ATHEROSCLEROSIS, CHOLESTEROL AND EGG
– REVIEW –

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Summary

The pathogenesis of atherosclerosis can not be summarized as a single process. Lipid infiltration hypothesis and endothelial injury hypothesis have been proposed and investigated. Recent developments show that there are many points of potential interactions between them and that they can actually be regarded as two phases of a single, unifying hypothesis. Among the many risk factors of atherosclerosis, plasma homocysteine and lipoprotein(a) draw a considerable interest because they are independent indicators of atherogenicity.

Triglyceride (TG)-rich lipoproteins (chylomicron and VLDL) are not considered to be atherogenic but they are related to the metabolism of HDL cholesterol and indirectly related to coronary heart disease (CHD). LDL can of itself be atherogenic but the oxidative products of this lipoprotein are more detrimental. HDL cholesterol has been considered to be a favorable cholesterol. The so-called 'causalist view' claims that HDL traps excess cholesterol from cellular membranes and transfers it to TG-rich lipoproteins that are subsequently removed by hepatic receptors. In the so-called 'noncausalist view', HDL does not interfere directly with cholesterol deposition in the arterial wall but instead reflects the metabolism of TG-rich lipoproteins and their conversion to atherogenic remnants.

Approximately 70-80% of the human population shows an effective feedback control mechanism in cholesterol homeostasis. Type of dietary fat has a significant effect on the lipoprotein cholesterol metabolism and atherosclerosis. Generally, saturated fatty acids elevate and PUFA lower serum cholesterol, whereas MUFA have no specific effect. EPA and DHA inhibit the synthesis of TG, VLDL and LDL, and may have favourable effects on some of the risk factors. Phsopholipids, particularly lecithin, have an antiatherosclerotic effect. Essential phospholipid (EPL) may enhance the formation of polyunsaturated cholesteryl ester (CE) which is less sclerotic and more easily dispersed via enhanced hydrolysis of CE in the arterial wall. Also, neutral fecal steroid elimination may be enhanced and cholesterol absorption reduced following EPL treatment. Antioxidants protect lipoproteins from oxidation, and cells from the injury of toxic, oxidized LDL. The rationale for lowering of serum cholesterol is the strong association between elevation of plasma or serum cholesterol and CHD. Cholesterol-lowering, especially LDL cholesterol, to the target level could be achieved using diet and combination of drug therapy.

Information on the link between cholesterol and CHD has decreased egg consumption by 16-25%. Some clinical studies have indicated that dietary cholesterol and egg have a significant hypercholesterolemic effect, while others have indicated no effect. These studies differed in the use of purified cholesterol or cholesterol in eggs, in the range of baseline and challenge cholesterol levels, in the quality and quantity of concomitant dietary fat, in the study population demographics and initial serum cholesterol levels, and clinical settings. Cholesterol content of eggs varies to a certain extent depending on the age, breed and diet of hens. However, egg yolk cholesterol level is very resistant to change because of the particular mechanism involved in yolk formation. Egg yolk contains a factor or factors responsible for accelerated cholesterol metabolism and excretion compared with crystalline cholesterol. One of these factors could be egg lecithin. Egg lecithin may not be as effective as soybean lecithin in lowering serum cholesterol level due probably to the differences of fatty acid composition. However, egg lecithin may have positive effects in hypercholesterolemia by increasing serum HDL level and excretion of fecal cholesterol. The association of serum cholesterol with egg consumption has been widely studied. When the basal or control diet contained little or no cholesterol, consumption of 1 or 2 eggs daily increased the concentration of plasma cholesterol, whereas that of the normolipemic persons on a normal diet was not significantly influenced by consuming

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2 to 3 eggs daily. At higher levels of egg consumption, the concentration of HDL tends to increase as well as LDL. There exist hyper-and hypo-responders to dietary (egg) cholesterol. Identifying individuals in both categories would be useful from the point of view of nutrition guidelines.

Dietary modification of fatty acid composition has been pursued as a viable method of modifying fat composition of eggs and adding value to eggs. In many cases beneficial effects of PUFA enriched eggs have been demonstrated. Generally, consumption of n-3 fatty acids enriched eggs lowered the concentration of plasma TG and total cholesterol compared to the consumption of regular eggs. Due to the highly oxidative nature of PUFA, stability of this fat is essential. The implication of hepatic lipid accumulation which was observed in hens fed on fish oils should be explored. Nutritional manipulations, such as supplementation with iodine, inhibitors of cholesterol biosynthesis, garlic products, amino acids and high fibre ingredients, have met a limited success in lowering egg cholesterol.
(**Key Words** : Atherosclerosis, Lipoproteins, Cholesterol, Egg Cholesterol)

Introduction

The pathogenesis of atherosclerosis which is the primary cause of coronary heart disease (CHD) in the human has long been known. Today the causative factors are well established but interactions between the risk factors and their mechanism at the molecular level are still to be elucidated.

One of the key risk factors in this disease is hypercholesterolemia. Cholesterol is a biologically important compound which is either synthesized internally or absorbed from dietary sources. The blood cholesterol level is controlled by a homeostatic mechanism in most normal individuals but not in some hypersensitive individuals. Cholesterol is carried as a fraction of lipoprotein in the body. Because the atherogenicity of the different lipoproteins differs, that is, some of them are 'good' ones and some others are 'bad' ones, the allegation that total cholesterol is the major cause of CHD is no longer valid.

In spite of the extensive and continuing controversy over the quality of dietary fat and incidence of CHD, the consumption of animal fat does not necessarily predispose to health problems. The quantity (total energy intake) and fatty acid composition of the fat are important. The recent reduction in animal fat and total cholesterol consumption caused a significant erosion of the previously accepted dietary image of the egg as a nutritionally near perfect food. So firmly entrenched are the present views on animal fats and cholesterol consumption, that to reinstate the egg with the consumer will require more efforts of egg industry as well as scientists. A more positive approach is the attempts to change lipid factors of eggs to align with present consumer perception and requirements.

This review focuses on the basic theories and recent developments in the areas of atherosclerosis, lipoproteins, cholesterol, egg cholesterol and modified eggs, and their interrelationships.

Factors Associated with Coronary Heart Disease

1. Atherosclerosis

Crawford (1960) defined atherosclerosis as "the widely prevalent arterial lesion characterized by patchy thickening of the intima, the thickenings comprising accumulations of fat and layers of collagen-like fibres, both being present in widely varying proportions". It is also an extremely common form of arteriosclerosis in which deposits of yellowish plaques containing cholesterol, lipoid material, and lipophages are formed within the intima and inner media of large and medium sized arteries.

The wall of an artery consists typically of an outercoat (tunica adventitia), external elastic lamina, a middle coat (tunica media), internal elastic lamina, and an inner coat (tunica intima). The innermost layer of the arterial wall, the intima, is a single layer of endothelial cell at infancy. The intima begins to thicken and remodel commencing in gestation as a result of smooth muscle cell (SMC) proliferation which have migrated from the media and proliferated between the endothe-lium and the internal elastic lamina. Normally blood-endothelial cell (EC) contact does not provoke a clotting response. When the endothelial layer is injured, a complex repair response is initiated.

The manifestation of atherosclerosis leads to the development of coronary heart disease (CHD), also synonymously termed coronary artery disease (CAD) or cardiovascular disease (CVD), which is announced by one of the three major manifestations: angina pectoris, myocardial infarction, or sudden cardiac death (Connor and Bristow, 1985).

Morphology

The earliest recognizable lesion of atherosclerosis is the fatty streak. The lesion may be present from childhood onwards in large elastic and muscular arteries. Histological

examination of fatty streaks shows slight thickening of the intima, this being associated with the presence of fat droplets. The localization of the fat appears to be predominantly intracellular though in some lesions there is a "dusting" of sudanophilic material along the internal elastic lamina (Woolf, 1988).

The advanced atherosclerotic plaque has two major components. First there is the cholesterol- and lipid-rich acellular part of the plaque, where much of the lipid is deposited. This is a major portion of the *atheroma*, a term derived from the Greek stem meaning "soft, grumous, or porridge-like". The surrounding *sclerotic* area of the plaque is composed of collagen and compressed cells, as well as elastin and other matrix elements. This part often forms a firm, fibrous cap over the advanced atherosclerotic plaque (Wissler, 1991).

Fundamental morphological aspects of the general pathology of atherosclerosis are emphasized as follows (Woolf, 1988):

1) The focal distribution of the lesions, which is almost certainly governed by haemodynamic factors.

2) The intima is predominantly involved in the lesions.

3) The complex nature of the elements of atherosclerotic lesions, consisting of lipid derived mostly from the plasma, necrotic connective tissue at the plaque bases, and proliferated fibromuscular tissue forming a covering or "cap" that separates the other constituents from the flowing blood in the arterial lumen.

Etiology

The pathogenesis of atherosclerosis cannot be summarized as a single process; the physiopathological duality established as early as the 19th century, with the apparently contradictory theories of Von Rokitanski and Virchow continued until recently.

In 1844, Von Rokitanski suggested that the lesion resulted from the deposit of substances contained in the blood on the internal walls of the arteries. In 1856, Virchow, who disagreed with this theory, considered that intima hyperplasia was a locally originating inflammatory phenomenon. These two theories were both backed up with much experimental evidence (Jacotot, 1994).

Since then, two hypothesis, the *lipid infiltration hypothesis* and *response to injury hypothesis*, have been proposed and investigated in efforts to elucidate this unique pathogenesis. However, the disease almost certainly in one of multiple etiologies and a common end-result can be reached by different pathways.

A constant feature of most atherosclerotic lesions is the accumulation of lipids. This is evident even in the earliest lesions (fatty streak) and becomes striking in late lesions. This, and a wealth of experimental evidence led to formulation of the *lipid infiltration hypothesis*, which proposes that infiltration of lipids from bloodstream into the vessel wall initiates the process and, in some way, triggers the subsequent events.

Other investigators, impressed by the lesions that develop after mechanical or chemical injury to the endothelial lining and by the marked proliferation of smooth muscle cells accompanying the atherogenic process, formulated the *endothelial injury hypothesis*.

For many years, these two hypotheses tended to be regarded as conflicting and essentially mutually exclusive. However, recent developments show that there are many points of potential interaction between them and that they can actually be regarded as two phases of a single, unifying hypothesis (Steinberg, 1987).

The *lipid infiltration hypothesis* includes the following steps leading to pathogenesis of atherosclerosis; High low density lipoprotein (LDL) level ➡ High rate of LDL infiltration to subendothelial space ➡ Increased cellular uptake of LDL(EC, SMC, macrophagy) ➡ Accumulation of cholesterol esters ➡ Atheroma.

The *endothelial injury hypothesis* includes the following steps; Endothelial injury ➡ Platelet adherence and aggregation ➡ Release of platelet-derived growth factor (PDGF) ➡ SMC proliferation with secretion of matrix elements ➡ Atheroma.

The end result of both hypotheses is the formation of atheroma. Some of the interactions between endothelial injury and lipid infiltration in the pathogenesis of atherosclerosis is as follows (Wissler, 1991):

1) Hyperlipidaemia – especially as oxidized LDL – may injure endothelium.
2) Platelet aggregation and release of PDGF may be initiated by high LDL level.
3) Sustained injury of the endothelium as produced by immune complexes or by autoimmune reactions to oxidized LDL may increase the rate of lipid infiltration.
4) LDL from hypercholesterolemic serum can stimulate proliferation of arterial SMC under many types of conditions.
5) LDL receptors on SMC are increased when growth factors are present.
6) The synthetic state SMC make more proteoglycans, which in turn can bind more LDL in the subintimal space.

