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Amy Sarver Ward for the degree of Master of Science in
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Title: The Effect of Egg Consumption on Cholesterol Distribution
Among Lipoproteins and the Ratio of Apo A-I/A-II in Hyper and Hyporesponders

Abstract approved:                        Dr. Suk Y. Oh

The effect of increased egg consumption on plasma cholesterol levels and its distribution among lipoproteins and Apolipoprotein A-I/A-II ratios of high density lipoprotein subfractions in individuals who did and did not demonstrate response in plasma cholesterol levels was studied in 20 healthy middle-aged men (30-55 years of age) for three months. Lunch containing 3 eggs was fed all subjects during the first month classification period. Subsequently, subjects were divided into hyper and hyporesponders according to their blood cholesterol level. Thereafter, 6 eggs were fed to hypo (n=12) while hyper (n=8) continued to consume 3 eggs with lunch. The average plasma cholesterol level of the 20 subjects was increased from 185±34 to 195±34 mg/100ml during the classification period. The mean plasma cholesterol level of the hyperresponders was significantly (p<0.025) increased from 170±42 to 199±40 mg/100ml during classification while that of the hyporesponders fell slightly. The additional 6 eggs consumed by the hyporesponders for 6 weeks did not increase the mean plasma
cholesterol level. The apoprotein ratios did not change as a result of the increased cholesterol diet and were not significantly different between the two response groups. The hyper and hyporesponders differed significantly in initial plasma cholesterol parameters that diminished as the subjects were challenged with increased cholesterol consumption. These initial differences included a significantly lower low density lipoprotein cholesterol/high density lipoprotein cholesterol ratio of 2.26±0.6 for hyperresponders compared to 3.31±0.9 for hyporesponders. There were also initial differences in the percentage of whole plasma cholesterol found in the HDL fractions: hyperresponders had a greater percentage of total and free cholesterol in the HDL and HDL-2 fractions and a greater percentage of total cholesterol in the HDL-3 fraction. The present data demonstrate that increased cholesterol consumption will increase plasma cholesterol levels and that there is individual variability of plasma cholesterol response as a result of feeding cholesterol.
The Effect of Egg Consumption on Cholesterol Distribution among Lipoproteins and the Ratio of Apo A-I/A-II in Hyper and Hyporesponders

by

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ABBREVIATIONS USED IN TEXT

Apo= Apolipoprotein
CHD= Coronary heart disease
HDL= High density lipoprotein
IDL= Intermediate density lipoprotein
LCAT= Lecithin:cholesterol acyl transferase
LDL= Low density lipoprotein
LPL= Lipoprotein lipase
P/S= Polyunsaturated/saturated fat ratio
RCT= Reverse cholesterol transport
VLDL= Very low density lipoprotein
THE EFFECT OF EGG CONSUMPTION ON CHOLESTEROL DISTRIBUTION AMONG LIPOPROTEINS AND THE RATIO OF APO A-I/A-II IN HYPER AND HYPORESPONDERS

INTRODUCTION

The relationship between coronary heart disease (CHD) and plasma cholesterol concentrations has been studied for many years. Epidemiological studies have shown that elevated plasma cholesterol levels are positively correlated with risk of developing CHD. Although many investigators have demonstrated that higher intake of dietary cholesterol increased plasma cholesterol concentration (Mattson et al., 1972; Roberts et al., 1981; Mistry et al., 1981), this issue remains controversial due to considerable differences between individuals in the response of plasma cholesterol concentration to dietary cholesterol (Quintao et al., 1971; Nestel and Poyser, 1976). Mahley et al. (1978) observed changes in plasma cholesterol of 0-23% in 11 individuals when 4-6 eggs per day were added to a normal diet. The physiological significance for the differences in individual response is as yet not understood and little investigated.

Alterations in plasma cholesterol induced by dietary cholesterol can be accounted for by changes in the plasma lipoproteins. The effects of dietary cholesterol on the distribution of cholesterol varies among mammalian species (Tan et al., 1980). Other nutrients, particularly fats, have been shown to influence the
effect of dietary cholesterol on lipoprotein cholesterol (Tan et al., 1980; Jackson et al., 1980; Oh and Monaco, 1983). Reported effects include increases in low density lipoprotein cholesterol (LDL-C) only (Connor and Lin, 1980), increased LDL-C and high density lipoprotein -2 (HDL-2) cholesterol (Mistry et al., 1981), and increased LDL-C, HDL-C and Apo A-I (Tan et al., 1980).

HDL has recently been the focus of many studies since HDL was shown to have a protective role against CHD (Miller and Miller, 1975, Gordon et al., 1977a). HDL removed cholesterol from a variety of cell types (Stein & Stein, 1975a and b) and is believed to play an important role in cholesterol transport from peripheral tissues to the liver (Glomsett, 1979). HDL and its major apolipoprotein, Apo A-I, have been quantitatively altered by dietary cholesterol (Mistry et al., 1981; Tan et al., 1980). Little information is available on the effect of dietary cholesterol on the levels of HDL-C and Apo A-I in individuals who show a wide range of plasma cholesterol level response to dietary cholesterol.

This study was designed to study the effect of dietary cholesterol on the plasma lipoproteins, cholesterol distribution among lipoproteins, and the Apo A-I/Apo A-II ratio in the HDL subfractions in the plasma of individuals who do and do not demonstrate a plasma cholesterol response to dietary cholesterol.
REVIEW OF LITERATURE

Cholesterol Metabolism

Cholesterol Absorption

Dietary (exogenous) cholesterol is absorbed in the small intestine by passive diffusion (Dietschy and Wilson, 1970b). In the lumen, exogenous cholesterol mixes with cholesterol from endogenous sources. Cholesterol from these two sources may exist in either the free form or esterified with a long chain fatty acid. Pancreatic cholesterol esterase hydrolyzes the cholesterol esters to free cholesterol which can then be solubilized in mixed micelles with monoglycerides and free fatty acids. In the intestinal mucosal cell, a large proportion of the free cholesterol is re-esterified and packaged into the largest of the lipoproteins, the chylomicron, and released into the lymph (Dietschy and Wilson, 1970b).

Cholesterol absorption has long been known to be facilitated by the presence of bile acids and long chain fatty acids. Elimination of bile acids from the intestinal tract 24 hours before \(^{14}\)C-cholesterol was fed resulted in none (<0.01%) of the \(^{14}\)C being recovered in the thoracic or lacteal lymph (Siperstein et al., 1952). Similarly, adding 6 g of fat to a previously fat free diet resulted in a 38% increase of cholesterol absorption (Kim and Ivy, 1952).

Exogenous cholesterol absorption increases with increased cholesterol intake and there appears to be no saturation of the cholesterol absorption mechanism (Borgstrom, 1969). Kudchodkar et al. (1973) found that amounts of cholesterol absorbed are
proportional to cholesterol intake, and also reported that cholesterol absorption did not level off with intakes of up to 1.2g cholesterol per day. Hyper and normocholesterolemic subjects appear to absorb cholesterol to similar extents. In two studies by Connor and Lin (Connor and Lin, 1974; Lin and Connor, 1980), the percentage of cholesterol absorbed was not affected by background diets of high or low cholesterol content in either hyper and normocholesterolemic subjects. Percentage of cholesterol absorption remained relatively constant at approximately 40 percent. Quintao et al. (1971) reported cholesterol absorption was increased with increases in intake; however, absorption ranged from 50 percent with a normal cholesterol intake (approx. 500mg/day) to 33 percent with a cholesterol intake of 3g per day.

Cholesterol Biosynthesis

All nucleated mammalian cells can synthesize cholesterol to some degree. However, many cells, such as the major cell types of the vascular bed, take up cholesterol from the plasma lipoproteins rather than carrying out de novo synthesis (Fielding and Fielding, 1982) to meet their requirement. In primates, the liver, GI tract and skin make up 99 percent of whole body cholesterol synthesis. The liver is the major organ of cholesterol biosynthesis and it alone contributes 82% of cholesterol biosynthesis in man (Dietschy and Wilson, 1970a).

There are approximately 26 biochemical reaction steps in cholesterol biosynthesis. These involve a series of polymerizations
and modifications of the basic building block, the isoprene unit, of which acetyl-CoA is the precursor. These steps can be separated into three major groups: 1) Acetyl-CoA $\rightarrow$ mevalonic acid, 2) mevalonic acid $\rightarrow$ squalene, 3) squalene $\rightarrow$ cholesterol. The enzymes involved in this pathway are both microsomal and cytoplasmic (Ott and Lachance, 1981; Lehninger, 1975).

All carbon atoms in the cholesterol molecule can be derived from acetate (acetyl-CoA). The major source of acetyl-CoA for cholesterol synthesis is in the mitochondria from the oxidation of pyruvate and fatty acids. Since the mitochondrial membrane is impermeable to acetyl-CoA, citrate is used to shuttle acetyl-CoA to the cytoplasm, where it is cleaved by citrate lyase to yield oxaloacetate and acetyl-CoA. Other possible sources of carbon inputs for cholesterol biosynthesis include: 1) leucine, yielding $\beta$-hydroxy-$\beta$-methylglutaryl-CoA (HMG-CoA) and 2) fatty acid metabolism, yielding acetoacetyl CoA. These two products are both intermediates in the pathway of cholesterol biosynthesis (Gibbons et al., 1982; Ott and Lachance, 1981).

The amount of cholesterol synthesized in humans varies greatly and is affected by many factors. In normal humans consuming an average diet containing 616 mg cholesterol per day, liver cholesterogenesis was reported to be $309\pm45$ pmoles/mg (112±17 pgrams) of tissue/2 hours (Bhattathiry and Siperstein, 1963). Grundy and Aherns (1969) estimated cholesterol synthesis by the sterol balance method. Five subjects who received less than 40mg cholesterol/day in
the diet synthesized between 689mg-1080mg cholesterol/day (mean 840mg/day) while six subjects fed "moderate" cholesterol diets (295-577mg/day) synthesized between 367-1407 mg/day (mean 706mg/day). It should be noted that all subjects, with one exception, had abnormal lipoprotein patterns.

It is well known that cholesterol feeding does affect cholesterol biosynthesis (Dietschy and Wilson, 1970a). In a study of moderate cholesterol intake (250-750mg cholesterol/day) with 2 normo- and 7 hyperlipidemic subjects, total body cholesterol synthesis was significantly decreased by 11-420mg/day as cholesterol absorption increased (Nestel and Poyser, 1976). Lin and Connor (1980) demonstrated feedback inhibition of cholesterol biosynthesis in a 25 week study. In men proceeding from a low to moderately high cholesterol diet, total body cholesterol biosynthesis decreased from 700±27.6 mg to 335±87.7 mg cholesterol/day (52.1% decrease).

The rate limiting enzyme in the cholesterol biosynthetic pathway is HMG-CoA reductase (Dietschy and Brown, 1974; Mistry et al., 1981). The major effect of cholesterol feeding on cholesterol biosynthesis appears to be on this enzyme (Gibbons et al., 1982; Dietschy and Wilson, 1970a; Ott and Lachance, 1981). Thirty-seven subjects fed 1500mg cholesterol/day showed suppression of HMG-CoA reductase by 32% in blood mononuclear cells (Mistry et al., 1981). Dietschy and Brown (1974) demonstrated that HMG-CoA reductase activity correlated closely with cholesterol synthesis in rat liver slices. Both newly synthesized cholesterol and cholesterol absorbed
from the intestine have been demonstrated to inhibit HMG-CoA reductase (Nervi and Dietschy, 1975; Mitropolous et al., 1978; Edwards et al., 1977).

Enterohepatic circulation of bile acids also affects cholesterol biosynthesis (Dietschy and Wilson, 1970a). Increasing the bile acid pool by oral administration of sodium taurocholate resulted in decreases in cholesterol turnover rates (Grundy et al., 1966). Increases in cholesterol synthesis can be induced by ileal bypass which interrupts enterohepatic circulation of bile acids or by feeding cholestyramine, a cholesterol sequestering agent (Wilson, 1972; Dietschy and Brown, 1974). It has been suggested that this decrease in cholesterologenesis is mediated through HMG-CoA reductase (Dietschy and Brown, 1974).

Cholesterol Transport

The free cholesterol molecule is insoluble in the plasma. It is transported in lipid-protein complexes known as lipoproteins. These lipoprotein complexes are spheres made up of lipids and proteins. The center of the particle is made up of apolar lipids such as esterified cholesterol and triglycerides. The outer surface is composed of protein, phospholipids and free cholesterol (Levy, 1981). Lipoproteins have been classified by different methods of isolation. The two most commonly used methods are electrophoresis and ultracentrifugation (Jackson et al., 1977).

The compositions and the physical properties of the
lipoprotein classes are shown in table 1. As the proportion of lipid is decreased from that of the chylomicron to that of HDL-3, there is a progressive increase in the percent of protein and decrease in diameter.

The transport of cholesterol may be divided into two overlapping systems of exogenous (dietary) and endogenous (synthesized and reabsorbed) cholesterol sources (Brown et al., 1981; Levy, 1981). A model has been proposed by Brown et al. (1981) which illustrates the differences between endogenous and exogenous cholesterol sources in the lipoprotein transport system.

