# Lipoproteins, nutrition, and heart disease[1–6]

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**ABSTRACT**     This article reviews the current status of our knowledge of lipoproteins, nutrition, and coronary heart disease (CHD). Special emphasis is placed on CHD risk assessment, dietary intervention studies, diet-gene interactions, and current dietary guidelines and the contributions of my laboratory to these areas. CHD remains a major cause of death and disability, and risk factors include age, sex, hypertension, smoking, diabetes, elevated serum LDL cholesterol, and low HDL cholesterol. Emerging independent risk factors include elevated serum concentrations of lipoprotein(a), remnant lipoproteins, and homocysteine. The cornerstone of CHD prevention is lifestyle modification. Dietary intervention studies support the concepts that restricting saturated fat and cholesterol and increasing the intake of essential fatty acids, especially n−3 fatty acids, reduces CHD risk. The variability in LDL-cholesterol response to diet is large, related in part to *APOE* and *APOA4* genotype. The use of antioxidants in intervention studies has not been shown to reduce CHD risk. Compliance with dietary recommendations remains a major problem, and directly altering the food supply may be the most effective way to ensure compliance. The available data indicate that the recommendation to use fats, oils, and sugars sparingly for CHD prevention should be modified to a recommendation to use animal, dairy, and hydrogenated fats; tropical oils; egg yolks; and sugars sparingly and to increase the use of vegetables, fruit, and whole grains. *Am J Clin Nutr* 2002;75:191–212.

**KEY WORDS**     Coronary heart disease, lipoproteins, LDL cholesterol, HDL cholesterol, VLDL cholesterol, lipoprotein(a), apolipoproteins, cholesterol, triacylglycerol, fatty acids, dietary recommendations

## INTRODUCTION

This lecture and review was given in honor of Elmer V McCollum to celebrate his significant contributions to the field of nutrition, especially in the area of vitamin A and its functions. The purpose was to review the current status of our knowledge of lipoproteins, nutrition, genetics, and coronary heard disease (CHD) and to review the contributions of my own research, in collaborations with others, to these areas over the past 25 y.

CHD remains the leading cause of death and disability in our society. About 13 million Americans have CHD, 1.5 million have a myocardial infarction (MI) each year, and ≈450 000 die of CHD each year (1). Although as many women as men die of CHD per year, the mean age at death from CHD is substantially higher in women. This is the major factor accounting for

women's greater longevity (1). The reason for this sex gap is the substantially higher concentrations of HDL cholesterol in women; such values are protective against CHD (2).

CHD is caused by atherosclerosis, a process characterized by endothelial dysfunction—in association with hypertension, diabetes, smoking, and elevated homocysteine concentrations—and cholesterol deposition in macrophages and smooth muscle cells in the arterial wall as the result of elevated LDLs, lipoprotein(a), and remnant lipoproteins and decreased HDLs. In addition, smooth muscle proliferation, inflammation, and calcification occur in this process. Thrombosis, occurring after plaque rupture and aggravated by elevated fibrinogen concentrations, is often the terminal event occluding the artery. Narrowing of coronary arteries causes angina pectoris or chest pain, especially with exertion, whereas occlusion can cause MI or death of heart muscle. Blood pressure is a key factor in the development of atherosclerosis; this process does not develop on the venous side of the circulation. Hypercholesterolemia is also critical; CHD is uncommon in societies with mean serum total cholesterol concentrations <4.6 mmol/L (180 mg/dL). The cornerstone of therapy for CHD is its prevention through the modification of risk factors.

A prediction table of the 10-y risk of CHD and angina in white men and women was developed by the Framingham Heart Study investigators on the basis of age, sex, total cholesterol, history of smoking, HDL cholesterol, and blood pressure with use of the National Cholesterol Education Program (NCEP) cutoffs (**Figure 1**) (2). High risk was defined as a risk of >20% over

Estimate of 10-y risk for men (Framingham point scores)

| Age (y) | Points |
|---|---|
| 20–34 | −9 |
| 35–39 | −4 |
| 40–44 | 0 |
| 45–49 | 3 |
| 50–54 | 6 |
| 55–59 | 8 |
| 60–64 | 10 |
| 65–69 | 11 |
| 70–74 | 12 |
| 75–79 | 13 |

| Total cholesterol (mmol/L) | (mg/dL) | Age 20–39 y | Age 40–49 y | Age 50–59 y | Age 60–69 y | Age 70–79 y |
|---|---|---|---|---|---|---|
| <4.1 | <160 | 0 | 0 | 0 | 0 | 0 |
| 4.1–5.2 | 160–199 | 4 | 3 | 2 | 1 | 0 |
| 5.2–6.2 | 200–239 | 7 | 5 | 3 | 1 | 0 |
| 6.2–7.2 | 240–279 | 9 | 6 | 4 | 2 | 1 |
| ≥7.2 | ≥280 | 11 | 8 | 5 | 3 | 1 |

| | Age 20–39 y | Age 40–49 y | Age 50–59 y | Age 60–69 y | Age 70–79 y |
|---|---|---|---|---|---|
| Nonsmoker | 0 | 0 | 0 | 0 | 0 |
| Smoker | 8 | 5 | 3 | 1 | 1 |

| HDL cholesterol (mmol/L) | (mg/dL) | |
|---|---|---|
| 1.6 | ≥60 | −1 |
| 1.3–1.6 | 50–59 | 0 |
| 1.0–1.3 | 40–49 | 1 |
| <1.0 | <40 | 2 |

| Systolic blood pressure (mm Hg) | If untreated | If treated |
|---|---|---|
| <120 | 0 | 0 |
| 120–129 | 0 | 1 |
| 130–139 | 1 | 2 |
| 140–159 | 1 | 2 |
| ≥160 | 2 | 3 |

| Point total | 10-y risk (%) |
|---|---|
| <0 | <1 |
| 0 | 1 |
| 1 | 1 |
| 2 | 1 |
| 3 | 1 |
| 4 | 1 |
| 5 | 2 |
| 6 | 2 |
| 7 | 3 |
| 8 | 4 |
| 9 | 5 |
| 10 | 6 |
| 11 | 8 |
| 12 | 10 |
| 13 | 12 |
| 14 | 16 |
| 15 | 20 |
| 16 | 25 |
| ≥17 | ≥30 |

Estimate of 10-y risk for women (Framingham point scores)

| Age (y) | Points |
|---|---|
| 20–34 | −7 |
| 35–39 | −3 |
| 40–44 | 0 |
| 45–49 | 3 |
| 50–54 | 6 |
| 55–59 | 8 |
| 60–64 | 10 |
| 65–69 | 12 |
| 70–74 | 14 |
| 75–79 | 16 |

| Total cholesterol (mmol/L) | (mg/dL) | Age 20–39 y | Age 40–49 y | Age 50–59 y | Age 60–69 y | Age 70–79 y |
|---|---|---|---|---|---|---|
| <4.1 | <160 | 0 | 0 | 0 | 0 | 0 |
| 4.1–5.2 | 160–199 | 4 | 3 | 2 | 1 | 1 |
| 5.2–6.2 | 200–239 | 8 | 6 | 4 | 2 | 1 |
| 6.2–7.2 | 240–279 | 11 | 8 | 5 | 3 | 2 |
| ≥7.2 | ≥280 | 13 | 10 | 7 | 4 | 2 |

| | Age 20–39 y | Age 40–49 y | Age 50–59 y | Age 60–69 y | Age 70–79 y |
|---|---|---|---|---|---|
| Nonsmoker | 0 | 0 | 0 | 0 | 0 |
| Smoker | 9 | 7 | 4 | 2 | 1 |

| HDL cholesterol (mmol/L) | (mg/dL) | |
|---|---|---|
| 1.6 | ≥60 | −1 |
| 1.3–1.6 | 50–59 | 0 |
| 1.0–1.3 | 40–49 | 1 |
| <1.0 | <40 | 2 |

| Systolic blood pressure (mm Hg) | If untreated | If treated |
|---|---|---|
| <120 | 0 | 0 |
| 120–129 | 1 | 3 |
| 130–139 | 2 | 4 |
| 140–159 | 3 | 5 |
| ≥160 | 4 | 6 |

| Point total | 10-y risk (%) |
|---|---|
| <9 | <1 |
| 9 | 1 |
| 10 | 1 |
| 11 | 1 |
| 12 | 1 |
| 13 | 2 |
| 14 | 2 |
| 15 | 3 |
| 16 | 4 |
| 17 | 5 |
| 18 | 6 |
| 19 | 8 |
| 20 | 11 |
| 21 | 14 |
| 22 | 17 |
| 23 | 22 |
| 24 | 27 |
| ≥25 | ≥30 |

**FIGURE 1.** Prediction table of the 10-y risk of coronary heart disease and angina in white men and women developed by the Framingham Heart Study investigators on the basis of age, sex, total cholesterol, history of smoking, HDL cholesterol, and blood pressure with use of the National Cholesterol Education Program cutoffs (2).

**TABLE 1**
Prevalence of risk factors in premature coronary heart disease[1]

| | Cases (n = 321) | Controls (n = 601) |
|---|---|---|
| | % | |
| Cigarette smoking (past year) | 67 | 29[2] |
| Low HDL cholesterol <0.9 mmol/L (35 mg/dL) | 63 | 19[2] |
| Hypertension | 41 | 21[2] |
| Elevated LDL cholesterol ≥4.1 mmol/L (160 mg/dL) | 36 | 26[2] |
| Elevated homocysteine | 28 | 10[2] |
| Elevated lipoprotein(a) | 19 | 10[2] |
| Diabetes | 12 | 1[2] |

[1] In subjects aged < 60 y. Elevated values for homocysteine and lipoprotein(a) are above the 90th percentile of control values. From reference 29.
[2] Significantly different from cases, $P < 0.05$.

10 y and low risk as <10%, which is the goal (2). Having CHD, other vascular disease, or diabetes has now been classified as a high-risk equivalent. In my view, reasonable goals of therapy in the general population for CHD prevention are to get the 10-y CHD risk to <10% by promoting smoking cessation, control of blood pressure (systolic <130 mm Hg and diastolic blood pressure <85 mm Hg), control of LDL cholesterol (<3.3 mmol/L, or 130 mg/dL), control of fasting glucose [glucose <6.0 mmol/L (<110 mg/dL) and glycated hemoglobin <7% (0.07)], HDL-cholesterol elevation (>1.0 mmol/L, or 40 mg/dL), and control of body mass index (in kg/m²: <27.0).

## LIPOPROTEINS AND CHD RISK

### LDL and HDL cholesterol

Total and HDL cholesterol can be assessed in the fasting or nonfasting state, and accurate finger stick methods are now available (3, 4). Until recently, a calculated value was recommended for the measurement of LDL cholesterol. This estimation was based on measuring cholesterol, triacylglycerol, and HDL cholesterol in serum in the fasting state and then calculating LDL cholesterol by subtracting the sum of HDL cholesterol and triacylglycerol divided by 5 for mg/dL or 2.2 for mmol/L from total cholesterol. We documented that this calculation is inaccurate in the nonfasting state or if triacylglycerol concentrations are >2.8 mmol/L (250 mg/dL) (5). The direct measurement methods now available for LDL and HDL cholesterol are much more accurate than the calculated values and can be carried out with the use of plasma or serum from nonfasting subjects (6).

### Lipoprotein(a)

Other important risk factors for CHD include elevated concentrations of lipoprotein(a), remnant lipoprotein particles, and homocysteine. Lipoprotein(a) is an apolipoprotein (apo) B-100 lipoprotein, usually LDL with another protein, apo(a), attached to apo B-100 by a disulfide bond. Elevated concentrations of lipoprotein(a) as determined by immunoassay, electrophoresis, or a lectin-based cholesterol assay that we developed in our laboratory are all associated with an ≈2-fold increased risk of CHD (7–13). Lipoprotein(a) concentrations are largely genetically determined in part due to marked variation in apo(a) isoforms

(9). High concentrations are associated with increased apo(a) secretion. Lipoprotein(a) values can be lowered with niacin (14) or hormone replacement therapy (15). In addition, in a large hormone replacement trial in postmenopausal women with established CHD, the only group that benefited significantly from hormone replacement therapy was the group of women with elevated lipoprotein(a) concentrations (16, 17). Studies are needed to evaluate the benefits of lipoprotein(a) lowering.