Risk Factors

The general risk factors for diseases due to atherosclerosis are widely studied. The relationship

between blood cholesterol and these diseases has been established due to a better knowledge of potentially atherogenic lipoproteins (LDL, Lp(a), modified LDL and certain postprandial particles) and the lipoproteins considered as being protective (HDL, LpAI).

Besides atherogenic dyslipidaemia, other clinical and biological parameters are statistically associated with the onset of ischaemic cardiovascular accidents. Non-modifiable risk factors are age, sex, family background, personal background, while modifiable risk factors are smoking, hypertension, diabetes mellitus, and obesity with insulin resistance (Jacotot, 1994). The risk factors such as hyperlipidaemia, cigarette smoking, hypertension, and diabetes reinforce each other and they have at the very least an additive effect. Perhaps the most important interaction is that between hyperlipidaemia and factors engendering arterial endothelial damage and inflammation.

Factors producing prolonged or repeated injury to arterial endothelium are summarized (Wissler, 1991) as follows:

1) Sustained mechanical injury – Prolonged hemodynamic, Repeated trauma
2) Sustained angiotensin effects
3) Norepinephrine and other vasoactive amines – Cigarette smoking, High-dosage cocaine use
4) Antigen-antibody reactions, autoimmunity, and immune complexes
5) Leukotrienes and free radicals
6) Chronic endotoxemia
7) Anoxia and CO – Cigarettes (CO and NO_2), Severe anoxia, Prolonged stasis
8) Chronic uraemia
9) Homocystinemia
10) Hypercholesterolemia (especially of LDL and β-VLDL)
11) Thromboxane (Henriksson et al., 1987)

Independent Risk Factors

Among the many established risk factors, *homocysteine* and *lipoprotein*(a) (*Lp*(a)) are considered as possible but still controversial independent risk factors.

Homocysteine theory

In homocystinuria, an inherited disorder of methionine metabolism, an extraordinarily large amount of homocysteine is found in blood and tissues. Vascular occlusive disease is the primary cause of premature death in homocystinuria. Substantial research activity in this area has resulted in the so-called *homocysteine theory* of arteriosclerosis (McCully, 1983) and it has been postulated that the accumulation of homocysteine in blood may enhance arteriosclerosis. The most frequent cause is a deficiency of cystathionine synthase, the enezyme that converts homocysteine to cystathionine. The other causes are more rare and are due to defects in the mechanism by which homocysteine is remethylated to methionine. 5-Methyl-tetrahydrofolate serves as the methyl donor for this reaction, which is catalyzed by methionine synthethase and involves the intermediate formation of an enzyme-bound methylcobalamin.

Experimental studies have suggested that homocysteine and its derivatives cause injuries and functional changes of vascular endothelial cells to precipitate arteriosclerosis and thrombosis. Although the disruption of endothelial cell integrity can be mediated by oxygen radicals and H_2O_2, well known products of autooxidation of thiol-containing amino acids, lipid peroxidation is not involved in homocysteine mediated toxicity (Jones et al., 1994). Theory of homocysteine as a risk factor for CVD is an attractive hypothesis because elevated and even normal plasma homocysteine levels can be normalized or decrased, respectively, by moderate doses of folic acid alone or in combination with vitamin B_{12} and pyridoxal 5-phosphate. Araki et al. (1993) suggested that plasma homocysteine levels could be one of a number of independent risk factors for macroangiopathy in patients with diabetes mellitus and that they can be reduced by parenteral treatment with methylcobalamin.

In a recent prospective Finnish population based study, however, the hypothesis that serum homocysteine is a risk factor for atherosclerotic disease was not supported. The lack of association between serum homocysteine and atherosclerosis may be due to the exceptionally low gene frequency predisposing to homocysteinemia in Finland (Alfthan et al., 1994).

Lipoprotein(a) theory

Lipoprotein(a) (Lp(a)) is a complex lipoprotein of glycoprotein known as apolipoprotein(a) or apo(a) linked to apolipoprotein B of LDL via disulphide bonds. The lipid composition of the main fraction of purified human Lp(a) which is isolated between a density of 1.05 and 1.08 g/ml was found to be similar to LDL. The protein content is greater due to the specific hydrophilic nature of glycoprotein. The apo(a) exhibited size polymorphism and the existence of 34 different isoforms was reported. The plasma concentration of total Lp(a) varies 1000-fold from almost zero to more than 1,000 mg/l. Unlike other lipoproteins, Lp(a) exhibits a very high degree of inheritability. Several studies have shown that the Lp(a) levels of black Americans are approximately twice of those of the whites.

There is strong evidence that supports an independent association between high Lp(a) levels and atherosclerotic

disorders (Dahlén, 1994). Among the patients with familial hyper-cholesterolemia some of them develop premature CHD and others do not follow that pattern. The difference that seems to distinguish these two groups is the level of Lp(a). The risk is higher, especially for early atherosclerosis in males, if the inherited plasma Lp(a) level exceeds 200-300 mg/l (Weizel, 1991).

The well known tendency of Lp(a) to self aggregate and precipitate *in vitro* when isolated, in contrast to other lipoproteins, as well as its greater capacity to bind to glycosamino-glycans in the presence of divalent cations and to fibrin and fibrinogen and other structures present in the arterial wall may be related to its documented preferential accumulation in the arterial intima *in vivo*. The hypothesis that immune processes may play a special role in Lp(a) associated atherosclerosis is an attractive possibility that, however, needs more extensive confirmation.

Body mass index, alcohol consumption, cigarette smoking, use of β-blockers or cholesterol-lowering medication and use of drugs for the treatment of diabetes and hypertension were not correlated with Lp(a) levels. It is interesting, however, to note that homocysteine, one of the other important risk factors, significantly increases the affinity of Lp(a) for fibrin and induces a 20-fold increase in affinity between Lp(a) and plasmin-treated fibrin and a 4-fold increase with unmodified fibrin.

Diet changes and drugs that change LDL levels have been found to have a negligible effect on Lp(a) (Brown et al., 1991). One reason for this difference may be the low affinity of Lp(a) for LDL receptor, compared with LDL. It has been suggested that the function of Lp(a) is the peripheral supply of cholesterol derived from the liver, which is transported independent of dietary influences and triglyceride metabolism. The most likely target tissues are endocrine organs with high steroid hormone production. Women have significantly higher values of serum Lp(a) than men and low level of serum testosterone may be correleated with high serum Lp(a) levels (Dionyssiou-Asteriou and Katimertzi, 1993). Nicotinic acid in combination with neomycin was found to reduce the Lp(a) levels considerably. It is also possible that regular physical exercise might decrease Lp(a) levels.

So far most facts indicate that it is mainly the inherited Lp(a) level that is associated with an increased risk of atherosclerotic disorder. There seems to be no doubt that Lp(a) is a marker of early atherosclerosis and true genetic risk factor for CVD. However, studies conducted with aged people of over 60 years old (Simons et al., 1993) and middle aged Finnish people (Alfthan et al., 1994) did not support the hypothesis that Lp(a) is a risk factor for atherosclerosis suggesting a more complex nature of this theory.

2. Lipoproteins

Lipoproteins play important roles in cholesterol metabolism. Plasma cholesterol is carried in lipoproteins of many different form and hypercholesterolemia, which is a major causative factor in atherosclerosis, can be due to an elevation of any one of them or any combination of several of them. The implications with respect to atherogenesis may be very different according to which particular lipoprotein fraction is elevated. Therefore, the allegation that cholesterol itself has a major role in the etiology of CVD is regarded as no longer valid. We will have to ask: what is the relative atherogenicity of the different lipoprotein fractions? (Steinberg, 1988)

Lipoproteins are any of the lipid-protein complexes in which lipids are transported in the blood. Lipoprotein particles consist of a spherical hydrophobic core of triglycerides or cholesteryl esters (CE) surrounded by an amphipathic monolayer of phospholipids, cholesterol, and apolipoproteins. The four principal classes are high-density, low-density, and very-low-density lipoproteins and chylomicrons.

Chylomicrons and very low density lipoproteins (VLDL) are triglyceride (TG)-rich lipoproteins. They are produced in both the gut (chylomicrons) and liver (VLDL) and have distinct roles in the transport of exogenous and endogenous lipids, respectively. The ratio of TG to cholesterol in these particles is greater than 10:1 (chylomicrons) and approximately 5:1 (VLDL), and when present in excess these particles may impart a distinct turbidity or creamy appearance to plasma. When isolated from plasma, chylomicrons contain a unique form of apoprotein B (B 48) as well as apoproteins E and C. In contrast, VLDL contain a distinct form of apoprotein B (B 100), which is also found in low-density lipoproteins (LDL). VLDL also contain apoproteins E and C, both of which are important in the metabolism of these particles. Intermediate-density lipoproteins (IDL) are formed in the degradation of VLDL; approximately half is cleared rapidly from the plasma into the liver by receptor-mediated endocytosis; the rest is further degraded to form LDL. The major apolipoproteins of IDL are B-100 and E.

Under normal circumstances, most of the cholesterol in plasma is carried as a constituent of LDL, and in patients with hypercholesterolemia the number of LDL particles in plasma is increased and changes in LDL cholesterol are similar to the changes in total cholesterol. Each LDL particle contains about 45% cholesterol (25% free and

75% esterified) with a smaller amount of protein (20%-25%, almost entirely apoprotein B) and phospholipids (22%). High-density lipoproteins (HDL) constitute the smallest of the lipoprotein particles and contain 25% to 28% cholesterol and 48% to 50% protein (apoproteins AI, AII, E and C) (Illingworth and Connor, 1985).

Chylomicrons

The chylomicrons themselves are probably not atherogenic at all. Patients with a very high concentration of chylomicrons (TG levels over 5,000 mg/dl and total cholesterol levels over 500 mg/dl) show no excess incidence of premature CHD. All of the cholesterol contained in chylomicrons do not appear to predispose to atherosclerosis. Still, even if the chylomicrons are not themselves atherogenic, the events that accompany their metabolism and/or the remnant products derived from them (through the action of lipoprotein lipase) may nevertheless be atherogenic. Because patients with familial hyperchylomicronemia lack lipoprotein lipase, they generate chylomicron remnants at a very slow rate. That may be why they do not suffer any deleterious consequences. On the other hand, patients with a less complete defect in lipoprotein lipase but with high levels of chylomicrons may be at an increased risk (Steinberg, 1988).

Plasma TG-rich lipoproteins (chylomicrons and VLDL) are related to HDL-cholesterol and risk of CVD. Rapid lipolysis of TG-rich lipoproteins produces increased lipid uptake, formation of HDL_2 which is larger, lipid-rich, less dense HDL and may protect the arterial wall. Delayed lipolysis of TG-rich lipoproteins increases the opportunity for the reciprocal transfer of TG from TG-rich lipoproteins into HDL and of CE from HDL into TG-rich proteins. This neutral lipid exchange reaction is catalyzed by cholesteryl ester transfer protein (CETP). TG transferred to HDL are hydrolysed by the enzyme hepatic lipase. In this way, HDL-cholesterol is lost to TG-rich lipoproteins, large HDL (HDL_2) are converted into small HDL (HDL_3), and the cholesterol-carrying capacity of HDL decreases. Hence, rapid lipolysis of TG-rich lipoproteins keeps HDL_2 levels (and thus HDL-cholesterol) high by promoting the formation of the larger HDL_2 as well as by preventing their catabolism (Patsch, 1994).