Exogenous Transport:

Cholesterol absorbed from the diet is packaged into the chylomicron along with dietary triglycerides (Dietschy and Wilson, 1970b). Exactly how cholesterol is "packaged" into chylomicrons is not known; however, Ways et al. (1967) demonstrated that individuals who are unable to synthesize chylomicrons (e.g. abetalipoproteinemics) do not show distention of the golgi after a fatty meal as is seen in normal individuals.

The molar ratio of free to esterified cholesterol is 1:1 in the chylomicron (Eisenberg and Levy, 1975). The free cholesterol can be esterified with long chain fatty acids by the action of the enzyme acyl:cholesterol acyl transferase (ACAT), which is present in the mucosal cells (Fielding and Fielding, 1982). The chylomicron is too large to cross the endothelial barrier (Brown et al., 1981). Instead, the chylomicron is depleted of its triacylglycerol core
<table>
<thead>
<tr>
<th>Class</th>
<th>density g/ml</th>
<th>electrophoretic mobility</th>
<th>diameter Å</th>
<th>% of molecules</th>
<th>major apoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron</td>
<td>0.95</td>
<td>origin</td>
<td>$10^3$-$10^4$</td>
<td>98</td>
<td>4-8, 2</td>
</tr>
<tr>
<td>Very Low Density Lipoprotein (VLDL)</td>
<td>0.95-1.006</td>
<td>pre-beta</td>
<td>250-270</td>
<td>90</td>
<td>15-25, 10</td>
</tr>
<tr>
<td>Intermediate Density Lipoprotein (IDL)</td>
<td>1.006-1.019</td>
<td>pre-beta₁</td>
<td>250</td>
<td>80</td>
<td>30, 15</td>
</tr>
<tr>
<td>Low Density Lipoprotein (LDL)</td>
<td>1.019-1.063</td>
<td>beta</td>
<td>200-250</td>
<td>75</td>
<td>42-50, 20</td>
</tr>
<tr>
<td>High Density Lipoprotein-2 (HDL-2)</td>
<td>1.063-1.125</td>
<td>alpha₁</td>
<td>70-120</td>
<td>57</td>
<td>23, 43</td>
</tr>
<tr>
<td>High Density Lipoprotein-3 (HDL-3)</td>
<td>1.125-1.210</td>
<td>alpha₁</td>
<td>50-100</td>
<td>41</td>
<td>14, 58</td>
</tr>
</tbody>
</table>

1 adapted from Gibbons et al., 1982. pp 236-237  
2 from Jackson et al., 1977  
3 from Schaefer et al., 1979  
4 % total dry weight
through the action of lipoprotein lipase (LPL) in the capillary beds of the bloodstream (Brunzell et al., 1973), and the free fatty acids and monoglycerides are released to adjacent cells. Some surface materials of the chylomicron, mostly protein and free cholesterol, are transferred to HDL (Havel, 1957; Gibbons et al., 1982). The chylomicron remnant is removed from circulation and degraded by the liver (Brown et al., 1981; Levy, 1981). Grundy and Mok (1976) measured the clearance of chylomicrons and found the rate of clearance to be $t_\frac{1}{2}=4.5\pm2.9$ minutes in normal subjects and $t_\frac{1}{2}=23\pm5.5$ minutes in hypertriglyceridemic subjects.

**Endogenous Cholesterol Transport:**

Endogenous cholesterol was at one time thought to be strictly of hepatic origin. It is now known that cholesterol from endogenous sources is packaged for transport by both the liver and intestine (Green and Glickman, 1981). Endogenous cholesterol is incorporated into the lipoprotein VLDL. While intestinal and hepatic VLDL do differ initially in peptide composition (Gangl and Ockner, 1975), intestinal VLDL that enters into the plasma is metabolized by the same mechanism as hepatic VLDL (Brunzell et al., 1973). VLDL particles are rich in triglycerides. This lipoprotein comes into contact with LPL in the capillary bed and releases its triglycerides into adjacent cells, much in the same manner as chylomicrons. As with chylomicrons, surface materials are taken up by HDL (Levy, 1981; Brown et al., 1981). As VLDL is depleted of core materials, it shrinks in size and increases in density. The free cholesterol that
was taken up by HDL is esterified by the enzyme lecithin:cholesterol acyl transferase (LCAT) (Fielding and Fielding, 1980) and the newly esterified cholesterol is transferred back to the VLDL particle (Brown et al., 1981). This resultant particle is termed IDL due to its increased density (Eisenberg and Levy, 1975). IDL loses most of its remaining triglycerides, resulting in conversion to the LDL (Brown et al., 1981). The half-life for the conversion of VLDL to LDL has been reported to be 1 to 3 hours (Bilheimer et al., 1972).

LDLs transfer cholesterol to liver and extrahepatic cells by binding to high-affinity receptors located on the cell surface. The LDL particle is then internalized to the lysosomes where the particle is degraded and cholesterol esters hydrolyzed (Brown et al., 1981). In this fashion, cholesterol is made available for cellular utilization.

Free cholesterol leaving the cells is carried on HDL (Wu and Bailey, 1980) and esterified by LCAT (Fielding and Fielding, 1981). This cholesterol can be transferred to VLDL or IDL and is thereby recycled through LDL. HDL and its role in reverse cholesterol transport will be discussed later.

Cholesterol Excretion and Degradation

Cholesterol output from the body is accomplished primarily through two major systems: excretion of cholesterol in the feces and the conversion of cholesterol to bile acids which are then partially excreted. Other minor pathways of excretion include conversion to
steroid hormones, losses through skin and hair, and elimination in the urine (Dietschy and Wilson, 1970c).

Unabsorbed cholesterol passes to the large intestine where it is degraded by bacteria. Therefore, cholesterol in the feces is mainly in the form of the neutral steroids, coprostanol and coprostanone, as well as some intact cholesterol. Coprostanol formation occurs by more than one pathway due to different bacterial populations. While the species responsible for cholesterol conversion are not known, coprostanol formation does not occur in germ free animals (Dietschy and Wilson, 1970c).

Conversion of cholesterol to bile acids is another major mechanism to eliminate cholesterol from the body. The formation of primary bile acids occurs in the liver (Gibbons et al., 1982). The first step is the introduction of a hydroxyl group at the 7 position of cholesterol to form cholest-5-ene-3β,7α-diol. From this point, the pathway diverges, resulting in intermediate compounds that may be conjugated to either of the amino acids glycine or taurine. These products, the result of cholesterol catabolism in the liver, are called primary bile acids: taurocholic acid, glycocholic acid, taurochenodeoxycholic acid and glycochenodeoxycholic acid (Dietschy and Wilson, 1970c). These bile acid salts are secreted by the liver into the bile and aid in lipid absorption. Ninety-five percent of bile acids are reabsorbed in the ileum and return to the liver via the portal vein (Gibbons et al., 1982). This recycling pathway of bile acids is termed enterohepatic circulation.
Bile acids may be further degraded by bacteria in the lower ileum and colon. This further modification forms the secondary bile acids: deoxycholic acid from cholic acid and lithocholic acid from chenodeoxycholic acid. Some of the secondary bile acids may also be reabsorbed (Gibbons et al., 1982; Dietschy and Wilson, 1970c).

Several factors effect the degradation and excretion of cholesterol. Bile acid formation is under feedback regulation by bile acids resorbed via enterohepatic circulation and by exogenous cholesterol absorption (Dietschy and Wilson, 1970c). Bile acid synthesis was increased four fold when enterohepatic circulation was halted (Grundy et al., 1966). Fecal bile acids have been reported to increase during increased cholesterol consumption, along with an increase in fecal neutral steroids (Nestel and Poyser, 1976; Lin and Connor, 1980). Quintao et al. (1971) observed an increase in endogenous fecal neutral steroids with increased consumption of cholesterol, however fecal bile acids remained unchanged.

Plasma Cholesterol

Free cholesterol comprises approximately one-third of the total cholesterol in whole plasma (Gibbons et al., 1982). It has been observed to freely exchange between the different lipoprotein fractions both in vivo and in vitro (Fielding and Fielding, 1982). Esterified cholesterol makes up the remaining two-thirds of total plasma cholesterol. An exchange of esterified cholesterol has been observed between each of the lipoprotein fractions (Barter and Lally,
1979). This exchange occurs only in the presence of a glycoprotein which is found in the 1.21 g/ml infranatant isolated from the serum (Pattnaik et al., 1978; Barter and Lally, 1979).

In the United States, plasma cholesterol rises from birth to peak at approx. 9-10 years, then falls during adolescence (Abrahams et al., 1978). During young adulthood, plasma cholesterol rises and continues to do so throughout the rest of life (Abrahams et al., 1977). From birth to young adulthood, females have slightly higher plasma cholesterol levels than males until the age of approximately 25 years, thereafter males have higher levels. After menopause, women again have higher cholesterol levels (Abrahams et al., 1977; Abrahams et al., 1978). Average serum cholesterol levels for men and women in the United States are presented in table 2.

There are no absolute standards for "normal" cholesterol levels. However, limits exist for cholesterol levels based on age, which if exceeded, indicate hyperlipidemia (US-DHEW, 1978). It is now generally accepted that the lower the plasma cholesterol level, the lower the risk of coronary heart disease (CHD) (Gordon et al., 1981; Shekelle et al., 1981).

In the 20 year Western Electric Study (Shekelle et al., 1981), a positive correlation existed between diet, serum cholesterol and death from CHD in American males. Similarly, the Framingham Study (Gordon et al., 1981) reported a significant positive correlation between low density lipoprotein cholesterol (LDL-C) levels and CHD in adult males.
Table 2. Total Serum Cholesterol Levels of Adults 18-74 years of age in the United States\textsuperscript{1} in mg/100ml

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Both Sexes</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-74</td>
<td>223.4±51.5*</td>
<td>221.8±49.9</td>
<td>224.9±52.8</td>
</tr>
<tr>
<td>18-24</td>
<td>189.4±39.0</td>
<td>186.9±36.7</td>
<td>191.7±41.0</td>
</tr>
<tr>
<td>25-34</td>
<td>206.6±43.2</td>
<td>210.3±44.0</td>
<td>203.2±42.2</td>
</tr>
<tr>
<td>35-44</td>
<td>223.6±45.3</td>
<td>231.3±45.9</td>
<td>216.5±43.5</td>
</tr>
<tr>
<td>45-54</td>
<td>241.1±49.7</td>
<td>239.4±47.0</td>
<td>242.6±52.0</td>
</tr>
<tr>
<td>55-64</td>
<td>248.9±50.3</td>
<td>240.2±51.2</td>
<td>256.8±48.2</td>
</tr>
<tr>
<td>65-74</td>
<td>250.7±54.2</td>
<td>236.2±53.8</td>
<td>261.6±51.9</td>
</tr>
</tbody>
</table>

\textsuperscript{1}\textit{adapted from Abrahams et al., 1977}  \hspace{1cm} *\textit{mean±SD}
The amount of cholesterol in the plasma is affected by all the factors which govern its metabolism: absorption, biosynthesis, transport and excretion. In addition, environmental factors are also known to affect plasma cholesterol. These include exercise (Schwane and Cundiff, 1979), alcohol consumption (Willett et al., 1980), obesity (Garrison et al., 1980), and, most notably, diet (Mattson et al., 1972; Roberts et al., 1981; Schaefer et al., 1981; Flynn et al., 1981).

Many dietary factors and their effect on plasma cholesterol have been examined. Exogenous cholesterol, polyunsaturated vs. saturated fat (P/S ratio), carbohydrate, fiber and specific foods such as eggs and red vs. white meat have been studied and reported to affect plasma cholesterol. However, the extent to which these factors contribute to plasma cholesterol is controversial. In addition, the effect of genetic factors and previously mentioned environmental factors on plasma cholesterol cannot be ignored when examining the controversial actions of diet on cholesterol levels in plasma.

In a study designed to examine the effect of dietary cholesterol on serum levels, 56 healthy men were placed on diets with four different cholesterol levels, but with identical fatty acid composition (Mattson et al., 1972). All subjects consumed a cholesterol-free formula diet for 21 days to establish a baseline value. Subjects were then split into four groups of differing cholesterol levels, otherwise diets were identical in percent
protein, percent carbohydrate, percent fat and fatty acid composition. Group I continued on a cholesterol-free diet, group II on 106 mg cholesterol/1000 kcal, group III on 212 mg cholesterol/1000 kcal, and group IV on 317 mg cholesterol/1000 kcal per day. These diets were fed for six weeks. The baseline cholesterol-free diet resulted in a decrease in serum cholesterol in all subjects. The decrease reached its lowest point at day 11 and remained at this point until day 21. On the diets differing in cholesterol level, subjects' serum cholesterol rose over baseline in each of the groups. Up to approximately day 28, there were increasing differences in serum cholesterol levels of the groups. The authors conclude that dietary cholesterol may have a major role in determining blood cholesterol levels, and that this increase is linear. An equation was formulated to describe the relationship between dietary cholesterol and the increase in serum cholesterol: each 100 mg cholesterol/1000 kcal consumed resulted in approximately 12 mg/100 ml increase in serum cholesterol.