### Triacylglycerol-rich lipoproteins and remnant lipoproteins

Triacylglycerol-rich lipoproteins, either of intestinal or liver origin, are metabolized to form atherogenic remnant lipoproteins by the action of lipoprotein lipase in the bloodstream. Once these particles have lost much of their triacylglycerol, they pick up cholesterol ester from other lipoproteins via the action of cholesterol ester transfer protein. New remnant-like particle cholesterol and triacylglycerol assays have been developed, and we evaluated these assays in the Framingham Offspring Study (18). In our view, an elevated remnant-like particle cholesterol value is a better CHD risk marker than is total serum triacylglycerol, especially in women (19). Remnant-like particle cholesterol can be lowered by weight loss, exercise, and various lipid-lowering agents, including fibric acid derivatives, niacin, and hydroxymethylglutaryl-CoA reductase inhibitors. High-risk CHD lipoprotein cholesterol values in the Framingham Heart Study were an LDL-cholesterol concentration >4.1 mmol/L (160 mg/dL), an HDL-cholesterol concentration <0.9 mmol/L (35 mg/dL), a total lipoprotein(a) concentration >300 mg/L (>30 mg/dL) or a lipoprotein(a) cholesterol concentration >0.25 mmol/L (10 mg/dL), and a remnant-like particle cholesterol value >0.20 mmol/L (8 mg/dL). In my view, these measurements will all be carried out in the future for more accurate CHD risk assessment.

### LDL particle size

There has been great interest in LDL particle size and its heritability and relation to CHD. We documented a strong inverse correlation between triacylglycerol concentrations and LDL particle size, in that women have larger LDL particles than do men, that weight loss increases LDL particle size, and that LDL particle size is not very heritable (20–28). Moreover, we found that whereas CHD patients have smaller LDL particles than do control subjects, LDL particle size is not an independent predictor of CHD risk after other established risk factors are controlled for (26). These findings remain to be confirmed in large prospective studies.

### Prevalence of risk factors and familial lipoprotein disorders in premature CHD

We have assessed the prevalence of risk factors in patients with premature CHD. The most prevalent CHD risk factors in these subjects were cigarette smoking, low HDL cholesterol, hypertension, elevated LDL cholesterol, and elevated homocysteine, followed by elevated lipoprotein(a) and finally diabetes (**Table 1**) (7, 29). In our view, these are all important independent risk factors. Familial lipoprotein disorders that we have observed in families afflicted with premature CHD include lipoprotein(a) excess, dyslipidemia with high triacylglycerol and low HDL cholesterol, combined hyperlipidemia associated with elevations in both triacylglycerol and LDL cholesterol and low HDL cholesterol, hypoalphalipoproteinemia, and hypercholesterolemia (**Table 2**) (30).

**TABLE 2**
Familial lipoprotein disorders in premature coronary heart disease[1]

|                                                                  | Percentage affected |
| ---------------------------------------------------------------- | ------------------- |
|                                                                  | %                   |
| Lipoprotein(a) excess                                            | 19                  |
| Dyslipidemia (high TG, low HDL)                                  | 15                  |
| Combined hyperlipidemia (high TG, high LDL, low HDL)             | 14                  |
| Hypoalphalipoproteinemia (low HDL)                               | 4                   |
| Hypercholesterolemia (high LDL)                                  | 3                   |

[1] In 101 kindreds comprising 599 subjects. Premature coronary heart disease defined as lipoprotein(a), triacylglycerol (TG), and LDL-cholesterol concentrations above the 90th percentile of control values and an HDL-cholesterol concentration less than the 10th percentile, with ≥2 affected family members. From reference 30.

## Lipoprotein metabolism

Using stable-isotope kinetic studies, we clearly defined human apolipoprotein kinetics in the constantly fed state (small, frequent, isoenergetic feeding) (**Figure 2**). Apo B-48 is essential for chylomicron formation in the intestine, the main pathway through which dietary fats enter the bloodstream. Apo B-48 is combined with lipid by the action of microsomal transfer protein. The production of apo B-48 is ≈1.3 mg·kg$^{-1}$·d$^{-1}$; intestinal triacylglycerol input varies on the basis of dietary fat intake,

but is often ≈50–100 g/d (step 1, Figure 2) (31, 33–36). Plasma apo B-48 concentrations are regulated by secretion rates that in turn are probably determined by the degree of intracellular degradation, which in turn is probably regulated by the amount of lipid in the enterocyte.

After chylomicrons enter the bloodstream, they rapidly undergo lipolysis via the action of lipoprotein lipase, lose much of their triacylglycerol, and acquire cholesterol ester from other lipoproteins via the action of cholesterol ester transfer protein (step 4). During this process they also acquire apo E from HDL and become chylomicron remnants. These remnants are taken up by the liver via receptors that bind apo E (step 6). The average residence time of apo B-48 in plasma is 4.8 h (31). Very high concentrations of chylomicrons secondary to defects in lipolysis can result in pancreatitis, whereas increases in chylomicron remnants resulting from apo E2 homozygosity or apo E deficiency increase CHD risk (37–39). With my colleagues at the National Institutes of Health, I was the first to describe apo E deficiency. Patients with such a deficiency have delayed apo B-48 clearance (38).

Apo B-100 is the major protein of VLDL and the sole protein of LDL. Apo B-100 is combined with lipid in the liver by the action of microsomal transfer protein. About 20.4 mg apo B-100·kg$^{-1}$·d$^{-1}$ is secreted into VLDL, which is also triacylglycerol rich (step 2) (31). VLDL rapidly loses much of its triacylglycerol via lipolysis (step 5). VLDL apo B-100 has a residence time of ≈3.6 h, and about one-half is converted to LDL apo B-100 in the
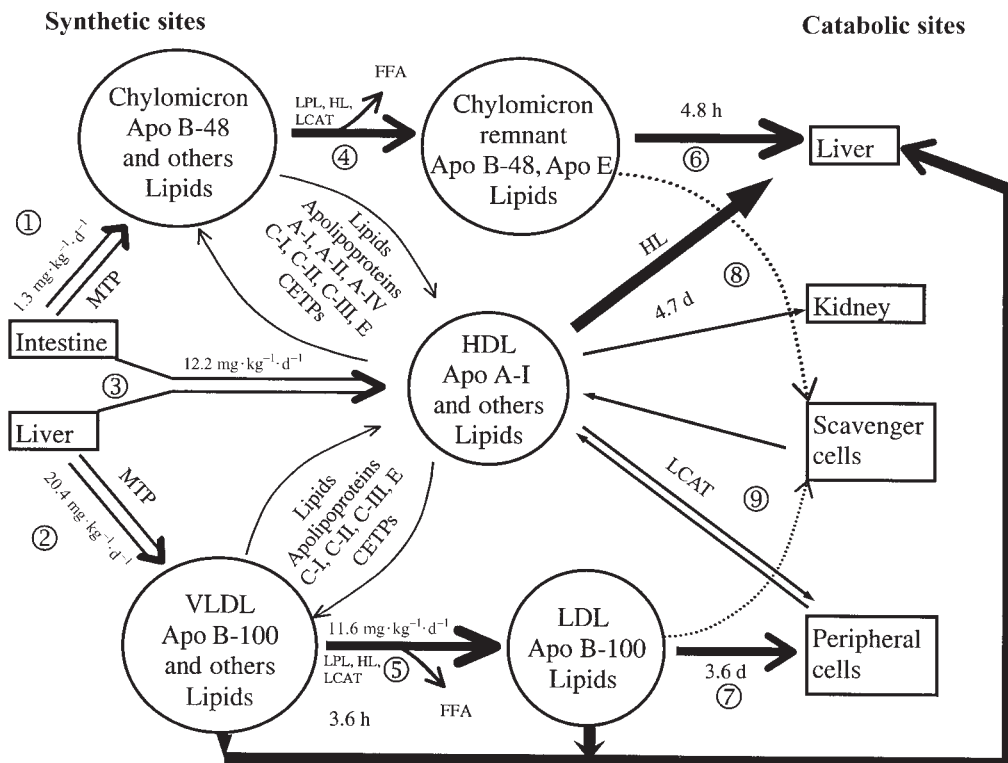


**FIGURE 2.** An overview of human apolipoprotein (apo) metabolism as assessed by endogenous labeling with a primed constant infusion of deuterated leucine in the fed state. CETP, cholesterol ester transfer protein (transfers cholesterol ester from LDL and HDL to remnants of triacylglycerol-rich lipoproteins); FFA, free fatty acid; HL, hepatic lipase (cleaves fatty acids from both phospholipid and triacylglycerol); LCAT, lecithin cholesterol acyltransferase (phosphatidylcholine-sterol *O*-acyltransferase; transfers fatty acid from phosphotidyl choline or lecithin to cholesterol to form cholesterol ester); LPL, lipoprotein lipase (cleaves fatty acids from glycerol backbone on triacylglycerol); MTP, microsomal transfer protein (joins lipid with apo B). Open arrows indicate secretion pathways (with secretion rates). Closed arrows indicate catabolic pathways (with mean residence times for apo B-48, apo A-I, and apo B-100, respectively). *See* references 31 and 32.

**TABLE 3**
Body mass index (in kg/m$^2$) and coronary heart disease risk factors[1]

| | Percentage affected | | |
| --- | --- | --- | --- |
| | BMI < 25 | BMI = 25–30 | BMI ≥ 30 |
| | | % | |
| Nonsmoking men ($n = 1147$) | 25.5 | 52.3 | 20.9 |
| Diabetes | 3.1 | 4.3[2] | 6.3[2] |
| Hypertension | 13.0 | 24.8[2] | 38.8[2] |
| Elevated LDL cholesterol | 14.8 | 25.7[2] | 25.0[2] |
| Low HDL cholesterol | 8.6 | 16.4[2] | 24.2[2] |
| Elevated TG | 7.0 | 17.7[2] | 27.1[2] |
| Nonsmoking women ($n = 1141$) | 55.5 | 27.4 | 17.0 |
| Diabetes | 0.3 | 2.2[2] | 6.7[2] |
| Hypertension | 16.3 | 25.0[2] | 41.8[2] |
| Elevated LDL cholesterol | 15.2 | 28.5[2] | 28.9[2] |
| Low HDL cholesterol | 1.1 | 2.5[2] | 7.7[2] |
| Elevated TG | 1.9 | 12.6[2] | 14.9[2] |

[1] Diabetes was defined as receiving insulin or an oral-glucose-lowering agent or having a fasting glucose concentration > 7.78 mmol/L (>140 mg/dL). Hypertension was defined as receiving an antihypertensive medicine or having systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg. Elevated LDL cholesterol was defined as > 4.1 mmol/L, low HDL cholesterol was defined as < 0.9 mmol/L, and elevated triacylglycerol (TG) was defined as > 2.2 mmol/L. The subjects' mean age was ≈53 y. From reference 58.

[2] Significantly different from group with BMI < 25, $P < 0.05$.

fed state. The residence time of LDL apo B-100 is ≈3.6 d, and LDL is the major cholesterol-carrying lipoprotein (31). LDL is cleared from plasma in part through the action of the LDL receptor (step 7) by both the liver and peripheral cells. Many patients with hypercholesterolemia have familial combined hyperlipoproteinemia, characterized by an overproduction of apo B-100 and premature CHD (30). Patients with marked elevations of LDL cholesterol and tendinous xanthomas generally have familial hypercholesterolemia resulting from a delayed catabolism of LDL apo B-100 associated with various defects in the LDL receptor (40, 41).

