesterolemic

Source: Clifton, P.M., et al. Arteriosclerosis 10: 394-401 (1990).

Reference Number: [40]

	Hypercholesterolemic group			
	Diet-sensitive (n=23)	Diet-insensitive (n=22)	Normocholesterolemic group (n=11)	Combined (n=56)
Plasma cholesterol	0.36±0.37	0.19±0.47†	0.06±0.47	0.23±0.44*
LDL cholesterol	0.30±0.36	0.15±0.46†	0.02±0.40	0.19±0.41*
HDL cholesterol	0.09±0.14	0.07±0.15	0.05±0.13	0.07±0.14*
VLDL cholesterol	−0.04±0.10	−0.03±0.12	−0.01±0.12	−0.03±0.11
Plasma triglyceride	−0.07±0.26	−0.10±0.29	0.02±0.31	−0.07±0.28

The values of changes in concentration are mmol/l±SD.
*$p<0.001$, comparisons with control values. †$p=0.06$, diet-sensitive versus diet-insensitive.

LDL = low density lipoprotein

HDL = high density lipoprotein

VLDL = very low density lipoprotein

TABLE 3. Characteristics and Plasma Lipoprotein-Lipid and Apolipoprotein Levels in ApoE Phenotype Groups.

Source: Pouliot, M.C., et al. J. Lipid Res. 31: 1023-1029 (1990).

Reference Number: [110]

Variable	ApoE Group		
	E2	E3	E4
N	22	24	17
Age (years)	35.6 ± 4.8	35.3 ± 4.7	35.0 ± 4.5
% Body fat	40.4 ± 8.2	38.2 ± 12.4	43.8 ± 8.8
Body mass index	30.9 ± 7.0	28.1 ± 8.0	32.7 ± 7.3
Waist:hip ratio	0.80 ± 0.04	0.78 ± 0.05	0.82 ± 0.06
VLDL-C (mmol/l)	0.45 ± 0.30	0.40 ± 0.27	0.49 ± 0.31
VLDL-TG (mmol/l)	0.98 ± 0.67	0.81 ± 0.60	1.02 ± 0.63
VLDL-apoB (g/l)	0.12 ± 0.15	0.10 ± 0.12	0.08 ± 0.07
LDL-C (mmol/l)	3.39 ± 0.81	3.52 ± 1.19	3.68 ± 0.88
LDL-TG (mmol/l)	0.28 ± 0.12	0.30 ± 0.16	0.32 ± 0.16
LDL-apoB (g/l)	0.75 ± 0.22	0.81 ± 0.29	0.87 ± 0.23
HDL-C (mmol/l)	1.22 ± 0.29	1.28 ± 0.26	1.09 ± 0.21
HDL-TG (mmol/l)	0.22 ± 0.09	0.22 ± 0.06	0.19 ± 0.05
HDL-apoA-I (g/l)	1.15 ± 0.21	1.14 ± 0.15	1.12 ± 0.19

E2, Subjects homozygous or heterozygous for the E2 isoform; E3, subjects homozygous for the E3 isoform; E4, subjects homozygous or heterozygous for the E4 isoform: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; C, cholesterol; TG, triglycerides; apo, apolipoprotein.

TABLE 4. Associations[a] Between Body Fatness Variables and Plasma Very Low Density Lipoprotein (VLDL) Components in Apolipoprotein E Phenotype Groups.

Source: Pouliot, M.C., et al. J. Lipid Res. 31: 1023-1029 (1990).

Reference Number: [110]

ApoE Group	Lipoprotein Components	Fat Mass	WHR	Abdominal Fat	
				Total	Deep
E2	VLDL-C	0.57***	0.58**	0.59**	0.64**
	VLDL-TG	0.59**	0.59**	0.62**	0.65***
	VLDL-apoB	0.42*	0.38	0.38	0.38
E3	VLDL-C	0.46*	0.54**	0.50**	0.56**
	VLDL-TG	0.53*	0.44*	0.58**	0.61**
	VLDL-apoB	0.74***	0.58**	0.80***	0.75***
E4	VLDL-C	0.10	0.13	0.32	0.11
	VLDL-TG	0.43	0.28	0.53*	0.33
	VLDL-apoB	-0.07	-0.17	0.08	-0.05

WHR = waist:hip circumference ratio

For other abbreviations, refer to Table 3.