A study designed to examine the interrelationships of cholesterol, P/S ratio, and fat levels on plasma cholesterol was carried out on 33 normo- and 28 hyperlipidemic subjects (Schaefer et al., 1981). Subjects were admitted to a metabolic ward while on ad libitum baseline diets: 15-20% protein, 35-40% carbohydrate, 35-40% fat, P/S 0.1-0.4 and 400-700 mg cholesterol per day. Thereafter, subjects were placed on one of three diets for a minimum of 14 days. Experimental diets were: I. isocaloric, 20% pro, 40% CHO, 40% fat,
normal P/S (0.1-0.3) and 250-300 mg cholesterol/day; Diet II, identical to diet I with the exception of the P/S ratio, which was changed from normal to high (1.8-2.2); Diet III, an isocaloric diet of 20% pro, 80% CHO, 5-10g fat/day, normal P/S (0.1-0.3) and low cholesterol 100-150 mg/day. Diet I, which differed from the baseline diet mainly in reduced cholesterol content, resulted in reductions of total plasma cholesterol by 5.9%, LDL-C was decreased by 5.6% and HDL-C by 6.3%. Diet II, which had the higher P/S ratio, resulted in even greater reductions in plasma cholesterol of 17%, LDL-C 16.2% and HDL-C 17.4% in normal patients and in hyperlipidemic subjects an 11% plasma cholesterol reduction, 10.8% LDL-C decrease and 17.1% decrease in HDL-C. Diet III, with normal P/S, low fat and low cholesterol, caused the greatest reductions in plasma cholesterol (26.2%), LDL-C 29.9% and HDL-C 27.9% in normal subjects and reductions of 22.6% in total cholesterol, 27.2% in LDL-C, and 28.6% in HDL-C in hyperlipidemic subjects. The authors concluded that lowering dietary cholesterol alone may cause modest decreases in plasma cholesterol levels, but greater reductions can be achieved by feeding diets low in cholesterol and fat and with a high P/S ratio.

An investigation to measure the effect of whole eggs on free living, normal subjects was carried out in a double blind study (Roberts et al., 1981). Sixteen subjects incorporated one-half cup of either homogenized whole egg or an egg substitute into their normal home diet for four weeks, and then consumed the other product for the same length of time. The homogenized whole egg added
approximately 500 mg cholesterol/day to an otherwise normal diet. The egg substitute contributed no cholesterol. The homogenized whole egg consumption resulted in a plasma cholesterol increase of 20mg/ml over baseline. The egg substitute resulted in a plasma cholesterol value 11% lower than the whole egg period. It was concluded that the addition of extra dietary cholesterol to a normal diet can significantly increase plasma cholesterol levels. The study also demonstrated the body's inability to completely compensate for extra cholesterol intake by increased excretion or decreased biosynthesis.

A study to determine the effect on serum cholesterol of removing whole egg from a habitually egg consuming population was undertaken (Bronsgeest-Schoute et al.,1979). Forty-four healthy subjects who normally consumed at least 1 egg/day, were requested not to eat any eggs or products containing large amounts of eggs for 3 weeks. All subjects were instructed to keep a food intake diary. The removal of eggs resulted in a decrease in cholesterol intake from 742±60 mg cholesterol/day to 264±13 mg cholesterol/day. This decrease in intake resulted in a small but significant decrease in serum cholesterol levels. The authors reported a wide individual variation in response and suggest that subgroups of hyper and hyporesponders exist. Subjects with a starting serum cholesterol level of <220mg/dl showed a decrease of 7.2±14.1 mg/dl, while subjects with a starting serum cholesterol level of >220 mg/dl had a cholesterol reduction of 4.9±17.6 mg/dl. Two conclusions were drawn related to the phenomena of hyper and hypo responders: 1) because an
absence of relationship between diet and serum cholesterol in a population is noted, it cannot be concluded that such a relationship does not exist; and 2) absolute serum cholesterol levels within a population may be determined by factors that are not equally important to all individuals. Tolerance tests to determine response type may give insight into this problem.

A summary of five additional studies measuring the effect of diet on plasma cholesterol levels in humans is presented in table 3. It is of interest to note that an overriding feature in these studies is the report of great individual variation.

The High Density Lipoproteins

Since the turn of the century, blood cholesterol has been related to coronary heart disease (CHD) (Gordon et al., 1977a) In 1954, a hypothesis was put forth that CHD could be predicted by certain lipoprotein patterns (Gofman et al., 1954). LDL-C and VLDL-TG were emphasized, and positively correlated with risk of CHD (Gordon et al., 1977a). Attention is now being focused on HDL-C. Numerous clinical and epidemiological studies have demonstrated an inverse relationship between HDL-C and risk of CHD (Gordon et al., 1977b; Gordon et al., 1981; Barboriak et al., 1979).

In a study of 400 male patients, the relationship between HDL-C levels and the extent of coronary artery occlusion was studied (Barboriak et al., 1979). It was reported that subjects with higher HDL-C levels had less extensive coronary artery occlusion than
<table>
<thead>
<tr>
<th>Source</th>
<th>Subjects</th>
<th>Diets(s)</th>
<th>Duration</th>
<th>Effects on Plasma Cholesterol</th>
<th>Conclusions Drawn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nestel &amp; Poyser, 1976</td>
<td>2 healthy; 7 hyperlipidemic men and women</td>
<td>Isocaloric diets</td>
<td>2 periods</td>
<td>0.5g additional C resulted in a sig. increase in plasma C in half of subjects</td>
<td>Great individual variation not understood. Dietary C strongly influences plasma C.</td>
</tr>
<tr>
<td>Mistry et al., 1977</td>
<td>I. 14 healthy men</td>
<td>Identical diets except:</td>
<td>I. 30d.</td>
<td>Increases in C content in all LP classes from both diets. Increases range 6-50%.</td>
<td>Dietary C does influence plasma C to differing increments due to wide individual variation.</td>
</tr>
<tr>
<td></td>
<td>II. 4 healthy men</td>
<td>I. 750mgC/day</td>
<td>II. 10d.</td>
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<td>II. 1500mgC/day</td>
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<tr>
<td>Mahley et al., 1978</td>
<td>I. 6 healthy men and women</td>
<td>I. ad lib. diet plus 4-6 eggs/d.</td>
<td>I. 4 wk</td>
<td>I. 0-25% increase in total plasma C. II. 0-6% increase in plasma C.</td>
<td>Wide individual variation regardless of change in plasma C. No conclusions drawn regarding CHD.</td>
</tr>
<tr>
<td></td>
<td>II. 5 healthy men and women</td>
<td>II. gradual incr. in egg consump-</td>
<td>II. 10 wk</td>
<td>плазма C increased sig. in all subjects in 5gC/d. LDL-C incr. in all subjects. C returned to baseline when return to ad lib. diet.</td>
<td>плазма C is altered as a result of dietary C. No strong conclusions drawn.</td>
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<td>tion to 3 eggs/d.</td>
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<tr>
<td>Applebaum- Dowden et al., 1979</td>
<td>4 healthy men and women</td>
<td>ad lib. then 5gC per day. Return 5gC 1 mo. to ad lib.</td>
<td>ad lib.1wk.</td>
<td>плазма C increased sig. in all subjects in 5gC/d. LDL-C incr. in all subjects. C returned to baseline when return to ad lib. diet.</td>
<td>плазма C is altered as a result of dietary C. No strong conclusions drawn.</td>
</tr>
<tr>
<td>Flaim et al., 1981</td>
<td>23 healthy men</td>
<td>Isocaloric diets of 400 or 1400mg</td>
<td>4wk on</td>
<td>No sig. differences between groups in LP fractions or total plasma C. Great individual variation emphasized.</td>
<td>Extra C in diets already containing moderate amounts of C does not alter plasma or LP C levels; individual response considerable.</td>
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<td>either the 400 or 1400</td>
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**Abbreviations:**
- **C** Cholesterol
- **LP** Lipoprotein
- **CHD** coronary heart disease
- **d** day
- **mo** month
- **wk** week
subjects with lower levels of HDL-C. In a five year study investigating the relationship between HDL-C and CHD (Goldbourt and Medalie, 1979), a significant inverse correlation was reported between HDL-C and incidence of myocardial infarction in males. When factors such as age, weight, diabetes mellitus, blood pressure, smoking and other cholesterol related factors were controlled by multivariant analysis, the inverse relationship between HDL-C and incidence of myocardial infarction remained significant. In the Framingham study of both men and women (Gordon et al., 1977b), low HDL-C was a risk factor in CHD for subjects over 50 years of age. A weaker positive association was noted for high LDL-C levels and incidence of CHD. As with the Goldbourt and Medalie study, HDL-C retained its negative association with CHD when the factors diabetes and relative body weight were controlled in data analysis.

While the exact mechanism by which HDL plays a protective role in CHD is not yet known, two theories have been proposed. 1) HDL may remove cholesterol from the tissues and transport it to the liver for catabolism and excretion. This theory is termed reverse cholesterol transport (Miller and Miller, 1975). 2) HDL may modify LDL metabolism by competing for cellular binding and cholesterol uptake (Carew et al., 1976).

Structure and Composition

The HDLs are the heaviest of the lipoproteins, defined by flotation in the ultracentrifuge at a density between 1.063 and 1.21
g/ml (Schaefer et al., 1979). HDLs are also defined as alpha-lipoproteins by electrophoretic mobility, corresponding to the migration distance of alpha globulins (Jackson et al., 1977). Composition of HDL by weight is about 50% protein, 25% phospholipids, 5% triglyceride and 20% cholesterol. The ratio of esterified to free cholesterol is approximately 3:1 (Gibbons et al., 1982; Eisenberg and Levy, 1975).

The HDL density fraction is quite heterogeneous (Krauss, 1982) and various subfractions have been isolated by using different isolation methods such as analytical or density gradient ultracentrifugation, gradient polyacrylamide gel electrophoresis, zonal ultracentrifugation and isoelectric focusing. Most commonly, HDL is divided into two subfractions by density gradient ultracentrifugation. HDLs in the density range 1.063-1.125g/ml are termed HDL-2 and those in the density range 1.125-1.210g/ml are termed HDL-3 (Schaefer et al., 1979). While the exact composition of the HDL subfractions depends upon the method of isolation, in general, HDL-2 is composed of (by weight) 43% protein, 28% phospholipid, 23% cholesterol and 6% triglyceride while HDL-3 is made of approximately 58% protein, 22% phospholipid, 14% cholesterol and 5% triglyceride (Schaefer et al., 1979). The composition of the HDL subfractions in comparison to the other lipoprotein fractions is presented in table 1.

HDL-C levels are approximately the same in both sexes from birth to 14 years with a mean of 53mg/dl. In males, HDL-C levels
drop after age 14 to 44-46mg/dl through age 54. After this age, HDL-C levels rise again to about 51mg/dl. In females the childhood level of 53mg/dl remains constant to age 25, then the level rises to 56-57mg/dl up to age 40 years. At around 65 years, HDL-C in females peaks at 65mg/dl, dropping at age 70 to a level of 60mg/dl (Rifkind et al., 1978).

HDL Apolipoproteins

The major apolipoproteins in the HDLs are apolipoprotein A-I (Apo A-I) and apolipoprotein A-II (Apo A-II). Ninety percent of the protein in the HDL fractions is comprised of these two apoproteins and ninety percent of the plasma Apo A-I and Apo A-II mass is found within HDL (Schaefer et al., 1982; Cheung and Albers, 1979).

Apo A-I is a single chain polypeptide of 245 amino acids (Jackson et al., 1977) and has a molecular weight of approximately 28,000 (Schaefer et al., 1982). Apo A-II is a dimer composed of two identical 77 amino acid polypeptide chains, connected by a single disulfide bond (Jackson et al., 1977). The molecular weight of Apo A-II is approximately 17,000 (Schäfer et al., 1982). The weight ratio of Apo A-I/Apo A-II has been reported to be greater in the HDL-2 subfraction than in HDL-3 (Schaefer et al., 1979; Cheung and Albers, 1979). It has been observed that the apolipoproteins A can exchange between the two HDL subfractions (Schaefer et al., 1979). In comparing the primary sequence of these two apolipoproteins, significant homology (47%) is reported at the carboxyl terminal
regions (Jeng, 1978). The carboxy terminal fragment of Apo A-I (residues 152-245) and residues 47-77 of Apo A-II are the minimum fragments required by each to associate with phospholipid (Pownall et al., 1978).

The formation of lipid-protein interactions of A-I and A-II is being studied to determine how they might affect HDL function. While both apoproteins form protein-phospholipid complexes (Pownall et al., 1978), Apo A-II demonstrates greater propensity for the lipid than does Apo A-I (Assman and Brewer, 1974). A specific functional role for Apo A-II has yet to be documented. Apo A-I has been demonstrated to activate the enzyme LCAT (Fielding and Fielding, 1972). The role that these two proteins play in HDL metabolism and CHD theories will be discussed later.

