THE EFFECT OF EGG CONSUMPTION IN SELF-SELECTED AND CONSTANT DIETS ON PLASMA LIPIDS OF HEALTHY YOUNG MEN

by

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A THESIS

Presented to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of Master of Science

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A B S T R A C T

The purpose of this study was to investigate the effect of egg addition to self-selected and constant diets in relation to pre-experimental plasma lipids of healthy young males. During the first 21 day period (Period A), 8 male university students ($\bar{X}$ = 23 years) consumed 3 eggs daily in addition to their usual diets. Following period A, the subjects continued their habitual eating patterns without the additional eggs for 7 days. They were then fed a constant diet which included 3 eggs daily for another 21 day period (Period B). Cholesterol (CHOL) intake was similar during the two periods. Fasted blood samples were collected at the beginning and end of each period, and analyzed for plasma total CHOL, high density lipoprotein CHOL, low density lipoprotein CHOL and triglycerides. Subjects mean initial values were 163 mg/dl (SD $\pm$ 27.2), 47 mg/dl (SD $\pm$ 9.7), 102 mg/dl (SD $\pm$ 34.5) and 76 mg/dl (SD $\pm$ 22.5), respectively. Total CHOL increased 1 mg/dl during period A and 5 mg/dl during period B. The change in high density lipoprotein CHOL was -2 and +4 mg/dl and in low density lipoprotein CHOL, -4 and +4 mg/dl

for periods A and B, respectively. Triglyceride changes were +24 and -23 mg/dl. Randomized block design analysis of variance revealed that the daily ingestion of 3 eggs, in the two periods, did not produce significant change in plasma lipids of the subjects.

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REVIEW OF LITERATURE

Part I

The Effect of Dietary Cholesterol on Total Blood Cholesterol

Cardiovascular disease is responsible for more than 50% of the deaths in most western industrialized countries (The Committee on Diet and Cardiovascular Disease, 1976). One of the risk factors associated with heart disease is a blood cholesterol (CHOL) concentration above 220 mg/dl (Kannel et al, 1971). Hence, the nutritionist is concerned with dietary factors that may influence blood CHOL. The effect of dietary CHOL on blood CHOL is controversial. Some researchers have demonstrated that CHOL consumed in the human diet raises blood CHOL significantly, while others have found that there is no physiological effect.

Factors which should be considered when evaluating the results of the research are the amount, source and method of incorporation or addition of the dietary CHOL, and the type and nutrient composition of the diet. Both formula and natural food diets have been utilized in investigative studies. The formula diet contains either

natural foods and/or isolated food components such as casein or dextrose, and is consumed as a liquid. Keys et al (1974) have suggested that the effect of exogenous CHOL in liquid formula diets may be overestimated as compared to the effect of CHOL in natural food diets. The CHOL source, added or incorporated into the diets, is either in the crystalline form or in the natural form, as egg. Researchers have questioned the influence of CHOL from various sources on plasma cholesterol (PCHOL) and on experimental atherosclerosis (Kritchevsky et al, 1979). In addition to the CHOL source, the age, sex and health of the subjects, the order and length of the feeding periods, and the specificity of diet and blood analysis are factors which have effect on the reported results. This review will emphasize research dealing with young adult humans who are free of metabolic disorders.

Formula Diets

The use of formula diets appeared to be an effective method of maintaining constancy in nutrient intake. However, this diet is free of fiber and thus may alter lipid metabolism. Kritchevsky (1977) has suggested that with the formula diet, there was reduced synthesis of bile

acids possibly because they were not excreted. For this reason, one might expect the formula diet, in itself, to have a hypercholesterolemic effect.

A. Cholesterol Source: Crystalline Cholesterol

The addition of crystalline CHOL to formula diets eliminated the problem of balancing fatty acid patterns necessary when CHOL was added in the form of egg. One problem arising from the use of crystalline CHOL, overlooked by many early investigators, was that the nature of the fat in which the CHOL was incorporated, appeared to have a significant effect on serum cholesterol (SCHOL).

Beveridge et al (1959) noted a relationship between the crystalline CHOL supplement and the fat in a study which was initially designed to identify the hypercholesterolemic substance in butterfat. Fifty-four male and 20 female university students consumed a fat-free isocaloric formula diet (14.74% protein, 0.63% fat, 84.63% carbohydrate of total energy) for 8 days during which PCHOL dropped rapidly from a mean of 193.7 to 141.2 mg/dl. They were then divided into 8 comparable groups and, for another 8 day period, they consumed diets in which 30% of the total caloric intake

was supplied by one of 8 fat mixtures. These consisted of a butterfat distillate supplying 1.36g CHOL/1000 kcal or various low CHOL butterfat fractions fed with or without crystalline CHOL to equal that of the original distillate. Other fat mixes used were saturated medium chain triglycerides, with or without the CHOL supplement, and a coconut oil supplement. The original butterfat distillate was related to an increase of 79 mg/dl in PCHOL. The ingestion of the remaining butterfat fractions resulted in similar increases when fed with CHOL while much smaller increases, 11.4 and 33.0 mg/dl were observed when a CHOL poor diet was consumed. The medium chain triglycerides did not produce changes in the PCHOL regardless of the CHOL supplement. The addition of the original coconut oil produced a mean increase of 23.1 mg/dl. The nature of the fat in which the CHOL was incorporated seemed to be an important element. The authors suggested that the specific positions of fatty acids on the glycerol molecule may determine interaction with CHOL consequently affecting the eventual absorption of the supplement. The importance of dietary fat was also observed by Connor et al (1961), who were not able to show any significant changes in blood CHOL

when up to 3600 mg crystalline CHOL per day was introduced into a 40% kcal fat formula diet (60% peanut oil, 30% cocoa butter, and 10% safflower oil). An increase in stool fat accompanied this test period which suggested absorptive changes. The use of healthy young subjects, constant caloric intake and comparable fatty acid patterns among diets, strengthened the reliability of the study of Beveridge et al (1959). The use of formula diets, which do not present a normal absorptive challenge for the gut, the subject variability due to limited observations of each subject (i.e., did not serve as their own controls), and the brevity of the regimen were not considered by Beveridge and his coworkers to have an effect on the results.

Having established that crystalline CHOL dissolved in a suitable fat increased PCHOL, Beveridge et al (1960) investigated the effect of dietary CHOL fed in varying amounts using a design previously described (Beveridge et al, 1959). Ninety-three university students were fed a fat-free formula diet for 8 days, which reduced the mean SCHOL from 201.0 to 145.5 mg/dl. They were divided into 8 groups and fed one of the test diets for another 8 days.

Crystalline CHOL was added in amounts from 0 to 1684 mg/1000 kcal to test diets comprised, 30% by calorie, of low CHOL (.02%) butter oil. Plasma cholesterol increased sharply when CHOL intake increased from 0 to 211 mg/1000 kcal but the dose response curve thereafter was relatively flat. The researchers assumed that an internal control mechanism prevented hypercholesterolemia at high levels of intake in healthy subjects. In both studies by Beveridge et al (1959, 1960), an increase in dietary CHOL was accompanied by an increase in fat, from 0 to 30% of caloric intake. The initial response of blood lipid to the low fat, low CHOL diet and 8-day stabilization periods made it difficult to determine what proportion of the plasma increase was due to dietary CHOL alone. The use of the formula diet and crystalline CHOL in these studies was reason for questioning whether the findings apply to subjects fed diets consisting of ordinary foods.

B. Cholesterol Source: Egg Yolk

Difficulty with introducing crystalline CHOL in an absorbable form, led numerous investigators to consider the use of a natural CHOL source, egg yolk, as a more

suitable alternative. As egg yolk varied, a fat similar to that of the egg yolk had to be added if CHOL were to be isolated as the sole dietary variable. The results of Wells and Bronte-Stewart (1963), for example, could not be attributed solely to dietary CHOL since an increase in fat accompanied the CHOL addition. In this experiment, the addition of 10 egg yolks to a low CHOL, low fat semi-synthetic diet produced consistent SCHOL increases of 50 to 60 mg/dl in a 46 year old man. The 10 egg yolks contributed significant fat as well as CHOL.