Very-Low-Density Lipoproteins (VLDL)

VLDL are secreted by the liver, and a portion of this lipoprotein fraction is converted to LDL. The rate at which this conversion occurs determines the LDL cholesterol production rate (Dietschy et al., 1991).

Most investigators do not consider VLDL to be atherogenic. Although epidemiologic studies have shown a significant positive correlation between triglyceride levels and incidence of coronary heart disease and a few studies find hypertriglyceridemia to be an independent risk factor, it is generally recognized that hypertriglyceridemia is not an independent risk factor (Steinberg, 1988).

There is one form of VLDL that is certainly atherogenic: the cholesterol-rich β-VLDL that accumulates in dysbetalipoproteinemia and in cholesterol-fed animals. Because β-VLDL is rich in triglycerides as well as cholesterol, these animals and patients have both hypertriglyceridemia and hypercholesterolemia. The very rapid rate at which β-VLDL is taken up by resident macrophages has been well documented. Affinity of the β-VLDL receptor of macrophages is extremely high for β-VLDL and this appears to account for the rapid uptake and degradation of β-VLDL compared to LDL (Steinberg, 1988).

Low-Density Lipoprotein (LDL)

In human subjects, most of the LDL in plasma is derived from intravascular metabolism of VLDL. LDL may therefore be regarded as the end product of VLDL metabolism and the rate at which VLDL fraction is converted to LDL determines the LDL cholesterol production rate. Catabolism of LDL occurs in both peripheral cells and in the liver (the latter being the major site of removal) and is facilitated by both receptor-and nonreceptor-mediated pathways. Therefore, the plasma LDL cholesterol concentration is essentially determined by two physiological process: the rate at which VLDL is converted to LDL and the rate at which LDL is removed from the plasma by both receptor-dependent (75%) and receptor-independent (25%) transport processes (Dietschy et al., 1991). A defect in LDL receptor activity has been identified as the primary genetic error in familial hypercholesterolemia. This defect results in a slowing of the clearance of LDL from plasma; the slower clearance then results in an elevation of plasma LDL concentration, and then the atherogenesis must somehow be the direct or relatively direct consequence of the hyperbeta-lipoproteinemia.

In some situations LDL can of itself be atherogenic, as in patients with hypercholesterolemia. However, the oxidation of lipoproteins has been suggested to play a major role in atherogenesis as well as in lesion propagation and pathogenesis. At early stages of atherosclerosis, macrophage-mediated oxidation of LDL is probably dominant, whereas at later stages, LDL oxidation by SMC may become more important (Aviram, 1993).

Oxidative modification of LDL contributes to the

generation of foam cells from monocyte/macrophages and the fatty streak by generating a form of LDL recognized and taken up more rapidly by way of the acetyl-LDL receptor on macrophages. The fatty streak usually develops under a structurally intact endothelium. Endothelial cells are particularly susceptible, and so the oxidized LDL could compromise the function and/or integrity of the endothelial lining. This might in some way favour monocyte/macrophages to penetrate between endothelial cells, take up residence beneath them, and there become converted to the lipid-loaded foam cell. The endothelial surface over a fatty streak lesion can occasionally appear to "lift-off", exposing underlying foam cells. The macrophage/foam cell could release active oxygen, lipase, collagenase, elastase, or other lytic enzymes that might be responsible for weakening cell-cell adhesion and eventually causing loss of intact endothelium. At that point, adherence of platelets to the exposed connective tissue matrix, or to the macrophage/foam cells themselves could lead to the release of platelet factors and trigger the series of events that cause SMC proliferation, deposition of extracellular connective tissue, and the other processes that give rise to the stenotic, advanced lesion. The above process bring the *lipid infiltration theory* and the *endothelial injury theory* together as elements in a unified theory (Steinberg, 1988).

Oxidation of LDL *in vitro* leads to the formation of numerous lipid oxidation products from the cholesterol, CE, phospholipids and triglycerides of LDL. Many of these oxidation products are known to be cytotoxic, including various oxysterols, e.g. cholestane-triol (Jacobson et al., 1985), oxidized fatty acids, aldehydes produced from fatty acid oxidation and various hydroperoxides. Lysophosphatidyl-choline is also produced during LDL oxidation by a phospholipase A_2 activity of apolipoprotein B-100, and it too can cause cell injury. Since lipases and oxygenases exist in cells of the arterial wall, several forms of modified LDL may exist *in vivo*. These modifications can occur either in parallel or along different stages of atherosclerosis. While the *in vitro* "biological" effects of oxidized LDL suggest a causal role in atherosclerosis lesion development, their relevances to *in vivo* mechanism remain to be resolved (Penn and Chisolm, 1994).

High-Density Lipoproteins (HDL)

High-density lipoproteins are heterogenous mixtures of particles which lie within the density interval 1.063-1.210 kg/l and represent the smallest of the lipid-protein complexes circulating within the bloodstream. Typical particles, 7 nm in diameter, have a volume of approximately 130 nm^3 and could be accommodated 3,000 times into the average VLDL. HDL are therefore, in terms of particle number, the most abundant lipoprotein in the circulation (Shepherd and Packard, 1993).

HDL are not formed as mature lipoproteins but appear in body fludis as precursor particles that have a discoidal shape and are composed of bilayers of phospholipids and unesterified cholesterol, and are stabilized by apoproteins. They are synthesized by the liver, intestine and cholesterol enriched macrophages. In the plasma compartment the nascent HDL undergo a disc to sphere transformation by the action of the enzyme lecithin cholesterol acyltransferase (LCAT). That is, newly formed small spherical HDL can undergo a conversion process while being in the circulation that causes enlargement of particles (conversion along the cascade $HDL_4 \rightarrow HDL_3 \rightarrow HDL_2 \rightarrow HDL_1$) or reverse conversion (depletion of core and surface molecules) along the same cascade (Eisenberg, 1993).

Apo A-I is the major protein constituent of spherical HDL. This protein is found in at least two particle types within the HDL density spectrum-one devoid in apo AII (LpAI) and the other containing it (LpAI/AII). The former is considered to be the primary tissue cholesterol acceptor and is therefore linked to cardioprotection. LpAI is found predominantly but not exclusively within the lighter HDL_2 particles (d = 1.036 − 1.125 kg/l) while LpAI/AII tends to favour HDL_3 (1.125 − 1.210 kg/l) (Shepherd and Packard, 1993).

The interrelationship among HDL cholesterol, plasma levels of TG-rich lipoproteins, and the risk of CAD is well established. There is a strong negative association between levels of HDL cholesterol and CAD risk and a weaker association between TG and CAD risk, while HDL and TG-rich lipoproteins exhibit a strong inverse association. Generally, a 1 mg/dl increment in HDL cholesterol is associated with decrease in CAD risk of 2% in men and 3% in women.

Two major hypotheses have been proposed for the role of HDL in the development of CAD:

1) The so-called 'causalist view' assigns HDL a protective effect against atherosclerosis. According to the theory, HDL trap excess cholesterol from cellular membranes by esterification and transfer the esterified cholesterol to TG-rich lipoproteins that are subsequently removed by hepatic receptors. This reverse cholesterol transport from peripheral cells to the liver counteracts the deposition of cholesterol at sites where an excess cholesterol load produces atherosclerosis. Thus, high HDL cholesterol levels signify a high rate of reverse cholesterol transport.

2) In the so-called 'noncausalist view', the relationship between the metabolism of TG-rich lipoproteins and that of HDL and its major subfractions, the smaller HDL_3 and the larger, more lipid-rich, and less dense HDL_2 is important. HDL do not interfere directly with cholesterol deposition in the arterial wall but instead reflect the metabolism of TG-rich lipoproteins and their conversion to atherogenic remnants. According to this view, high HDL cholesterol levels indicate an efficient metabolism of TG-rich lipoproteins and a low production rate of atherogenic remnants (Patsch and Braunsteiner, 1991).

The first hypothesis, which is also called the 'reverse cholesterol transport hypothesis', is popular but the evidence to support this theory is still limited, and to a large extent circumstantial. The biochemical and clinical data regarding HDL cholesterol and CAD support noncausalist hypotheses (Patsch, 1993).

During the maturation process of HDL, three plasma enzymes—lecithin:cholesterol acyltransferase (LCAT), CETP and hepatic lipase, contribute significantly. The action of LCAT upon discoidal HDL triggers a change in the disposition of cholesterol within the particle. The enzyme catalyses the transfer of a fatty acid from lecithin to the hydroxyl residue on cholesterol, leading to the generation of lysolecithin and CE. The polarities of these products are fundamentally different from those of their precursors. Lysolecithin is more hydrophilic and dissociates readily from the lipoprotein into the aqueous environment. On the other hand, esterification of the sterol increases its hydrophobicity and causes it to partition into the non polar interior of the particle. The surface site vacated by sterol is then available to accept additional cholesterol molecules either from other lipoproteins or from cell surfaces. Thus, LCAT has a dual action. It facilitates the sequestration of cholesterol within the hydrophobic core of LDL, generating a chemical potential gradient which leads to continuing uptake of the sterol. Acquisition of cholesterol by HDL proceeds until the nascent disc is fully transformed into a sphere. Obviously continued cholesterol assimilation is limited by the capacity of the HDL particles were it not possible for the sterol ester to undergo facilitated transfer to less dense lipoproteins via the agency of CETP (Shepherd and Packard, 1993). According to the causalist view, this protein exchanges the CE in the core of HDL for TG acquired from TG-rich lipoproteins (VLDL or chylomicron) and these lower density lipoproteins enriched with CE are removed through specific receptors by the liver (Patsch, 1993).

In the noncausalist view, TG-rich lipoproteins affects HDL_2 levels in two ways:

1) Rapid lipolysis of TG-rich lipoproteins by the enzyme lipoprotein lipase (LPL) generates excess surface materials, predominantly phospholipids, which is assimilated by HDL. This uptake of lipid promotes the formation of large HDL_2 which allows transport of twice as many CE molecules per mole of apoproteins than HDL_3. The assimilation of lipolytic surface remnants by HDL not only affects the formation of HDL_2 but may also protect arterial wall cells. Surface remnants, when not incorporated into HDL, are cytotoxic to macrophages in culture.

2) Delayed lipolysis of TG-rich lipoproteins increases the opportunity for the reciprocal transfer of TG from TG-rich lipoproteins into HDL and CE from HDL into TG-rich lipoproteins. This neutral lipid exchange reaction is catalyzed by lipid transfer protein-1 (LTP-1). Triglycerides transferred to HDL are hydrolysed by the enzyme hepatic lipase. In this way, HDL cholesterol is transferred into TG-rich lipoproteins, large HDL are converted into small HDL and the cholesterol-carrying capacity of HDL decreased. Hence, rapid lipolysis of TG-rich lipoproteins keeps HDL_2 levels (and thus HDL cholesterol) high by promoting the formation of the larger HDL_2 as well as by preventing their catabolism (Patsch and Braunsteiner, 1991).