The remaining apoproteins in HDL make up approximately 8% of HDL protein by weight. These are apoprotein C (Apo C), apoprotein D (Apo D) and apoprotein E (Apo E). Apoproteins D and E are present in trace amounts (Pownall et al., 1978). These apolipoproteins and their relation to the differing lipoprotein fractions are presented in table 1. The apo C proteins are a related group of low molecular weight peptides designated apo C-I, apo C-II and apo C-III. All three are soluble in aqueous solutions and bind phospholipids (Schaefer et al., 1978). Higher levels of C apoproteins have been found in HDL-2 than HDL-3 (Schaefer et al., 1979). Apo C-II has been shown to activate LPL (Havel et al., 1973). The role the C apoproteins play in HDL metabolism is as yet poorly understood.
Apo E, the arginine rich protein, is found in the HDL-2 subfraction (Shaefer et al., 1979). It has been suggested that this apoprotein enhances HDL binding activity to LDL receptors on cell surfaces (Mahley et al., 1978). Apo D, the "thin-line peptide", had been isolated from HDL-3, has a MW of approx. 22,700 and its function may be to activate LCAT but is yet poorly understood (Schaefer et al., 1978).

HDL Metabolism

Circulating HDL appears to arise from three sources: the parenchymal cells of the liver (Jackson et al., 1977), the intestine (Green and Glickman, 1981), and from triglyceride rich lipoproteins during their hydrolysis by LPL (Havel, 1978). Nascent HDL can be isolated from rat liver perfusates (Jackson et al., 1977). These particles are discoidal rather than spherical as is plasma HDL. When the enzyme LCAT, which is also produced in the liver, is inhibited, the particles isolated in the perfusate are discoidal, containing phospholipid, protein and mostly unesterified cholesterol. By contrast, spherical HDL in plasma contains 2/3 of its cholesterol in the esterified form (Gibbons et al., 1982). It has been suggested that the action of LCAT on these particles results in spherical HDL (Havel, 1978). LCAT esterifies most of the surface free cholesterol, forcing it to the hydrophobic core of the particle. The rate of esterification of free cholesterol by LCAT is seven times faster in discoidal than spherical HDL (Havel, 1978). Individuals with LCAT
deficiency have circulating HDL that are disc shaped, similar to the particles seen in the rat liver perfusates (Jackson et al., 1977).

HDL particles isolated from intestinal lymph have been found to contain both spherical and discoidal HDL (Green and Glickman, 1981). When HDL was isolated from lymph in the presence of an LCAT inhibitor, more discoidal particles were produced (Green et al., 1978). These discs are similar to hepatic nascent HDL, but contain more Apo A-I and lower free cholesterol/phospholipid ratio.

Complexes containing phospholipid, Apo A-I, Apo C and free cholesterol from VLDL and chylomicron remnants have been observed to transfer to the HDL fraction of plasma when acted upon by LPL (Havel, 1978). While it is unknown whether these particles are transitory or incorporated into the HDL particle, it is suggested that they contribute to mature HDL formation by acting as substrate for LCAT (Gibbons et al., 1982). Patsch et al. (1978) demonstrated a transfer of apo C, phospholipid and cholesterol from VLDL to HDL-3 when the two lipoproteins were incubated with LPL.

Triglyceride-rich lipoproteins and their relationship to HDL-2 and HDL-3 have been studied. In an in vivo human study (Baggio et al., 1980), a shift of components from TG-rich lipoproteins to HDL-3, then to HDL-2 as lipolysis proceeded was suggested. At 4.5 hours post-prandial, TG-rich lipoproteins, and total plasma TGs were significantly increased, while lipid levels in total HDL did not change. However, the lipids and proteins in HDL-2 were increased and those of HDL-3 were decreased. At 9 hours the HDL-2/HDL-3 ratio was
significantly increased over the 4.5 hour time period, especially in women.

Uptake and catabolism of HDL in vivo is poorly understood. In the rat, the liver is the major site of HDL catabolism (Sigurdsson et al., 1979; Sparks et al., 1981). Sparks et al. (1981) reported no difference in catabolic rates of the individual apoproteins and fractional catabolic rates of abnormal and normal nephrotic HDL in rats, suggesting that HDL is catabolized as a particle. In humans, Blum et al. (1977) studied HDL kinetics using radioisotopes in normal subjects and concluded that the fractional catabolic rates of apolipoprotein A-I and A-II were identical. However, Schaefer et al. (1982) reported that apo A-I is catabolized at a higher fractional rate than apo A-II, in both whole plasma and HDL. It is uncertain whether the liver is the major organ of uptake and catabolism of HDL in man in light of in vitro evidence that it is taken up and degraded by fibroblasts, aortic smooth-muscle cells, and vascular endothelial cells (Gibbons et al., 1982) as well as in hepatocytes (Sherrill et al., 1980).

Theories on the Protective Effect of HDL

As has been previously mentioned, the major route for cholesterol excretion is through the liver (Dietschy and Wilson, 1970c). Therefore, during cholesterol turnover, cholesterol in tissues must return to the liver (Miller and Miller, 1975). It is this process that has been termed reverse cholesterol transport
(RCT). Glomsett (1979) recently proposed a hypothesis of RCT. Removal of free cholesterol from cell surface membranes by the action of LCAT on HDL could result in either a controlled recycling of cholesterol or in a net removal of cholesterol.

As has been mentioned, both free and esterified cholesterol exchange between the lipoprotein fractions. Free cholesterol exchanges freely, while esterified cholesterol transfer requires the presence of an exchange protein (Barter and Lally, 1979; Pattnaik et al., 1978). In an in vitro study, the esterified cholesterol exchange was observed between each of the lipoprotein fractions only in the presence of a protein which is found only in the 1.21g/ml infranatant of the serum (Barter and Lally, 1979). This exchange protein has been described by Pattnaik et al. (1978) to be a glycoprotein with a MW of approximately 80,000. The mechanism through which this exchange protein functions is not yet understood. Pattnaik and Zilversmit (1979) reported that the exchange protein formed a complex with HDL which could be isolated, but not VLDL or LDL. It has been suggested that the exchange protein exists bound to HDL in plasma.

The enzyme LCAT is necessary for net efflux of cholesterol from cells (Fielding and Fielding, 1981) and, as mentioned, is intimately involved in HDL metabolism. A brief discussion of this enzyme follows. LCAT is responsible for nearly all of the esterified cholesterol in human plasma. Individuals with familial LCAT deficiency have nearly no esterified cholesterol in the fasting state
(Glomsett, 1968). The enzyme transfers a fatty acid from lecithin to the 3-B position of free cholesterol forming cholesterol ester and lysolecithin (Pownall et al., 1978).

The preferred substrate for LCAT appears to be HDL; the HDL-3 subfraction is a greater activator of the enzyme than HDL-2 (Fielding et al., 1972). Nascent HDL appears to be preferred over mature HDL (Miller, 1980) and its involvement with LCAT has been mentioned. Apo A-I, Apo D and Apo C-I to a lesser extent stimulate LCAT (Pownall et al., 1978). The rate of the LCAT reaction is apparently dependent upon the molar ratio of lecithin/free cholesterol (Nichols and Gong, 1971). When an artificial bilayer of lecithin/free cholesterol was utilized, the LCAT reaction rate was increased with increasing the lecithin/free cholesterol molar ratio. This ratio in mature HDL is high and even higher in nascent HDL (Gibbons et al., 1982). That is why nascent HDL is an efficient substrate.

Wu and Bailey (1980) demonstrated an in vitro flux of cholesterol from cells to HDL. The authors concluded that HDL-catalyzed efflux is about three fold more rapid than influx, so that normally HDL catalyzes a net efflux of cholesterol. In contrast, LDL promoted a net influx. Cholesterol removal from cells by HDL has been reported by others (Stein et al., 1976 and 1977). Fielding and Fielding (1981) demonstrated the importance of LCAT in net efflux of cholesterol from cells. In an in vitro study, when LCAT activity was inhibited, no net efflux of cholesterol from incubated cells occurred.
Apoprotein transfer between the lipoproteins also occurs. When plasma from LCAT deficient subjects was incubated in the presence of LCAT, Apo C was lost from VLDL and taken up by HDL, while Apo E was transferred from HDL to VLDL (Gibbons et al., 1982). Mahley et al. (1978) has suggested that Apo E enhances HDL binding activity, and it has been demonstrated that a cholesterol induced lipoprotein (HDLc) containing Apo E is taken up with high affinity by the liver (Sherrill et al., 1980) suggesting that Apo E may serve as a signal for hepatic recognition.

In view of the evidence concerning HDL and RCT, it appears that HDL may function indirectly in cholesterol removal by redistributing cholesterol ester and apoproteins to the lipoproteins. Further evidence for HDL involvement in RCT is a strong, negative correlation between HDL-C concentrations and the size of both rapidly and slowly equilibrating tissue cholesterol pools (Miller and Miller, 1975).

Carew et al. (1976) have proposed a different theory concerning HDL and its protective effect on CHD. This theory states that HDL can compete with LDL for tissue receptors. It has been demonstrated that cultured porcine smooth muscle cells increase in cell cholesterol content during uptake and degradation of LDL (Weinstein et al., 1976). Carew et al. (1976) suggested that factors reducing uptake of LDL may reduce the atherogenic process. They demonstrated that labelled LDL incubated with cultured porcine smooth muscle cells in the presence of HDL exhibited decreased binding,
internalization and degradation. The decrease was 30% at equal concentrations of HDL and LDL and 60% when HDL concentrations were five times that of LDL. In other studies utilizing cultured rat aortic smooth muscle cells, both human LDL and HDL were able to be bound and internalized by the cultured cells, but there was a greater degradation of LDL per unit time (Stein and Stein, 1975a and 1975b)

Diet and Other Factors Effecting HDL

Several factors are known to affect HDL levels and composition. These include exercise, obesity, weight loss, smoking, alcohol consumption, drugs and diet composition. These factors and their combinations must be considered in studying HDL, as they may influence results and thereby affect interpretation.

The effect of cardiorespiratory fitness and HDL-C was investigated in 152 men and women (Schwane and Cundiff, 1979). In both sexes, cardiorespiratory fitness was positively correlated with HDL-C. In a related investigation, men undergoing regular physical training had HDL-C and Apo A-I levels of 1.77±0.39 mmole/l and 2.16±0.29g/l respectively. This is significantly greater than controls whose HDL-C level was 1.42±0.31mmole/l and Apo A-I concentration of 1.65±0.28g/l. Apo A-II levels were similar in both groups, resulting in a greater Apo A-I/Apo A-II ratio in the athletes (Lehtonen et al., 1979). An inverse relationship between obesity and HDL-C is noted in both sex groups between the ages 16 and 49 years (Garrison et al., 1980) while weight loss shows a different effect on
HDL-C between the sexes. Men have an increase in HDL-C during weight loss, while women show a decreased level (Brownell and Stunkard, 1981).

Alcohol consumption increases HDL-C levels (Willett et al., 1980) while cigarette smoking is associated with lower HDL-C levels (Berg et al., 1979). Smokers have also been noted to have Apo A-I and Apo A-II levels that are significantly less than non-smokers (Berg et al., 1979).

Estrogens are known to increase HDL levels (Krauss et al., 1979) and premenopausal women have higher HDL levels than men (Gibbons et al., 1982). To assess the effect of estrogens on the HDL subspecies, 11 menopausal estrogen users were compared to 16 non-users. Estrogen users had 20% greater HDL levels than the non-users and the major difference was an increased HDL-2 level (Krauss et al., 1979). Progestins have an opposite effect. HDL levels, particularly HDL-2 levels, are lower in progestin users than in non-users (Krauss et al., 1979).

Nicotinic acid therapy of 3g/day in 5 healthy young adults increased HDL-C levels by 23% and the HDL-2/HDL-3 ratio by 345% over periods of non-therapy (Shepard et al., 1979). This increase in the HDL-2/HDL-3 ratio was the result of a substantial increase in HDL-2 of 646% and a mild drop in HDL-3 of 47%. Nicotinic acid therapy also influenced the metabolism of apoproteins A. Apo A-I levels were increased by 7% due to a decrease in fractional catabolic rate, and Apo A-II levels dropped. While the fractional catabolic rate of Apo
A-II was slightly decreased (8%), its synthetic rate was decreased by 22%. Circulating levels of A-I transferred from the HDL-3 to the HDL-2 subfraction as a result of the nicotinic acid therapy. The authors suggest changes in apoprotein metabolism may regulate distribution of HDL subfractions.