The use of a simulated yolk fat was initiated by Connor et al (1961) during the investigation of the effect of egg yolk on serum lipids of 6 male subjects (age 40 to 45 years). A fat mixture of 60% peanut oil, 30% cocoa butter and 10% safflower oil identical to egg yolk in percentages of saturated, monounsaturated, and polyunsaturated fatty acids which was employed to maintain constant fat in the diets. The incorporation of egg yolk in a formula diet (40% kcal fat), which provided CHOL intakes from 1650 to 4800 mg/day, resulted in increases in blood CHOL which did not relate directly to CHOL intake. In an ensuing study in 1961, egg yolk was added in smaller amounts ranging rom 475 to 1425 mg CHOL/day. The various levels of egg yolk CHOL resulted

in similar increases, approximately 69 mg/dl in the SCHOL. Individual variability when subjects are not used as their own controls and the large weight change reported may have masked possible differences. The authors hypothesized that absorption was the limiting factor. More recent work, however, has shown that the absorption of CHOL seems to increase proportionately to increases in dietary CHOL, with no absolute upper limit clearly defined (Connor and Lin, 1974).

The effect of egg yolk incorporated into a formula diet but given to young subjects considered to have normal lipid patterns uncomplicated by early stages of cardiovascular disease was reported by Erickson et al (1964). Partially hydrogenated and unhydrogenated fat blends, with and without dried egg yolk powder (CHOL range 0 to 306 mg/1000 kcal) which constituted 41% of total calories in formula diets were fed to 42 males (mean age 35 years). Fat A (P/S ratio 1.6) was partially hydrogenated soybean oil and fat B (P/S ratio 1.5) was a vegetable fat mix comprised of 52.5% olive oil, 39.5% safflower oil and 8% cocoa butter. Fat B was similar to fat A except it did not contain any hydrogenated fat. Simulated egg yolk

(36% olive oil, 31% palm oil, 25% cocoa butter, 8% cottonseed oil) was used when egg was not added to keep total fat and fatty acid patterns constant. The subjects were gradually introduced to the formula diet, divided into 7 groups and fed the test diets for 5-week periods using an incomplete latin square design. A mean increase of 24 mg/dl in PCHOL was reported for fat A and 27 mg/dl for fat B, when the CHOL source (egg) was incorporated, however, PCHOL values did not differ greatly from pretest values. Phospholipids increased 18.4 and 22.2 mg/dl, respectively for both diets. Triglycerides increased 4 and 14 mg/dl, respectively. Researchers concluded that there was no interaction between dietary CHOL and the isomeric unsaturated fats. Plasma cholesterol increases were greater than those reported by researchers using crystalline CHOL. Keys et al (1965) had also noted slightly increased responses to egg CHOL as compared to crystalline CHOL when fat intakes were similar.

The effect of graded amounts of egg CHOL was studied by Mattson et al (1972). Moderate amounts of dried egg yolk were incorporated into formula diets designed to represent the composition of the average U.S. diet

(40% kcal fat, P/S ratio 0.3). A simulated egg yolk fat was designed in order to maintain constant fat intake. Fifty-six men (median age 26 years) were divided into 4 groups according to the decrease in SCHOL when CHOL-free diets were fed, weight and absolute CHOL values. Subjects were fed for 42 days with formula diets which contained 0, 106, 212 or 317 mg CHOL/1000 kcal. Serum cholesterol increased in a linear fashion described by the regression equation:

SCHOL (mg/dl) = 1.6 + 0.118 (diet CHOL, mg/1000 kcal).

An increase of 12 mg/dl for each additional 100 mg dietary CHOL/1000 kcal was observed. Notably, only the highest CHOL supplement produced SCHOL levels which were above the pretest values. Hegsted et al (1965) were able to produce a slightly greater response using natural foods and egg yolk in older subjects who were fed similar levels of cholesterol. The data reported by Mattson et al (1972) resulted in a steeper slope than that shown by Erickson et al (1964) and Beveridge et al (1960) suggesting a more dramatic relationship within the normal range of CHOL intake.

From these studies, it can be seen that the addition

or incorporation of a CHOL source in a formula diet generally is followed by an increase in blood CHOL. The dietary CHOL source and manner of incorporation appears to be a factor in the magnitude of outcome. Beveridge et al (1959, 1960) and Connor et al (1961) agree that for optimal absorption of a purified CHOL form, attention must be paid to the type and amount of accompanying fat. Utilization of egg yolk in formula diets was found, by Erickson et al (1964), Connor et al (1961), and Mattson et al (1972), to produce a more elevated SCHOL response than crystalline CHOL. This relationship was thought to only be true at moderate intakes of CHOL and may not apply at extreme levels of dietary intake. Recently, Kritchevsky et al (1979) reported varied, but not distinctly different, influences of different CHOL sources: crystalline, fresh egg yolk and dried egg yolk powder on SCHOL in rabbits. These researchers suggested that a longer study (i.e., greater than 3 months) might reveal distinct differences in SCHOL response. In addition, spontaneously produced angiotoxic derivatives which develop in crystalline and dehydrated CHOL sources stored in air at room temperature may produce greater rises in SCHOL than those from fresh sources

(Taylor et al, 1979). The frequency of occurrence of such derivatives has not been documented.

Mixed Solid Food Diets

The use of mixed solid food diets, instead of formula diets provided a normal absorptive challenge for the gut. The added fiber has specific physiochemical properties dependent on the structure and composition of its components (Eastwood et al, 1979) that may exert an influence on lipid absorption or excretion. Lignin and pectin, specifically, exert a physiological action on bile-acid absorption which may modify fecal steroids and CHOL turnovers. Cooney O'Brien and Rieser (1979), in a comparison of the effects of formula and mixed food diets on CHOL metabolism in the rat, reported greater SCHOL increases on addition of CHOL to the formula diet. The largest SCHOL increases were associated with the diet lowest in fiber. This may not, however, be applicable to humans. Furthermore, control of fat composition in studies utilizing mixed diets was more difficult due to the nonhomogeneous nature of the total diet.

A. Cholesterol Source: Crystalline Cholesterol

Although most researchers who used natural food diets also used a natural CHOL source, a few used crystalline CHOL to facilitate the maintenance of identical fat composition among the experimental diets. Keys et al (1965) compared egg yolk CHOL to crystalline CHOL. Egg yolk produced higher SCHOL than crystalline, however, the difference was not statistically significant. For this reason, Keys et al (1965) proceeded to utilize the easily incorporated crystalline form. Graded amounts of crystalline CHOL were dissolved in 100g cottonseed oil and incorporated into a constant diet containing 40% kcals as fat. Each man served as his own control. A crossover design compensated for possible subject-time variability. In addition, the 21-day experimental periods which allowed for stabilization of lipid patterns, and equivalent fat composition in pretest and test periods represented controlled features of the design. Resulting serum increases were similar to those observed by Beveridge et al (1960). The greatest increases were reported at the lower ranges of CHOL intake and decreased as intake increased. The authors concluded that intakes of CHOL within the

range of normal U.S. diets resulted in only minimal, easily masked differences in SCHOL.