*p = < 0.05; **p = < 0.01; ***p < 0.001

[a]All values are log 10.

TABLE 5. Associations Between Body Fatness Variables and Plasma Low Density Lipoprotein (LDL) Components in Apolipoprotein E Phenotype Groups.

Source: Pouliot, M.C., et al. J. Lipid Res. 31: 1023-1029 (1990).

Reference Number: [110]

ApoE Group	Lipoprotein Components	Fat Mass	WHR	Abdominal Fat	
				Total	Deep
E2	LDL-C	0.09	0.27	0.05	0.20
	LDL-TG	0.57**	0.34	0.54*	0.54*
	LDL-apoB	0.08	0.38	0.07	0.29
E3	LDL-C	0.56**	0.46*	0.57**	0.55**
	LDL-TG	0.66***	0.51*	0.69***	0.68***
	LDL-apoB	0.43*	0.43*	0.45*	0.47*
E4	LDL-C	0.16	0.15	0.44	0.51*
	LDL-TG	0.16	0.23	0.45	0.27
	LDL-apoB	0.29	0.34	0.58*	0.50*

For other abbreviations, refer to Tables 3 and 4.

*p = < 0.05; **p = < 0.01; ***p < 0.001

TABLE 6. Changes in Total Plasma Cholesterol and Low Density Lipoprotein Cholesterol During Dietary Study. Source: [112]

Source: Tikkanen, M.J., et al. Arteriosclerosis 10: 285-288 (1990).

Reference Number: [110]

Changes	Plasma lipid	E4/E4 (n=8)	E4/E3, E3/E3, or E3/E2 (n=102)	*P*
Decrease during intervention	Cholesterol	−1.84	−1.13	0.0097
	LDL chol	−1.51	−0.91	0.0164
Increase during switchback	Cholesterol	1.52	0.92	0.0141
	LDL chol	1.17	0.68	0.0221

The values are given in mmol/l.
LDL, low density lipoprotein.

TABLE 7

Lipid and Lipoprotein Changes from Baseline Diet to Low Fat Diet

Variable	Pattern A (N=32) Baseline Diet	Pattern A (N=32) Low Fat Diet	Pattern B (N=9) Baseline Diet	Pattern B (N=9) Low Fat Diet
	mg/dL Mean ± SEM			
Cholesterol	175 ± 43	177 ± 37	180 ± 43	192 ± 45
Triglyceride	91 ± 44	80 ± 27	128 ± 45	172 ± 78**,†
HDL-Cholesterol	48 ± 7.0	47 ± 9.6	43 ± 12	39 ± 10**
LDL S_f^o 0 - 7 mass	146 ± 59	152 ± 62	182 ± 54	201 ± 36
LDL S_f^o 7 - 12 mass	114 ± 41	115 ± 43	83 ± 32	78 ± 40
IDL S_f^o 12 - 20 mass	19 ± 17	22 ± 15	31 ± 23	38 ± 27
VLDL S_f^o 20 - 400 mass	55 ± 51	46 ± 37	95 ± 49	134 ± 66*†
HDL_3 $F^o_{1.20}$ 0 - 3.5 mass	191 ± 29	184 ± 34	183 ± 47	181 ± 44
HDL_2 $F^o_{1.20}$ 3.5 - 9 mass	52 ± 28	52 ± 35	34 ± 40	34 ± 40

Changes with diet significant: * $p < 0.05$; ** $p < 0.01$.

† Change in pattern B subjects significantly different from change in pattern A subjects at $p < 0.01$.

FIGURE LEGENDS

FIGURE 1. Difference Between Predicted (Keys formula) and Observed Changes in Serum Cholesterol, by Baseline Serum Cholesterol and Time After Randomization

Source: Boyd, N. F., et al. Am. J. Clin. Nutr. 52: 470-476 (1990).

Reference Number: [38]

FIGURE 2. Percent Change From Before to After Fish Oil in Lipoprotein Chemical Composition.

Source: Inagaki, M., et al.: Atherosclerosis 82: 237-246 (1990).