The effect of diet composition on HDL offers conflicting results. In a study of the effect of dietary cholesterol on serum lipids (Mistry et al., 1977), 18 normal subjects were fed 750 or 1500 mg cholesterol/day in the form of egg yolk. Both intakes increased cholesterol in all of the lipoprotein fractions. HDL-C was increased by 19%. While it was not the largest increase in the lipoprotein fractions, HDL-C remained elevated four weeks after a return to a normal diet. The other lipoprotein cholesterol levels returned to baseline only 10 days after cessation of extra cholesterol consumption. In a similar study of cholesterol feeding (Mistry et al., 1981), subjects again consumed 750 or 1500 mg cholesterol/day from egg yolk. In subjects consuming 1500 mg/day, HDL was subfractionated into its two major subclasses HDL-2 and HDL-3. While HDL-C levels rose 20% with the rise due to increases in HDL-2. HDL-3 cholesterol was unchanged. As with the previous study, HDL-C levels fell more slowly than other lipoprotein classes. The authors suggest this slow return of HDL-C to baseline may be due to "centripetal" transport of cholesterol from the tissue pools. Subjects consuming 750 mg/day also had significant increases in HDL-C, but the HDL subclasses were not measured. In a study of an even larger amount of
cholesterol feeding (Applebaum-Bowden et al., 1979), intakes of 5000mg cholesterol/day did not significantly increase HDL-C levels. However Apo A-I was raised slightly (p<0.07).

While cholesterol feeding does not always change plasma cholesterol levels, an effect may still be noted on HDL. In a study by Mahley et al. (1978) six healthy men and women were fed a high cholesterol diet in the form of 4 to 6 eggs/day for four weeks. HDL was isolated by ultracentrifugation in the density range of 1.093-1.210 g/ml. Total plasma cholesterol remained at prediet levels for three subjects and was increased by 20-25% in the others. While the HDL composition did not change as a result of diet, the reactivity of HDL with high affinity cell receptors was significantly affected in all subjects. Prior to diet, HDL displaced 7.5±1.2% of labelled LDL bound and internalized by human fibroblasts. After the cholesterol feeding, HDL displaced 20.2±7.4% of labelled LDL. When 5 other subjects were fed a gradual increase in eggs to 3 daily, similar results were reported. During this 18 week study, plasma cholesterol increased 0-6%. Competitive binding and degradation of LDL by HDL was increased in all subjects. An HDL subfraction was isolated from the 1.093-1.21 density range. This minor subfraction contained the Apo E for the entire density range and accounted for the receptor binding. It is not yet understood what these changes in cell receptor binding mean in terms of HDL-C and CHD.

The type of fat, saturated or unsaturated, also affects HDL composition. Jackson et al. (1980) have investigated the effect of
diets with a P/S ratio of 4.0 or 2.0 followed by a P/S ratio of 0.4 in three normal healthy men for two weeks per diet. The caloric sources of the diets from CHO, pro, and fat were 40%, 20% and 40% respectively. Cholesterol intake was constant at 400 mg/day. Plasma cholesterol and triglycerides decreased in going from a low to high P/S ratio and HDL-C was increased by 6% and 7% in two of the subjects. Fatty acid composition was altered in HDL. The high P/S ratio diet resulted in an increase in percent linoleate and a decrease in oleate when compared to the saturated fat diet. The subfractions of HDL showed no significant changes in lipid or protein composition as a result of the two diets. When the whole HDL fraction was incubated with ascites cells to determine the effects of the two diets on cholesterol removal, both types of diets resulted in the same amounts of cholesterol removal. The two HDL subfractions removed similar amounts of cholesterol from the cells, and the type of diet was reported to have little effect on cholesterol removal. Apparently, the changes in HDL composition induced by high P/S was not great enough to cause changes in the HDL subfractions or cholesterol removal from cells.

In a related study (Tan et al., 1980) of six healthy subjects, two isocaloric diets were designed for each: 1) low cholesterol-high P/S ratio, and 2) high cholesterol-low P/S ratio diet. During the low cholesterol-high P/S diet, HDL-C did not change significantly, even though total serum cholesterol decreased. The high cholesterol-low P/S ratio diet resulted in a 30% increase in
HDL-C after two weeks on the diet. Apo A-I was significantly increased after 3 weeks on the high cholesterol-low P/S ratio diet.

In a study designed to separate the effects of dietary fat and cholesterol on the lipoproteins, 16 Rhesus monkeys were assigned to one of four diets (Erschow et al., 1981). The monkeys were fed a semi-purified diet containing corn oil or coconut oil with or without cholesterol from birth to three years of age. The cholesterol content of the diet was 300 mg/1000 kcal. The monkeys fed coconut oil and coconut oil plus cholesterol had significantly higher levels of HDL protein, triglycerides, phospholipids and total lipids than monkeys fed the two corn oil diets. The effect of cholesterol in the diet was apparent in monkeys fed coconut oil and cholesterol because of their consistently higher levels of all HDL lipids and protein than monkeys fed coconut oil alone. Type of fat or level of cholesterol did not affect the composition of HDL in any of the groups. However, the coconut oil diets increased the circulating mass of both HDL lipid and protein. This suggests that the increased HDL mass was due to an increased number of circulating HDL molecules. The HDL fraction was not divided into subclasses.
MATERIALS AND METHODS

Subjects

Twenty male subjects between the ages of 30 and 55 were recruited by poster from the Corvallis area. The subjects had normal plasma triglyceride and cholesterol levels and were free from known metabolic disease. A description of subjects by age and baseline cholesterol levels is presented in table 4. Each man freely gave his consent to participate in the study and signed a consent form approved by the Human Subjects Committee of Oregon State University.

Periods of study

The entire investigation period was 77 days, which was divided into three periods: Baseline (B), Classification (C), and Experimental (E) (Figure 1).

I. Baseline

The baseline period consisted of 7 days. Subjects took fecal marker (200mg FD&C blue) with breakfast on days B-1 and B-7. Subjects were instructed on procedures for recording diets and recorded food intakes and collected fecal samples between the markers. Fasting blood was drawn on day B-7 (Fig. 1).

II. Classification

The classification period consisted of 30 days. During this period, subjects were required to eat a luncheon provided in the metabolic kitchen in Milam Hall on the Oregon State University campus. The luncheons were served buffet style and had several daily
Table 4. Description of Subjects

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Age (years)</th>
<th>Body Weight (kg)</th>
<th>Height (cm)</th>
<th>Baseline Plasma Cholesterol (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>64</td>
<td>173</td>
<td>202</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
<td>87</td>
<td>193</td>
<td>189</td>
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<td>3</td>
<td>31</td>
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<td>4</td>
<td>34</td>
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<td>21</td>
<td>55</td>
<td>74</td>
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<td>22</td>
<td>30</td>
<td>73</td>
<td>183</td>
<td>137</td>
</tr>
<tr>
<td>23</td>
<td>33</td>
<td>89</td>
<td>185</td>
<td>191</td>
</tr>
<tr>
<td>mean±SD</td>
<td>38±8</td>
<td>82±15</td>
<td>181±9</td>
<td>185±34</td>
</tr>
</tbody>
</table>
choices of food items. There was a requirement that subjects consume three whole eggs per day. Subjects were required to eat luncheon on the campus site Monday through Friday. On week-ends subjects were given 6 eggs, to consume 3 each day. Subjects were free-living and therefore allowed to eat their regular diet. Fecal markers were taken on classification days C-23 and C-30 (refer to fig. 1) and complete stool samples collected between markers. Diets were recorded between classification days C-23 and C-30. Fasting blood was drawn on day C-30.

III. Experimental Period

The experimental period lasted 40 days. Subjects were classified as hyper- or hypocholesterol responders as a result of cholesterol levels determined from fasting plasma drawn on C-30. Subjects with an 8% or greater plasma cholesterol increase over the baseline values were classified as hyperresponders and continued to receive 3 eggs/day during the entire study period. Subjects whose plasma cholesterol did not rise or rose less than 8% over baseline were classified as hyporesponders and received 6 eggs/day in place of the previous 3 eggs/day. Subjects were instructed to continue normal eating patterns and received the appropriate number of eggs to consume on week-ends. Fecal markers were taken on experimental days E-33 and E-40. Subjects recorded daily dietary intake from experimental period days E-33 through E-40. Fasting blood was drawn on experimental days E-26 and E-40.

Procedure for Blood Drawing
Blood was drawn between 7:00 and 9:00 a.m. from fasting subjects. Subjects were instructed not to eat or drink any fluids except water, starting from 8:00 p.m. the night previous to a blood draw. Breakfast, not lunch, was provided on the days that blood was drawn and the appropriate number of eggs were consumed. 35 ml blood was collected by venipuncture using sodium EDTA (1mg/ml blood) as anti-coagulant. Plasma was separated by centrifuging at 4°C for 30 minutes at 2500 rpm.

Methods of Analysis

Lipoprotein Isolation

Immediately after the separation of plasma, lipoproteins were isolated by sequential density ultracentrifugation (Havel et al., 1955). VLDL was isolated at plasma density (d<1.006g/ml) by centrifugation in a fixed angle rotor (Ti-50) at 50,000 rpm for 12 hours at 5°C using a Beckman Model L5-75 ultracentrifuge. Isolated VLDL was washed in saline with a salt density of 1.006g/ml to remove albumin. LDL, HDL-2 and HDL-3 lipoprotein fractions were isolated sequentially raising the plasma density with KBr granules to 1.063 g/ml, 1.125 g/ml and 1.210 g/ml respectively. All fractions were spun at 50K rpm for times of: LDL, 18 hours; HDL-2, 24 hours; HDL-3, 36 hours. HDL-3 was also washed in saline of the same density solution (d=1.21) for 24 hours to remove traces of albumin. All fractions were removed by the tube-slicing technique.

Cholesterol Determination

Total and free cholesterol levels were determined on whole
plasma as well as all lipoprotein fractions. Cholesterol was assayed colorimetrically using an enzymatic method as described by Allain et al. (1974). For total cholesterol determination, cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (Miles Laboratories, Elkhart, Indiana). The free cholesterol produced is oxidized by cholesterol oxidase (Miles Laboratories, Elkhart, Indiana) to cholest-4-en-3-one with the concurrent production of hydrogen peroxide. The peroxide in the presence of peroxidase (Miles Laboratories, Elkhart, Indiana), couples with 4-amino-antipyrine (Sigma Chemical Co., St. Louis, Missouri) and phenol to produce a chromagen with maximum absorption at 500 nm. Free cholesterol is measured by omitting the cholesterol ester hydrolase treatment.

Apolipoprotein Ratio Measurements

The apoprotein A-I:A-II ratios were determined in 6 subjects by gel scanning of apoprotein bands. The protein bands were resolved by SDS-polyacrylamide gel electrophoresis as described by Kane et al. (1975). HDL fractions were dialyzed against 0.15M NaCl solution containing 0.01% Na-EDTA at pH 7.0. Total protein concentrations were determined by the Lowry procedure (Lowry et al., 1951). Based on Lowry protein values, sample volumes containing 16ug of protein were lyophilized. They were then delipidated by the addition of 2.0 ml of chloroform:methanol, 2:1(v/v). The samples were allowed to stand for 60 minutes at 4°C before 4 ml. of anhydrous ether were added. Samples were mixed and then centrifuged at 2500 rpm for 15
minutes. Most of the solvent was removed by suction and samples were dried under nitrogen.

To each sample, 100ul of incubation solution containing 2.5mM Tris, 19.2 mM glycine, and 0.3% sodium dodecylsulfate (SDS) and bromophenol blue were added and the mixture incubated at 37 °C for 90 minutes. The 11% polyacrylamide gels were prepared by using 0.1% SDS (w/v), 0.19% N,N,N',N'-tetramethylethylenediamine (v/v) and 0.5% ammonium persulfate (w/v) in a buffer containing 24mM Tris in 19.2mM glycine (pH 8.3). Electrophoresis was carried out at 150 volts until half the bromophenol blue marker had eluted. Gels were stained by immersion for 1.5 hours in a Coomassie brilliant blue solution of 20 parts 50% aqueous methanol, 1 part glacial acetic acid and 0.1% (w/v) Coomassie blue.

Gel scanning was using a Beckman model DU spectrophotometer equipped with a Gilford gel scanner. Peak area values were determined using a polar planimeter (Keuffel and Esser, No. 4236).

Statistical Analysis

Paired and student t-tests were performed on mean differences for cholesterol content of whole plasma, lipoprotein fractions and ratios of Apo A-I/Apo A-II, between sampling periods (pre and post treatment), to compare hyper and hyporesponders, and to compare the effect of the egg feeding diet throughout the investigation (over time). The accepted level of significance was p<0.05.
RESULTS

Reported Dietary Intake

Average daily cholesterol intake of all subjects was computed from dietary records and is presented in table 5. Average daily cholesterol consumption was 180±67 mg/1000kcal while on baseline ad libitum diets. This intake was increased significantly (p<0.001) when 3 eggs per day were added to the diet. Hyporesponders consumed an average of 202±69 mg cholesterol/1000kcal per day, whereas hyperresponders consumed 149±57; a difference not statistically significant. When 3 eggs were added to the diet, daily cholesterol intake of hyperresponders increased significantly (p<0.001) to a mean of 470±160 mg/1000kcal. The 3 egg addition resulted in a significant (p<0.001) increase in intake for hyporesponders as well. During the experimental period, cholesterol intake for hyperresponders remained unchanged. Hyporesponders consumed 6 eggs/day with their regular diet resulting in a mean cholesterol intake of 761±172 mg/1000kcal. This increase was significantly (p<.001) greater than cholesterol intake during the classification period.