In order to study the effect of saturation of fat in SCHOL, Anderson et al (1976) used a dietary CHOL supplement of 291 mg/day of purified cholesterol dissolved in 40g of the oil supplements. The total amount of CHOL was low to avoid the possibility of exceeding the intestinal capacity to absorb CHOL. The effect on serum lipids of the added cholesterol to mixed diets, which contained 3 mg of CHOL and equal fat but dissimilar fat composition, was examined in 12 young men. Feeding periods were 14 days. Dietary fat supplements consisted of 97g/day of a saturated oil (2 parts of palm oil and one part of coconut oil), or 97g/day of safflower oil, both fed with and without the CHOL supplement. The CHOL addition produced a mean elevation of SCHOL of 9 mg/dl for the saturated fat diet and 8 mg/dl for the polyunsaturated fat diet. Phospholipids increased 8 and 10 mg/dl, and triglycerides 4 and 6 mg/dl, respectively. The authors concluded that the effect of CHOL was independent of the degree of saturation of the dietary fat. Keys et al (1965) had previously reported greater blood CHOL responses which may have been due to the dissolution of cholesterol in a larger amount (100g) of oil utilized in that

experiment.

B. Cholesterol Source: Egg Yolk

The use of a natural CHOL source, such as egg yolk was more prevalent among researchers who fed mixed diets to the subjects. The more closely the experiment simulated a normal living situation, the less the diet was controlled.

Of this group of investigators, Connor et al (1964) maintained the most rigid control over the composition of the natural food diets. Six male prisoners, age 24 to 48 years, with a mean basal SCHOL of 213 mg/dl, consumed 40% kcals as fat. The diets were designed to compare intakes between 0 and 265 mg cholesterol/1000 kcals. Egg yolk and beef round resulted in mean SCHOL increase of 35 and 26 mg/dl for saturated and unsaturated fat diets, respectively. These results were greater than those observed by Keys et al (1965) with crystalline CHOL and were more in agreement with the findings of Mattson et al (1972).

Less rigid dietary control was exercised by Steiner and Domanski (1941) and Messinger et al (1950) who also used institutionalized subjects. Food supply was regulated for quality and portion size which facilitated nutrient estimation. Steiner and Domanski (1941) reported a mean

increase of 101 mg/dl in SCHOL with the addition of dry egg yolk to regular hospital diets. Interpretation of their work was complicated by the abnormal health of their subjects and the overestimation of SCHOL using the Bloor method of analysis. In an analogous manner, Messinger et al (1950) added dry egg yolk, which provided 3750 mg CHOL/day, to the habitual hospital diet of several groups of atherosclerosis patients (mean age 60 years) for 42 days. An average increase of 36 mg/dl from an initial SCHOL of 199 mg/dl was observed. The researchers found large differences among individuals and were unable to return SCHOL levels to their initial values in the 21 to 28 day post-feeding periods. Since the subjects had diagnosed atherosclerosis, lipid metabolism cannot be considered to be identical to healthy subjects. Nutrient composition of the diets was not closely regulated and the experimental period was accompanied by many dietary variables. The statistical significance of this study has been questioned due to the modest effect of such a dramatic increase in dietary CHOL. Since it is known that adding CHOL to the food has the greatest effect with a low CHOL diet, the absence of a CHOL-free period will reduce the response to dietary treatment.

There has been recent interest concerning CHOL intakes for free living subjects consuming self-selected diets. In an attempt to reproduce these situations, such designs deviated markedly from the controlled conditions of previous experiments. Studies using self-selected diets examined the effect of the addition of eggs to the diet rather than the effect of dietary CHOL. The most striking aspect of these studies is the lack of a significant effect in contrast to the linear or curvilinear relationship between the dietary CHOL and blood CHOL as has been indicated in laboratory controlled experiments.

The effect of whole eggs superimposed on the habitual diets of healthy free living male groups was investigated by Slater et al (1976). In the first study, the subjects were 15 male university students, 20 to 30 years old, with PCHOL less than 220 mg/dl. All meals were consumed in the university cafeteria to facilitate accurate dietary records. The initial 2 weeks consisted of ad libitum food selection followed by 6 weeks during which 2 whole eggs were included in breakfast. Carbohydrate was altered for weight maintenance. Paired differences showed a significant increase during the third and fourth weeks when eggs were

added, however, analysis of variance revealed no significant change overall either in PCHOL or triglycerides. In another experiment, 25 healthy male students, mean age 24 years, were selected on the basis of absence of personal and family history of heart disease, diabetes or hypertension, as well as an initial low PCHOL value (mean 171 mg/dl). The study plan consisted of a 2 week control period, followed by an 8 week period in which subjects were instructed to consume 2 eggs per day, and finally, a 2 week period in which the subjects excluded all visible eggs from their diets. Three day diet records revealed an average intake of CHOL from all sources of 314, 793 and 343 mg/day, for the three periods, respectively. Triglyceride levels increased after 5 weeks when 2 whole eggs were consumed but returned to normal thereafter. Analysis of variance showed no difference in PCHOL levels. It was concluded that eating 2 eggs per day in an otherwise normal diet does not change plasma lipids over an 8 week period in healthy young men. The authors attributed these results to the lesser effect of added dietary CHOL to a diet already containing optimal CHOL. The plateau effect of high intakes of CHOL on SCHOL had been observed earlier by Beveridge

et al (1960) and Connor et al (1963). This physiological mechanism has not yet been satisfactorialy explained.

Following Slater's reports, Porter et al (1977) and Flynn et al (1979) confirmed the lack of effect of a maximum of 2 eggs/day in older groups of males consuming their usual diets and fed 0 and 1 or 2 eggs daily for 3 month periods, in crossover designs. Kummerow et al (1977) noted similar results when 2 whole eggs were fed in addition to hospital diets to patients for up to 54 days. Decreases of 6 to 19 mg/dl in SCHOL were observed by Porter et al (1977) and Flynn et al (1979) when the diet was changed from one containing 1 or 2 eggs, respectively, to one which did not contain eggs. This change was not statistically significant and did not occur when the order of feeding was reversed. High individual variability in lipid pattern response was observed. The enzymatic determination of SCHOL used by Flynn et al (1979) gave significantly lower values for the same group of subjects used by Porter et al (1977) two years earlier indicating a need for standardized analysis.

It appears that when a moderate amount of CHOL, in the form of whole egg, is added to diets in this uncontrolled

way, its potential hypercholesterolemic effect is greatly reduced. The problem of monitoring changes in dietary pattern, especially when extrapolating from dietary records, make it difficult to isolate dietary variables. The pre- and post-experimental PCHOL of the subjects varied significantly from each other, as reported by Slater et al (1976), despite the assumption of similar dietary intake. Also, the basis on which the degree of change is calculated may be influential. Most controlled experiments measure the increase by comparison with lipid levels during a standard CHOL-free period, since change appears to be of greatest magnitude within this range. Because free-living subjects begin the experiment at their normal blood lipid level, and usual individual CHOL intake, the addition of 1 to 2 eggs may not be enough to increase blood CHOL significantly. Individual variability and internal control mechanisms for CHOL metabolism may be significant factors.

The lack of consistency in the reported results of these studies suggest the need for further evaluation of the effect of egg consumption in relation to the habitual diets of human subjects. Does the ingestion of additional eggs by free living subjects consuming regular diets have

the same effect on PCHOL as the same number of eggs incorporated into a diet which is constant in calories and nutrients? The same subjects, experimental protocol and dietary CHOL source, as well as similarity of diets, for each experiment would reduce variability. A reasonable amount of dietary CHOL, 3 whole eggs daily, which is above the mean daily intake of 44g egg/day reported by the Health Protection Branch (1977) for adult males (20 to 39 years) may be adequate to produce detectable change.

REVIEW OF LITERATURE

Part II

The Effect of Dietary Cholesterol on Blood Lipoproteins

Researchers have found difficulty correlating dietary cholesterol (CHOL) with blood CHOL in a consistent manner. For example, Simons et al (1978) reported a linear relationship between dietary and absorbed CHOL but could find no correlation between absorbed CHOL and plasma cholesterol (PCHOL). The well known fact that both dietary and nondietary factors interact in the regulation of total CHOL metabolism was reconfirmed. Biochemical studies, concentrating on CHOL transport, synthesis and utilization, have focused on lipoprotein fractions as a more reflective parameter of plasma lipid patterns. Limited research has suggested that dietary CHOL may not affect all the lipoprotein fractions, and thus may be better correlated with one or two specific moieties.