The levels of HDL_3 in plasma are generally constant among individuals, whereas the HDL_2 levels vary greatly and thus account for most of the variability of total HDL cholesterol. Hence, the negative association of HDL-cholesterol and CAD risk is essentially due to the highly variable plasma levels of HDL_2. The rate-limiting factor for the formation of HDL_2 is presumably the supply of surface remnants released from TG-rich lipoproteins through lipolysis. Thus, the powerful negative association of HDL-cholesterol and CAD is in reality a positive relation between CAD and TG-rich lipoproteins or some of their subfractions. The close inverse relationship between TG and HDL-cholesterol appears to be caused by the influence which the metabolism of TG-rich lipoproteins exerts on HDL.

In addition to the functions of HDL described above, it has been suggested that HDL also take part in the protection of plasma lipid (LDL) from peroxidation. This will extend our knowledge of HDL rightly referred to as 'antiatherogenic' (Klimov et al., 1993).

3. Cholesterol

Cholesterol is a eukaryotic sterol that in higher animals is the precursor of bile acids, vitamin D_3 and steroid hormones, and a key constituent of cell

membranes, mediating their fluidity and permeability. Most is synthesized by the liver and other tissues, but some is absorbed from dietary sources. Dietary cholesterol enters the body by way of the chylomicron pathway and is removed from the plasma by the liver as a component of chylomicron remnants. Only about 40% of ingested cholesterol is absorbed, and the remaining 60% passes out in the faeces (Connor and Connor, 1985). Cholesterol in plasma is transported by specific lipoproteins. Cholesterol can accumulated or deposit abnormally, as in some gallstones and in atheromas. There no longer is any doubt that hypercholesterolemia is one of the major causative factors in atherosclerosis because cholesterol is a major ingredient of atherosclerotic plaque and cholesterol feeding has been shown to induce atherosclerosis in animals.

Some population studies indicate that, particularly in men, the curve relating cholesterol to mortality is U-shaped, with higher mortality rates at both end of the cholesterol distribution (< 160 or > 240 total cholesterol mg/dl). Thus, low cholesterol as well as high cholesterol can be detrimental. The low cholesterol-mortality relationships may possibly be an effect-cause variety in which non-cardiovascular diseases may have resulted in a low cholesterol level. It is also possible that some of the dietary changes that result in a low cholesterol also result in other unfavourable metabolic changes that predispose individuals to other diseases (LaRosa, 1994).

Cholesterol Metabolism and Homeostasis

The degree of precision in regulatory responses to a dietary cholesterol challenge in the general human population remains unknown. But it is conclusive that hereditary factors play a major role in the regulation of serum cholesterol and the predisposition to atherosclerosis (Farriaux et al., 1987).

It is generally suggested that an average 'threshold amount', the amount of dietary cholesterol necessary to produce an increase in the plasma cholesterol concentration from a baseline with cholesterol-free diet, is 100 mg/day; an average 'ceiling amount' of dietary cholesterol is in the neighbourhood of 300 to 400 mg/day in adult human (Connor and Connor, 1985). Serum cholesterol levels of individuals differed by 100 mg/dl when all received the same diet. The group of men had a mean serum cholesterol level of about 225 mg/dl when consuming a diet similar to that of the average American (Hegsted, 1991).

Cholesterol in the diet of man is normally associated with other dietary lipids such as triglycerides and phospholipids. Only a relatively small proportion of dietary cholesterol is esterified and this esterified cholesterol must be hydrolysed before absorption can occur. Hydrolysis is catalyzed in the lumen of the small intestine by pancreatic CE hydrolase. Free cholesterol of dietary origin or biliary origin is incorporated into mixed micelles. The incorporation of cholesterol into such micelles is a prerequisite step in the process of cholesterol absorption by the mucosal cells of the small intestine. Absorption efficiency of cholesterol as a natural component of normal diet varies between 25-50%, averaging 37-38% (Moore, 1987). Absorbed cholesterol is transported from the intestinal mucosa through the lymphatic system to the blood in mammal. This cholesterol is carried in chylomicrons mainly as CE synthesized by acyl CoA acyl transferase (ACAT) in the mucosal cells; these are carried in the core of chylomicron particles together with dietary TG. After the bulk of the TG have been removed by the action of lipoprotein lipase in extrahepatic tissue (mainly adipose tissue and striated muscles), the CE which remain with the resulting chylomicron 'remnant' are taken up chiefly by the liver via endocytosis into hepatocytes. Thus dietary cholesterol, transported in chylomicron chiefly as CE, is delivered efficiently to the liver to be excreted via bile and contributes little to the pool of circulating cholesterol. However, to the extent that it is retained in the liver, chylomicron cholesterol contributes to down-regulation of hepatic LDL receptors and thus has an important indirect effect in raising the concentration of cholesterol in VLDL and especially LDL. Hepatic cholesterol enters the blood chiefly as CE contained within VLDL; most of these esters are returned to the liver with VLDL remnants or LDL, and they do not contribute to the transport of extrahepatic cholesterol to the liver (reverse cholesterol transport) (Havel, 1991).

In most animal species the intestine accounts for approximately 10 to 20% of total body cholesterol synthesis. In contrast, the relative contribution of the liver to total body sterol synthesis varies significantly among species. In the absence of dietary cholesterol, the liver may account for as much as 40 to 50% of total body synthesis in species such as the rat and squirrel monkey but only 10 to 15% in other species such as the hamster, cynomolgus monkey, and humans. It is those species with very low relative rates of hepatic cholesterol synthesis that respond most unfavourably to the addition of lipids to the diet. Within a single species, the individual with the lowest rate of hepatic cholesterol synthesis responds with the most exaggerated elevation in LDL cholesterol to lipid feeding (Dietschy et al., 1991).

Numerous studies in free-living outpatients suggest that a majority have a relatively precise feedback control

mechanism, since an increased dietary cholesterol intake fails to significantly increase plasma cholesterol levels in the majority of subjects. Approximately 75% (Eberhardt, 1991) or 80% (Kralj and Bidin, 1993) of the population showed cholesterol-compensating mechanisms. For them dietary cholesterol has little biological significance. For the remaining 20 to 25%, which includes genetic disorders, various addicts, diabetic, renal patients, and patients in the post-apoplectic state, increased dietary cholesterol could produce unfavourable health effects. In 75 studies, 69% of the male subjects compensated for the increased cholesterol intake by decreasing cholesterol fractional absorption and/or endogenous cholesterol synthesis. In sharp contrast to the relatively minor effect of dietary cholesterol on plasma cholesterol levels, the quality of dietary fat has shown to have a more consistent influence on plasma lipid levels: a shift from a saturated to a mono- or polyunsaturated fat diet will lower plasma cholesterol levels in the majority of subjects. The responses to dietary cholesterol and fat are therefore highly individualized and most individuals have effective feedback control mechanisms (McNamara et al., 1987).

In a study conducted with baboons, both high cholesterol and saturated fat treatments independently raised both LDL and HDL, and apolipoproteins in serum compared to low cholesterol and unsaturated fat treatments. However, the values of liver cholesterol, bile cholesterol, neutral steroid excretion, bile acid excretion and cholesterol turnover rate were lower in the group fed saturated fat diets while those were higher in the group fed high cholesterol diets. Dietary cholesterol greatly suppressed whole-body cholesterol synthesis, but type of fat did not affect cholesterol synthesis or percent of cholesterol absorption. These results suggest that dieatry cholesterol and saturated fat increase plasma lipoprotein concentrations through different physiological mechanisms (Mott et al., 1992).

Plasma LDL cholesterol is mostly removed by receptor-dependent transport processes. Saturated fatty acids routinely decrease receptor activity whereas unsaturated fatty acids increase receptor-dependent LDL transport. The effect of saturated fatty acids on the plasma LDL cholesterol concentration, however, are always much greater than changes associated with manipulation of the unsaturated fatty acid content of the diet. The effects of either saturated or unsaturated fatty acids on plasma LDL cholesterol levels are critically dependent upon the concentration of cholesterol in the diet. For example, feeding 20% hydrogenated coconut ail (HCO) in the absence of dietary cholesterol increases the plasma LDL cholesterol concentration by approximately 25 mg/dl whereas feeding the same quantity of HCO in the presence of 0.12% cholesterol increases the LDL cholesterol concentration by over 125 mg/dl. Not all saturated fatty acids behave in an identical manner, however. Medium-chain-length triacylglycerols (MCT) has relatively little effect of the plasma cholesterol level or on any of the parameters that regulate this level whereas HCO has a marked effect. It has been demonstrated that stearic acid is less hypercholesterolemic than some of the others and only one or two specific saturated fatty acids have the detrimental effects of decreasing receptor activity and increasing LDL cholesterol production rates (Dietschy et al., 1991).

Effect of Type of Fat on Lipoprotein Cholesterol Metabolism and Atherosclerosis

There are only few studies with humans that show an effect of type of fat alone on atherosclerotic lesion development or regression, even though fat effects on lipoprotein concentrations are well documented.

Epidemiological and clinical studies have demonstrated a relationship between the intake of saturated fatty acids and atherosclerosis. Among these fatty acids, stearic acid has the smallest effect on cholesterol levels. It was thought that monounsaturated fatty acids (MUFA) did not affect lipoprotein metabolism. However, recently it has been shown that MUFA have the same hypocholesterolemic effect as polyunsaturated fatty acids (PUFA); moreover they do not induce a decrease in HDL cholesterol. Therefore, the overall metabolic effect of MUFA seems to be beneficial (Mancini and Parillo. 1991).

As far as the metabolic effect of MUFA is concerned, a contradictory result has been obtained in a more recent population-based study in which associations of dietary fat and cholesterol with carotid artery wall thickness (as a measure of atherosclerosis) were investigated. Wall thickness was measured with B-mode ultrasound. After adjustment for age and energy intake, animal fat, saturated fat, monounsaturated fat, cholesterol, and Key's score were positively related to wall thickness, while vegetable fat and PUFA were inversely related to wall thickness (Tell et al., 1994).

Intake habit of nine fats were identified from dietary mannerism involving the consumption of red meat, fat on meat, fried fish/chicken, butter, eggs, whole milk, bacon/sausage, cheese and the use of solid fats when cooking vegetables. The total number of habits did not correlate well with serum cholesterol. These nine habits explained less that 10% of the variability of serum cholesterol values (Lackland and Wheeler, 1990).

In animal studies New Zealand White male rabbits were fed with a 1% cholesterol-enriched and/or 10% fish oil supplemented diet for 6 weeks. Fish oil treated rabbits showed a beneficial effect on the reduction of atherosclerotic lesions and tissue cholesterol levels in the aorta and pulmonary artery, but not in the left ventricle (Chen et al., 1992).

In general, the severity of atherosclerosis is inversely related to the level of fat unsaturation. Two exceptions are cocoa butter which is much less atherogenic than expected, most probably due to its high content of stearic aicd, and peanut oil, while relatively unsaturated, is surprisingly atherogenic for rats, rabbits and monkeys. This latter effect is not related to the level (6%) of long-chain saturated fatty acids (arachidic, behenic, lignoceric) present in peanut oil, but rather to its TG structure. Randomization of peanut oil, which modifies its TG structure, significantly reduces its atherogenicity (Kritchevsky, 1991).