Plasma Cholesterol

Subject classification

During the four week classification period on 3 eggs/day with ad libitum diet, the mean plasma cholesterol levels of all subjects increased from 185±34 mg/100ml to 195±34 mg/100ml. This increase was statistically significant at p<0.05 by paired t-test. Consumption of
Table 5. Average Daily Cholesterol Intake of Habitual (Baseline) and Experimental Diets (mean±SD)

<table>
<thead>
<tr>
<th>Dietary Period</th>
<th>Cholesterol (mg/1000kcal)</th>
<th>Energy (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all subjects</td>
<td>180±67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2644±667</td>
</tr>
<tr>
<td>hyperresponders</td>
<td>149±51&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2717±983</td>
</tr>
<tr>
<td>hyporesponders</td>
<td>202±69&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2596±384</td>
</tr>
<tr>
<td>Classification:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all subjects</td>
<td>433±118&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2767±692</td>
</tr>
<tr>
<td>hyperresponders</td>
<td>470±160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2683±920</td>
</tr>
<tr>
<td>hyporesponders</td>
<td>409±77&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2824±530</td>
</tr>
<tr>
<td>Experimental:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hyperresponders</td>
<td>450±135&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2840±933</td>
</tr>
<tr>
<td>hyporesponders</td>
<td>761±172&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>2617±472</td>
</tr>
</tbody>
</table>

Levels of significant differences between data with same letter by paired t-test

<sup>a</sup> p 0.001  <sup>b</sup> p 0.001  <sup>c</sup> p 0.001  
<sup>d</sup> p 0.001  <sup>e</sup> p 0.001  <sup>f</sup> p 0.001

<sup>1</sup>Baseline indicates habitual diet; classification indicates 3 eggs/day plus habitual diet; experimental indicates habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo).
3 eggs/day with ad libitum diets resulted in a plasma cholesterol increase of 8% or greater over baseline values in 8 of the 20 subjects. These 8 were classified as hyperresponders and continued on 3 eggs/day during the experimental period. The other 12 subjects whose plasma cholesterol decreased or was increased less than 8% over their baseline value were classified as hyporesponders and consumed 6 eggs/day with their ad libitum diet during the following 6 week experimental period.

Whole Plasma Cholesterol

Hyperresponders: Whole plasma total cholesterol values of hyperresponders are presented in table 6 and figure 2. The additional 3 eggs per day resulted in a significant (p<0.025) increase in total plasma cholesterol from 170.4±41.7 mg/100ml at baseline to 198.7±40.3 mg/100ml at classification. This level remained relatively unchanged, falling slightly during the experimental period. Free cholesterol values for hyperresponders' whole plasma are presented in table 6 and figure 2. While total cholesterol increased from baseline to classification, free cholesterol remained unchanged, indicating the rise in total plasma cholesterol was due to an increase in the amount of esterified cholesterol in the plasma.

Hyporesponders: Whole plasma total cholesterol values for hyporesponders are presented in table 7 and figure 2. The additional 3 eggs per day resulted in a small, non-significant decrease in average total plasma cholesterol. Consumption of 6 eggs/day during
Figure 2. Mean whole plasma cholesterol concentration (mg/100ml) by week for hyper (solid line) and hyporesponders (dotted line). Open circles represent free cholesterol means, closed circles represent total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
Table 6. Hyperresponders Mean Plasma Cholesterol Concentrations in mg/100ml in Three Periods

<table>
<thead>
<tr>
<th></th>
<th>mean±SD</th>
<th>week 1</th>
<th>week 5</th>
<th>week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>170.4±41.7 a</td>
<td>198.7±40.3 a</td>
<td>191.4±44.7</td>
<td></td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>51.2±12.3</td>
<td>53.9±10.7</td>
<td>51.8±13.1</td>
<td></td>
</tr>
<tr>
<td><strong>VLDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>3.1±1.6</td>
<td>10.7±9.8</td>
<td>7.4±7.2</td>
<td></td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>2.2±1.4</td>
<td>4.7±3.9</td>
<td>3.4±3.5</td>
<td></td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>109.3±36.4 b</td>
<td>113.8±40.1</td>
<td>117.1±35.1 b</td>
<td></td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>32.6±13.0</td>
<td>33.1±9.9</td>
<td>21.2±11.1</td>
<td></td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>48.6±9.8 c</td>
<td>53.8±12.4 c</td>
<td>50.0±11.7</td>
<td></td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>11.2±3.4</td>
<td>10.3±3.2</td>
<td>10.4±2.8</td>
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<tr>
<td><strong>HDL-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>21.6±7.5</td>
<td>24.0±10.5</td>
<td>21.0±9.3</td>
<td></td>
</tr>
<tr>
<td><strong>FC</strong></td>
<td>5.7±2.1</td>
<td>6.0±2.8</td>
<td>5.4±2.3</td>
<td></td>
</tr>
<tr>
<td><strong>HDL-3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TC</strong></td>
<td>27.0±4.5</td>
<td>29.8±3.9</td>
<td>29.0±4.5</td>
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</tr>
<tr>
<td><strong>FC</strong></td>
<td>5.5±2.8</td>
<td>4.4±0.7</td>
<td>5.0±1.0</td>
<td></td>
</tr>
</tbody>
</table>

Key to footnotes:

1 n=8
2 week 1 indicates baseline values; week 5, 4 weeks on habitual diet plus 3 eggs/day; week 11, 6 weeks on habitual diet plus 3 eggs/day.
3 TC (Total cholesterol); FC (Free cholesterol)
4 levels of significant differences between data with same letter by paired t-test.
   a p 0.025  b p 0.050  c p 0.025
5 Very low density lipoprotein
6 Low density lipoprotein
7 High density lipoprotein
8 High density lipoprotein-2
9 High density lipoprotein-3
<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 5</th>
<th>Week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Whole Plasma</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TC</td>
<td>194.6±25.8</td>
<td>192.5±30.7</td>
<td>205.0±27.3</td>
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<tr>
<td>FC</td>
<td>56.7±7.5</td>
<td>52.7±9.1</td>
<td>53.5±5.5</td>
</tr>
<tr>
<td><strong>VLDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>8.0±7.8</td>
<td>9.6±12.6</td>
<td>8.2±7.8</td>
</tr>
<tr>
<td>FC</td>
<td>5.8±7.2</td>
<td>4.3±5.4</td>
<td>4.1±3.8</td>
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<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>127.6±15.9</td>
<td>132.6±21.2</td>
<td>132.2±17.4</td>
</tr>
<tr>
<td>FC</td>
<td>36.8±7.1</td>
<td>32.4±4.4</td>
<td>35.4±4.0</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>41.2±12.2</td>
<td>47.3±11.7</td>
<td>46.5±11.3</td>
</tr>
<tr>
<td>FC</td>
<td>8.7±2.5</td>
<td>8.8±2.3</td>
<td>9.3±3.1</td>
</tr>
<tr>
<td><strong>HDL-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>16.1±6.6</td>
<td>21.2±8.3</td>
<td>19.9±8.6</td>
</tr>
<tr>
<td>FC</td>
<td>4.3±1.8</td>
<td>4.9±1.6</td>
<td>5.0±2.4</td>
</tr>
<tr>
<td><strong>HDL-3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>25.2±6.3</td>
<td>26.2±4.8</td>
<td>26.5±4.4</td>
</tr>
<tr>
<td>FC</td>
<td>4.4±0.9</td>
<td>3.9±1.0</td>
<td>4.3±0.9</td>
</tr>
</tbody>
</table>

Key to footnotes:

1n=12

2Week 1 indicates baseline values; week 5, 4 weeks on 3 eggs/day plus habitual diet; week 11, 6 weeks on 6 eggs/day plus habitual diet

3TC (Total cholesterol); FC (Free cholesterol)

4Levels of significant differences between data with same letter by paired t-test

a p 0.01 b p 0.05

5Very low density lipoprotein

6Low density lipoprotein

7High density lipoprotein

8High density lipoprotein-2

9High density lipoprotein-3
the 6 week experimental period resulted in an average increase of 12mg/100ml in total plasma cholesterol, which is not statistically significant. Free cholesterol had little change throughout the investigation.

Plasma cholesterol concentration of hyporesponders was greater than that of hyperresponders initially by approximately 25mg/100ml. However this difference was not significant and hyper and hyporesponders did not differ significantly in free or total whole plasma cholesterol throughout the investigation period.

VLDL Cholesterol

Hyperresponders: VLDL cholesterol values for hyperresponders are presented in tables 6 and 8, and in figure 3. When 3 eggs/day were added to the ad lib. diet, the average VLDL cholesterol value increased but not significantly in the hyperresponders. Continuing this feeding pattern for six weeks resulted in a small, non-significant decrease in plasma total cholesterol in this fraction (fig. 3a). The average values for free cholesterol in this group followed a pattern similar to that seen in total cholesterol. When the mean values for the levels of cholesterol in the lipoprotein fractions are expressed as a percent of whole plasma cholesterol, hyperresponders initially carried an average of 2.0±1.2% (fig. 3b, table 8) of their total cholesterol in the VLDL fraction. This percentage increased slightly at week 5 when subjects had consumed 3 eggs/day with their diet for 4 weeks. At the end of the
Figure 3. (a) mean plasma cholesterol concentration (mg/100ml) in the VLDL fraction by week. (b) mean values for VLDL cholesterol expressed as percentage of whole plasma cholesterol concentrations. Solid lines represent hyperresponders, dotted lines hyporesponders. Open circles indicate free cholesterol, closed circles indicate total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
Table 8. Hyperresponders\(^1\) Mean Values for Levels of Lipoprotein Cholesterol Expressed as Percent (%) of Whole Plasma Cholesterol in Three Periods\(^2\)

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Week 1 Mean±SD</th>
<th>Week 5 Mean±SD</th>
<th>Week 11 Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL (^5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (^3)</td>
<td>2.0±1.2</td>
<td>5.8±6.1</td>
<td>4.0±4.0</td>
</tr>
<tr>
<td>FC</td>
<td>4.6±3.1</td>
<td>8.9±7.4</td>
<td>6.8±7.0</td>
</tr>
<tr>
<td>LDL (^6)</td>
<td></td>
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</tr>
<tr>
<td>TC</td>
<td>63.3±8.3</td>
<td>56.5±13.6</td>
<td>60.8±7.9</td>
</tr>
<tr>
<td>FC</td>
<td>62.2±12.1</td>
<td>61.8±13.8</td>
<td>61.1±8.0</td>
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<tr>
<td>HDL (^7)</td>
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<tr>
<td>TC</td>
<td>29.1±5.2 (^a)</td>
<td>27.6±6.4</td>
<td>26.5±5.5 (^a)</td>
</tr>
<tr>
<td>FC</td>
<td>22.8±7.8</td>
<td>19.6±5.8</td>
<td>20.4±4.6</td>
</tr>
<tr>
<td>HDL-2 (^8)</td>
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<td></td>
</tr>
<tr>
<td>TC</td>
<td>12.8±3.7 (^b)</td>
<td>12.1±4.1</td>
<td>10.9±3.3 (^b)</td>
</tr>
<tr>
<td>FC</td>
<td>11.2±3.3</td>
<td>11.2±4.5</td>
<td>10.5±3.6</td>
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<tr>
<td>HDL-3 (^9)</td>
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<tr>
<td>TC</td>
<td>16.2±3.2</td>
<td>15.5±3.6</td>
<td>15.8±3.7</td>
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<tr>
<td>FC</td>
<td>11.5±7.0</td>
<td>8.5±2.3</td>
<td>9.9±2.5</td>
</tr>
</tbody>
</table>

Key to footnotes:
\(^1\) n=8
\(^2\) Week 1 indicates laseline values; week 5, 4 weeks on 3 eggs/day plus habitual diet; week 11, 6 weeks on habitual diet plus 3 eggs/day.
\(^3\) TC (Total cholesterol); FC (Free cholesterol)
\(^4\) Levels of significant differences between data with same letter by paired t-test.
\(^a\) p 0.025 \(^b\) p 0.025
\(^5\) Very low density lipoprotein
\(^6\) Low density lipoprotein
\(^7\) High density lipoprotein
\(^8\) High density lipoprotein-2
\(^9\) High density lipoprotein-3
investigation hyperresponders carried an average of 4% of their total cholesterol in the VLDL fraction. Hyperresponders carried a greater percentage of free cholesterol (Table 8) in the VLDL fraction than that seen in total cholesterol. The percentage of free cholesterol found in this lipoprotein followed the trend noted in total cholesterol.

Hyporesponders: VLDL cholesterol values for hyporesponders is presented in tables 7 and 9, and in figure 3. Plasma cholesterol in the VLDL fraction of hyporesponders did not change significantly throughout the investigation. The percentage of cholesterol carried by the VLDL fraction in hyporesponders also did not change significantly throughout this investigation.

Although the hyporesponders initially carried more cholesterol in the VLDL fraction, this difference was not statistically significant (p<0.10). There were no significant differences in VLDL cholesterol between the response groups.