Reference Number: [71]

FIGURE 3. Cholesterol Absorption (left), Bile Acid Synthesis (left middle), Cholesterol Synthesis (right middle), and Serum Lathosterol (right) in the Subjects With Various ApoE Phenotypes. The Values Are Expressed as Mean ± SEM of 4 (E2/2, E2/3, E2/4), 22 (E3/3), and 13 (E4/4, E4/3) Subjects.*, Different From the Subjects E2/2, E2/3, and E2/4; , + Different From the Subject E3/3; ($P < 0.05$ or less; Analysis of Variance; F Values of 3/39 for Cholesterol Absorption and 3.17 for Serum Lathosterol).

Source: Kesäniemi, A.Y., et al. J. Clin. Invest. 80: 578-581 (1987).

Reference Number: [120]

FIGURE 4. RP Peak Heights (in micrograms per liter) of Total Plasma, Chylomicron, and Nonchylomicron Fractions During the Vitamin A-Fat Loading Test in Individual Subjects With Different Apo E Phenotypes.

Source: Weintraub, M.S., et al. J. Clin. Invest. 80: 1571:1577 (1987).

Reference Number: [118]

FIGURE 5. Relationships Between Levels of HDL-C (mg/dl) and Apo A-I, TR (mg/kg per d), and FCR (pools/d). Results are Plotted as Change vs. Change (Low-Fat Diet Value - High-Fat Diet Value) and as Within-Diet Results for High and Low Fat Intake.

Source: Brinton, E. A., et al. J. Clin. Invest. 85:144-151 (1990).

Reference Number: [142]

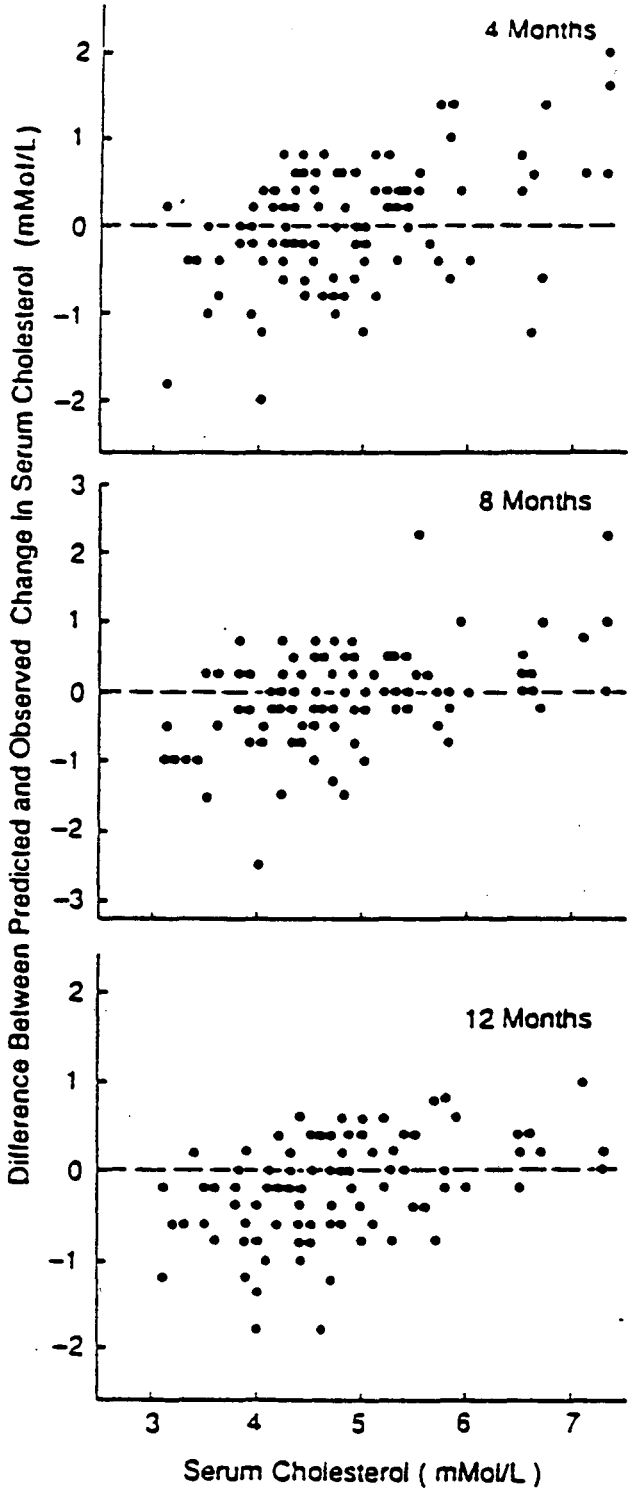
4 Months
8 Months
12 Months
Difference Between Predicted and Observed Change In Serum Cholesterol (mMol/L)
Serum Cholesterol (mMol/L)