African green monkeys given diets enriched in PUFA, either from safflower oil (n-6) or fish oil (n-3), had generally lower total plasma LDL and HDL cholesterol concentrations compared to those given saturated fat diets. Plasma TG concentrations were generally lower in the n-6 PUFA group, but they were significantly higher in the n-3 PUFA group than in the saturated fat group. Thus, both types of PUFA appeared to lower the concentration of the favorable cholesterol (HDL and apoA-I), while the effect to lower the unfavorable cholesterol (LDL and apoB) was also present (although more modest in degree). Compositional alterations in LDL are important in the development of atherosclerosis in these animals. LDL particle size is a measure of the change in LDL composition that correlates to the extent of atherosclerosis. Diet-induced alterations in CE metabolism increase the content of higher-melting point cholesterol esters, particularly in the saturated fat group, resulting in larger LDL particles with higher average liquid crystalline to liquid transition temperature. Because the oxidation of LDL lipids is known to be a major factor in the initiation or exacerbation of atherosclerosis, the increased number of double bonds in the LDL in the PUFA groups might well result in more atherosclerosis. But the changes in lipoproteins that occurred in the PUFA groups actually resulted in less rather than more atherosclerosis. The oxidation of LDL during atherosclerosis development is apparently quite well buffered by antioxidant status in the animal, so that atherosclerosis is less extensive in animals fed PUFA. Instead other factors that might predispose to atherosclerosis, such as the physical state of the LDL core lipid, appear to be more important. Clearly, the studies support the hypothesis that the enlargement of LDL particle size and enrichment of LDL particles with higher-melting point CE (oleate) represent atherogenic alterations in LDL particle composition. In animals fed PUFA, atherosclerosis was less extensive in spite of decreased HDL cholesterol concentrations. It would appear that HDL are needed to protect against atherosclerosis only when LDL are atherogenic, as defined by particle concentration or composition. Regardless of the mechanism, however, PUFA in the diet are likely to have a beneficial rather than detrimental effect on the development of atherosclerosis (Rudel et al., 1991).

Some studies showing substantial drops in HDL when PUFA have been fed were based on diets containing excessive amounts of PUFA – diets that no one eats. Several authors reported that low-fat diets lower HDL and are therefore presumably undesirable. But there appear to be abundant epidemiologic data showing that low-fat diets protect against CHD. This, after all, is the primary concern, regardless of serum lipoprotein levels (Hegsted, 1991).

The past decade has seen a veritable explosion of knowledge about the biological role of the n-3 fatty acids, especially eicosapentaenoic aicd (EPA) and docosahex-aenoic acid (DHA), which are derived only from marine organisms. These fatty acids have diverse biological actions, which include:

1) Prostaglandins and leukotriene precursors (and inhibitors)
2) Anti-platelet aggregation; anti-thrombotic action
3) Inhibit the synthesis of TG, VLDL, and LDL
4) Hypolipidemic action
5) Anti-atherosclerotic (promotes endothelial-derived relaxing factor and inhibits cellular growth factors)
6) Immunologic effects; anti-cancer action?
7) Essential fatty acids, especially for the brain, retina, and spermatozoa

Several of these actions can affect the atherosclerosis process; others are important in infancy. When fish oil (salmon) was administered at 6 to 30% of total calories in the metabolically controlled diet, profound hypolipidemic effects in normal human subjects and in hypertrigl-yceridemic patients were shown. There were marked reductions in plasma cholesterol and TG concentration; TG lowering was especially great (which is opposite to the result obtained with African green monkey). There were also reductions in VLDL, chylomicron, remnants, LDL, apoB, and apoE. The HDL changes were inconsistent and varied from subject to subject. Whereas the mechanism of the hypolipidemic action of the n-6 rich vegetable oils containing linoleic acid such as corn or

safflower oil still remains obscure, the mechanism of action of the n-3 fatty acids in fish oil has been well documented. The synthesis of TG and VLDL in the liver is greatly reduced by n-3 fatty acids. At the same time, the turnover of VLDL in plasma is greatly shortened, and LDL production is decreased (Connor, 1991).

In addition to their effects on plasma lipoproteins, dietary n-3 fatty acids have been reported to exert potentially favourable effects on blood pressure, platelet function and viscosity (Schmidt et al., 1993). Further, EPA and DHA inhibited the proliferation of vascular SMC (Shiina et al., 1993). These effects could partly explain the anti-atherosclerotic effect of n-3 fatty acids or especially those of marine lipids.

Trans-fatty acids have physical properties that are similar to the saturated fatty acids. They are found in milk, certain margarines, and shortenings and fats used for frying. The trans-fatty acids are formed during hydrogenation of PUFA. Roughly 6 to 8% of total fat is consumed as trans-fatty acids. Despite limited data, there has been reason to believe that they might have effects on serum cholesterol more like saturated fatty acids. In one study, it was shown that trans-fatty acids raised LDL cholesterol and lowered HDL cholesterol (Weizel, 1991).

Effects of Phospholipids on Lipoprotein Cholesterol Metabolism and Atherosclerosis

Phospholipids, particularly lecithin, have been the object of numerous studies, both in view of their postulated activity in lipid metabolism and in experimental atherosclerosis. Supplementing 3% of soybean lecithin to atherogenic rabbit diet promoted a return to normal of the lipoprotein distribution profile and removal of lipid from established atherosclerotic plaque (Hunt and Duncan, 1985).

Rats were fed on a hypercholesterolemic diet (5% lard, 0.5% cholesterol and 0.25% sodium cholate) containing 5% of dietary phospholipid as safflower phospholipid (SAP), soybean phospholipid (SOP), or egg yolk phospholipid (EGP), or 5% soybean oil (SO) as a control for 4 weeks. The concentration of plasma cholesterol and chylomicron plus VLDL cholesterol were higher in rats fed on the EGP. The phospholipid diets induced a significant increase of HDL cholesterol in comparison with the SO diet. The activity of LCAT and excretion of faecal neutral steroids into faeces was increased in rats fed on phospholipid diets. Among the phospholipid-fed rats, the SAP and SOP diets increased the excretion of faecal neutral steroids compared with the EGP diet. It is suggested that SAP and SOP inhibit the absorption of dietary cholesterol in the small intestine of hypercholesterolemic rats and that the effect of those on plasma cholesterol metabolism is different from that of EGP (Iwata et al., 1993). Most recently, polyene phospholipids (i.e. phospholipids with linoleic acid predominantly in the 2 position), better known as 'essential phospholipid' (EPL), have drawn attention, both because of the possibility of enteral and parenteral administration, and also because of the apparent capacity of these molecules to modify lipid and lipoprotein metabolism to a more marked extent and with better selectivity vs. standard lecithin.

A marked decrease in the severity of atherosclerotic lesions in rabbits, repeatedly infused with the phosphatide suspensions, was noticeable, whether they were derived from animal or vegetable sources. The results could be related to a direct transport of cholesterol out of extrahepatic tissues into the plasma, possibly after tissue cholesterol solubilization. Other studies conducted with rabbits showed that cholesterol implants induced an inflammatory response, ameliorated by EPL administration. Cholesterol arachidonate was the least sclerogenic of all the esters tested and egg lecithin was definitely less effective in inducing resorption than EPL. In a human study, cholesterol and phospholipids in serum were significantly lower after consuming soybean phospholipids compared with egg phospholipids (O'Brien and Andrews, 1993).

In other animals such as rats, minipigs, quails and baboons, antiatherosclerotic effects of EPL were significant. Quails may be the animal species most sensitive to atherosclerosis regression induced by EPL.

The suggested hypotheses for the antiatherosclerotic effect of EPL are that it may enhance the formation of polyunsaturated CE, less sclerotic and more easily dispersed, possibly via the action of tissue LCAT and enhance hydrolysis of CE in the arterial wall via stimulation of cholesteryl esterase activity. Cholesterol esterification and hydrolysis in the arterial wall may be a balanced process: if EPL enhances CE hydrolysis, then free cholesterol may be easily transported across cell membranes, with ensuing atherosclerosis regression. Further possible models of action of EPL have been proposed. EPL can markedly modify the arterial wall metabolism, both by reducing plasma membrane EPL and by inhibiting arterial wall lipid biosynthesis. They may also interact with the binding of atherogenic lipoprotein fractions with the arterial wall polyanions. A further mode of action of EPL may be at the level of plasma LCAT, thus allowing cholesterol removal from tissues. In addition, cholesterol balance studies have suggested that neutral faecal steroid elimination may be enhanced and

cholesterol absorption reduced following EPL treatment (Sirtori, 1993).

Antioxidants, Antioxidant Enzymes in Hypercholesterolemia

The elimination of free radicals and their derivatives from the organisms depends to a high extent on the concentration of antioxidative vitamins (vitamin, E, C and β-carotene) and on the activity of antioxidative enzymes, mainly superoxide dismutase (SOD), catalase (CT) and glutathione peroxidase (GSH-Px), and the concentration of reduced glutathione (GSH).

The antioxidant inhibition of LDL modifications may arrest the development of the atherosclerotic lesion. Antioxidants, such as vitamin E, probucol (Bocan et al., 1994), and butylated hydroxytoluene (BHT), show two protective effects, one in which the lipoproteins are protected against oxidation and one in which cells are protected against the injury of toxic, oxidized LDL (Penn and Chisolm, 1994).

It is suggested that the decrease in the activity of antioxidant enzymes increases susceptibility of an organ to oxidative injury while the increase in the activity would have a protective effect against oxidant injury. Antioxidant enzyme activities may affect the hemostatic processes and lipid peroxidation in patients with CHD. Platelet glutathione peroxidase activity of the CHD patients was significantly lower than controls. Other antioxidative platelet enzymes:catalase ratio and superoxide dismutase were also significantly decreased in CHD patients (Buczynski et al., 1993).

Vitamin E-induced amelioration of atherosclerosis in cholesterol-fed rabbits was associated with decreases in blood and aortic tissue malondialdehyde, a lipid peroxidation product. In rabbits, the activity of antioxidant enzymes in blood is affected differently from that in aortic tissue. There appears to be a mutually supportive interaction among the antioxidant enzymes which provide defence against oxidant injury. The protective effects of Vitamin E against hypercholesterolemic atherosclerosis may not due to changes in the antioxidant enzymes but may be mainly mediated through its chain-breaking antioxidant activity (Mantha et al., 1993).

Susceptible and resistant quail are biochemically distinct in terms of alteration in antioxidant components produced by dietary cholesterol. In the susceptible group, significant negative correlations were noted between severity of atherosclerotic lesions and aortic SOD and glutathione reductase activities. Cholesterol feeding was associated with increased activity of plasma GSH-Px and SOD activities in both strains (Godin et al., 1994).

A study was conducted to assess the intensity of peroxidation processes during experimental hypercholesterolemia, and whether supplementation of diet with vitamin C, E and/or β-carotene has an influence on atheromatous lesions and on peroxidation indices in the organism of guinea pigs. Biochemical investigations showed a lower concentration of lipid peroxides in the animals receiving the atherogenic diet with added vitamins. The studied antioxidative vitamins restored the activity of antioxidative enzymes (SOD, CAT) which had been decreased by the atherogenic diet. The results suggest a protective action of antioxidative vitamins preventing the development of atheromatous lesions, and an inhibitory effect of vitamins on lipid peroxidation in experimental hypercholesterolemia (Ziemlanski and Panczenko-Kresowska, 1994).

Cholesterol Lowering

The rationale for lowering of serum cholesterol is the strong association between elevations of plasma or serum cholesterol and CHD, and that lowering of cholesterol by whatever means possible can help to prevent CHD.