LDL Cholesterol

Hyperresponders: Values for LDL cholesterol in hyperresponders are presented in tables 6 and 8, and in figure 4. The LDL cholesterol levels of hyperresponders rose gradually throughout the investigation from week 1, the baseline value (109.3±36.4mg/100ml) to week 11, the experimental value (117.1±35.1mg/100ml). This increase in LDL cholesterol was significant (p<0.05). Free cholesterol in the LDL fraction did not
<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Week 1</th>
<th>Week 5</th>
<th>Week 11</th>
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</thead>
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<tr>
<td>VLDL</td>
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<td>TC</td>
<td>4.0±3.5</td>
<td>4.5±5.1</td>
<td>3.5±3.2</td>
</tr>
<tr>
<td>FC</td>
<td>9.3±9.9</td>
<td>7.2±7.4</td>
<td>7.2±6.0</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TC</td>
<td>65.8±5.6</td>
<td>69.1±6.2</td>
<td>64.7±5.6</td>
</tr>
<tr>
<td>FC</td>
<td>64.8±10.0</td>
<td>62.4±8.5</td>
<td>66.3±5.5</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>21.2±5.8</td>
<td>24.8±6.0</td>
<td>22.9±5.8</td>
</tr>
<tr>
<td>FC</td>
<td>15.5±4.7</td>
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</tr>
<tr>
<td>HDL-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>8.2±3.1</td>
<td>10.9±3.9</td>
<td>9.8±4.4</td>
</tr>
<tr>
<td>FC</td>
<td>7.7±3.0</td>
<td>9.4±3.0</td>
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<tr>
<td>HDL-3</td>
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<tr>
<td>TC</td>
<td>13.0±3.1</td>
<td>13.9±2.9</td>
<td>13.1±2.2</td>
</tr>
<tr>
<td>FC</td>
<td>7.8±2.1</td>
<td>7.6±2.4</td>
<td>8.2±2.0</td>
</tr>
</tbody>
</table>

Key to footnotes:

1 n=12
2 Week 1 indicates baseline values; week 5, 4 weeks on 3 eggs/day plus habitual diet; week 11, 6 weeks on 6 eggs/day plus habitual diet
3 TC (Total cholesterol); FC (Free cholesterol)
4 Levels of significant differences between data with same letter by paired t-test.

\[ \begin{align*}
& a_p 0.05 \\
& b_p 0.025 \\
& c_p 0.025 \\
& d_p 0.01 \\
& e_p 0.05
\end{align*} \]

5 Very low density lipoprotein
6 Low density lipoprotein
7 High density lipoprotein
8 High density lipoprotein-2
9 High density lipoprotein-3
Figure 4. (a) mean plasma cholesterol concentration (mg/100ml) in the LDL fraction by week. (b) mean values for LDL cholesterol expressed as percentage of whole plasma cholesterol concentrations. Solid lines represent hyperresponders, dotted lines hyporesponders. Open circles indicate free cholesterol, closed circles indicate total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet, plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
change significantly in the investigation. The percentage of total cholesterol carried in the LDL fraction fell slightly from week 1 (baseline) to week 5 (classification) but rose to baseline values by the end of the investigation. None of these changes were significant. The percentage of free cholesterol carried in this lipoprotein fraction remained unchanged throughout the investigation.

Hyporesponders: Values for LDL cholesterol in hyporesponders are presented in tables 7 and 9, and in figure 4. Total cholesterol in the LDL fraction rose slightly but not significantly over the investigation. However the amount of free cholesterol in the lipoprotein fell significantly (p<0.01) when 3 eggs were added to the diet. As 6 eggs were consumed per day by the hyporesponders, LDL free cholesterol again rose and returned approximately to the baseline value. The percentage of total cholesterol carried in the LDL fraction rose slightly (N.S.) when 3 eggs/day were added to the diet, then fell to below the baseline value at the end of the experimental period. The percentage of free cholesterol that was carried in the LDL fraction followed a reverse trend of that seen in total cholesterol carried in this fraction; percentage free cholesterol initially fell, then rose as 6 eggs were added to the diet (table 9, figure 4b).

While hyporesponders had consistently greater amounts of total cholesterol in the LDL fraction, these differences were non-significant. However free cholesterol values carried by both groups were nearly identical (fig. 4b). The percentage of
cholesterol carried in the LDL fraction was initially similar in hypo and hyperresponders. When three eggs were added to the daily diet, hyporesponders increased the percentage of total cholesterol carried in the LDL fraction whereas the percentage was decreased in hyperresponders resulting in a significant difference ($p<0.025$) between groups (fig 4b). While the percentage of free cholesterol carried in LDL followed different patterns between groups (fig. 4b), amounts of free cholesterol carried in the LDL did not differ significantly.

HDL cholesterol

Hyperresponders: HDL cholesterol values were obtained by adding the two HDL subfractions: HDL-2 and HDL-3. HDL cholesterol values are presented in tables 6 and 8, and in figure 5. Total HDL cholesterol rose significantly ($p<0.025$) from week 1 (baseline) to week 5 (classification). Continuing on 3 eggs/day to the end of the experimental period resulted in a small decrease in HDL total cholesterol. Free cholesterol in the HDL fraction remained relatively constant throughout the investigation. The percentage of total cholesterol carried in this lipoprotein fraction fell slightly by the end of the classification period (week 5) and continued to fall to the end of the investigation resulting in a significant decrease ($p<0.025$) from week 1 to week 11. The percentage of free cholesterol in this fraction did not change significantly throughout the investigation.
Figure 5. (a) mean plasma cholesterol concentration (mg/100ml) in the HDL fraction by week. (b) mean values for HDL cholesterol expressed as percentage of whole plasma cholesterol concentrations. Solid lines represent hyperresponders, dotted lines hyporesponders. Open circles indicate free cholesterol, closed circles indicate total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
Hyporesponders: HDL cholesterol values are presented in tables 7 and 9, and figure 5. When three eggs/day were added to the diet, the hyporesponders HDL cholesterol rose slightly ($p<0.10$). At the end of the experimental period of 6 eggs/day, the HDL total cholesterol remained at approximately the classification level. The amount of cholesterol in the HDL fraction at the beginning and end of the investigation (weeks 1 and 11) differed significantly ($p<0.05$). The amount of free cholesterol in the HDL fraction remained unchanged throughout the investigation. The percentage of total and free cholesterol carried in this lipoprotein fraction rose significantly ($p<0.05$ and $p<0.025$ respectively) when hyporesponders went from an ad libitum diet to one that included 3 eggs/day. The initial rise was not continued when 6 eggs were added to the daily diet, but remained at the enhanced level of the classification period (week 5) when the investigation ended.

The percentage of cholesterol carried initially in the HDL fraction by hyper and hyporesponders was significantly different in both free ($p<0.025$) and total ($p<0.01$) cholesterol. Hyperresponders carried $29.1\pm5.2\%$ of their total cholesterol in the HDL fraction, whereas hyporesponders carried $21.2\pm5.8\%$. The amount of free cholesterol carried in the HDL fraction was $22.8\pm7.8\%$ in hyperresponders and only $15.5\pm4.7\%$ in hyporesponders. During the classification and experimental periods, hyperresponders maintained greater percentages of cholesterol in this lipoprotein fraction and greater levels of mean total and free plasma cholesterol, but these
differences were not significant (figure 5).

HDL-2 Cholesterol

Hyperresponders: The cholesterol values for the HDL-2 subfraction are presented in tables 6 and 8, and in figure 6. The amount of total cholesterol in the HDL-2 fraction increased slightly when 3 eggs were added to the diet. This level fell below (N.S.) baseline averages at the end of the feeding period. Free cholesterol did not change significantly throughout the investigation. The percentage of total cholesterol carried in the HDL-2 subfraction decreased to a small but significant degree (p<0.025) from average baseline values to the end of the investigation. The percentage of free cholesterol carried in this subfraction did not change throughout the investigation.

Hyporesponders: The cholesterol values for this subfraction are presented in tables 7 and 9 and in figure 6. The amount of total cholesterol in the HDL-2 subfraction increased from week 1 to week 5, followed by a slight drop at week 11. None of these changes were significant. Free cholesterol in this lipoprotein fraction did not change throughout the investigation. The percentage of total cholesterol carried in this density fraction increased significantly (p<0.01) when 3 eggs were added to the daily diet. When 6 eggs/day were fed, the level did not change but remained at approximately the classification (week 5) levels. This end value was significantly (p<0.05) greater than the baseline (week 1) average. The percentage
Figure 6. (a) mean plasma cholesterol concentration (mg/100ml) in the HDL-2 fraction by week. (b) mean values for HDL-2 cholesterol expressed as percentage of whole plasma cholesterol concentrations. Solid lines represent hyperresponders, dotted lines hyporesponders. Open circles indicate free cholesterol, closed circles indicate total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
of free cholesterol carried by hyporesponders increased non-significantly from week 1 to 5, and remained unchanged at week 11.

Hyper and hyporesponders differed significantly in the percentage of total and free cholesterol (p<0.005 and p<0.025, respectively) initially carried in this lipoprotein fraction. As the groups were challenged with extra cholesterol consumption, hyperresponders continued to carry a greater percentage of cholesterol in this fraction, but the differences were small (fig. 6b). Hyperresponders had greater amounts (mg/100ml) of total and free cholesterol in this subfraction throughout the investigation (fig. 6a) than did hyporesponders, but the differences were not significant.

HDL-3 Cholesterol

Hyperresponders: Values for cholesterol in the HDL-3 subfraction are presented in tables 6 and 8, and in figure 7. Total cholesterol in this lipoprotein subfraction rose slightly but did not change significantly when 3 eggs/day were added to the ad lib. diet. The amount of free cholesterol in this subfraction remained relatively unchanged throughout the investigation. The percentage of total cholesterol carried in the subfraction remained unchanged during the investigation. The percentage of free cholesterol in this subfraction fell slightly at the classification period but did not change significantly throughout the investigation.
Figure 7. (a) mean plasma cholesterol concentration (mg/100ml) in the HDL-3 fraction by week. (b) mean values for HDL-3 cholesterol expressed as percentage of whole plasma cholesterol concentrations. Solid lines represent hyperresponders, dotted lines hyporesponders. Open circles indicate free cholesterol, closed circles indicate total cholesterol. Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day for 6 weeks.
Hyporesponders: The values for cholesterol in the HDL-3 subfraction are presented in tables 7 and 9, and in figure 7. Total and free cholesterol did not change significantly throughout the investigation in the amount of cholesterol (mg/100ml) found in this subfraction or in the percentage of total and free cholesterol carried in this density group.

Hyper and hyporesponders differed significantly (p<0.05) in the amount of cholesterol initially carried in this subfraction. Thereafter the differences became non-significant. However, hyperresponders had greater amounts of cholesterol and carried a greater percentage of cholesterol in this subfraction than did hyporesponders throughout the study (figure 7).

LDL-C/HDL-C

The LDL-C/HDL-C ratio allows us to compare the changes in LDL cholesterol relative to the changes of cholesterol in the HDL fraction. Hyporesponders had consistently higher ratios throughout the experiment indicating that there were relatively greater amounts of cholesterol in the LDL fraction and lesser amounts of cholesterol in HDLs as compared to hyperresponders. This is reflected in the significant differences between groups in the first and fifth weeks (Table 10). The LDL-C/HDL-C ratio in free cholesterol is significantly different only at week 1. Thereafter the differences are not significant. Hyporesponders have consistently greater ratios than are found in hyperresponders. Neither group had a significant
Table 10. Mean LDL-C/HDL-C Ratios in Three Periods

<table>
<thead>
<tr>
<th></th>
<th>week 1</th>
<th>week 5</th>
<th>week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hyperresponders</strong> (n=8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC^2</td>
<td>2.26±0.6^a^3</td>
<td>2.17±0.7^b</td>
<td>2.37±0.6</td>
</tr>
<tr>
<td>FC</td>
<td>3.14±1.2^c</td>
<td>3.27±0.7</td>
<td>3.14±0.8</td>
</tr>
<tr>
<td><strong>hyporesponders</strong> (n=12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>3.31±0.9^a</td>
<td>2.96±0.9^b</td>
<td>3.04±1.0</td>
</tr>
<tr>
<td>FC</td>
<td>4.51±1.5^c</td>
<td>4.02±1.5</td>
<td>4.23±1.7</td>
</tr>
</tbody>
</table>

Key to footnotes:

1. Week 1 indicates baseline values; week 5, 4 weeks on habitual diet plus 3 eggs/day; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.

2. TC (Total cholesterol); FC (Free cholesterol)

3. Levels of significant differences between same letters by student's t-test

^a p 0.01 ^b p 0.05 ^c p 0.05
change in the ratio as the study progressed.

HDL-2/HDL-3 Cholesterol ratio

The HDL-2/HDL-3 cholesterol ratio of hyper and hyporesponders differed initially in that the ratio of hyporesponders was significantly lower than that of the hyperresponders. As the groups consumed 3 eggs per day, these values became nearly identical (Table 11). The ratios from free cholesterol were not significantly different between groups. The HDL-2/HDL-3 cholesterol ratio of the hyperresponders did not change significantly in free or total cholesterol throughout the investigation. Hyporesponders had a significant (p<0.005) increase in the ratio as they consumed 3 eggs/day during the classification period. The ratio did not significantly change further as the hyporesponders consumed 6 eggs per day during the experimental period. The free cholesterol ratio of the hyporesponders also increased significantly (p<0.001) at week 5, but did not change significantly at week 11.