Apo A-I is essential for HDL formation because in its absence no HDL is present in plasma (step 3) (42–46). With my colleagues at the National Institutes of Health, I was the first to describe familial apo A-I deficiency. Patients with such a deficiency have premature CHD. The normal production of apo A-I is ≈12.2 mg·kg$^{-1}$·d$^{-1}$, and this protein in very small HDL particles can be produced both in the intestine and the liver (32). Patients with familial hypoalphalipoproteinemia have decreased apo A-I production and premature CHD, whereas patients with familial combined hyperlipidemia or familial dyslipidemia (high triacylglycerol concentrations) have low HDL and apo A-I concentrations as a result of enhanced apo A-I fractional catabolism (steps 6 and 7) (47, 48). Patients with defects in cellular cholesterol efflux and defects in the ATP binding cassette protein A1 gene (ABC A1 gene) have very small pre-β HDL particles, marked hypercatabolism of apo A-I, and premature CHD (step 9) (49–51). These patients have homozygous Tangier disease with cholesterol ester deposition in their tonsils, liver, spleen, and elsewhere in the body (49–51).

### Lipoprotein alterations associated with aging, sex, obesity, and diabetes

Fasting triacylglycerol concentrations increase significantly with age: by ≈80% between the ages of 20 and ≈50 y (52).

LDL-cholesterol concentrations also increase, by ≈30% (52). We investigated the reasons for these alterations and noted delayed chylomicron remnant clearance in the elderly compared with the young. In addition, older subjects have elevated VLDL apo B-100 secretion and delayed LDL apo B-100 clearance, accounting for the increases in triacylglycerol and LDL cholesterol (53, 54). In the very elderly (those aged >80 y), both triacylglycerol and LDL cholesterol are significantly lower than in middle-aged persons (52). These reductions mirror the reductions in body mass index that occur and may be due to decreased apo B-100 production in these subjects (52).

It is well known that women have significantly higher concentrations of HDL cholesterol and apo A-I than do men, and we documented that premenopausal women also have higher apo A-I secretion rates than do men (55). We also documented that estrogen increases HDL apo A-I production and liver *APOA1* gene expression (56, 57).

Obesity, of course, is an important factor in increasing the prevalence of CHD risk factors. At a body mass index (in kg/m$^2$) of >30 compared with <25, there are substantial increases in both men and women in the prevalence of the major modifiable risk factors other than cigarette smoking: hypertension, diabetes, elevated LDL cholesterol, and low HDL cholesterol (**Table 3**) (58). Moreover, in subjects with diabetes, there are substantial increases in triacylglycerol and remnant-like particles, decreases in HDL cholesterol, and alterations in LDL particle size (59).

### Postprandial lipoprotein alterations

After a fat-rich meal, plasma triacylglycerol concentrations increase ≈2-fold, whereas chylomicron apo B-48 concentrations increase 4-fold and VLDL apo B-100 and cholesterol concentrations increase 20%. There is significant variability in this response, in part because of alterations in *APOE* genotype (**Figure 3**). At the same time, LDL and HDL cholesterol decrease ≈7% as the result of the transfer of cholesterol esters from LDL and HDL to triacylglycerol-rich lipoproteins. We assessed the mechanisms whereby these changes occur and examined subjects in the constantly fed state compared with the fasted state. In the constantly fed state, we noted higher VLDL apo B-100 secretion, lower conversion of VLDL apo B-100 to LDL, and delayed LDL apo B-100 clearance (60). Therefore, with an influx of chylomicron particles from the intestine with
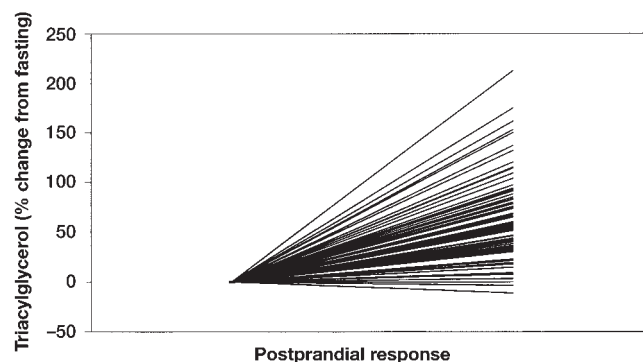


**FIGURE 3.** Variability in plasma triacylglycerol response to a meal rich in fat, saturated fat, and cholesterol [2 sausages, 2 eggs, and 2 muffins from McDonald's (Oak Brook, IL) containing 56 g fat and 510 mg cholesterol] in 88 normal subjects ($\overline{x}$: 71% increase) sampled in the fasting state (12 h) and 4 h after the fat meal (unpublished observations, 2001).

**TABLE 4**
National Cholesterol Education Program guidelines on dietary therapy[1]

| Nutrient | Average US diet[2] | Therapeutic lifestyle changes |
|---|---|---|
| Total fat (% of energy) | 34 | 25–35 |
| Saturated fat (% of energy) | 12 | <7 |
| Monounsaturated fat (% of energy) | 13 | <20 |
| Polyunsaturated fat (% of energy) | 7 | <10 |
| Cholesterol (mg/d) | 270 | <200 |
| Total energy | — | To achieve and maintain a desirable body weight |
| Carbohydrate (% of energy) | 51 | 50–60 |
| Protein (% of energy) | 15 | 15 |

[1] From reference 2.

[2] Total population data from the third National Health and Nutrition Examination Survey, excluding children aged <2 y.

rapid lipolysis and uptake of these particles by the liver, there is a need to export triacylglycerol-rich particles by the liver, resulting in an increase in apo B-100 secretion. Excess cholesterol is also delivered to the liver, resulting in down-regulation of LDL receptor activity and therefore delayed LDL clearance (33–35, 60). With fasting for >12 h, we found markedly lower intestinal triacylglycerol input, lower hepatic VLDL apo B-100 secretion, enhanced conversion of VLDL apo B-100 to LDL apo B-100, and higher LDL apo B-100 fractional catabolism (60).

### Genetic variation affecting plasma lipoprotein concentrations and therapeutic response

We have tested diets meeting NCEP Step II diet criteria (**Table 4**) and US Department of Agriculture (USDA) food guide pyramid guidelines (**Table 5**) under isoweight conditions, and have found these diets to be well tolerated and palatable and to result in mean LDL-cholesterol reductions of 15–20%; however, HDL cholesterol also is reduced by a mean of 15% (62–70). These reductions are stable within 4 wk of implementing the program and for up to 24 wk. We have also noted marked variability in LDL-cholesterol-lowering response (**Figure 4**) (67–69). We ascribed this to ≥2 major gene polymorphisms, the most important of which is in the *APOE* gene (71, 72).

Apo E is crucial for the liver uptake of remnant lipoproteins (38). The apo E-4 variant, resulting from an arginine for cysteine substitution at residue 112, occurs in 21% of the population. The apo E-2 variant, resulting from a cysteine for arginine substitution at residue 158, occurs in 14% of the population, mainly in the heterozygous state (73, 74). Homozygotes for the *E2* allele (*E2/E2*) or apo E deficiency have markedly delayed chylomicron remnant clearance (37, 38). We and others associated the presence of the *E4* allele with greater LDL-cholesterol lowering in response to a Step II diet and also noted less response to the use of hydroxymethylglutaryl-CoA reductase inhibitors (67, 71, 75). Others reported greater intestinal cholesterol absorption in subjects carrying the *E4* allele; we documented that these subjects' higher LDL-cholesterol concentrations are associated with delayed clearance (76, 77). Moreover, we and others documented that the *E4* allele is associated with a greater risk of CHD, Alzheimer disease, and dementia (78–80). In contrast, in the Framingham Heart Study, the *E2* allele was associated with lower LDL-cholesterol concentrations, elevated concentrations of remnant lipoproteins, an increased

risk of CHD in men, and a decreased risk of Alzheimer disease and dementia (74, 80). Moreover, patients with the *E2* allele are more responsive to statin therapy (75).

We also documented that men with the *APOA4* genotype resulting from a glutamine for histidine amino acid substitution at residue 360, present in 16% of the population in the heterozygous state, have a decreased LDL-cholesterol response to a Step II diet (72). Variations in other gene loci involved in lipoproteins and heart disease include the lipoprotein lipase 447X mutation, which occurs in ≈17% of the population and is associated with increased lipoprotein lipase activity, lower triacylglycerol concentrations, higher HDL-cholesterol concentrations, and lower CHD risk (81). In addition, genetic variation at the cholesterol ester transfer protein locus, specifically the *Taq*IB polymorphism, which is also common in the population and is associated with increased cholesterol ester transfer protein activity and low HDL cholesterol, is associated with increased CHD risk (82). We also documented that a scavenger receptor B1 exon mutation can affect HDL-cholesterol concentrations (83). This receptor plays an important role in HDL cholesterol uptake by the liver. We and others observed that mutations in the *ABC A1* gene cause a rare, severe form of HDL deficiency known as Tangier disease, and we documented that 3 common mutations at this gene locus are more common in CHD patients than in control subjects (50, 51). It is now clearly known that *ABC A1* plays an important role in cholesterol efflux from cells.

**TABLE 5**
US Department of Agriculture food guide pyramid[1]

| Daily food intake recommendations |
|---|
| 1) 6–11 servings of bread, cereal, rice, or pasta |
|     1 serving is 1 slice of bread, 1 oz ready-to-eat cereal, or 0.5 cup cereal, rice, or pasta. |
|     (Emphasis should be placed on whole-grain foods prepared with little or no sugar.) |
| 2) 3–5 servings of vegetables |
|     1 serving is 1 cup leafy vegetables, 0.5 cup other vegetables (cooked or chopped raw), or 0.75 cup vegetable juice. |
|     (Use canola or soybean oil on salads daily.) |
| 3) 2–4 servings of fruit |
|     1 serving is 1 apple, banana, or orange; 0.5 cup chopped, cooked, or canned fruit; or 0.75 cup fruit juice. |
|     (Use items with little or no added sugar.) |
| 4) 2–3 servings of milk, yogurt, or cheese |
|     1 serving is 1 cup of milk or yogurt, 1.5 oz natural cheese, or 2 oz processed cheese. |
|     (Use nonfat or low-fat items such as skimmed or low-fat milk or cheeses made from these products. Replace butter with soft *trans* fatty acid–free margarine.) |
| 5) 2–3 servings of meat, poultry, fish, dried beans, eggs, or nuts |
|     1 serving is 2–3 oz lean meat, poultry (white meat without skin), or fish; 1 egg; or 1 cup beans or nuts. |
|     (Replace lean red meat with fish or poultry as much as possible, and restrict use of egg yolks.) |
| 6) Use fats, oils, and sugars (including syrup) sparingly |
|     (Replace with "Use animal, dairy, and hydrogenated or *trans* fats; tropical oils; red meat; egg yolks; and sugars sparingly.") |

[1] My proposals for change to these guidelines are in parentheses. Additional proposed modifications: ≥8 servings of water or fluids/d and calcium, vitamin D, and vitamin B-12 supplements (61). To convert to SI units, 1 oz ≈ 28 g and 1 cup ≈ 236 mL.
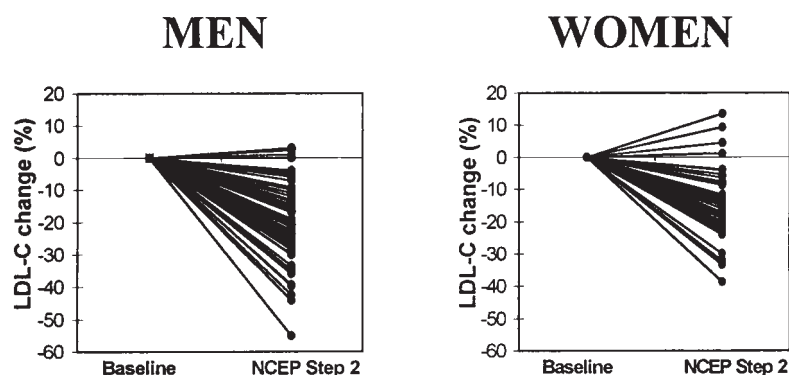
## MEN WOMEN



**FIGURE 4.** Variability in plasma LDL-cholesterol (LDL-C) response to a National Cholesterol Education Program Step II diet (containing <7% saturated fat and <200 mg dietary cholesterol/d) compared with an average US diet under isoenergetic conditions in 72 men and 48 women. Reprinted with permission from reference 75.