The effects of diet, especially lipids, and heredity on the hypercholesterolemia, and importance of lipoproteins in cholesterol metabolism have been discussed in the earlier chapters. As far as diet is concerned, there is no doubt that a statistical association exists between the intake of cholesterol and the amount of saturated fatty acids, and the incidence of CHD caused by hypercholesterolemia. Therefore, it seems logical to change the plasma cholesterol levels by modification of diet. The principles of a lipid-lowering diet are low cholesterol intake, partial replacement of saturated fatty acids with unsaturated fatty acids, and reduction of total fat calories. This recommendation is not without problems. As far as dietary cholesterol is concerned, it is known that great interindividual variations exist in response to dietary cholesterol. The maximum level of 300 mg of dietary cholesterol/day that is recommended is an arbitrary level that is not supported by experimental evidence (Weizel, 1991).

Because dietary therapy has limitations, many lipid-lowering drugs such as cholestyramine, nicotinic acid, fibrates and 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase inhibitors, have been tested and used. Cholestyramine, a bile acid sequestrant, is an effective lipid-lowering drug. A group of men treated with this drug for 7 years showed a 19% decrease in the incidence of CHD death and/or nonfatal myocardial infarction when compared to the control group. Reduction in total cholesterol and LDL cholesterol were proportional to adherence to the cholestyramine by the treatment group

(Gotto, Jr. and Jones, 1986). Although this drug is very effective in lowering lipids at higher doses, it is also very unpleasant to take so the only one-half of the participants were able to take the prescribed dose.

Nicotinic acid could be an alternative drug. Niacin-treated men had shown a decreased incidence of definite nonfatal myocardial infarction but no significant decrease in cardiac or total mortality. However, with a mean follow-up of 15 years, nearly 9 years after termination of the study, all-cause and cardiovascular mortality in the niacin group was 11% lower than in the placebo group. This late benefit may be a result of early favourable effects of niacin in decreasing nonfatal infarction, or the cholesterol-lowering effect of niacin, or both (Rifkind, 1988). Niacin may be an effective drug but many patients can not tolearte the side effects at higher doses.

Fibrates have been the most widely used drug in Europe for the treatment of hyperlipidaemia. The side effects with these drugs are fewer than with other drugs. Unfortunately, however, these drugs exert their main effect on the VLDL fraction and to a lesser degree on the LDL fraction. Gemfibrozil, a fibric acid derivative, has useful TG-lowering properties. It produced changes in plasma lipids, namely moderate reductions in total and LDL cholesterol (8%), more pronounced reductions in TG (35%), and a moderate increase in HDL cholesterol (> 10%). The cumulative combined incidence of fatal and nonfatal myocardial infarction and cardiac death were reduced by 34% but the total mortality rate was not significantly altered by treatment (Rifkind, 1988).

HMG CoA-reductase is an enzyme that catalyses the reduction of HMG to mevalonate, which is a key rate-limiting step in the biosynthesis of cholesterol. HMG CoA-reductase inhibitors have been available as a new class of cholesterol-lowering drugs. This group of drugs fulfils the requirements for cholesterol lowering by lowering the LDL cholesterol fraction and raising the HDL cholesterol fraction at the same time. They seem to be ideal drugs provided long-term toxicity does not prevent the application of these drugs (Weizel, 1991).

There is agreement on the need for the reduction of CHD risk through cholesterol lowering and the management of other CHD risk factors. Target cholesterol levels of about 200 mg/dl are set for adults. Levels above 240-250 mg/dl are used to define high-risk groups (Rifkind, 1988). Cholesterol-lowering, especially LDL cholesterol, to the target level could be achieved using diet and combination drug therapy.

Relationships Between Coronary Heart Disease and Consumption of Eggs

1. Egg cholesterol

U.S. per capita shell egg consumption has declined steadily since 1955 despite a falling real rice. A fixed coefficient model indicated that information on the link between cholesterol and heart disease had decreased per capita shell egg consumption by 16 to 25% by the first quarter of 1987 (Brown and Schrader, 1990). According to the guidelines of the U.S. National Cholesterol Education Program (NCEP), diet counsellors recommend consumption of fewer than 4 eggs per week to reduce high circulating cholesterol concentrations in adult Americans (Garwin et al., 1992).

Some clinical studies in the literature have indicated that dietary cholesterol and eggs have a significant hypercholesterolemic effect, while others have indicated no effect. These studies differed in the use of purified cholesterol or cholesterol in eggs, in the range of baseline and challenge cholesterol levels, in the quality and quantity of concomitant dietary fat, in the study population demographics and initial serum cholesterol levels, and clinical settings (e.g. metabolic ward vs. free-living populations).

Egg Yolk Composition and Yolk Cholesterol Metabolism

The yolk comprises 30 to 33% of the total egg weight. Solids form about 50% of yolk. Yolk is composed of protein (15.7-16.6%), lipid (31.8-35.5%), carbohydrate (0.2-1.0%), ash (1.1%) and water. The composition of yolk lipid is 65.5% triglyceride, 28.3% phospholipid and 5.2% cholesterol. Yolk phospholipids are composed of 73.0% phosphatidylcholine (lecithin), 15.0% phosphatidylethanolamine, 5.8% lysophosphatidylcholine, 2.5% sphingomyelin, 2.1% lysophosphatidylethanolamine, 0.9% plasmalogen, and 0.6% inositol phospholipid (Powrie and Nakai, 1986). Fatty acid composition of fat of ordinary egg yolk is 20.1% PUFA, 45.7% monounsaturated fatty acids and 34.2% saturated fatty acids (Noble et al., 1990). The three major constituents of yolk are triglyceride-rich lipoprotein, lipovitellin and phosvitin. Minor components include immunoglobulins, serum albumin and binding proteins for a variety of vitamins.

Cholesterol content of eggs varies with age; it increases with age of hen (Shafey and Cham, 1994) or decreases up to 30 weeks of age and then remains constant until 70 weeks of age (Hall and McKay, 1993). It also varies between and within breeds and eggs of crossbreds were lower than in pure lines (Rhode Island Red, New Hampshire and White Leghorn) (Hall and McKay, 1992). Cholesterol content of eggs can be

manipulated by diet to a certain extent, e.g. 10 to 15% decrease at most, however (Austic, 1994). Over 95% of yolk cholesterol is associated with the yolk triglyceride-rich lipoproteins. The remainder is bound to lipovitellin, a protein/lipid complex that contains about 20% lipid of which only 4% is cholesterol. Most cholsesterol in yolk is in non-esterified form, although about 20% is present as cholesterol esters in hens fed normal commercial diets (Griffin, 1992).

Of the non-esterified yolk cholesterol eaten, only 4% is esterified in the duodenum and 35% of the total esterified cholesterol is absorbed. Of the absorbed cholseterol 31 to 68% is secreted into the gut through bile and excreted in feces. Only 0 to 26% of the absorbed cholesterol is retained in the body. In contrast, 750 to 1,250 mg of unesterified cholesterol is secreted in the bile daily, and of this 50% is re-absorbed in the intestines. It means that a very minute quantity of exogenous cholesterol enters the blood stream of human (Lakhotia et al., 1991).

Determination of Egg Cholesterol

The traditional colorimetric method was found to overestimate the yolk cholesterol concentration when compared with the enzymatic, gas chromatographic (GC), or HPLC methods (Jiang et al., 1991).

A study was conducted to investigate the efficacy of direct sample saponification versus saponification of a lipid extract for analysis of yolk cholesterol. When the results were compared with the National Institute of Standard and Technology (NIST) reference (cholesterol in whole egg powder), the direct sample saponification method was more accurate. Egg yolk cholesterol value determined with this method was 196 ± 42 mg/egg (Van-Elswyk et al., 1991).

Cholesterol of eggs from White Leghorn hens across Canada was studied using HPLC. Cholesterol concentration ranged from 12 to 15 mg/g yolk, and the mean value for all eggs was 13.0 mg/g or 221 mg/egg. Large eggs contained 214 mg cholesterol/egg on average. Cholesterol content (mg/egg) was found to correlate positively with hen age, egg weight and yolk weight and negatively with dietary protein and fat but the correlations were not significant (Chung et al., 1991).

The USDA recently changed its estimate of the cholesterol content of the average egg from 274 to 213 mg. This new estimate was due to an improved testing method, not to the fact that egg producers have been able to develop a lower cholesterol egg as some producers claim (Liebman, 1989).

Mean cholesterol contents, estimated by spectrophotometry, in yolks of eggs from different species of poultry were 1.581 (hens), 1.759 (ducks), 1.715 (turkeys), 1.542 (quail) and 1.336% (partridge) (Villanua and Villanua, 1988). Concentration of egg yolk cholesterol of different lines of Japanese quails were significantly different ranging 1.620 to 2.052% of yolk (Baumgartner et al., 1992).

Crystalline Cholesterol vs. Egg Yolk Cholesterol

Crystalline cholesterol in the diet of chickens produced significantly higher serum cholesterol than did egg yolk powder (EYP). The faecal excretion by rats given EYP with lipoprotein labelled with [^{14}C] cholesterol or a mixture of EYP and [^{14}C] cholesterol was similar, 98.1 and 92.7% of [^{14}C] cholesterol compared with 47% for rats on a control stock diet. Results suggested that egg yolk contains a factor or factors responsible for accelerated cholesterol metabolism and excretion. It was concluded that eggs are wrongly considered to be cholesterogenic (Sim, 1987).

Egg Lecithin

Egg yolk contains approximately 7.2% of lecithin. Egg phospholipids may not be as effective as soybean phospholipids in lowering serum cholesterol levels (O' Brien and Andrews, 1993; Iwata et al., 1993) probably due to the difference of fatty acid composition. However, many research results indicates that egg lecithin may have positive effects on hypercholesterolemia.

The comparative influence on plasma and tissue lipids of soybean and egg lecithins, which have contrasting fatty acid compositions, was studied in hypercholesterolemic guinea pigs. The polyunsaturated to saturated fatty acid (P:S) ratios of soybean and egg lecithins were 3.4 and 0.38, respectively. Hypercholesterolemia was induced by feeding guinea pigs on a purified diet that contained 15% lard with 0.5% cholesterol. Guinea pigs were then fed for 6 weeks on the same diet supplemented with soybean or egg lecithin at the level of 7.5% of the diet. The results showed that there was a 49% decrease in total plasma cholesterol in the soybean lecithin group without decreasing HDL cholesterol and a 177% increase in HDL cholesterol in the egg lecithin group without a significant increase in total cholesterol compared with those values in the control group (O'Brien and Corrigan, 1988).

Rats were given diets containing 5% purified phospholipids from rapeseed, soybean or egg yolk with or without added cholesterol. There were no significant differences among the 3 phospholipid groups in lowering serum and liver cholesterol concentrations. Apparent excretion ratios of fecal cholesterol to ingested cholesterol

in all phospholipid groups were 2 to 3 times greater than in the 5% olive oil or soybean oil control. Serum total and HDL cholesterol and liver cholesterol were decreased in the rapeseed and soybean phospholipid groups compared with the controls given soybean oil (Hosoyamada et al., 1993).