Apo A-I/Apo A-II Ratios

The A-I/A-II ratios in the HDL-2 and HDL-3 subfractions are presented in table 12. While the hyperresponders have consistently higher ratios of the two apoproteins in both HDL-2 and HDL-3 fractions, there were no statistically significant differences either between or within groups due to the small degree of freedom.
Table 11. Mean HDL-2/HDL-3 Cholesterol Ratios in Three Periods

<table>
<thead>
<tr>
<th></th>
<th>mean±SD</th>
<th>week 1</th>
<th>week 5</th>
<th>week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hyperresponders</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=8)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>TC</td>
<td>0.81±0.3</td>
<td>0.80±0.3</td>
<td>0.72±0.3</td>
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</tr>
<tr>
<td>FC</td>
<td>1.17±0.5</td>
<td>1.36±0.6</td>
<td>1.10±0.4</td>
<td></td>
</tr>
<tr>
<td><strong>hyporesponders</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(n=12)</td>
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</tr>
<tr>
<td>TC</td>
<td>0.63±0.2</td>
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<tr>
<td>FC</td>
<td>0.99±0.3</td>
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</table>

Key to footnotes:
1 Week 1 indicates baseline values; week 5, all subjects on habitual diet plus 3 eggs/day for 4 weeks; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.
2 TC (Total cholesterol); FC (Free cholesterol)
3 Levels of significant differences between same letters by paired t-test

*a p 0.005  b p 0.001
Table 12. Mean Apo A-I/A-II Ratios

<table>
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<th></th>
<th>mean±SD</th>
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<th>week 5</th>
<th>week 11</th>
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</thead>
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<td>hyperresponders (n=3)</td>
<td>7.09±3.5</td>
<td>4.82±0.7</td>
<td>7.26±2.7</td>
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<td>hyporesponders (n=3)</td>
<td>3.18±0.6</td>
<td>4.3±0.7</td>
<td>5.10±0.3</td>
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</tr>
<tr>
<td>HDL-33</td>
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</tr>
<tr>
<td>hyperresponders (n=3)</td>
<td>4.66±0.9</td>
<td>5.03±1.2</td>
<td>4.71±1.5</td>
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</tr>
<tr>
<td>hyporesponders (n=3)</td>
<td>3.61±0.8</td>
<td>3.90±1.6</td>
<td>3.36±0.2</td>
<td></td>
</tr>
</tbody>
</table>

No significant differences between or within groups

1Ratios measured week 1 indicates baseline values; week 5, 4 weeks on habitual diet plus 3 eggs/day; week 11, subjects consumed habitual diet plus 3 eggs/day (hyper) or 6 eggs/day (hypo) for 6 weeks.

2High density lipoprotein-2

3High density lipoprotein-3
DISCUSSION

The purpose of the present study was to investigate the individual variability of plasma cholesterol levels and the distribution of cholesterol among lipoproteins in response to egg ingestion in middle-aged normolipidemic males. Ever since the turn of the century, when blood cholesterol was related to coronary heart disease (CHD), the effect of diet on this sterol in blood has been under extensive study. While it is generally accepted that dietary cholesterol strongly influences serum cholesterol levels, the degree to which this effect is exerted is the subject of much controversy. When cholesterol was added to a cholesterol-free diet, plasma cholesterol levels rose linearly (Mattson et al., 1972). However, adding cholesterol to a diet that habitually contains a moderate amount of cholesterol has not produced consistent results (Roberts et al., 1981; Flaim et al., 1981). The addition of two or three eggs to the diet of normal, free-living volunteers has led to a plasma cholesterol of no response (Flaim et al., 1981) or a significant rise (Roberts et al., 1981). Accompanying these observations is the report of wide individual variation. There is currently little information regarding dietary cholesterol and individual response.

The results of this study demonstrated that an increase in dietary cholesterol to a diet already containing moderate amounts of cholesterol will result in a small increase in mean plasma cholesterol concentration. There were wide individual variations of
plasma cholesterol concentrations in response to dietary cholesterol. The plasma cholesterol levels in response to an additional 3 eggs per day ranged from a decrease of 17 to a rise of 53 mg/100ml. Individual variation of this magnitude has been observed by many others (Mistry et al., 1981; Flaim et al., 1981). Mistry et al. (1981) added 1500mg cholesterol per day to the habitual diet of 37 subjects for 14 days, resulting in plasma cholesterol response that ranged from -6 to +75 mg/100 ml. Similarly, Flaim et al. (1981) reported that adding 1000mg cholesterol per day to a diet already containing a moderate amount of cholesterol did not significantly increase the mean plasma cholesterol level of 12 subjects, but 5 out of 12 (40%) had a marked rise in plasma cholesterol after one week on the experimental diet. In the present study, 8 out of 20 subjects (40%) had a marked increase in plasma cholesterol concentrations in response to increased cholesterol intake.

The increases in plasma cholesterol of both groups (the mild increase on 6 eggs/day of hyporesponders and the significant increase in hyperresponders on 3 eggs/day), can be accounted for by small increases in both the LDL and HDL fractions. In this study, hyperresponders had a significant increase in LDL-C after 10 weeks of 3 eggs/day and a significant increase in HDL-C after 4 weeks on this diet. Hyporesponders had a slightly different response. A small, non-significant increase in LDL-C after 4 weeks on 3 eggs/day and a significant increase in HDL-C after consuming 6 eggs/day for 6 weeks. This is consistent with the findings of others who reported that
ingesting 1000mg cholesterol per day resulted in an increase in total plasma cholesterol levels that could be attributed to a significant increase in LDL-C and a slight non-significant increase in HDL-C (Lin and Connor, 1980). Mistry et al. (1981) found that increases in plasma cholesterol concentrations due to increased cholesterol consumption were due to increases in LDL-C and HDL-2 cholesterol. It is interesting to note that in this study, the small increases in plasma cholesterol in both groups were caused by increases in esterified cholesterol. The significant increases in total plasma cholesterol of hyperresponders in our study were followed by increases in LDL-C and HDL-C but were not accompanied by increases in free cholesterol. In hyporesponders, the delayed increase in HDL-C was not followed by a rise in free cholesterol. While the total cholesterol in LDL-C did not increase significantly with 3 eggs per day, free cholesterol in this fraction did fall significantly indicating that an overall rise in the esterified cholesterol occurred. Free cholesterol values are not often reported in human feeding studies; however, in two animal studies involving monkeys (Ershow et al., 1981) and rats (Bony et al., 1980), increases in plasma cholesterol in lipoprotein fractions as a result of dietary cholesterol were reported to be from increases in esterified cholesterol. The plasma cholesterol values reported in the lipoprotein fractions are within the normal range (Heiss et al., 1980) and are consistent with the values noted in other feeding studies (Roberts et al., 1981). The values reported here for the Apo
A-I/A-II ratios are similar to those reported by Schaefer et al. (1979) and Cheung and Albers (1977).

The habitual intake of dietary cholesterol in hyporesponders did not differ significantly from that of hyperresponders. When 3 eggs were added to the habitual diet, cholesterol intake of hyporesponders increased from 202±69 mg/1000kcal to 409±77 mg/1000kcal, whereas hyperresponders had an increase in intake from 149±51 mg/1000kcal to 470±160 mg/1000kcal. This is an increase of 207 mg/1000kcal for hyporesponders compared to 321 mg/1000kcal for hyperresponders. However, a greater increase in cholesterol consumption does not alone explain the plasma cholesterol response seen in the 8 hyperresponders. When the hyporesponders added 6 eggs per day to their habitual diet, an increase of 559 mg/1000kcal, the plasma cholesterol of these 12 subjects still did not rise significantly. A normal daily intake of cholesterol had been reported to be 500 mg per day (Dairy Council Digest, 1979). The subjects in this study had a habitual intake of 474±193 mg cholesterol per day at the onset of this investigation, which is within the normal range. This out-patient feeding study does have limitations regarding subjects' dietary intake. Self-reported dietary intakes have been reported to be underestimated (Roberts et al., 1981). However, subjects in our study reported a normal caloric intake for their age and sex group, which would indicate that intake was not underestimated.

The differences between the two groups of cholesterol
responders are most pronounced in baseline values, before subjects were challenged with a high cholesterol intake. Hyperresponders had a significantly lower initial LDL-C/HDL-C ratio (maintaining a lower LDL-C and higher HDL-C level throughout the study), and carried a greater percentage of their total cholesterol in the HDL, HDL-2, HDL-3 fractions. Hyperresponders also had a lower initial plasma cholesterol level, and while not statistically significant, maintained a greater Apo A-I/A-II weight ratio than the hyporesponders throughout the study. This ratio trend noted here should be studied with a larger population to measure whether ratio differences do exist between the two response groups.

When factors that have been correlated with CHD are considered, hyperresponders would appear to be at less risk for CHD. As noted above, hyperresponders had greater mean HDL-C values, a greater percentage of total cholesterol in the HDL fractions, in addition to a greater Apo A-I/A-II weight ratio. These are all inversely correlated to risk of death from CHD. This group also had a lower LDL-C, lower LDL-C/HDL-C ratio and a slightly lower initial mean plasma cholesterol, which are positively correlated with risk of CHD.

Several investigators have demonstrated that the body reacts to increased cholesterol consumption by two mechanisms: increasing steroid excretion and/or decreasing cholesterol synthesis (Lin and Connor, 1980; Nestel and Poyser, 1976; Quintao et al., 1971). Decreasing cholesterol absorption has not been found to be a defense
mechanism against the overloading of dietary cholesterol (Lin and Connor, 1980). Mistry et al. (1981) noted that individuals whose plasma cholesterol did not increase as a result of dietary cholesterol showed greater LDL receptor activity and less HMG-CoA reductase activity. Consistent with these findings are the results of Nestel and Poyser (1976) who observed that individuals whose plasma cholesterol was not altered or only slightly increased by dietary cholesterol showed greater suppression of whole body cholesterol synthesis, whereas individuals whose plasma cholesterol rose as a result of dietary cholesterol showed a greater excretion of fecal steroids and less suppression of cholesterol synthesis. Changes in lipoprotein composition and distribution are also known to occur with high cholesterol diets. These include the appearance of two abnormal lipoproteins, β-very low density lipoprotein and a cholesterol induced high density lipoprotein, HDLc (Mahley, 1982). A more detailed compositional and structural analysis of the plasma lipoproteins should be studied to detect effects from dietary components.

As has been mentioned, the liver is the only organ which can excrete cholesterol in quantitatively important amounts (Dietschy & Wilson, 1970c; Miller & Miller, 1975). The reverse cholesterol transport theory contends that HDL functions to return cholesterol from extrahepatic cells to the liver. One possible explanation for increased HDL-C and greater percentage of whole cholesterol in the HDL fractions in the hyperresponders is responding to increased
dietary cholesterol through greater steroid excretion. This could support the findings of Nestel and Poyser (1976) whose hyperresponders mentioned above responded with greater steroid excretion. Since steroid excretion was not measured here, it is unknown whether this truly occurred. However when hyperresponders consumed 3 eggs per day in addition to their habitual diet, the HDL-C level increased significantly. This increase was due to a small increase in the cholesterol content in both the HDL-2 and HDL-3 subfractions. Only after consumption of 6 eggs/day did the HDL-C of the hyporesponders increase significantly due to small increases in both the HDL-2 and HDL-3 subfraction. However, contrary to this, when mean values of HDL-C are expressed as a percentage of whole plasma cholesterol; hyperresponders decreased the percentage of total cholesterol carried in this fraction during egg consumption and the percentage was increased in hyporesponders. This paradox may be explained by the fact that the magnitude of the significant increases in plasma total cholesterol levels over the diet period was much greater than that in HDL-C increases in hyperresponders. In contrast, the plasma total cholesterol levels of hyporesponders were not greatly increased over the same period while the HDL-C levels were increased as with the hyperresponders. Further studies to give greater insight into individual response should include an initial measurement of fecal steroid excretion and HMG-CoA reductase.
Summary and Conclusions

Mean plasma cholesterol level was increased to a small, but significant, degree as a result of consuming 3 eggs per day in addition to habitual diet. The plasma cholesterol of the 20 subjects was 185±34 mg/100ml at baseline increasing to 195±34 mg/100ml after four weeks on the diet with 3 eggs. Forty percent of the subjects had a plasma cholesterol increase of 8% or greater. The addition of 6 eggs per day to the habitual diet of the hyporesponders did not significantly increase plasma cholesterol. There were initial lipoprotein cholesterol differences between hyper and hyporesponders. These included a greater percentage of total and free plasma cholesterol in the HDL and HDL-2 lipoprotein fractions of the hyperresponders; a greater percentage of total cholesterol in the HDL-3 subfraction of hyperresponders; and a lower ratio of LDL-C/HDL-C in hyperresponders. Further study is necessary to determine differences in response groups of steroid excretion, HMG CoA reductase activity and ratios of Apo A-I/Apo A-II.
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