Figure 1

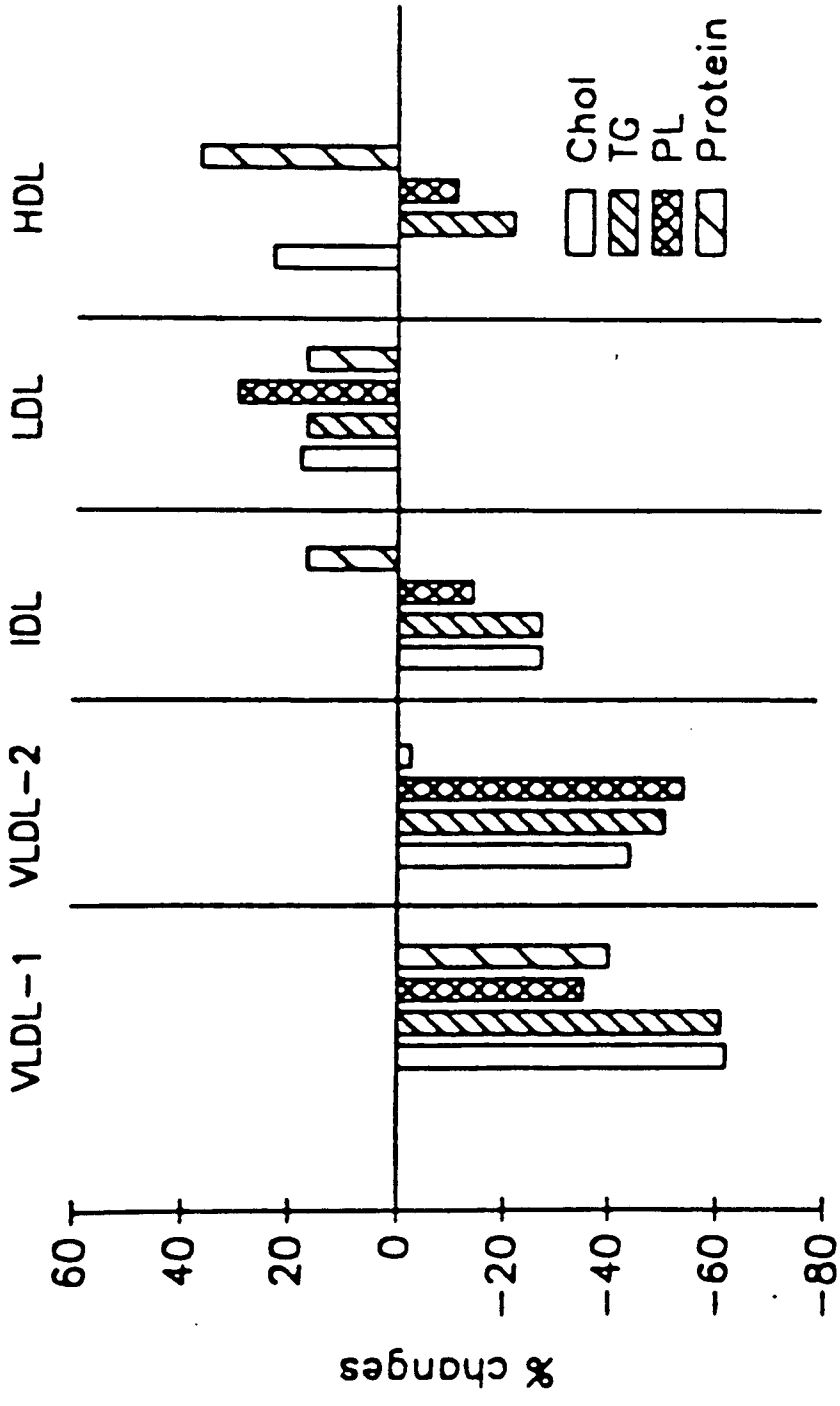
% changes
60
40
20
0
-20
-40
-60
-80
VLDL-1
VLDL-2
IDL
LDL
HDL
Chol
TG
PL
Protein