Structure and Function of Lipoproteins

Lipoproteins are associations of lipids with proteins by hydrophobic interaction. Protein components called

apoproteins, and polar lipids in a surface-film surrounding a neutral lipid core provide the basic structure. They act as a vehicle for transport of water insoluble lipids, such as CHOL,and may specify the tissue to which the lipids are to be delivered. Chylomicrons carry triglycerides from the intestine to nonhepatic tissues for utilization or storage whereas very low density proteins (VLDL) carry endogenous triglycerides from the liver to extra hepatic tissues. The low density lipoproteins (LDL) are derived from VLDL catabolism. These proteins are not homogenous but also include intermediate lipoprotein (IDL or IDL_1) and LDL_2 which differ in structure and function (Eisenberg and Levy, 1975). High density lipoproteins (HDL), synthesized in the liver, are composed of several protein-lipid combinations.

Biochemical studies of HDL have revealed three characteristics of their structure:

(i) the phospholipid polar head groups may be located at the surface of the HDL.

(ii) the apoproteins are at or near the surface.

(iii) the interior of HDL is viscous and contains lipid components in amounts too small for co-operative melting. (Smith et al, 1978).

Similarly, LDL protein is at or near the surface in association with a phospholipid monolayer. Low density lipoproteins contain cholesteryl esters in separate entities in which some triglycerides are probably solubilized. Little is known about the structure of VLDL.

The liver and intestine are the chief sites of apoprotein assembly. The liver secretes VLDL and HDL whereas the intestine secretes chylomicrons and some VLDL. Once secreted, the nascent lipoproteins undergo rapid modification in the plasma by physical transfer of lipid and apoprotein components and by enzymatic modification by lecithin CHOL acyl transferase and lipoprotein lipase.

Three processes are involved in the catabolism of plasma lipoproteins: (i) physical transfer and exchange of components, (ii) enzymatic changes, and (iii) cellular uptake at receptor sites and passive endocytosis. By observing human mutations, Brown and Goldstein (1977) have outlined a pathway by which extra hepatic cells acquire CHOL for plasma membrane synthesis. Initially, LDL binds to a receptor site of the cell in human fibroblasts, lymphoid cells and aortic smooth muscle cells. Internal

vesicles begin to form, and the LDL is internalized by endocytosis. Vesicles then merge with the lysosomes whose enzymes hydrolyze LDL into amino acids and free CHOL. The free CHOL is used by the cell to synthesize membranes and acts in a regulatory capacity:

(i) it inhibits 3-hydroxy-3-methylglutaryl CoA reductase and thus decreases the synthesis of more CHOL.

(ii) it stimulates acyl-coenzyme A: cholesterol acyl transferase which reesterfies the free CHOL to form CHOL oleate for storage.

(iii) it diminishes the synthesis of LDL receptors.

Thus, the uptake of CHOL is balanced with cellular synthesis to satisfy the requirements for membrane structure and for synthesis of steroid hormones. Mutations occurring in any of these steps may cause problems in normal CHOL metabolism, failure of the regulatory processes and elevation of plasma LDL. In westernized populations, Goldstein and Brown (1977) have postulated, the mean level of LDL in plasma and interstitial fluid appears to be fivefold higher than needed for delivery of CHOL to body cells. When plasma leaks into the artery wall, through areas of

endothelial damage, this unphysiologically high level of LDL exceeds the clearance capacity of the smooth muscle cell's receptor mediated process. The LDL then is taken up by a phagocytic receptor independent response resulting in uncontrolled accumulation of CHOL esters within the cells, the heart of the atheromatous plaque.

High density lipoproteins, on the other hand, appear to transport CHOL from the peripheral tissue to the liver for recycling or excretion. Schwartz et al (1978) observed that the major portion of bilary CHOL originated from free CHOL which suggested involvement of HDL. To investigate the possibility of liver selectivity, they labelled the free CHOL of HDL and LDL with ^{14}C or ^{3}H. The label associated with the HDL fraction appeared most rapidly in bilary CHOL. The red blood cell showed lesser preference for HDL CHOL. The authors suggested that the HDL acts as a shuttle for the transport of free CHOL from tissues to liver. Low levels of HDL might lead to excessive accumulations of CHOL in body tissues.

Epidemiological Studies Correlating Lipoproteins With Diet

Because the relationship of lipoprotein and dietary CHOL has only recently been appreciated, few epidemiological

studies have monitored lipoproteins. Frank et al (1978) attempted to monitor lipoprotein fractions in the Bogalusa Heart Study with 10 year-old children. They found that eggs were the chief dietary CHOL source (mean intake 324 mg CHOL/day, range 0 to 1536); breakfast and dinner contributed the majority of CHOL intake. A weak positive correlation was found between CHOL intake and LDL-CHOL but none with VLDL-CHOL, HDL-CHOL and total CHOL. The lowest HDL levels (25th percentile) were found for children with the highest CHOL intake when grouped for risk factor variable level. Serum cholesterol level was significantly higher for students with the longest eating span. This study suggested that some interesting relationships may be found in future studies and confirms the difficulty of relating blood CHOL to dietary factors within a homogenous population.

In contrast, Connor et al (1978) were able to find high correlation between dietary and blood CHOL, and between PCHOL and LDL-CHOL within a homogenous group, the Tarahumara Indians of Mexico renowned for their competitive running. Direct correlation between CHOL intake and PCHOL may have been facilitated by the low range of CHOL

intake, 17 to 144 mg/day - below the "threshold level" above which differences in intake may not affect PCHOL. Mean PCHOL was 125, LDL-CHOL was 87, VLDL-CHOL 21 and HDL-CHOL 25 mg/dl. The PCHOL, LDL-CHOL and especially HDL-CHOL were much lower than those of North American societies. The authors attributed this to low dietary CHOL ($\bar{X}$ = 71 mg/day), low fat (12% of calories) and low saturated fat (2% of calories). Low PCHOL levels, and notably low HDL-CHOL, may reflect lifelong low intakes of dietary CHOL and saturated fat. In contrast, a group of rural Africans were found to have a mean HDL CHOL of 42 mg/dl regardless of similarity in health, activity, diet and total PCHOL to that of the Mexican group (Walker and Walker, 1978).

Recent epidemiological studies have documented a negative correlation between HDL-CHOL concentration and the incidence of coronary heart disease (Miller, 1979) however, the relationship of HDL with other risk factors such as sex, cigarette smoking, obesity, sedentary lifestyle, and diet patterns are not documented. If, as Goldstein and Brown (1977) have suggested, LDL-CHOL concentration in most western subjects are inappropriately elevated so as to

completely saturate the LDL receptors, CHOL metabolism in peripheral tissues may be influenced more by the reverse CHOL transport than by changes in LDL concentration.

Metabolic Studies

There is limited information about the effect of dietary CHOL on plasma lipoproteins in healthy humans. Animal models present problems in application to humans. The review will discuss studies of healthy humans but will include animal trials. Researchers have looked for dietary induced alterations in many aspects of lipoprotein metabolism, some of which are: changes in lipid profile, structure, composition and/or function of lipoproteins.