Egg Consumption and Plasma Cholesterol

Previous studies suggesting that dietary cholesterol has a greater effect on the serum cholesterol concentration have been made against a background of a higher fat intake or have contrasted extreme cholesterol intakes. There was no apparent difference in total serum cholesterol level in the group eating 7 eggs a week compared with that in the group eating 2 eggs a week in those people who have already reduced their intake of saturated fat and increased the P/S ratio and fibre-rich carbohydrate (Edington et al., 1987).

In a human study, 30 healthy men and women, 67 to 91 years old, were given a diet with 2,000 to 2,300 kcal daily with 8 eggs weekly completely replacing beef and pork for 28 to 45 days. Diets included chicken meat, fish and tripe. Total cholesterol, TG and total lipids in blood decreased or were unchanged in 83%, 90% and 72% of the people tested, respectively. HDL cholesterol increased or was unchanged in 60% of the people. It was postulated that egg lecithins and phosphatidyl-choline were responsible for the hypocholesterolemic effect (Vizioli et al., 1988).

In metabolic ward experiments the basal or control diets contained little or no cholesterol. Consumption of 1 or 2 eggs daily resulted in an increase of about 10 or 20%, respectively, in the concentration of cholesterol in plasma (Moore, 1987).

The associations of serum total cholesterol (TC) with egg, coffee and tea consumption were examined in a survey of 658 men in Israel. Serum TC values were not higher at higher intakes of whole egg. A significant positive association was found between coffee consumption and serum TC, mainly reflecting a difference in the LDL cholesterol. There was an even stronger negative association between tea intake and TC (Green and Jucha, 1986).

The influence of a low-energy diet when associated with high cholesterol intake was studied in 17 normal men during an 8-wk cross-over experiment. The subjects were given a daily supplement of 2 whole eggs and 2 egg yolks (about 1 g cholesterol) with their usual diet for 4 weeks or with a low-energy diet for 4 weeks. Each subject took part randomly in both dietary periods. During the first part of the study, there were no changes in the plasma cholesterol of the subjects given eggs with their usual diet. In contrast, the low energy diet and associated weight loss considerably decreased the tolerance to high cholesterol intake resulting in increased plasma cholesterol. The mean rise was 22.7% but with wide individual variations in the response. This was almost completely normalized when the subjects returned to their usual energy intake indicating the involvement of weight reduction in the increase observed. Changes in LDL cholesterol were parallel to those of total plasma cholesterol with an increase after the low-energy diet and normalization after body weight recovery. The opposite effect was shown with the low-energy diet after previous adaptation to the eating of 4 eggs daily. This diet resulted in a decrease in plasma cholesterol. The results emphasized that the possible adverse effect of slimming diets when associated with high cholesterol (egg) intake (Lacombe et al., 1987).

Normolipemic, healthy man and women of 30 to 68 years old were divided to 2 groups in a cross-over experiment to study the effects of eating eggs on the serum lipids. Group A showed significant increases in mean serum cholesterol after eating 3 eggs daily for 10 weeks and a significant decrease within 2 weeks of crossover to eating no eggs. Group B showed a significant decrease in mean serum cholseterol after eating no eggs for 12 weeks but, after crossing over to eating 3 eggs daily for 10 weeks, showed no longitudinally significant increases. Only group A showed an significant increase of HDL cholesterol at the end of 12 weeks of eating 3 eggs daily. Mean serum TG showed no significant change (Flynn et al., 1986).

Egg Consumption and Plasma Lipoproteins

Weanling Beagle dogs were fed on diets containing the equivalent of 0, 2, 4, 8, or 16 raw eggs/kg of air-dry diet for 15 weeks. Eggs replaced soybean protein, maize oil and sucrose in the basal diet to maintain similar energy and nitrogen intakes in all groups. Total plasma cholesterol increased with age and amount of egg in diet. Inclusion of 2 eggs/kg diet had minimal effect on plasma cholesterol. Increasing amount of egg consumption with 4, 8 or 16 eggs/kg resulted in significant increase of total plasma and HDL cholesterol concentrations. Small increases in LDL cholesterol and VLDL cholesterol were also noted. No changes in plasma TG were observed based on the progressive age of puppies or level of egg diet (Cho et al., 1984).

The effects of increase of dietary cholesterol (6 eggs daily) on the metabolism of LDL were studied in 7 healthy subjects. Egg increased HDL and LDL cholesterol values by 18 and 40%, respectively. Kinetic studies

indicated that increased synthesis and decreased removal rate were the primary causes of increased LDL. The decrease in LDL catabolism seemed to be due to a decrease in receptor activity (Packard et al., 1983).

Mean fasting plasma total cholesterol, LDL cholesterol and total TG values were 6, 7 and 19% lower in 36 vegetarian men than in 18 nonvegetarian men of similar age, weight, height, alcohol intake and physical activity. Although the vegetarian were characterized by widely differing egg intake, no relationship was observed between dietary or egg cholesterol intakes and plasma lipids. Total fat intake, however, seemed to exert an important influence on plasma lipids within the vegetarian group. Total mean cholesterol and TG values were 11 and 21% lower and mean HDL cholesterol values were 14% higher in low-fat vegetarians (23 to 33% kcal from fat) than in high-fat vegetarians (35 to 48% kcal from fat) (Liebman and Bazzarre, 1983).

In addition to their usual diet, 17 lactovegetarian college students consumed 400 kcal of test foods daily containing one extra-large egg for 3 weeks and similar isocaloric eggless foods for an additional 3 weeks in a randomized double-blind crossover trial. Ingestion of the egg diet increased dietary cholesterol from 97 to 418 mg daily. Mean plasma LDL cholesterol was 12% higher and mean plasma apolipoprotein B was 9% higher when eggs were being consumed than during the eggless period. Mean plasma HDL cholesterol, apolipoprotein A-I and A-II, VLDL cholesterol and total TG did not change significantly. Plasma LDL may be more sensitive to cholesterol at low intakes than at moderate to high intakes (Sack et al., 1984).

Male students who had eaten 2 eggs daily while on a controlled diet for 12 weeks continued on a controlled diet but abstained from eating eggs for 12 weeks. Significantly lower total cholesterol values were obtained at the end of 6 weeks and 12 weeks when they were under emotional stress due to final examination. Mean values for HDL cholesterol in abstainers were significantly lower at the end of 6 weeks, but did not change further by 12 weeks. Mean serum TG values were variable. Blood pressure was not changed significantly (Flynn et al., 1984).

In normolipemic persons of 20 to 50 years old, dietary cholesterol was increased from 311 to 1,430 mg by the intake of 6 eggs daily. LDL cholesterol increased from 102 to 120 mg/100 ml but there was no significant relation between increases in LDL cholesterol to Lp(a) (Brown et al., 1991).

An experiment was conducted with persons having chronic kidney failure. Serum cholesterol and HDL cholesterol tended to increase with change from none to 3 eggs daily and to decrease with the change from 3 eggs to none; serum TG was high initially and did not change significantly with diet. It was concluded that taking a high-cholesterol (egg) diet for 4 weeks was not associated with any considerable increase in serum cholesterol in persons regularly undergoing hemodialysis. Eggs are a source of protein of high biological value and might tend to increase serum HDL cholesterol and serum cholesterol-binding reserve (Green et al., 1985).

The possibility that dietary eggs and ascorbic acid have a positive and synergistic influence on HDL cholesterol was investigated. Twenty men, 36 to 60 years old, followed each of four 12-wk modifications of their daily habitual diet: no visible eggs or supplemental ascorbic acid (basal); 3 eggs (EG); 1.5 g ascorbic acid (AA); 3 eggs and 1.5 g ascorbic acids. Average HDL cholesterol concentrations were unchanged by these diet modifications but HDL cholesterol was positively correlated with plasma ascorbic acid. Total cholesterol was the only plasma lipid that was affected significantly by any of the treatments, and the difference was shown only between diets EG and AA; 209 and 194 mg/100 ml, respectively (O'Brien and McMurray, 1988).

The effect of the intake of 3, 7 or 14 eggs/wk on biochemical risk markers of CHD was examined in 70 young men who followed a high-fat diet. Except for increased LCAT activities and total serum protein concentrations, no significant differences in lipoproteins or coagulation factors occurred between groups. It seems that egg intake in this range did not influence CHD risk markers in these subjects. It was concluded that recommendations to lower risk should probably concentrate on a reduction in fat and not cholesterol intake (Voster et al., 1992).

Hyper- or Hyporesponders to Egg Cholesterol

There appear to exist hyper-responders and hypo-responders to dietary cholesterol, but large fluctuations in serum cholesterol between individuals make such responders difficult to identify with precision (Katan et al., 1983).

After eating 3 eggs daily in addition to their habitual diets for 28 days, 21 subjects were divided into 8 hyper- and 13 hypo-responders. The average plasma cholesterol of the 21 subjects changed from 188 ± 36 to 199 ± 36 mg/100 ml during the 28 days. During the same period the mean plasma cholesterol of the hyper-responders was increased from 170 ± 41 to 199 ± 29 mg/100 ml whereas that of the hypo-responders fell slightly. The addition of 6 eggs to the daily diet of the hypo-responders did not alter the mean plasma cholesterol concentrations

but resulted in a wide difference in response of plasma cholesterol concentration. This study illustrated the variabilities of plasma cholesterol level among free-living subjects who demonstrated two-stage thresholds of response to dietary intake of cholesterol (Oh and Miller, 1985).

Individuals consuming beef or pork, or poultry and fish mainatined similar blood cholesterol and HDL contents; adding eggs to the habitual diets of most people or cessation of egg consumption, on average, does not affect blood cholesterol. However, there are hyper- and hypo-responders who react differently to the addition or removal of eggs from their diet. Indentifying individuals in both categories would seem useful from the point of view of prevention and nutrition guidelines (Brisson, 1987).

In hypercholesterolemic children it was recommended that dietary saturated fat sources be reduced and those of PUFA be increased, that the ratio of P/S should not exceed 1 and that no more than 3 eggs weekly should be eaten (Farriaux et al., 1987).

In a study to determine whether hyperresponse to dietary cholesterol occurs in individuals consuming a low-fat diet, hyperresponsive subjects were given 9, zero, and 9 eggs per week over 3 consecutive months. Plasma total, LDL, and HDL cholesterol did not differ signifiantly. Consistent hyperresponse to moderate cholesterol intake is not apparent in people eating a low-fat, high-fibre diet. Reduction in dietary cholesterol below 400 mg/d produces no further substantial cholesterol lowering (Edington et al., 1989).

A case study described the serum cholesterol, absorbed cholesterol, bile acid pool and daily bile acid synthesis of an elderly man who had consumed approximately 25 eggs each day. His normal serum cholesterol level was attributed to an adaptive mechanism in which this patient had significantly lower cholesterol absorption and double the usual rate of bile acid synthesis (Kern, 1991).

Apolipoprotein (apo) E occurs in all classes of lipoproteins; it may be involved in the conversion of VLDL to LDL and its clearance from the circulation. The hypothesis that apo E polymorphism modulates an individual's response to cholesterol-rich diet was tested in normolipemic young people with apo E phenotypes E3/2, E3/3, E4/3 and E4/4. Consuming a high cholesterol diet (750 mg/day from egg yolks) increased total cholesterol concentration by 13, 18 and 12% in groups E3/2, E3/3 and E4/3, respectively. Strong responses were observed in the small group of E4/4 subjects, in whom the increases in total cholesterol, LDL cholesterol, and apo B were approximately 2.3-fold compared with all the other phenotypes studied (Lehtimaki et al., 1992).