Figure 2

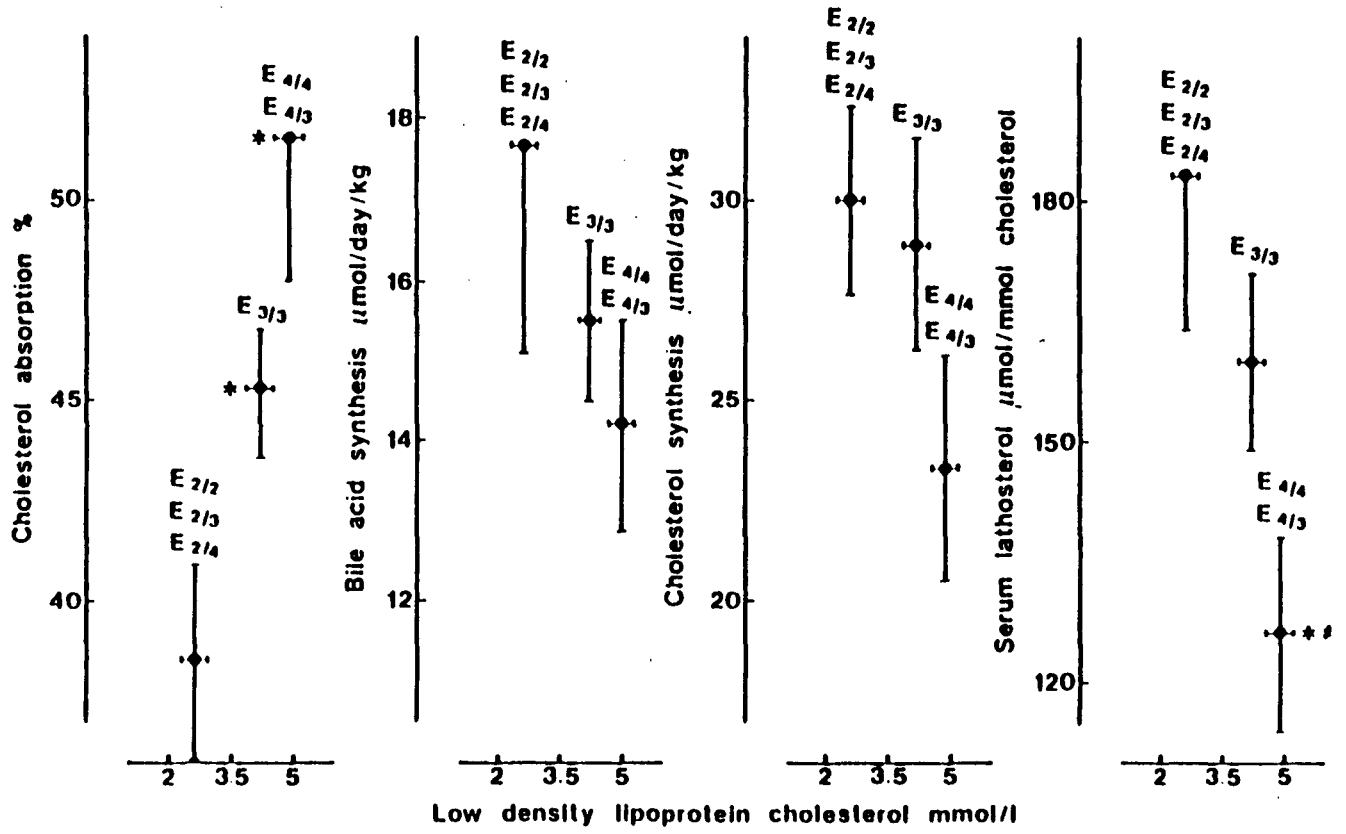
Cholesterol absorption %
50
45
40
E 4/4
E 4/3
E 3/3
E 2/2
E 2/3
E 2/4
Bile acid synthesis µmol/day/kg
18
16
14
12
Cholesterol synthesis µmol/day/kg
30
25
20
Serum lathosterol µmol/mmol cholesterol
180
150
120
2
3.5
5
Low density lipoprotein cholesterol mmol/l