Increases in SCHOL reflected mainly in percentage increases in VLDL and IDL, and absolute increases in LDL_2, have been reported to follow the addition of egg yolk to regular diets. Changes in plasma lipoproteins in 18 humans, age 19 to 62 years, fed 750 to 1500 mg CHOL daily in the form of egg yolk, were observed by Mistry et al (1977). Fourteen subjects received 750 mg CHOL for 30 days and the remainder 1500 mg CHOL for 10 days in addition to their regular diet. Large individual variability of response to

the dietary supplement was noted; generally a rise in SCHOL reflected in various lipoprotein classes was found at both intakes. Although the largest absolute CHOL increment occurred in LDL_2, percentage changes were most pronounced in VLDL and particularly IDL which showed a 2 to 6 fold increase. Very low density protein and IDL increases were highest in subjects fed 1500 mg CHOL/day. The authors suggested these latter changes might be due to primary secretion of CHOL-rich chylomicrons and to accumulation of chylomicrons and VLDL remnants. Low density lipoprotein (LDL_2) and HDL fractions increased 14% and 19%, respectively. Notably, HDL took longer to return to initial values in the post test period. More than 4 weeks were required as compared to 10 days in other fractions and was possibly due to CHOL transport away from expanded pools.

Significant increases in total SCHOL, LDL and triglycerides were also reported by Honda and Sumikura (1976) when 3 or 5 eggs were added to the habitual diet of students for 7 to 9 weeks. No change was apparent at the 3 egg level, in contrast to substantial increases at the 5 egg level, after 1 week of feeding. Levels returned to pretest values by 4 weeks after feeding. Notable here

is the longer than traditionally accepted test periods required for response and lesser effect of lower egg intake.

The CHOL content of HDL remained essentially unchanged although total PCHOL increased about 23% in one half of subjects fed 4 to 6 eggs for a 4 week period (Mahley et al, 1978). High density lipoprotein CHOL appears, on the whole, less responsive to dietary induced hypercholesterolemia than other human lipoprotein fractions.

In many species, including human, "abnormal" lipoproteins can be identified in plasma after a change in lipid homeostasis produced by cholesterol feeding. The effect of eggs on human HDL was studied by Mahley et al (1978) in response to the occurrence of an "abnormal" lipoprotein, designated HDL_c, observed on addition of CHOL to the diets of laboratory animals. The HDL_c is a larger, more CHOL-rich lipoprotein with increased amounts of arginine-rich lipoprotein E which may be present normally in trace amounts (Eisenberg and Levy, 1975). Six healthy subjects added 4 to 6 eggs per day to their diet for a 4 week period. A second group of 5 subjects gradually increased their egg intake to 3 per day in an 18 week period. Plasma cholesterol increases were

variable; they ranged from 0 to 23% in the first group and from 0 to 6% in the second. Although, as previously mentioned, there was no change in HDL-CHOL, HDL showed increased binding activity displacing 2.6 times more ^{125}I-LDL after 4 weeks. Apparent increase of apoprotein E in HDL_c which binds with higher affinity than apoprotein B of LDL to cell surface receptors was thought to be responsible for the higher activity, however, the relationship to atherosclerosis remains unclear.

Another "abnormal" lipoprotein that has been reported is β-VLDL. Eisenberg and Levy (1975) observed the presence of β-VLDL in CHOL-fed animals. This lipoprotein was described as a CHOL-rich, triglyceride poor particle in the VLDL density range but with β-electrophoretic mobility and containing excessive amounts of arginine-rich apoprotein E. Mistry et al (1977) reported that VLDL in 6 subjects showed mobility, decreased particle size distribution and increased arginine-rich apoprotein E content similar to earlier observations in animals.

From these few human studies, it appears that the addition of 3 or more eggs to an uncontrolled diet produces some changes in lipoproteins which may or may not be

apparent in CHOL fraction determinations and are subject to much individual variability.

With respect to animal studies, nonhuman primates have proved most useful due to their close phylogenetic relationship to man. It must be cautioned, though, that the magnitude and distribution among different classes of lipoproteins of different species is variable and may not simulate human response (Scrinivasan et al, 1976). The nature of animal studies allow the researcher to maintain more rigid dietary controls than seen in the free-living human subjects. However, in some, increases in dietary CHOL are accompanied by alterations in dietary fat so that CHOL is not the sole dietary variable.

Scrinivasan et al (1976) attempted to identify the nonhuman primate species most representative of man by feeding 6 species increasing amounts of CHOL (.05% to 1.5% w/w) for 3 week periods intermittently with 3 week low-CHOL rest periods. The species were ranked as follows according to their mean SCHOL response at 0.5% dietary CHOL: squirrel $>$ green $>$ spider $\simeq$ rhesus $\simeq$ patas $>$ chimpanzees. Individual lipoprotein classes also varied; response was greatest in LDL of chimpanzee followed by VLDL fraction

whereas spider monkeys showed major increase in HDL. The authors concluded that the chimpanzee appeared most comparable to humans. In a longer 6 week study period, Scrinivasan et al (1978) noted a dramatic increase in SCHOL with 0.36% (1mg/kcal) exogenous CHOL in squirrel and spider monkeys with the observed changes mainly in LDL and HDL fractions. The HDL response again varied among species. Increases in serum LDL_2, VLDL and IDL, as shown by Mistry et al (1977), were also reported by Lee and Morris (1976) in rhesus monkeys fed a high CHOL (1.2% w/w from egg yolk), high fat diet for 3 weeks. Since the basis of comparison was a low CHOL, low fat diet period, a change in fat composition accompanied the increase in CHOL. Very low density lipoprotein and IDL-CHOL fractions increased most dramatically followed by LDL_2 which was the predominant CHOL carrying fraction. The HDL fraction remained relatively the same with percentage CHOL content actually decreasing in contrast to percentage increases reported by Mistry et al (1977). The increase in total SCHOL correlated with an increase in CHOL esters and a decrease in protein content of LDL_2 not present in monkeys with naturally occurring hypercholesterolemia; this change did not appear to reflect

dietary fat composition. The authors felt that the enzyme systems which synthesize LDL components are specific and incorporate preferred fatty acids. Cholesterol oleate, the chief substance in early atherosclerosis lesions increased significantly which may have some implication in the relation to heart disease and suggests a dietary influence.

In a longer term study, Illingworth et al (1975) fed monkeys for approximately one year in order to investigate the effects of dietary induced hyperlipidemia specifically with regard to LDL. One group of squirrel monkeys were made hypercholesterolemic when fed a diet containing 25% butter and 0.5% CHOL w/w for 9 to 12 months. The lipoprotein response was restricted to increases in LDL CHOL with other fractions remaining normal. Further long term studies by Portman et al (1976) led to the conclusion that PCHOL and LDL-CHOL were greater in squirrel monkeys fed CHOL than in those not. These studies demonstrate several trends in the dietary CHOL-lipoprotein relationship. First, the addition of egg yolk is often followed by increases in SCHOL reflected mainly in percentage increases in VLDL and IDL, and LDL_2 absolute increases in LDL_2.

The fractional changes in HDL, on the other hand, appear to be affected by many variables such as individual and species differentiation, experimental period, fatty acid composition and amount of CHOL. The behaviour of HDL may have an influence on susceptibility to atherosclerotic lesions. Secondly, the addition of CHOL with or without increased fat to the diet is associated with the appearance of two "abnormal" lipoproteins, β-VLDL and HDL_c. The arginine-rich apoprotein E is the predominant protein of both in addition to apoprotein B in β-VLDL and apoprotein A-1 in HDL_c. The ratio of β-VLDL to HDL_c is thought to reflect the degree of cholesterolemia (Eisenberg and Levy, 1975).

R E S E A R C H P A P E R

The Effect of Egg Consumption in Self-Selected and Constant Diets on Plasma Lipids of Healthy Young Men

Introduction

There is lack of agreement in the research literature as to the relative effect of dietary cholesterol (CHOL) on blood CHOL. For experiments which have utilized egg as the CHOL source in mixed food diets fed to healthy young subjects, researchers have drawn varied conclusions. Generally, the data have shown a linear or curvilinear response between dietary and blood CHOL when the dietary variables are constant. Whereas less controlled experiments, designed to show the effect of additional CHOL to habitual diets, have produced no significant response.