## DIETARY FATS

Dietary fats and cholesterol play a major role in CHD development, mostly by modulating plasma lipoprotein concentrations. Dietary modification remains the cornerstone of CHD prevention. The major constituents of the diet are proteins, carbohydrates, and fats. The average US diet contains ≈34% of energy as total fat (Table 4). Fats are further classified on the basis of the number and type of chemical bonds that they possess.

### Saturated fatty acids

Saturated fatty acids contain no double bonds and generally vary in chain length from 12 to 18 carbons. The average US intake is ≈12% of energy (Table 4). The most prevalent saturated fatty acid in the diet is palmitic acid (16:0), followed in order of abundance by stearic (18:0), myristic (14:0), and lauric (12:0) acids. Major sources of saturated fat in the US diet include dairy, beef, pork, poultry, and lamb products. These foods have almost as much monounsaturated fats as saturated fat. Not all saturated fatty acids affect total cholesterol concentrations in the same manner. Stearic acid has little effect on plasma cholesterol concentrations (84, 85), whereas myristic and palmitic acids have been reported to have the greatest cholesterol-raising potential (86). It is postulated that the relatively neutral effect of stearic acid is due to its rapid conversion in the body to oleic acid (*cis* 18:1n−9), a monounsaturated fatty acid (87). Saturated fatty acids increase LDL-cholesterol concentrations by decreasing LDL receptor–mediated catabolism (88, 89). This effect in our view is mediated both by decreased LDL receptor messenger RNA (mRNA) expression and decreased membrane fluidity (90). We postulate that this latter effect causes less receptor recycling across the cell membrane.

When dietary cholesterol is kept constant, saturated fatty acids relative to monounsaturated fatty acids and polyunsaturated fatty acids (PUFAs) or carbohydrate also increase HDL-cholesterol concentrations in both animals and humans, primarily by delaying the clearance of HDL apo A-I from the plasma compartment (91–93). Conversely, when dietary cholesterol is reduced along with total and saturated fat, decreased HDL apo A-I production and hepatic apo A-I mRNA expression are observed (94–96). In our view, this latter alteration in HDL cholesterol and apo A-I is compensatory because there is less need for reverse cholesterol transport when dietary cholesterol is restricted. Therefore, it is important to reduce saturated fat even though HDL cholesterol decreases because LDL cholesterol decreases much more and overall CHD risk decreases as a result. It is recommended that dietary saturated fat intake be <7% of energy to reduce CHD risk (Table 4).

### Monounsaturated fatty acids

The major monounsaturated fatty acid in the diet is oleic acid, which contains one double bond at the number 9 carbon and is consumed in the US diet at ≈13% of energy. An upper limit of 20% of energy is advocated by the NCEP (Table 4). Early work conducted in the 1960s suggested that monounsaturated fatty acids, as compared with dietary carbohydrates, were neutral with respect to their effects on plasma total cholesterol concentrations (85, 97). Many investigators have shown that, when substituted for dietary saturated fatty acids, monounsaturated fatty acids have a hypocholesterolemic effect (62, 98–106). In our own animal studies, monounsaturated fats enhanced LDL apo B clearance relative to saturated fat (107). The overall data indicate that monounsaturated fats do not lower LDL or HDL cholesterol relative to saturated fat as much as does polyunsaturated fat (104–108). The major sources of oleic acid in the diet are the same as those previously listed for saturated fatty acids, except for canola oil (**Table 6**). For this reason, the saturated fat and monounsaturated fat contents of most natural diets are similar, and when saturated fat is restricted, the monounsaturated fat content of the diet decreases.

### *trans* Fatty acids

A relatively new concern with respect to monounsaturated fatty acid consumption involves the concentration of *trans* fatty acids in the diet (63, 64, 110, 111). In *trans* isomers of fatty acids, the carbon moieties on the 2 sides of a double bond provide a straight, closely packed configuration because the attached hydrogen moieties are on opposite sides of the carbon chain. In contrast, the *cis* isomers found in vegetable oils have a bent configuration because the hydrogen moieties are on the same side of the carbon chain (**Figure 5**). Elaidic acid (*trans* 18:1n−9) is the principal *trans* fatty acid in the diet. *trans* Fatty acids are formed during the hydrogenation process, a process that converts vegetable oils to a semisolid state. During this process, α-linoleic acid (18:2n−6) is converted to either stearic acid, oleic acid, or elaidic acid. Studies have shown that, compared with diets enriched in α-linoleic or

**TABLE 6**
Comparison of dietary fats in order of saturated fat content[1]

| Fat | SFA | MUFA | PUFA | Cholesterol |
|---|---|---|---|---|
|  | % | % | % | g/L |
| Canola oil[2] | 6 | 62 | 31 | 0 |
| Safflower oil | 9 | 12 | 78 | 0 |
| Sunflower oil | 11 | 20 | 69 | 0 |
| Corn oil | 13 | 25 | 62 | 0 |
| Peanut oil | 13 | 49 | 33 | 0 |
| Olive oil | 14 | 77 | 9 | 0 |
| Soybean oil[2] | 15 | 24 | 61 | 0 |
| Margarine[3] | 18 | 48 | 29 | 0 |
| Cottonseed oil[2] | 27 | 19 | 54 | 0 |
| Vegetable shortening[3] | 25 | 43 | 25 | 0 |
| Chicken fat | 30 | 47 | 22 | 2.2 |
| Animal fat shortening[3] | 43 | 48 | 6 | 1.8 |
| Lard | 41 | 47 | 12 | 2.4 |
| Beef fat | 51 | 44 | 4 | 2.8 |
| Palm oil | 51 | 39 | 10 | 0 |
| Butter[3] | 54 | 30 | 12 | 6.6 |
| Coconut oil | 77 | 6 | 15 | 0 |

[1] Values for SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids) represent the percentage of total energy from fat. SFA is the sum of lauric, myristic, palmitic, and stearic acids (109).

[2] Contains a considerable amount (>5%) of α-linolenic acid.

[3] Can be high in *trans* fatty acids: vegetable shortening > margarine > animal fat shortening > butter; however, new soft margarines are very low in *trans* fatty acids.

oleic acid, diets enriched in elaidic acid do not have a beneficial effect on the plasma lipoprotein profile, elevating LDL-cholesterol and reducing HDL-cholesterol concentrations (63, 64, 110–115). There is also evidence that increased *trans* fatty acid consumption, in contrast with consumption of saturated fat, increases plasma concentrations of lipoprotein(a), an independent risk factor for CHD (64, 114, 115).

We have carefully examined the effects of dietary oils at 20% of energy and documented that canola and soybean oils are superior to other oils in terms of their effects on LDL cholesterol and the ratio of total to HDL cholesterol (62). We also observed that hydrogenated soybean oil margarine increases LDL cholesterol by 7% compared with native soybean oil. Therefore, hydrogenation and *trans* fatty acid formation should be kept to a minimum. Soft margarine that is low in *trans* fatty acids is superior to butter in terms of LDL lowering (11% lower), and the ratio of total to HDL cholesterol is 5% lower (64). Clearly, soybean oil is the best
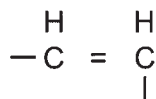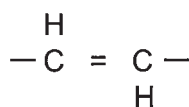


FIGURE 5. *cis* and *trans* Fatty acid double bond configurations. Unlike the *cis* configuration, the *trans* configuration results in no angle in the carbon chain. The *trans* configuration occurs with hydrogenation.

compared with butter, resulting in 12% lower LDL-cholesterol concentrations and having a 6% lower ratio of total to HDL cholesterol (64). Hydrogenated margarines and butter should be avoided, and either oil or soft margarine low in *trans* fatty acids should be used as a spread. Major sources of *trans* fatty acids in the diet are baked products, processed foods, and margarines rich in hydrogenated fat or oil (Table 6).

**Polyunsaturated fatty acids**

Dietary PUFAs are subclassified as n−6 and n−3, indicating the location of the carbon involved in the first double bond from the omega end of the carbon chain (**Figure 6**). The major n−6 fatty acid in the diet is α-linoleic acid, which serves as the precursor for arachidonic acid (20:4n−6), which has important biological effects in the body. α-Linoleic acid is not synthesized by the body and is therefore an essential fatty acid. Food sources rich in α-linoleic acid include vegetables and vegetable oils (corn, soybean, safflower, and sunflower), with the exception of coconut and palm oils (Table 6). The other major essential fatty acid in the diet is α-linolenic acid (18:3n−3). This fatty acid can be rapidly converted in the body to eicosapentaenoic acid (20:5n−3), which can be further elongated, desaturated, and β oxidized to docosahexaenoic acid (22:6n−3) (116). Certain patients with genetic retinal disorders appear to have a defect in the formation of docosahexaenoic acid (117, 118).

Linoleic acid clearly has a hypocholesterolemic effect when substituted for dietary saturated fatty acids, reducing both LDL- and HDL-cholesterol concentrations. However, dietary arachidonic acid has little or no effect on plasma lipoprotein concentrations (119). n−3 Fatty acids found in fish oil, especially eicosapentaenoic acid, lower triacylglycerol concentrations significantly and reduce CHD risk as well, in part, independently of their influence on lipoprotein concentrations (120, 121). Specifically, high intakes of n−3 fatty acids are associated with lower platelet aggregation, lower immune response, and lower blood pressure (122–126). Our data indicate that it is eicosapentaenoic acid and not docosahexaenoic acid that lowers the immune response. Adequate intake of essential fatty acids is 5% of total energy, with ≥1% derived from n−3 fatty acids and the remainder from n−6 fatty acids. We observed a daily intake of 12–13% in a Taipei population in Taiwan that had one-third the age-adjusted CHD rates of Americans (127). It is recommended that the polyunsaturated fat intake be <10% of energy (2). Major sources of polyunsaturated fats in the US diet are soybean oil, other vegetable oils, and foods containing these products (Table 6). An optimal ratio of n−6 to n−3 fatty acids in the diet is believed to be ≈4:1.

**Cholesterol**

In 1913 Anitschkow (128) first documented the important role of dietary cholesterol in the pathogenesis of atherosclerosis by using a rabbit model. Since that time, a myriad of studies have shown that raising dietary cholesterol results in higher plasma cholesterol concentrations (129–132) and is associated with

$$CH_3 - (CH_2)_3 - (CH_2 - CH = CH)_2 - (CH_2)_7 - COOH$$
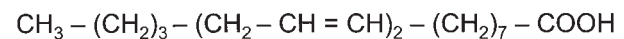
Omega end                                                          Alpha end

FIGURE 6. Structure of linoleic acid, the major essential fatty acid, showing the location of the double bond.
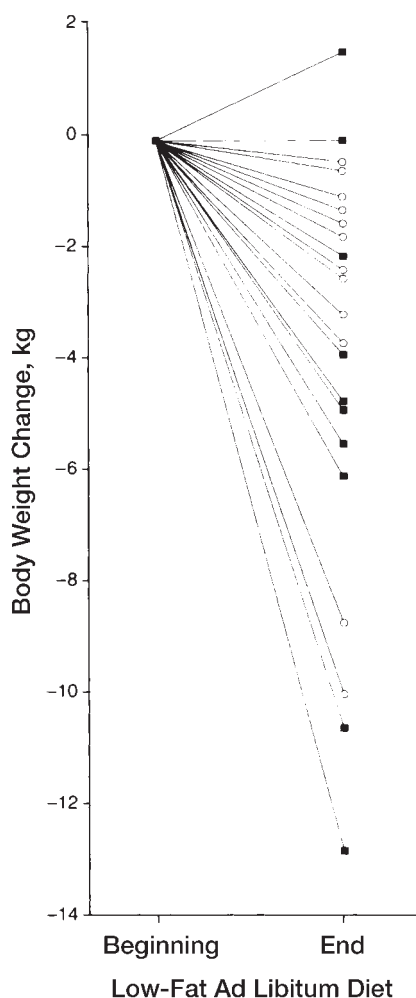
**FIGURE 7.** Variability in body weight change ($\bar{x}$: 3.2 kg decrease) with consumption of an ad libitum diet containing 15% fat over 12 wk in 29 subjects. Reprinted with permission from reference 153.

increased risk of CHD (133–142). Less well defined are the interactive effects between dietary fatty acid composition and cholesterol with respect to the plasma lipoprotein response (143, 144). Most studies have shown that dietary cholesterol is a less potent regulator of plasma cholesterol concentrations than are dietary fatty acids, but that the former may act synergistically with the latter in the mediation of plasma cholesterol concentrations. Responsiveness to dietary cholesterol varies over a wider range than does responsiveness to other dietary variables, further complicating the interaction between fatty acids and cholesterol (66, 130, 131, 144).