Figure 3

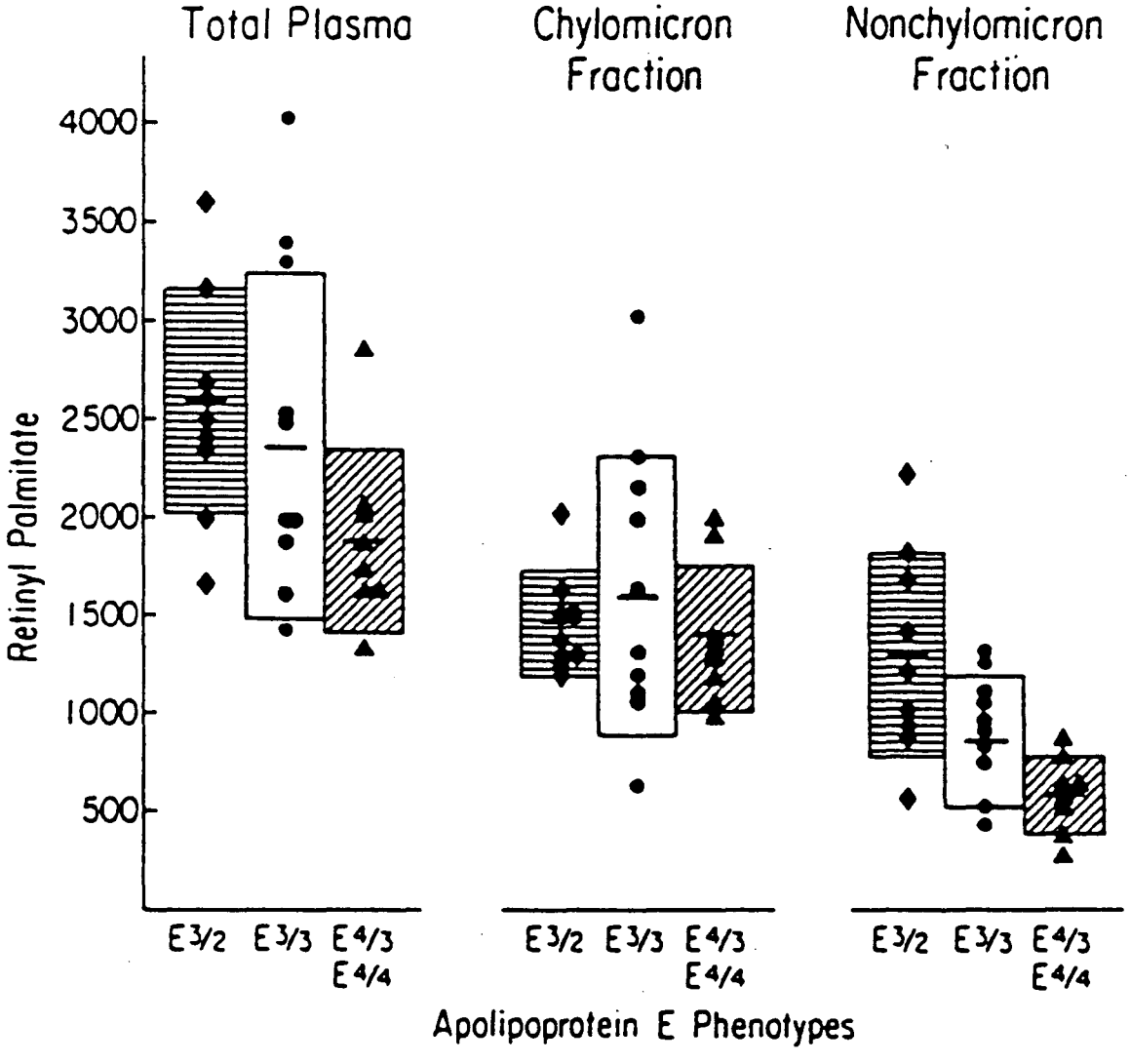
Total Plasma
Chylomicron Fraction
Nonchylomicron Fraction
Retinyl Palmitate
4000
3500
3000
2500
2000
1500
1000
500
E3/2
E3/3
E4/3 E4/4
Apolipoprotein E Phenotypes