In laboratory controlled trials, Connor et al (1964) reported distinct increases in mean serum cholesterol (SCHOL) when 265 mg egg yolk CHOL/1000 kcal was incorporated into the low CHOL mixed food diets of 6 healthy young males. These results were confirmed in the authors' laboratory

(Morris, 1977), when 0 to 6 whole eggs provided dietary CHOL, and compared closely to those observed by Mattson et al (1972) who used formula diets. An increase of 12 mg/dl SCHOL accompanied each additional 100 mg dietary CHOL/1000 kcal within a range of 0 to 317 mg CHOL/1000 kcal. Such research assigns considerable importance to this lipid component of the diet.

There has been recent interest concerning the effect of egg rather than the effect of CHOL per se, added to the diets habitually consumed by free living subjects. The most striking aspect of these studies is the lack of a significant effect on blood lipids. No change in plasma cholesterol (PCHOL) was found by Slater et al (1976) when 2 whole eggs were included in the habitual diets of healthy male university students. Similar results were reported by Porter et al (1977) and Flynn et al (1979) with an older group of subjects. The data shows that when a moderate amount of CHOL, as in whole egg, is added to habitual diets, the hypercholesterolemic effect is reduced.

Variables inherent in the experimental design may influence blood CHOL so that it is difficult to isolate

dietary CHOL as the sole variable. The problem of monitoring change in dietary patterns, especially when extrapolating from dietary records, is exemplified by significant variation of pre and post experimental PCHOL, as noted by Slater et al (1976), despite the assumption of similar dietary intakes. Also, the basis on which the degree of change is calculated may be influential. Most laboratory controlled experiments monitor the change by comparison with lipid levels during a standard CHOL-free period, since change appears to be of greatest magnitude within this range. One to 2 eggs may not be sufficient to produce significant blood CHOL increases for subjects who are consuming habitual amounts of CHOL. Individual variability and regulatory control mechanisms may be significant factors in maintaining blood CHOL levels. The lack of consistency in the reported results of such studies suggest the need for further evaluation of the effect of egg consumption in reference to the customary diets of subjects.

Furthermore, perhaps a more reflective parameter, for example, lipoprotein fractions, is needed for interpreting the complex dietary and non-dietary factors

that interact in regulating blood CHOL metabolism. It is possible that dietary CHOL could affect one or more of the various lipoprotein fractions without changing the SCHOL concentration (Mahley et al, 1978) or that the changes in individual fractions may be more meaningful than the total change (Mistry et al, 1977). To date, there is limited data which examines human lipoprotein response to dietary variables. This, in itself, is adequate reason to explore the lipoprotein profile in further metabolic investigations of blood lipids.

The primary objective of this study was to examine the effect of the ingestion of additional eggs in self-selected and constant diets on human plasma lipids. The experiment consisted of 2 parallel studies. In one, the subjects continued their regular dietary patterns with the addition of 3 eggs daily. In the other, the subjects were fed a constant weighed mixed food diet with 3 eggs incorporated into the total diet. The same subjects and experimental protocol were utilized in both studies in order to reduce variability, and a mixed diet to ensure a normal absorptive challenge. A reasonable amount of dietary CHOL, 3 whole eggs daily, which is in excess of

the mean daily intake of 44g egg/day (approximately one egg) reported by the Health Protection Branch (1977) for adult males (20 to 39 years), was thought to be adequate to produce detectable blood lipid change. This was supported by the mean change of +19.8 mg/dl reported by Mattson et al (1972) when subjects' pre-test mean SCHOL was compared with their SCHOL values during the 317 mg CHOL/1000 kcal feeding period.

Materials and Methods

A. Subjects

The subjects were 8 healthy male university students, age 20 to 25 years ($\bar{X}$ = 23 years) recruited by notices posted on the campus which advertised free meals plus a cash bonus. Physical data is shown in Table 1. They were selected on the basis of a personal interview and a medical examination. Their mean height was 181 cm and their mean weight was 73 kg. They had an initial mean PCHOL of 163.4 mg/dl (range 141 to 223 mg/dl). None of the subjects had a personal history of cardiovascular disease but 3 had a family history of premature cardiovascular disease (occurring before the age of 55

Table 1

Subject Physical Data

Subject	Age	Height	Initial Weight	Weight Change	
				Period A	Period B
	yrs	cm	kg	kg	kg
CM	23	179	74	+2.3	-0.9
SD	21	176	72	-2.5	+0.8
BG	20	180	75	+1.3	-0.9
PG	25	169	65	+0.9	-0.9
BR	23	178	67	+0.9	-0.9
PGal	25	180	74	+0.5	-1.1
KH	24	179	74	0.0	-1.4
DR	21	185	78	+1.0	-0.9
$\bar{X}$	23	181	73	+0.6	-0.8

in either parent). All subjects remained in good health during the course of the study. Subject mean weight change during period A was +0.6 kg and -0.8 kg during period B.

B. Experimental Design

During the first 21 day period (period A), subjects continued their regular diets and consumed an additional 3 whole eggs daily. Contact was maintained with the subjects by triweekly visits to collect eggs, to give blood or for dietary recalls. The subjects were weighed weekly. Following period A, subjects continued their habitual eating patterns without the additional eggs for a 7 day period. They were then fed a constant diet (with 3 eggs/day incorporated) for a 21 day period (period B). Meals were served in the apartment area of the Home Economics Building. The periods were ordered in this manner to eliminate possible effects of laboratory feeding periods on subsequent food habits that might have occurred with a crossover design.

C. Diet

In period A, subjects consumed their regular diets and were instructed to add 3 eggs daily. They were asked to

consume no other visible eggs outside of those supplied. All eggs used in the study were from Shaver hens housed in a single pen in the Animal Science Department at the University of Manitoba. Twenty-four hour dietary recalls were taken on days 6, 9, 15 and 22 of this period. Nutrient calculations were derived with the use of USDA Handbook No. 8[1] and the 1979 Revised Nutrient Values of Some Common Foods[2], and are summarized in Table 2. The calculation of diet A revealed an intake of 41% kcal fat, 17% kcal protein, 2752 kcal and 1245 mg CHOL. Other nutrients satisfied the 1975 revised Canadian Dietary Standard recommendations for 16 to 35 year old males.

In period B, two day rotating menus of selected foods were designed to simulate that of the typical Canadian diet. Three eggs were incorporated into the diet. Diet B was found, by analysis, to contain 116g fat, 110g protein, and 656 mg CHOL (Table 2). The gross energy content of the diet without eggs was 2884 kcal and the physiological energy content of the total diet was 2881 kcal/day.

1 Composition of Foods (Watt and Merrill, 1975).

2 Published by authority of the Minister of National Health and Welfare. Health Services and Promotion Branch and Health Protection Branch.

Table 2

Composition of Diets

Period	Method	Energy	Protein	Fat	Cholesterol	Campesterol	β-sitosterol
		kcal	g	g	mg	mg	mg
A	Calculation	2752	115	127	1245		
B	Calculation	2881	122	117	1275		
	Analysis						
	Diet Excluding Eggs[1]	2884[2]	90	101	161	56	350
	Eggs[3]	254	20	15	495		
	Total	3138	110	116	656	56	350

1 Mean value of quadriplicate analysis of both menus.

2 Gross energy.

3 Protein and energy values for 3 eggs calculated from USDA Handbook 8.

Amounts of campesterol and β-sitosterol are given in Table 2. Amounts of α-tocopherol and stigmasterol were less than 15 mg/day and are not included in the table. The percent fatty acid composition is found in Table 3. Estimated vitamin and mineral content satisfied the 1975 revised Canadian Dietary Standard. Calories were adjusted for weight maintenance by adding a 515 kcal supplement with 33% calories as fat and 11% as protein.