We have examined the effects of dietary cholesterol as well, and noted that 1.3 egg yolks/d containing 272 mg cholesterol increases LDL cholesterol by a mean of 8% with a high–corn oil diet and by a mean of 11% with a high–beef tallow diet (when either corn oil or beef was fed at 20% of energy) (66). Note that egg yolks are a rich source of lutein and zeaxanthin and increase the plasma concentrations of these constituents by 28% and 142%, respectively, which may be important in preventing macular degeneration (145). However, lutein can now be readily obtained in vitamin supplements.

The plasma LDL-cholesterol response to dietary cholesterol, as well as to dietary fatty acids, can be influenced by genetic factors, such as *APOA4* and *APOE* genotype (71, 72). *APOE* genotype has been reported to affect cholesterol absorption, with subjects carrying the *E4* allele having the highest cholesterol absorption rates (76). Although the relation between dietary cholesterol and plasma cholesterol concentrations is complex, what remains clear is that cholesterol, along with saturated fat, should be restricted in the diet to ≤200 mg/d to decrease CHD risk (Table 4). Major sources of cholesterol in the US diet are egg yolk and the sources of saturated fatty acids previously mentioned (Table 6). In our studies, when saturated fat was reduced to <7% of energy and cholesterol to <200 mg/d, LDL cholesterol was reduced by ≈15–20% compared with an average US diet. However, the variability in response was great (Figure 4) (67, 68). Increasing fish intake with such a diet decreases postprandial plasma triacylglycerol concentrations (69).

### Level of fat

An article by Katan et al (146) has created controversy over the recommendation for dietary fat restriction in the prevention of CHD. The controversy involves whether a diet in which the total fat content is held constant, but is relatively enriched in monounsaturated fatty acids, offers better protection against CHD than does a low-fat diet. This argument has stemmed primarily from the observation that low-fat diets often reduce both HDL-cholesterol and LDL-cholesterol concentrations (147). However, a study of coronary artery atherosclerosis in African Green monkeys showed that, when isoenergetically substituted for saturated fat, monounsaturated fat failed to provide protection against the development of atherosclerosis (148, 149). This was despite the fact that the monounsaturated fat diet was associated with the most favorable plasma lipoprotein profile, specifically, the lowest ratio of LDL to HDL cholesterol. Because nonhuman primate models of atherosclerosis have been good predictors of human outcomes, we believe that the recommendation to increase dietary monounsaturated fat content, as made by Katan and colleagues, is not justified. Additionally, the studies of Ornish et al (150–152), in which oils were largely eliminated from the diet, showed that dietary fat restriction did not significantly lower HDL-cholesterol concentrations, most likely because of the significant weight loss that occurred simultaneously in the experimental group. Moreover, the studies of Ornish et al (150–152) showed significant benefit on coronary atherosclerosis with a very-low-fat diet along with exercise, yoga, and meditation.

Another important issue regarding the recommendation for diets enriched in monounsaturated fats diets concerns the fact that fats rich in saturated fat often contain a substantial amount of monounsaturated fat as well (Table 6). Thus, restricting foods rich in saturated fat (eg, beef, pork, and dairy fat) leads to a concomitant decrease in monounsaturated fat. Supplementing the diet with monounsaturated fat leads to increased energy intake, promoting weight gain. In contrast, low-fat diets may be beneficial for promoting weight loss because of their reduced energy density (fat = 37.66 kJ/g; protein and carbohydrate = 16.74 kJ/g). Under controlled dietary circumstances, we documented that a diet containing 15% of energy as fat promotes weight loss when subjects are allowed to adjust their own energy intake between 66% and 133% of energy needed to maintain weight (70). However, there is wide variability in response (**Figure 7**). Dietary fat restriction (<30% of energy) along with exercise appears to be

**TABLE 7**
Predictive equations for estimating changes (Δ) in plasma cholesterol and lipoprotein cholesterol in response to dietary fatty acids and cholesterol[1]

Total cholesterol in mg/dL
  Hegsted et al (85)
    $\Delta TC = 2.16\ \Delta S - 1.65\ \Delta P + 0.067\ \Delta C - 0.53$
  Keys et al (97)
    $\Delta TC = 1.35(2\ \Delta S - \Delta P) + 1.52\ \Delta Z$
Total, LDL, and HDL cholesterol in mg/dL
  Mensink and Katan (104)
    $\Delta TC = 1.51\ \Delta S - 0.12\ \Delta M - 0.60\ \Delta P$
    $\Delta LDL\text{-}C = 1.28\ \Delta S - 0.24\ \Delta M - 0.55\ \Delta P$
    $\Delta HDL\text{-}C = 0.47\ \Delta S + 0.34\ \Delta M + 0.28\ \Delta P$
  Hegsted et al (158)
    $\Delta TC = 2.10\ \Delta S - 1.16\ \Delta P + 0.067\ \Delta C$
    $\Delta LDL\text{-}C = 1.74\ \Delta S - 0.77\ \Delta P + 0.044\ \Delta C$
  Yu et al (157)
    $\Delta TC = 2.02\ \Delta12{:}0\text{-}16{:}0 - 0.03\ \Delta18{:}0 - 0.48\ \Delta M - 0.96\ \Delta P$
    $\Delta LDL\text{-}C = 1.46\ \Delta12{:}0\text{-}16{:}0 + 0.07\ \Delta18{:}0 - 0.69\ \Delta M - 0.96\ \Delta P$
    $\Delta HDL\text{-}C = 0.62\ \Delta12{:}0\text{-}16{:}0 - 0.06\ \Delta18{:}0 + 0.39\ \Delta M + 0.24\ \Delta P$
LDL cholesterol expressed in terms of percentage change[2]
    $\Delta LDL\text{-}C\ (\%) = 1.34\ \Delta S - 0.59\ \Delta P + 0.033\ \Delta C$

[1] Where ΔTC, ΔLDL-C, and ΔHDL-C are changes in plasma total, LDL, and HDL cholesterol; ΔS is change in percentage of daily energy from saturated fatty acids; ΔM is change in percentage of daily energy from monounsaturated fatty acids; ΔP is change in percentage of daily energy from polyunsaturated fatty acids; ΔZ is change in the square root of daily dietary cholesterol in ≈60 ng/kJ (mg/1000 kcal); and ΔC is change in dietary cholesterol in mg/d.

[2] Based on Hegsted et al (158), converted to percentage change assuming an average LDL-C concentration of 3.4 mmol/L (130 mg/dL).

very important in the prevention of obesity, which is so common in our society (70, 154). A reasonable fat intake in my view is 15–30% of energy, and not 25–35% as recommended by the NCEP (Table 4) (2). The ideal natural oil may be canola oil because of its low saturated fat content and its reasonable balance of n−6 to n−3 fatty acids (ratio of 2:1) (Table 6).

We carried out experiments in outpatients and observed weight losses of ≈0.7 kg/wk when subjects with CHD were given a low-fat NCEP Step II diet meeting the USDA food guide pyramid criteria (70). When the subjects with CHD received 2 meals and 2 snacks, a 3-kg weight loss and a 14% lowering of LDL cholesterol were noted; when the subjects received 1 meal and 2 snacks over 4 wk, only a 1-kg weight loss and a 6% reduction in LDL cholesterol were noted. Clearly, compliance with diet is a major problem in the free-living state, and the most reliable way to achieve dietary modification is by providing the food. Such an approach may be important in the future for those in whom dietary compliance is essential.

## RATIONALE FOR DIETARY THERAPY

### Animal model evidence

Animal models have shown a direct relation between LDL-cholesterol concentrations and atherosclerosis. Animals consuming diets high in saturated fat and cholesterol have elevated LDL-cholesterol concentrations and develop intimal lesions that progress from fatty streaks to ulcerated plaques, resembling those of human atherosclerosis (155). In monkeys, severe atherosclerosis regresses when blood cholesterol is lowered through diet therapy. Such studies support a causal relation between LDL cholesterol

and atherosclerosis and, further, suggest reversibility of the process when plasma LDL-cholesterol concentrations are reduced.

### Human study evidence

Beginning in the 1950s, systematic investigations were conducted to determine how dietary fatty acids affect plasma cholesterol concentrations. Regression analyses reported in 1965 by Hegsted et al (85) and Keys et al (97) characterized the effects of dietary fat type and individual fatty acids on plasma total cholesterol concentrations, leading to the generation of predictive equations. More recently, these equations have been modified to account for the effects of dietary fatty acids on LDL- and HDL-cholesterol concentrations (104, 156–158), with only the new Hegsted equation including dietary cholesterol (158) (**Table 7**). All of these equations calculate an absolute change in LDL cholesterol, assuming that the magnitude of change is the same regardless of the baseline value, which in our own studies is clearly not the case (67). We documented that hypercholesterolemic subjects have greater absolute reductions in LDL cholesterol than do subjects with lower initial values (67). Therefore, we modified the new Hegsted equation to calculate the percentage change in LDL cholesterol on the basis of an average value (Table 7). The data indicate that for every 1% decrease in energy as saturated fat there is a 1.34% decrease in LDL cholesterol. For every 1% increase in energy as polyunsaturated fat there is a 0.59% decrease in LDL cholesterol, and for every 100-mg/d decrease in dietary cholesterol, there is a 3.3% decrease in LDL cholesterol under isoenergetic conditions.

### Epidemiologic evidence

Epidemiologic studies have led to the identification of key dietary components that are etiologic factors in the pathogenesis of CHD. In these studies, comparisons are made either between populations (international or cross-populational) or within populations (cross-sectional or longitudinal). The strength of epidemiologic studies lies in the number of people involved and in the duration of the follow-up. Of the dietary components examined, both total and saturated fat, as well as cholesterol, have shown the most consistent significant associations with CHD mortality (159).

Cross-population studies allow for the analysis of individuals that differ greatly with respect to diet and CHD incidence. The most classic of the cross-population studies are the Seven Countries Study (160) and the Japan-Honolulu–San Francisco or Ni-Hon-San Study (161, 162). Both of these studies examined the relations between dietary intake and CHD in large populations of middle-aged men. In the Seven Countries Study, dietary intake was assessed with 7-d food records and, in some instances, by chemical analysis of the diet. Total fat intake ranged from 40% of energy in the United States and Finland to <20% of energy in Japan, with saturated fat intake varying from 20% to <10% of energy. This study, which involved >12000 men from 16 populations, was the first to show a strong correlation between saturated fat intake and CHD mortality (r = 0.84). It was shown that this relation extended through 25 y of follow-up (160).

Similar results were obtained in the Ni-Hon-San Study. This study involved 3 cohorts of middle-aged men of Japanese ancestry living in Japan, Honolulu, or San Francisco (161, 162). Dietary intakes in these 3 groups differed greatly, with the group residing in Japan having the lowest total fat intake (15%), followed by the Honolulu (33%) and San Francisco (38%) groups

**TABLE 8**
Characteristics of men participating in the Ni-Hon-San Study[1]

|  | Residence | | |
| --- | --- | --- | --- |
|  | Japan | Honolulu | San Francisco |
| Age (y) | 57 | 54 | 52 |
| Weight (kg) | 55 | 63 | 66 |
| Serum cholesterol (mmol/L) | 4.60 | 5.61 | 5.87 |
| Total fat (% of energy) | 15 | 33 | 38 |
| Protein (% of energy) | 14 | 17 | 16 |
| Carbohydrate (% of energy) | 63 | 46 | 44 |
| Alcohol (% of energy) | 9 | 4 | 3 |
| 5-y CHD mortality rate (/1000) | 1.3 | 2.2 | 3.7 |

[1] CHD, coronary heart disease. From references 140, 161, and 162.