Oxidized Egg Cholesterol

Oxidation products of cholesterol, oxisterols, which have been observed to be formed in powered eggs (Tsai and Hudson, 1985; Bosinger et al., 1993) and other dehydrated cholesterol-containing foods, have been suspected as the probable primary cause of atherosclerotic lesion. Powdered or fresh egg yolks given to young male chickens up to 30 and 43 weeks old produced about the same increase in plasma cholesterol, liver fat and liver cholesterol, compared with a soybean-based cholesterol-free diet. Aortic atherosclerosis was more server with fresh egg yolks than with powdered egg or the cholesterol-free diet. Enhanced feedback inhibition of cholesterol synthesis by oxidized cholesterol derivatives such as 7-ketosterol and other oxisterols (Naber et al., 1985; Turchetto et al., 1994) may at least partly explain these results (Griminger and Fisher, 1986).

2. Modified Eggs

Concern about the cholesterol content of the human diet has led to many attempts to reduce the cholesterol content of eggs. Although this has involved a number of different approaches, including genetic selection and nutritional or pharmacological manipulation, the overwhelming evidence is that egg yolk cholesterol level is very resistant to change because yolk formation is the major pathway of plasma lipids of laying hens.

PUFA Eggs

Because the reduction of the cholesterol content of poultry products has met with little success, dietary fatty acid modification has been pursued as a viable method of adding value to poultry products. Especially, due to the numerous proposed cardiovascular benefits associated with consumption of n-3 fatty acids, marketing of eggs enriched by n-3 fatty acid may benefit the producer.

Hens fed soybean oil laid eggs with elevated 18:2n6 and 18:3n3 fatty acid concentrations compared to those from hens given tallow. The 18:1 fatty acid concentration in egg yolks increased proportionately with the addition of olive oil in the diet. However, the lipid treatments did not influence cholesterol concentrations in the egg (Watkins and Elkin, 1992).

Recent studies have centered on manipulation of specific fatty acids such as n-3 fatty acids (especially EPA and DHA) found in marine products. The n-3 fatty acid content of eggs can be readily increased by inclusion of marine oils/meals in the diet. Effects on yolk composition

of a standard laying hen diet enriched with 3% menhaden oil (test diet), versus an isocloric (control) diet containing no added fat, were evaluated for 18 weeks. Dietary menhaden oil did not alter egg production, egg weight, total yolk fat, or yolk cholesterol. However, yolk content of n-6 and n-3 fatty acids were influenced by diet. Arachidonic acid decreased and EPA increased in eggs from hens fed the test diet following 1 wk of dietary treatment. DHA and linolinate increased in eggs from hens fed the test diet at 2 and 3 week of the trial, respectively. Increased microscopic hepatic lipid infiltration was observed with dietary n-3 administration. This histological change may have significance for flocks predisposed to fatty liver syndrome and may also provide a unique system in which to study the effects of dietary n-3 fatty acids on liver lipid metabolism (Hargis et al., 1991). In another experiment, reproductively acitve hens but not cockerels exhibited increased hepatic lipidosis following 6 month of feeding of 3% menhaden oil, confirming earlier findings. Hens fed 3% animal-vegetable oil did not exhibit hepatic lipid accumulation. Serum triglyceride and cholesterol concentrations were significantly reduced in hens fed menhaden oil (Van-Elswyk et al., 1994).

Effects of dietary n-3 or n-6 fatty acid-enriched chicken eggs on plasma and tissue cholesterol and fatty acid composition of rats were studied. Consumption of n-3 PUFA-enriched egg yolks which were produced by feeding 10% flaxseed significantly reduced both plasma and liver total cholesterol. The plasma cholesterol of rats fed on yolk powders enriched with n-6 PUFA (mainly linoleic acid) was reduced to the same extent as in those fed on the n-3 enriched powders, but the liver cholesterol was significantly increased, indicating differential effects of dietary n-3 and n-6 PUFA (Jiang and Sim, 1992).

One group of people consumed four n-3 eggs per day during the first 4-wk period and four control eggs for the second 4-wk period. The other group ate the same number of eggs in the reverse order. Mean plasma cholesterol concentration was significantly increased by control eggs but unchanged by n-3 eggs. Mean plasma TG concentration was decreased by n-3 eggs but increased by control eggs. Blood pressure was significantly decreased by n-3 egg but was not changed by control eggs (Oh et al., 1991).

However, the beneficial effects of n-3 PUFA fortified eggs in humans have not always been consistent. In some cases the consumption of the modified eggs lowered plasma total cholesterol and TG values compared to the consumption of regular eggs (Sim and Jiang, 1994) confirming earlier results while in other cases it had no effect (Farrell, 1993, 1994). The potential for using egg yolk as a carrier of these fatty acids for use in infant bottle formula was also discussed (Farrell, 1993). Although some experiments failed to detect any differences between n-3 fatty acids enriched eggs produced by supplementing fish oils and those produced by vegetable oils in flavour, taste, colour or overall assessment (Farrell and Gibson, 1991; Farrell, 1994), off-flavour problems associated with fish oil supplementation have prompted studies on the use of other sources of n-3 fatty acids, e.g. vegetable oils. While effective in enriching egg products (and meat as well) with linolenic acid (18:3n-3), plant sources result in only minor changes in the content of C20 n-3 fatty acids. Also, linseed which is a major plant source of n-3 fatty acid caused hens to lay eggs with fishy flavour as well (Jiang and Sim, 1994). Various methods of oil processing and the use of dietary antioxidants have been examined to improve flavour quality and storage stability of n-3 fatty acid enriched products (Hargis and Van-Elswyk, 1993).

Stability of PUFA is essential. As the number of double bond in fatty acids increases the speed of oxidation is accelerated. PUFA, especially EPA and DHA, in fish oils are very unstable to oxidation. Therefore, the benefits of feeding PUFA could be negated if they are not properly stabilized. When a group of rabbits was fed purified fish oil concentrate plus 1.5% cholesterol, aortic atherosclerosis was significantly higher as compared with that fed 1.5% cholesterol alone. Total serum peroxide levels, expressed as malondialdehyde eguivalents were significantly elevated in the fish oil-treated group. This may be due to malondialdehyde modification of the lipoproteins and may be responsible for the enhanced development of atherosclerosis (Thiery and Seidel, 1987).

Iodine and Vitamin E Eggs

Feeding a controlled diet to laying hens produced modified eggs that contained more vitamin E and iodine, and more unsaturated fat than eggs from hens fed normal diet. Adult human beings with initial undesirably high (5.17-7.76 mmol/L) concentration of serum total cholesterol were randomly assigned to a National Cholesterol Education Program (NCEP) diet including either no whole eggs or 12 whole modified eggs a week. Subjects in both groups significantly reduced their serum LDL and HDL cholesterol over the 6-wk study. It was concluded that the modified eggs did not adversely affect measured lipid concentrations when added to a low fat diet that favourably alters lipid profiles in hypercholesterolemic subjects (Garwin et al., 1992; Morgan et al., 1993).

Iodine-enriched (IE) eggs produced by feeding kelp have been reported to reduce plasma cholesterol in

humans and experimental animals. One IE egg/day was ingested by borderline and hyperlipidemic individuals (> 5.7 mmol/L) ingesting a low-fat, low-cholesterol control diet. Subjects in both the egg group and the diet control group had a significant reduction in total plasma cholesterol at the end of the study compared with study entry. However, paired comparisons of total and lipoprotein cholesterol levels at the end of egg intervention period demonstrated that the egg group had a significantly greater increase than the diet control group in total plasma cholesterol and LDL cholesterol. This effect was most pronounced in subjects with higher initial cholesterol levels and subjects with mixed hyperlipidemia of elevated cholesterol and triglyceride (Garber et al., 1992).

Rats were fed for one week on a commercial diet supplemented with propylthiouracil (PTU) 10 mg/100 g or thyroxine-Na 240 mμg/100 g diet, respectively, to induce hypo- or hyperthyroidism, and then further fed for 4 weeks on the respective drug-supplemnted diets, containing 1% (w/w) ordinary or high-iodine egg powder. There were no essential differences between rats given high-I and ordinary eggs in hypo- or hyperthyroid state, although effects of PTU treatment on thyroid and serum TG value seemed to be slightly less in rats given high-I eggs than those given ordinary eggs (Katamine et al., 1985).

Low Cholesterol Eggs

Lovastatin, a competitive inhibitor of HMG CoA reductase, was administered to laying hens. Cholesterol content per g of yolk was not affected at the dose level of 35 mg/kg (Luhman et al., 1990) but significantly lowered with each higher successive level (59-265 ppm) of lovastatin, and showed a plateau at approximately 151 mg, a 15% reduction from the basal value (Elkin and Rogler, 1990). PD 132301-2 (Elkin et al., 1993), an inhibitor of ACAT (acyl CoA: cholesterol O-acyltransferase), or dichloroacetate (Beyer and Jensen, 1993a), an inhibitor of cholesterol biosynthesis, however, were not effective in reducing egg yolk cholesterol content.

Fresh or ethanol-extracted garlic supplement to the layer diets of Japanese quail decreased serum, egg yolk and liver cholesterol, as well as blood glucose. Antibody titre also increased. Egg production decreased and egg weight increased. Fresh cloves were more effective and this effect was time-dependent (El-Habbak et al., 1989). However, supplementation of garlic oil at the level of 0.02% to the diet of White Leghorn hens did not affect total plasma lipids, plasma cholesterol and yolk cholesterol (Reddy et al., 1991).

Three experiments were conducted to determine the effect of supplemental α-keto-isocaproic (KIC) or leucine on layer performance and plasma and egg cholestrol levels. Only in one experiment, in which 0.09% KIC and 0.09% leucine, and 0.27% KIC were supplemented reduced egg cholesterol significantly below the controls (Beyer and Jensen, 1992).

Eggs of hens given LFA3 (described as a lower fat additive) showed an increase in shell weight/egg weight 14.0%, shell thickness 9.3%, shell density 8.4%, albumin weight/egg weight 1.6% and Haugh units by 4.1% but a decrease in yolk weight 19.9%, yolk weight/egg weight 9.4% and cholesterol/yolk by 31.5% (Cao et al., 1993).

Inclusion of 30% oat bran or 3% cottonseed hulls in a Leghorn layer diet significantly lowered egg yolk cholesterol concentration by 6 to 7% (Lirette et al., 1993) but other fibre-rich high-protein barley flour (HPBF: brewer's spent grains that have been dried, milled and blended to provide a product with 35% fibre, 35% protien and 9% fat) up to 10% or supplementation with α-tocotrienol up to 200 mg did not affect egg cholesterol concentration (Beyer and Jensen, 1993b).

Supplementation of dried alga *Chlorella vulgaris* at a level of 0.3% increased colour intensity and β-carotene content of yolk. With intake of algae meal in alternate weeks there was an increase in egg shell thickness and hardness and a higher ratio of egg shell weight:total egg weight. However, proportion of yolk in whole egg and cholesterol content of yolk were not affected by intake of algae meal (Kotrbacek et al., 1993).

Addition of chromium picolinate (Page et al., 1991), luceme saponins (Gorobets, 1992), or sorbose (Beyer and Jensen, 1993c) to layer diets reduced total cholesterol concentration in blood serum but not in egg yolk.

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