Figure 4

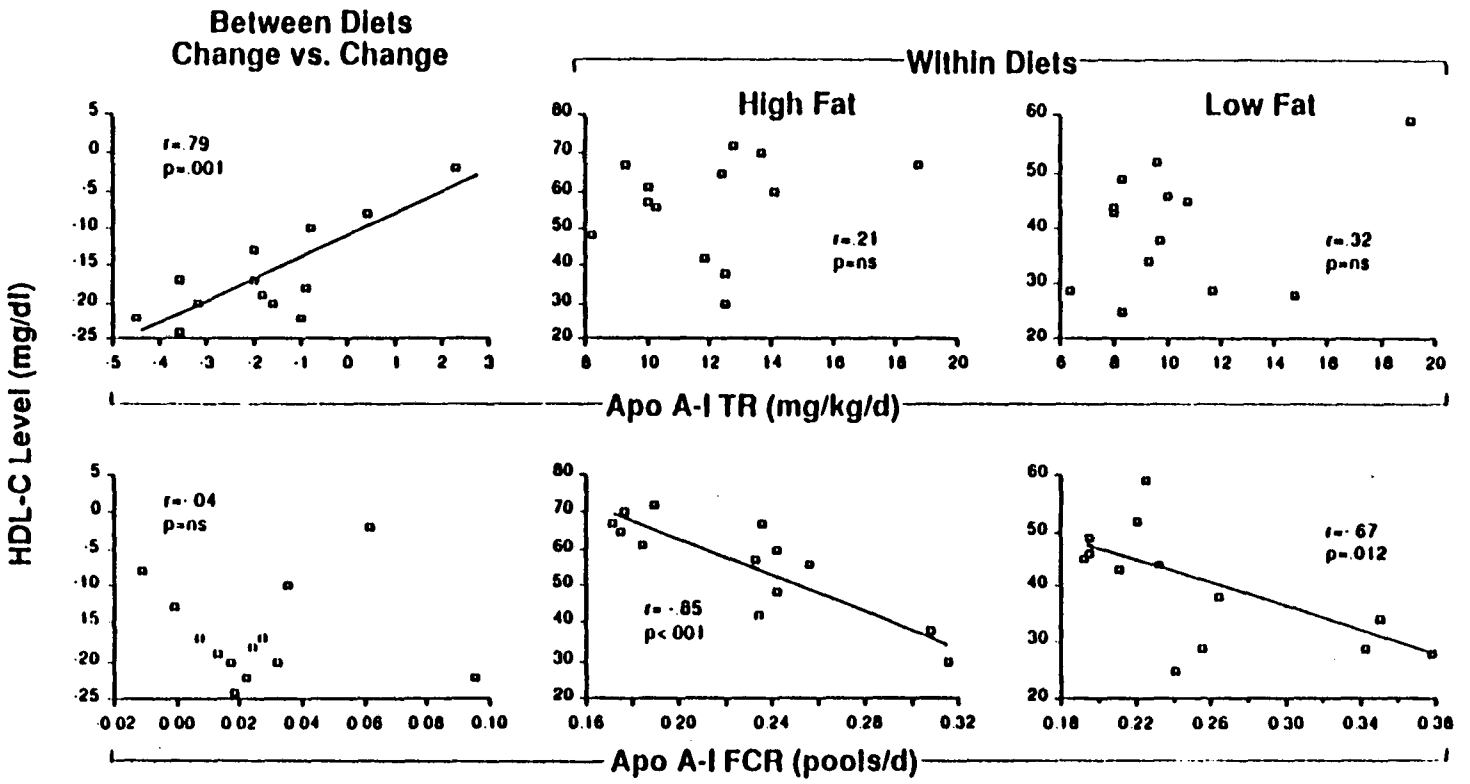
Between Diets
Change vs. Change
Within Diets
High Fat
Low Fat
HDL-C Level (mg/dl)
r=.79
p=.001
r=.21
p=ns
r=.32
p=ns
r=-.04
p=ns
r=-.85
p<.001
r=-.67
p=.012
Apo A-I TR (mg/kg/d)
Apo A-I FCR (pools/d)

Figure 5

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
TECHNICAL INFORMATION DEPARTMENT
BERKELEY, CALIFORNIA 94720