(**Table 8**). Intake of saturated fat paralleled that of total fat. CHD mortality rates were significantly higher for those living in Honolulu and San Francisco than for those living in Japan, leading to the conclusion that both total and saturated fat intakes are directly associated with CHD risk.

In the Twenty Countries Study, Stamler (140) compared the consumption of several dietary variables from 1954 to 1965 with CHD mortality data for men and women during the period of 1971 and 1973. As shown in **Table 9**, significant positive associations were observed between CHD mortality and the consumption of butter, all dairy products, and meat and poultry, all of which are relatively enriched in saturated fat. Conversely, a significant inverse association was noted between CHD mortality and the consumption of grains, fruit, and starchy and nonstarchy vegetables, foods relatively rich in complex carbohydrates. However, a positive association was noted with sugar and syrup consumption, indicating that all carbohydrates are not the same regarding CHD risk. In 1988 Hegsted and Ausman (163) examined the relations between diet, alcohol, and CHD by using dietary and mortality data from 18 of the countries studied by Stamler. Their analyses showed that saturated and polyunsaturated fat consumption were significant factors in CHD risk, with saturated fat having a detrimental effect and polyunsaturated fat a protective effect. The regression coefficient for saturated fat (0.71) was nearly twice that of polyunsaturated fat ($-0.34$), consistent with the early predictive equations of Hegsted et al (85) and Keys et al (97). A significant inverse association was also observed between CHD mortality and total alcohol consumption ($r = -0.58$).

In contrast with cross-population studies, within-population studies allow for adjustments to be made in the analyses for many of the confounding variables that can also contribute to CHD risk, such as blood pressure and body weight, and groups from within a similar environment can be studied. Despite having differences in design, within-population and cross-population studies are generally consistent in showing that saturated fat and cholesterol intakes are positively correlated with CHD risk. However, the magnitude of the dietary effects on CHD risk is much less, probably because of greater dietary homogeneity within populations than between populations. Within-population studies showing a positive association between saturated fat consumption and CHD risk include the Ireland-Boston Diet-Heart Study, the Honolulu Heart Program Study, the Belgium Study, and the Atherosclerosis Risk in Communities Study, or ARIC, among others (133, 136–141, 164). In most cases, associations with monounsaturated fats were not reported, with the exception of a cohort of the Framingham Study in which a positive relation

was observed between monounsaturated fat intake and CHD risk in men (165). No other significant dietary relations were noted in this study. A strong inverse association between the intake of polyunsaturated fat and CHD risk was reported in 2 studies: the Western Electric Study (139) and the Belgium Study (166), the latter of which involved >21 000 men and women.

The relation between *trans* fatty acid intake and CHD risk was examined in the Nurses' Health Study (167). After adjustment for total energy consumption, the relative risk of CHD was 50% ($P = 0.001$) greater in those consuming the highest (5.7 g/d) percentage of *trans* fatty acids than in those consuming the lowest (2.4 g/d) percentage, but a dose-response relation was not observed at intermediate intakes.

Investigators of the Physicians' Health Study, a prospective cohort study of US male physicians, have examined the potential benefits of n−3 fatty acids with respect to CHD risk (168, 169), as originally proposed by Kromhout et al in 1985 (135, 136). Fish consumption was assessed by use of semiquantitative food-frequency questionnaires, which required respondents to indicate how often on average during the past year they had consumed selected foods. After control for age, randomized aspirin and β-carotene assignment, and CHD risk factors, dietary fish intake was associated with a reduced risk of sudden death ($-52\%$, $P = 0.03$), with an apparent threshold effect at a consumption of one fatty fish meal per week, the equivalent of ≈1.5 g n−3 fatty acids.

The difficulties with these cross-sectional studies is that, within populations, there is often much dietary homogeneity, and genetic variation may be more significant. Moreover, in our own studies, food-frequency questionnaires are much less accurate in assessing actual chemically defined intakes than are 3-d food records (170). With food-frequency questionnaires, no assessment of energy intake is obtained and assumptions about this variable are made from body weight. For these reasons, estimates of percentage of energy intake from various macronutrients are highly inaccurate. Therefore, more emphasis must be placed on studies in which diet is assessed with the use of actual food records.

### Dietary intervention trial evidence

Many studies of cholesterol lowering by dietary means have been carried out. In 1946 Morrison (171, 172) initiated a dietary trial consisting of an experimental group of 42 men and 8 women who were survivors of a MI and a control group of 43 men and 7 women. Alternate patients were placed on a low-fat (25 g), low-cholesterol (50–70 mg/d) diet and were followed for 3 y. Patients in the experimental group were found to have significant reductions in total cholesterol concentrations and less CHD mortality

**TABLE 9**
Associations observed between the percentage of energy derived from specific foods and coronary heart disease mortality in the Twenty Countries Study[1]

| Food source | Correlation coefficient |
| --- | --- |
| Butter | 0.546 |
| All dairy products | 0.619 |
| Eggs | 0.592 |
| Meat and poultry | 0.561 |
| Sugar and syrup | 0.676 |
| Grains, fruit, and starchy and nonstarchy vegetables | −0.633 |

[1] $P < 0.0001$ for all. From reference 140.

**TABLE 10**
Selected dietary intervention trials with coronary heart disease (CHD) morbidity or mortality or both as endpoints[1]

| Study | Subjects | | Study characteristics | | | Percentage change in TC from baseline[2] | Comments |
|---|---|---|---|---|---|---|---|
| | n | Age | Design | Random-ized | Dura-tion | | |
| | | y | | | y | % | |
| Finnish Mental Hospital (180–182)[3] | 5115 M + F 5497 M + F | >15 | Crossover | No | 6 | −12 to 18 | 53% decrease in CHD mortality in men (P < 0.002), with a 34% decrease in women (NS). |
| Oslo Diet-Heart (183) | 412 M with CHD | 30–64 | Unblinded | Yes | 5 | −14 | 33% decrease in MI (P < 0.05). 26% decrease in CHD mortality (NS). |
| Los Angeles VA (184) | 846 M | 50–89 | Double-blinded | Yes | 5–8 | −13 | 31% decrease in the endpoints of MI, CHD mortality, CVA, ruptured aneurysm, and ischemic gangrene (P < 0.01); 20% decrease in primary endpoints of MI and sudden death (NS). |
| Minnesota Mental Hospital (185) | 9057 M + F | All | Double-blinded | Yes | <4.5 | −14 | No significant differences or trends were noted in MI or sudden death. |
| DART (186) | 2033 M | <70 | Factorial | Yes | 2 | −2.8[4] | 29% decrease in 2-y all-cause mortality in CHD subjects advised to eat fish (P < 0.05) as a result of 33% decrease in CHD mortality (P < 0.01). |
| Lyon Diet Heart (187, 188) | 605 M + F with CHD | <70 | Single-blinded | Yes | 5 | −7.5 | 65% decrease in CHD mortality in post-MI patients fed an α-linolenic–rich diet (P < 0.01). |
| GISSI (189) | 11 324 M + F with CHD | Not defined | Factorial | Yes | 3.5 | 7–9 | 15% decrease in relative risk of all-cause death, nonfatal MI, and nonfatal stroke in the 2 groups supplemented with 1 g n−3 PUFA. No benefit was seen in the group given vitamin E. |
| HOPE (190, 191) | 9541 M + F with CHD | ≥55 | Double-blinded | Yes | 4–6 | NR | 400 mg vitamin E taken daily had no beneficial effects on cardiovascular outcomes in a high-risk patient population. |

[1] CVA, cerebrovascular accident; DART, Diet and Reinfarction Trial; GISSI, Gruppo Italiano per lo Studio della Sopravivenza nell'Infarto miocardico; HOPE, Heart Outcomes Prevention Evaluation; MI, myocardial infarction; NR, not reported; PUFA, polyunsaturated fatty acids; TC, total cholesterol.

[2] The percentage change in plasma total, rather than LDL, cholesterol is reported because of the unavailability of the latter value in the early intervention trials.

[3] Values are for the periods of 1959–1965 and 1965–1971, respectively.

[4] Value represents decrease in those given fat advice only (NS). No changes from baseline were noted in either the fiber or fish advice groups.

at the 12-y follow-up. Several other relatively small trials were conducted between 1956 and 1965, which similarly concluded that restriction of dietary fat and cholesterol, under controlled conditions, significantly reduces CHD mortality (173–179).

The results of several other dietary intervention trials conducted after 1965 in which CHD morbidity or mortality was reported as the endpoint are summarized in **Table 10**. These studies were larger than those reported in earlier years. The Finnish Mental Hospital Study used a crossover design and involved >10 000 male and female inpatients at 2 mental hospitals (180–182). The experimental diet, in which milk was replaced by an emulsion of soybean oil in skim milk and butter by margarine, resulted in mean reductions in plasma total cholesterol concentrations of 12–18%. In men, CHD mortality was reduced by 53% (P < 0.002) by the experimental diet, whereas a 34% reduction was observed in women (NS). The first Oslo Diet-Heart Study involved 412 survivors of an MI, aged 30–64 y, who were randomly assigned to a diet low in saturated fat (8.4% of energy) and cholesterol (264 mg/d) but high in polyunsaturated fat (15.5% of energy), with 28% of total energy derived from soybean oil rich in linoleic acid (183). After 5 y of follow-up, the intervention group had a mean decrease in total cholesterol of 14%, a 33% reduction in MIs (P < 0.05), and a 26% decrease in CHD mortality (NS).

The Los Angeles Veterans Administration Study also used a diet relatively high in polyunsaturated fat (184). In this trial, men living in a veterans' home were randomly assigned to either treatment or control groups. The design of the treatment diet involved substitution of vegetable oils (corn, cottonseed, safflower, and soybean) for about two-thirds of the animal fat in the diet. The 2 groups were fed a diet containing 40% of total energy as fat, with the treatment diet containing 3 times more polyunsaturated fat (16% of energy) and 40% less cholesterol (365 mg/d) than the control diet. Saturated fat intake was ≈11% of energy in the treatment group and 18% of energy in the control group. A trend in favor of the treatment group for the primary endpoints of MI and sudden death was observed. When other atherosclerotic events were included, dietary treatment was found to be of significant benefit in CHD risk reduction.

Another trial of significantly greater size, the Minnesota Coronary Survey, also assessed diets of comparable total fat content (185). This double-blind, randomized trial involved 9057 patients at 6 mental hospitals and 1 nursing home in Minnesota. Treatment and control diets included 39% of energy from fat, but the former diet was relatively enriched in polyunsaturated fat (15% compared with 5%) and lower in saturated fat (9% compared with 18%) and cholesterol (166 compared with 446 mg/d).

Despite a mean 14% reduction in plasma cholesterol concentrations, no significant differences were noted in the study's primary endpoints of MI and sudden death. The negative results may have been due, in part, to the low mean cholesterol concentration of the subjects at baseline (207 mg/dL, or 5.3 mmol/L), as well as the low mean age of the study participants (192).

In the Diet and Reinfarction Trial, 2033 men who had recovered from an MI were allocated to receive or not to receive advice on each of 3 dietary factors: *1*) a reduction in fat intake with an increase in the ratio of PUFAs to saturated fatty acids, *2*) an increase in fish intake, and *3*) an increase in cereal fiber intake (186). Members of the fish group could consume fish oil capsules (two 1-g capsules/d) as a partial or total substitute for fatty fish. A net reduction of 2.8% in total cholesterol was observed in the group that received advice on fat intake over the 2-y period, whereas no significant differences were noted in either total or HDL-cholesterol concentrations of the groups who received advice on fish and fiber intakes. Decreased mortality was not seen in those advised on either fat or fiber intake. In contrast, total mortality was reduced by 29% in those advised to increase their fish intake relative to those not so advised, with the difference being entirely attributable to a decrease in CHD deaths.