Comparison of CHOL intake during the two periods is complicated by the fact that the AOAC method used yielded lower values (Punwar, 1975; Sheppard et al, 1977) than those found in the revised 1979 Nutrient Value of Some Common Foods (Feeley, 1972) used for calculations. These tables gave a value of 333 mg CHOL for the 20.7g yolks used in this study. This value is higher than the currently accepted value of 250 mg CHOL/large egg (Slater et al, 1976) and much higher than the 165 mg CHOL/yolk derived by the AOAC method. However, since the 3 eggs provided the major portion of dietary CHOL, and the calculated values between periods were comparable, it is assumed that CHOL intakes in period A and B were similar. Fat decreased from 41% in period A to 36% of total

Table 3

Percent Fatty Acid Methyl Esters of Diet B[1]

Fatty Acid	Percent of Total Fatty Acids	
	Diet Excluding Eggs[1]	Egg Yolk
C10:0	0.56	
C10:1	Tr[2]	
C12:0	0.70	Tr
C12:1	Tr	
C13:1	Tr	
C14:0	2.74	Tr
C14:1	0.56	Tr
C14:2	Tr	Tr
C15:1	Tr	
C16:0	16.42	26.49
C16:1	1.77	4.55
C16:2	Tr	Tr
C17:1	Tr	Tr
C18:0	7.18	8.53
C18:1	37.85	47.13
C18:2	27.21	10.75
C18:3	Tr	Tr
C20:1	Tr	
C20:4		1.09

1 Values are means of quadriplicate determinations of both menus.

2 Assigned to any value less than 0.5%.

physiological energy. Fatty acid composition of diet B, shown in Table 3, modelled after that used in other metabolic studies (Morris, 1976), was designed to simulate the fatty acid composition of the average Canadian diet. Protein decreased from 17 to 15% and carbohydrate increased from 42 to 49% of total physiological energy from period A to B. Caloric intake was higher in the latter period. Combined with the fact that 3 subjects required the 515 kcal supplement and that all maintained their weight during period B, the dietary recalls of period A are thought to underestimate energy intake.

D. Collection and Analysis of Samples

1. Diets from Period B

Duplicate composites of one half of each menu, excluding eggs, were weighed on a Sartorius top-loading balance and homogenized with 200 ml glass distilled water in a Waring commercial blender. Weighed aliquots of this homogenate were lyophilized in a Virtis freeze dryer[3]. Pulverized samples were stored in whirl-pak plastic bags at -10C for later analysis. Weighed aliquots of homogenized egg yolk samples were stored in sealed containers at -10C for later

[3] Model 10-140 MR-BA.

analysis. Total lipid was extracted according to the method of Bligh and Dyer (1959) using a monophasic mixture of chloroform: methanol: water. The chloroform layer was dried and total lipid determined. An aliquot of the lipid, dissolved in hexane, was flushed with nitrogen and stored at -10C for fatty acid analysis. This was saponified and methyl esters were prepared according to the method of Metcalfe et al (1966). The esters were resolved on 6' x 1/8" O.D. stainless steel column packed with 10% EGSS-Y on 100/120 mesh Gas Chrom Q in a Varian Aerograph gas chromatograph[4] equipped with a Hewlett Packard digital integrator to determine the percent fatty acid methyl esters. Sterol analysis of the food composite and egg samples were performed by the official method of the AOAC (1975). A Perkin Elmer gas chromatograph[5] equipped with a dual flame ionization detector and a Hewlett Packard reporting integrator was used. The sterols were resolved using the alternative column described in the method. Total nitrogen content of the diet was measured by the modified Kjeldahl procedure (AACC, 1962). Titanium dioxide was used as the catalyst (Williams, 1973). A factor of 6.25 was used to calculate the quantity of protein. Gross energy of the diet samples was determined using a Parr Adiabatic

4 Model 1740-1

5 Model 3920-B

Calorimeter.[6]

2. Blood Samples

Venous blood samples from fasted subjects were drawn on day 1, 8, 15 and 22 of each period. Blood was drawn into a 7 ml BD vacutainer tube, containing 15% EDTA solution, for whole blood analysis at the Hematology Laboratory, Health Science Center, Winnipeg. On day 1 and 22, two additional 10 ml tubes, containing powdered EDTA were collected and sent on ice to the Metabolism and Endocrinology Laboratory, Health Science Centre, Winnipeg, for triglyceride, total PCHOL, low density lipoprotein cholesterol (LDL-CHOL) and high density lipoprotein cholesterol (HDL-CHOL). The analysis was done on the Beckmann Auto Analyzer using the enzymatic assay for total CHOL (Sontrop et al, 1978), the method of Albers et al (1978) for HDL-CHOL and the method of Kessler and Lederer (1965) for triglycerides. The LDL-CHOL was calculated by formula (Friedwald et al, 1972). A randomized block design analysis of variance was applied to each lipid parameter to reduce

6 Model 1241

the experimental error and to see whether evidence existed which indicated a difference in the mean response for blocks or subjects.

Results and Discussion

Individual and mean plasma total CHOL, HDL-CHOL, LDL-CHOL and triglyceride values for subjects for the first and last day of each dietary period are shown in Table 4. Three eggs, fed either in addition to the regular diet or included in a constant diet, did not have any significant effect on plasma total CHOL, HDL-CHOL and LDL-CHOL; the population treatment means were not significantly different. The population treatment means of the triglycerides were different at $p = 0.01$. Tukey's Test revealed that the triglyceride mean on day 22A was significantly greater than the mean triglyceride level on day 22B at the 5% level. There was a mean increase in PCHOL of 1 mg/dl in period A and 5 mg/dl in period B. The HDL-CHOL decreased 2 mg/dl in period A and increased 4 mg/dl in period B. The change in LDL-CHOL was -4 and +4 mg/dl, respectively. Plasma triglycerides increased 24 mg/dl in A and decreased 23 mg/dl in period B. The mean decrease in PCHOL of 8 mg/dl during the 7 day rest period, utilized to reestablish the baseline, was

Table 4

Plasma Total CHOL, HDL-CHOL, LDL-CHOL, and Triglycerides for Subjects Fed Three Eggs in Two Different Experimental Regimes

Subject	PCHOL mg/dl				HDL-CHOL mg/dl				LDL-CHOL mg/dl				Triglycerides mg/dl			
	Day				Day				Day				Day			
	1A[1]	22A	1B	22B	1A	22A	1B	22B	1A	22A	1B	22B	1A	22A	1B	22B
CM	160	139	143	139	65	52	53	63	84	76	75	66	54	57	74	52
SD	135	137	141	134	55	49	53	55	68	67	70	68	59	103	88	56
BG	163	159	164	161	44	45	45	42	105	95	109	106	71	95	48	64
PG	149	162	141	161	46	58	45	56	87	102	84	105	81	76	61	44
BR	223	225	201	200	32	34	49	47	176	147	134	133	76	124	92	54
PGal	141	159	143	175	45	34	44	50	71	93	77	110	127	109	112	74
KH	163	147	153	155	42	47	42	43	108	84	93	96	66	143	88	78
DR	173	183	164	166	44	46	42	53	115	118	106	101	71	90	104	61
$\bar{X}$	163	164	156	161	47	45	47	51	102	98	94	98	76	100	83	60
S.D.	±27.2	±28.7	±20.5	±20.7	±9.7	±6.7	±4.5	±7.1	±34.5	±25.3	±21.7	±22.1	±22.5	±26.9	±21.4	±11.4

[1] 1A = day 1, period A.

greater than the change with egg addition. This may suggest a voluntary reduction in usual egg consumption or a possible sequence effect. For example, Flynn et al (1979) observed that the sequence of egg to no egg produced a greater effect than no egg to egg.