The Lyon Diet Heart Study, a randomized secondary prevention trial, compared the effects of a Mediterranean diet enriched in α-linolenic acid with a diet similar to that of the NCEP Step I diet in MI survivors (187). The experimental group, which comprised 302 men and women, consumed significantly less saturated fat, linoleic acid, and cholesterol, but more oleic acid and α-linolenic fatty acids than did the 303 men and women of the control group. A canola oil–based margarine (5% α-linolenic acid, 5% elaidic acid, 15% saturated fatty acids, 16% linoleic acid, and 48% oleic acid) was supplied to all members of the experimental group. Serum lipids, blood pressure, and body mass index remained similar in the experimental and control groups during the 2-y trial, with the trend with time being a decrease in LDL cholesterol and an increase in HDL cholesterol in the experimental group. After a mean follow-up of 27 mo, there were 3 cardiac deaths in the experimental group compared with 16 in the control group. After adjustment for prognostic variables, this difference represented a 76% decrease in risk of cardiac death ($P < 0.02$), although the number of cardiac deaths was small. Recently, the final results of the Lyon Diet Heart Study were reported by de Lorgeril et al (188), representing a mean of 46 mo of follow-up per patient. These data confirmed those of the 2-y follow-up, with the protective effect of the Mediterranean dietary pattern maintained up to 4 y after the initial infarction. Specifically, 6 cardiac deaths occurred in the experimental group relative to 19 in the control group, translating into a 65% reduction in risk of cardiac death ($P < 0.01$).

The independent and combined effects of n−3 PUFA and vitamin E (α-tocopherol) supplementation on morbidity and mortality in MI survivors were examined in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial (189). Patients surviving a recent MI (3 mo) were randomly assigned to one of the following dietary supplement groups: *1*) 1 g n−3 PUFAs/d (*n* = 2836), *2*) 300 mg vitamin E/d (*n* = 2830), *3*) 1 g n−3 PUFAs + 300 mg vitamin E/d (*n* = 2830), or *4*) control (*n* = 2828). The dietary supplements, which were consumed by patients for ≈3.5 y, were provided in capsule form. The primary combined efficacy endpoints were the cumulative rate of all-cause death, nonfatal MI, and nonfatal stroke. Compared with baseline values, there were no significant changes in plasma total cholesterol, LDL-cholesterol, or HDL-cholesterol concentrations in any of the groups; however, a small decrease (−3.4%) in plasma triacylglycerol in the group receiving n−3 PUFA capsules without vitamin E was statistically significant. The analysis of study data showed that treatment with n−3 PUFA capsules over a 3.5-y period was associated with a significant reduction (−15%) in relative risk for the combined endpoints. Benefit was attributable to a decrease in the risk of all-cause death, as well as cardiovascular death (−17%), with the combined treatment of n−3 PUFAs + vitamin E yielding results similar to those for the group supplemented with n−3 PUFAs alone. Conversely, no beneficial effects were observed in the group supplemented solely with vitamin E.

Consistent with the previous report, a relation between vitamin E supplementation and cardiovascular outcomes was not observed in the Heart Outcomes Prevention Evaluation (HOPE) Study (190, 191). HOPE, a randomized, double-blind trial, was designed to evaluate the effects of ramipril, an angiotensin-converting enzyme inhibitor, and vitamin E in 9541 patients at high risk of cardiovascular events. Eligible patients were randomly assigned to receive either 400 IU (400 mg) vitamin E from natural sources (*n* = 4761) or an equivalent placebo (*n* = 4780) daily for 4–6 y, with a mean duration of 4.5 y. Patients were examined every 6 mo for a variety of outcomes during this period. The primary outcome was a composite of MI, stroke, and death from cardiovascular causes. By design, the baseline characteristics of the vitamin E and placebo groups were similar, with ≈80% of patients in each group having a history of CHD and a similar mean age. A total of 772 (16%) of the patients who were assigned to the vitamin E group had a primary cardiovascular event compared with 739 (15.5%) of those assigned to the placebo group.

There were no significant differences between the groups receiving vitamin E and placebo, respectively, in the numbers of deaths from either all (535 compared with 537) or cardiovascular (342 compared with 328) causes or in the incidence of MIs (532 compared with 524) or strokes (209 compared with 180). Moreover, among those who were receiving ramipril, vitamin E had no significant effect on primary outcomes. An examination of secondary cardiovascular outcomes also failed to detect any differences in the number of hospitalizations for unstable angina or heart failure between the vitamin E and placebo groups. Thus, the preceding 2 studies support the results of others, where no significant effects of vitamin E on CHD were observed (193). No benefit for β-carotene was noted in this latter trial (193).

In contrast, the previous results conflict with those of the Cambridge Heart Antioxidant Study (194), in which a large reduction (−47%) in the relative risk of nonfatal MI was observed in the vitamin E group. Note, however, that there were imbalances in several baseline characteristics in this trial that raise questions as to whether randomization resulted in comparable groups. Moreover, the number of events was small and the mean duration of follow-up (1.4 y) was relatively short.

## Selected dietary intervention trials with angiography as an endpoint

Dietary intervention trials in which angiography was used as an endpoint have also shown that reduction of plasma cholesterol concentrations is of great benefit for coronary atherosclerosis,

**TABLE 11**
Selected dietary intervention trials with angiography as an endpoint[1]

| Study | Subjects | | Study characteristics | | | LDL-C reduction[3] | Comments |
|-------|------|-----|--------|------------|----------|--------|----------|
|       | n[2] | Age | Design | Randomized | Duration |        |          |
|       |      | y   |        |            | y        | %      |          |
| Lifestyle Heart Trial (150, 151) | 41 M + F | 35–75 | Prospective, controlled | Yes | 1 | 37 | Average percentage diameter stenosis regressed 1.7% in the experimental group and progressed 2.2% in the control group (P < 0.02). |
| Lifestyle Heart Trial (152) | 35 M + F | 35–75 | Prospective, controlled | Yes | 5 | 20 | Average percentage diameter stenosis regressed 3.1% from baseline in the experimental group and progressed 11.8% from baseline in the control group (P = 0.001). |
| CLAS (195) | 162 M | 40–59 | Placebo, controlled | Yes | 2 | 7[4] | Increased intake of total fat and polyunsaturated fat associated with development of new lesions. Three fatty acids emerged as significant fisk factors for new lesions: lauric (at >0.22% of energy), oleic (at >11.3% of energy), and linoleic (at >9.7% of energy). |
| STARS (196) | 74 M | <66 | Endpoint, blinded | Yes | 3 | 16 | Overall CHD progression decreased in 15% of intervention subjects (P < 0.02). Overall CHD regression increased in 38% of intervention subjects (P < 0.02). |
| SCRIP (197, 198) | 300 M + F | 56[5] | Placebo, controlled | Yes | 4 | 23 | Intervention group showed a rate of narrowing of diseased coronary segments that was 47% less than that of subjects in the usual care group (P < 0.02). |
| Heidelberg Trial (199) | 113 M | 35–68 | Prospective, controlled | Yes | 1 | 9 | Progression of coronary lesions was noted in 23% of intervention subjects compared with 48% of control subjects (P < 0.05). Regression occurred in 32% of intervention subjects compared with 17% of control subjects (P < 0.05). |

[1] CHD, coronary heart disease; CLAS, Cholesterol Lowering Atherosclerosis Study; LDL-C, LDL cholesterol; SCRIP, Stanford Coronary Risk Intervention Project; STARS, St Thomas' Atherosclerosis Regression Study.

[2] Represents sum of intervention and control groups (all subjects had CHD).

[3] All reductions were statistically significant (P < 0.05).

[4] Represents decrease in those who did not develop new lesions. No change was noted in LDL-C in those who developed new lesions.

[5] Mean value.

as shown in **Table 11**. The Lifestyle Heart Trial, conducted by Ornish et al (150–152), yielded particularly dramatic results, likely attributable to the use of a very-low-fat diet. Patients in the Lifestyle Heart Trial were asked to consume a low-fat vegetarian diet that included fruit, vegetables, grains, legumes, and soybean products without energy restriction. All oils and animal products were excluded from the diet, except for egg whites and nonfat milk or yogurt. The diet contained ≈7% of energy as fat, 15–20% of energy as protein, 70–75% of energy as complex carbohydrates, and 12 mg cholesterol/d. The intervention group also participated in an exercise and meditation program. In the experimental group, LDL cholesterol was reduced by 37% (P < 0.01), whereas HDL cholesterol did not change significantly. The lack of HDL lowering was likely due to the significant ≈10-kg (22-lb) weight loss that occurred in the experimental group. Patients in the experimental group reported a 91% reduction in the frequency of angina after 1 y, whereas those in the control group reported a 165% increase in frequency (151). After 1 y, average percentage

diameter stenosis regressed from 43.6% to 41.9% in the experimental group, yet progressed from 41.6% to 43.8% in the control group. After 5 y, the value continued to decrease in the experimental group (−3.1 absolute percentage points), with further progression noted in the control group (11.8 absolute percentage points) (152). The results of this study clearly show that lifestyle changes can cause regression of coronary atherosclerosis.

Both the Cholesterol Lowering Atherosclerosis Study (CLAS) and the St Thomas' Atherosclerosis Regression Study (STARS) showed that cholesterol lowering through diet can inhibit the progression of atherosclerosis. CLAS was a randomized, placebo-controlled, angiographic trial that examined 162 men aged 40–59 y with progressive atherosclerosis who had undergone coronary bypass surgery (195). Dietary goals were to provide 26% of total energy as fat, with <5% of energy as saturated fat, <10% of energy each as monounsaturated and polyunsaturated fats, and <250 mg cholesterol/d within the context of a self-selected diet. Data analysis of 24-h dietary recalls, as well as
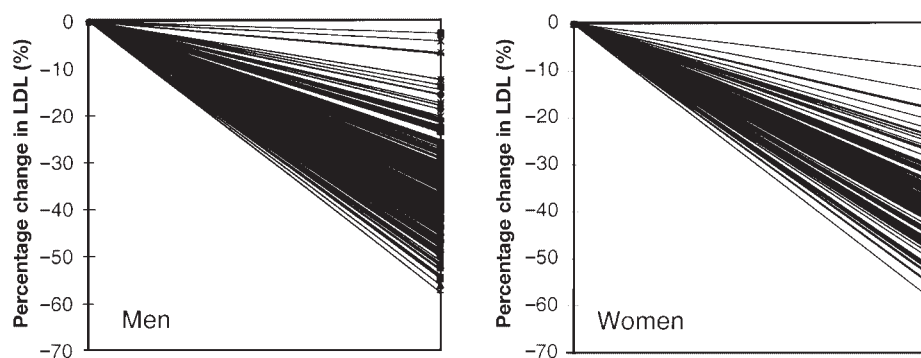
**FIGURE 8.** Variability in LDL-cholesterol response over a 1-y period to 10 mg oral atorvastatin daily ($\bar{x}$: 37% reduction; 36% in men, 38% in women) in 195 men and 133 women ($\bar{x}$ age: 59 y) with serum LDL-cholesterol concentrations sufficient to warrant drug therapy after consuming a diet based on the National Cholesterol Education Program Adult Treatment Panel II guidelines (unpublished observations, 2001).

assessment of angiograms, showed that increased consumption of either total fat or polyunsaturated fat was associated with a significant increase in risk of new lesions, as was increased intake of lauric, oleic, and linoleic fatty acids. Subjects in the diet phase of CLAS in whom new lesions did not develop had increased their dietary protein to compensate for a reduced fat intake by substituting low-fat meats and dairy products for high-fat meats and dairy products. These results suggest that protein and carbohydrate are preferable to monounsaturated fatty acids and PUFAs as replacements for fat energy in the diet.

STARS was designed to assess the effects of a practical lipid-lowering diet on the coronary arteries of patients with CHD (196). In the lipid-lowering diet, total fat intake was reduced to 27% of dietary energy, saturated fat to 8–10% of energy, polyunsaturated fat to 8% of energy, and cholesterol to 0.02 mg/kJ (100 mg/ 1000 kcal). Subjects in the intervention group had significant reductions ($P < 0.01$) in total cholesterol ($-14\%$), LDL-cholesterol ($-16\%$), and triacylglycerol ($-20\%$) concentrations, with no significant differences in HDL cholesterol. About 3 y after randomization, computerized image analysis of coronary arteries showed that dietary change had not only retarded progression of CHD in 15% of subjects in the intervention group, but had caused overall regression in 38% as well. Mean percentage diameter stenosis decreased 0.5% in the intervention group, whereas it increased 5.6% in the control group ($P < 0.001$).

The Stanford Coronary Risk Intervention Project followed 300 men and women with CHD for 4 y, comparing multifactor risk reduction with usual care (197, 198). The intervention group was counseled to consume a diet low in total fat (<20% of energy), low in saturated fat (<6% of energy), and low in cholesterol (<75 mg/d) and was encouraged to exercise. LDL cholesterol ($-22\%$) and triacylglycerol ($-20\%$) decreased significantly in the intervention group, whereas HDL cholesterol increased by 12%. Additionally, the rate of narrowing of diseased coronary segments in the intervention group was 47% less than that in the usual care group. Similar results were observed in the Heidelberg Study, which was also a diet and exercise intervention trial (199).

Therefore, dietary intervention trials have evaluated the effects of reduced intakes of total and saturated fat, or increased intakes of PUFAs (both n−6 and n−3), on cardiovascular morbidity and mortality. The results of these trials, as well as those assessing angiographic endpoints, clearly show that restriction of saturated fat significantly reduces CHD risk, whereas a relative increase in n−6 and n−3 fatty acid consumption (especially n−3 fatty acids) is beneficial in this regard. Conversely, the results of trials examining the cardioprotective effects of antioxidants, predominantly vitamin E, are less definitive and do not clearly support the concept that either vitamin E or β-carotene reduces CHD risk. Possibly, future studies that include vitamin C and selenium will be more beneficial, especially in primary prevention.

## Intervention trials with cholesterol-lowering medications

Studies of cholesterol-lowering medication have confirmed the concept that lowering total and LDL cholesterol is associated with significant CHD risk reduction. In the primary prevention setting, significant benefit was observed with cholestyramine in the Lipid Research Clinics Coronary Primary Prevention Trial (19% CHD risk reduction), with gemfibrozil in the Helsinki Heart Study (34% CHD risk reduction), with pravastatin in the West of Scotland Study (31% CHD risk reduction), and with lovastatin in the Air Force/Texas Coronary Artery Prevention Study (37% CHD risk reduction) (200–203). In studies in patients with established CHD, benefit was noted in the Coronary Drug Project with niacin (20% risk reduction), in the Scandinavian Simvastatin Survival Study with simvastatin (34% risk reduction), in the Cholesterol and Recurrent Events Trial with pravastatin (24% risk reduction), in the LIPID Trial with pravastatin (24% risk reduction), and in the Agressive lipid lowering Versus Revascularization Trial (AVERT) with atorvastatin (36% risk reduction) (204–212). These studies confirm the concept that LDL-cholesterol lowering is associated with significant risk reduction, and that for every 1% reduction in LDL cholesterol there is an $\approx 1\%$ reduction in CHD risk in both primary and secondary prevention in subjects with baseline LDL-cholesterol concentrations >3.4 mmol/L (130 mg/dL). In addition, in the secondary prevention studies, significant reductions in stroke risk and total mortality were also documented (205, 208, 211). Therefore, hydroxymethylglutaryl-CoA reductase inhibitors or statins are now the drugs of choice for CHD risk reduction after diet in subjects with elevated LDL-cholesterol concentrations.

Our own kinetic studies with stable isotopes clearly indicate that hydroxymethylglutaryl-CoA reductase inhibitors not only decrease cholesterol production, but also reduce the secretion of apo B-100–containing lipoproteins (213). Similar to dietary response, there is a wide variability in LDL-cholesterol response to statins, in part related to *APOE* genotype (**Figure 8**). In

**TABLE 12**
Treatment guidelines for patients with elevated LDL cholesterol[1]

| Initiate diet therapy | After diet therapy, initiate drug therapy | Goal of therapy |
|---|---|---|
| | *mmol/L (mg/dL)* | |
| <2 CHD risk factors | ≥4.9 (≥190) | <4.1 (<160) |
| ≥2 CHD risk factors or 10-y CHD risk of 10–20% | ≥4.1 (≥160) | <3.4 (<130) |
| CHD, vascular disease, diabetes, or 10-y CHD risk ≥20% | ≥3.4 (≥130) | >2.6 (<100) |

[1] CHD, coronary heart disease. From reference 2. CHD risk factors are shown in Table 13.

contrast with dietary responsiveness, subjects carrying the *E4* allele are less responsive to statins than are those not carrying this allele (75). Of note is that the dietary trials produced smaller reductions in LDL-cholesterol concentrations than did the drug studies, but in some cases greater reductions in mortality (eg, the Finnish Mental Hospital study). These data suggest that alterations in dietary fatty acids and cholesterol may have additional benefits above and beyond LDL-cholesterol lowering (181, 182, 205).

Some studies, such as the Helsinki Heart Study and the Veterans Administration High Density Lipoprotein Intervention Trial, also indicate significant benefit in CHD risk reduction from HDL-cholesterol raising via the use of gemfibrozil (214, 215). In my view, this strategy may well be the next frontier in CHD risk reduction: the use of pharmacologic agents that significantly increase HDL cholesterol, such as cholesterol ester transfer protein inhibitors that are well tolerated and can double HDL-cholesterol concentrations by preventing cholesterol ester transfer to apo B–containing lipoproteins.

Overall, the results of the preceding studies show that lowering LDL cholesterol to <3.4 mmol/L (130 mg/dL) is of significant benefit with regard to CHD risk reduction in patients with or without CHD having average or elevated concentrations of LDL cholesterol. Some of these studies suggest that it may be even more beneficial to reduce LDL-cholesterol concentrations to <2.6 mmol/L (100 mg/dL), or possibly to as low as 2.0 mmol/L (80 mg/dL), in those with CHD (210, 212). However, it is important to note that other lipid abnormalities, such as low HDL-cholesterol, elevated lipoprotein(a), and elevated triacylglycerol concentrations, also predispose patients to premature CHD and should therefore be considered when designing an optimal treatment strategy. The evidence from the intervention trials also indicates that for every 1% increase in HDL-cholesterol concentrations with medication there is a 3% reduction in CHD risk (201, 206, 214, 215). In my view, attempts should be made to increase HDL cholesterol to >1.0 mmol/L (40 mg/dL), especially in patients with CHD.

In contrast with the situation for LDL-cholesterol lowering and HDL-cholesterol raising, no significant benefit has been noted in large-scale, placebo-controlled, randomized prospective intervention studies with antioxidants, specifically β-carotene, vitamin E, or probucol. In our own studies in normal and diabetic hamsters fed atherogenic diets, we noted no protection from foam cell formation with either vitamin E or probucol (216). We did note protection with lovastatin or atorvastatin and disappearance of foam cells when the animals were returned to a regular diet. At the present time, the data support the concept that optimizing LDL-cholesterol and HDL-cholesterol concentrations is associated with significant benefit in terms of reduction from both CHD morbidity and mortality, as well as reduction in stroke risk in patients with established CHD.

## NATIONAL CHOLESTEROL EDUCATION PROGRAM GUIDELINES

LDL-cholesterol goals established by the Adult Treatment Panel of the NCEP are shown in **Table 12** (2). Dietary guidelines established by the NCEP are shown in Table 4 (2). CHD risk factors other than elevated LDL cholesterol established by the NCEP are shown in **Table 13** (2). Secondary causes of hypercholesterolemia need to be excluded; these include hypothyroidism, diabetes, liver disease, nephrotic syndrome, and use of medications that can increase serum lipids (prednisone, anabolic steroids, and progestins) (2). Secondary goals established by an American Heart Association panel are to get triacylglycerol values <2.2 mmol/L (200 mg/dL) and HDL-cholesterol values >0.9 mmol/L (35 mg/dL) in patients with CHD (217). The European Atherosclerosis Society has also generated cholesterol guidelines. They recommend an LDL-cholesterol goal of <2 mmol/L (115 mg/dL) in all subjects who have CHD or a CHD risk of ≥20% per decade (Figure 1). Their secondary goals are to get triacylglycerol concentrations to <3 mmol/L (175 mg/dL) and HDL cholesterol to >1.0 mmol/L (40 mg/dL) (218).

## CONCLUSIONS

CHD remains a leading cause of death and disability. Prevention through risk factor control—smoking cessation and control of blood pressure, blood glucose, and LDL cholesterol and raising of HDL cholesterol—remains the most effective long-term option for treatment. Obesity has an adverse effect on all these risk factors except smoking and therefore requires treatment. Emerging risk factors requiring attention are elevated concentrations of lipoprotein(a), remnant lipoproteins, and homocysteine. Lifestyle modification remains the cornerstone of CHD prevention. Hydroxymethylglutaryl-CoA reductase inhibitors can be added in high-risk individuals to further control LDL cholesterol.

Dietary treatment can clearly decrease CHD risk, especially when the food supply is altered. I recommend decreasing saturated fat to <7% of energy, decreasing total fat to 15–30% of

**TABLE 13**
National Cholesterol Education Program coronary heart disease (CHD) risk factors in addition to diabetes and elevated LDL cholesterol[1]

*1)* Male ≥45 y
*2)* Female ≥55 y
*3)* Family history of premature CHD[2]
*4)* Hypertension
*5)* Cigarette smoking
*6)* HDL cholesterol <1.0 mmol/L (40 mg/dL)

[1] Subtract one risk factor for HDL cholesterol ≥ 1.6 mmol/L (60 mg/dL). Diabetes has been defined as a CHD risk equivalent. From reference 2.

[2] Defined as CHD in a male first-degree relative aged <55 y or a female first-degree relative aged <65 y.

**TABLE 14**
World Health Organization dietary recommendations for prevention of chronic disease[1]

| Nutrient | Recommendation |
| --- | --- |
| Total fat | 15–30% of total energy |
| Saturated fat | <10% of total energy |
| Polyunsaturated fat | 3–7% of total energy |
| Protein | 10–15% of total energy |
| Carbohydrate | |
|   Complex | 55–75% of total energy |
|   Simple | <10% of total energy |
| Fiber (nonstarch polysaccharides) | 16–24 g/d |
| Fruit and vegetables | >400 g/d |
| Salt | <6 g/d |
| Cholesterol | <300 mg/d |

[1] From reference 219.

energy, decreasing dietary cholesterol to <200 mg/d, decreasing sugar intake to <10% of energy, having an n−6 fatty acid intake of 5–10% of energy, having an n−3 fatty acid intake of ≥1% of energy, minimizing *trans* fatty acid intake, and increasing the intake of vegetables, fruit, and grains, as recommended in the USDA food guide pyramid. I particularly agree with the dietary recommendations of the World Health Organization for the prevention of chronic disease (**Table 14**). I do not see any compelling reason to markedly increase monounsaturated fat intake, but it is important to maintain an adequate intake of essential fatty acids while restricting intakes of saturated fat and cholesterol. Randomized clinical trials confirm that low-fat, low-energy diets coupled with exercise are the most effective for long-term weight loss. In my view, the USDA food guide pyramid (Table 5) should be modified to indicate that animal, dairy, and hydrogenated fats; tropical oils; egg yolks; and sugars be used sparingly, but not all oils. Recently, other Tufts colleagues suggested that the food pyramid be modified for the elderly (>70 y of age) by recommending ≥8 servings of water or fluids/d and the use of calcium, vitamin D, and vitamin B-12 supplements (61). I agree with these recommendations.

Both dietary and drug therapy to lower LDL cholesterol indicate a marked variability in response. The data suggest that subjects who overabsorb dietary cholesterol in the intestine are more likely to carry the *APOE E4* allele, are more responsive to diet, and are less responsive to statins in terms of cholesterol lowering. Such subjects, comprising 20% of the population, also have higher LDL cholesterol concentrations and are at increased risk of heart disease and dementia. Although dietary modification and drug therapy that lowers LDL cholesterol clearly decreases CHD risk, the use of antioxidants has not been conclusively shown to do so. In our view, diet assessment is best done with food records, and the most effective way to change dietary intake is to alter the food supply, by providing specific foods or supplements, or to provide prepared meals to the consumer.

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