Some individual variability in plasma lipid response was noted. Subjects PG and PGal responded with greater PCHOL increases (+13 and +18 mg/dl) in period A and even larger increases in B (+22 and +32 mg/dl) whereas the rest of the subjects were relatively unresponsive. No reason for this difference was obvious from subjects' weight, age, initial lipid values, medical history or compliance. In a recent paper, Brongeest-Schonte et al (1979) reported a small subgroup (7/44) of hyperresponders, in a similar study, who exhibited much greater decreases in SCHOL than the majority of the subjects when eggs were removed from the diet. Slater et al (1976) found, in one study, that 3 subjects out of a total of 22 accounted for 36% of the increase in PCHOL. Although many researchers have commented on the marked variability among subjects in response to dietary CHOL, the relative proportion of those hyperresponders has not been well documented.

There are several possible explanations for the lack

of effect of added eggs on the plasma lipids when compared to pre-experimental levels. Firstly, the amount of dietary CHOL consumed during the treatment periods may not have differed sufficiently from pretest intakes. Although the CHOL content of the regular and constant diets (1245 and 1275 mg/day, respectively) differs markedly from zero CHOL intake commonly used as a basis for comparison in controlled experiments, these intakes may not be adequate to produce significant difference when compared to the estimated average intake of 300 - 800 mg/day for North Americans (Flynn et al, 1979). Similar findings have been noted in other studies. For example, Connor et al (1961) reported that 475 to 1425 mg of egg CHOL daily produced similar increases in SCHOL when compared to serum levels for a CHOL-free diet but did not differ greatly from the subjects' pretest SCHOL levels. The PCHOL levels which resulted from the consumption of a formula diet containing 742 mg CHOL/day did not differ from those produced by the subjects' normal prison diet in a study conducted by Erickson et al (1964). Again, change was apparent only in comparison to blood CHOL during a low CHOL period. This supposition is supported by work done earlier in this laboratory (Morris,

1977) and can fit the linear relationship hypothesis presented by Mattson et al (1972). Morris (1977) found that initial mean SCHOL levels did not change appreciably over a 21-day laboratory feeding period for healthy young subjects fed 2 or 3 whole eggs when total CHOL intake was 623 mg or 826 mg/day. This does not mean that dietary CHOL has no effect on blood CHOL since both Morris (1977) and Mattson et al (1972) observed a linear relationship over the range of 0 to 6 egg level. One might therefore, expect that if a larger amount of CHOL had been fed to the subjects in this study, an increase in PCHOL may have occurred.

Secondly, the addition of CHOL may not be effective due to an internal synthesis control mechanism operating particularly efficiently in young healthy subjects (Beveridge et al, 1960). It is known that dietary CHOL inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase via a negative feedback mechanism. Increased excretion provides another mechanism of control. Beveridge et al (1960) have shown a "plateau" effect of dietary CHOL in SCHOL when 634 mg CHOL/day was ingested. As previously mentioned, Connor et al (1961) found that amounts of CHOL greater than 475

mg/day produced a similar response. In 1965, Keys et al pooled the results of these and of other investigators to formulate the curvilinear hypothesis of the dietary-blood CHOL relationship. One could predict substantial response at low levels of CHOL intake, for example, less than 300 mg/day, and a lesser effect at higher levels of intake. On this basis, it could be expected then that the addition of 3 eggs to regular diets would stimulate little response since these intakes would fall on the flat portion of Key's dose-response curve. The lack of serum response to dietary eggs observed by Slater et al (1976), Porter et al (1977), Flynn et al (1979) and Kummerow et al (1977) may also be attributed to these factors.

Additional factors may be in part responsible for the lack of response. Essentially, these revert to the difficulty of comparing constant to self-selected diets, with or without added eggs, and to a lesser extent comparing the self-selected diet with added eggs to that without. Some of the variables involved are changes that may have occurred in fat composition and fiber intake. In addition, intakes in the self-selected diet are not perceived in sufficient detail for accurate comparison

with the precise estimates of the constant diet.

A possible change in fatty acid composition may have effected the outcome. The P/S ratio of the constant diet was approximately 0.9. Although sufficient information about fat sources was not available from dietary recalls to calculate P/S ratio in the self-selected diet, it is thought that it may have been less than in the constant diet. Mattson et al (1972) utilized a P/S ratio of 0.3 (12% polyunsaturated and 40% saturated fatty acids of total fat) to simulate average consumption which was considerably lower than that utilized in period B of this study. Keys et al (1965) have demonstrated the hypocholesterolemic effect of decrease in percentage intakes of saturated fatty acids. Saturated fatty acids comprising less than 9% of total calories have been utilized to demonstrate such effect in relation to habitual diets (The National Diet-Heart Study, 1968); saturated fats comprised approximately 9% of calories in this study. On the other hand, Hegsted et al (1965) have emphasized that the P/S ratio is not valid for comparisons in view of the varied effect of individual fatty acids on serum cholesterol. In addition, Connor et al (1964) and Brongeest-Schonte et al (1979) have illustrated that the

effect of CHOL is dependent on the fatty acid composition whereas others (Anderson et al, 1976; Erickson et al, 1964) have shown the effect of CHOL to be independent. Consequently, the decrease in saturated fat and increase in polyunsaturated fat that may have occurred in period B may offset any increase in PCHOL due to an increase in dietary CHOL.

An increase in fiber during period B was noted from examination of food recalls versus laboratory diet and increase in fecal bulk observed by the subjects. It appeared to be due to increased consumption of vegetables and wholewheat bread in period B. Thus, increases in pectin in particular (Jenkins et al, 1979) may have masked the hypercholesterolemic effect of added eggs. It appears unrealistic to compare the constant diet to the self selected diet or either to pre-experimental diets as they represent entirely different diets; the effect of egg addition is only one of many variables.

The length of feeding period may influence outcome in 2 respects. First, the 7 day period without eggs may not have been adequate to achieve stabilization of lipid patterns since a minimum of 3 weeks is needed for blood lipid

adjustment (Keys et al, 1965). Although statistical analysis monitored for difference in mean response, the pre-experimental periods provided the basis for detection of change. The basis of comparison, with regard to duration as well as CHOL content and general composition, is a critical factor in such experiments. Secondly, periods longer than 3 weeks may be necessary to achieve equilibrium as noted by Mattson et al (1972) and Slater et al (1976). In addition, Mistry et al (1977) and Honda and Sumikura (1976) have reported 4 or more weeks are required after change in egg consumption for HDL-CHOL to return to pre-test values.

Much of the explanation for lack of response of total PCHOL explains the lack of change in lipoprotein fractions. Changes in lipoprotein fractions, reported by researchers who added eggs to the diet, were usually accompanied by concurrent increases in PCHOL. The amount of change in PCHOL required, if any, before alternations in lipoprotein CHOL fractions are observed, or vice versa, has not been documented. With regard to individual values rather than group means, LDL-CHOL generally paralleled total PCHOL whereas HDL remained stationary or changed in the opposite

direction. These trends are in agreement with current knowledge regarding the amount of CHOL carried by and the function of these lipoproteins.

In contrast to the findings of this study, several researchers have reported response in these lipoprotein fractions when eggs were added to a self-selected diet. Mistry et al (1977) reported change of -5% to over 20% in SCHOL reflected mainly in VLDL and IDL percentage increases and absolute increases in LDL for intakes of 750 and 1500 mg CHOL/day. High individual variability of response was noted but statistical analysis was not applied. The addition of 3 or 5 eggs to the habitual diet of students was found by Honda and Sumikura (1976) to produce significant increases in serum LDL, total CHOL and triglycerides after 7 to 9 weeks of feeding. Notably, significant change for 3 eggs took longer to occur than at the higher intakes. Mahley et al (1978) reported that PCHOL increased (approximately 23%) in about half of the subjects when 4 to 6 eggs were added daily to the subjects habitual diet for 4-week periods. High density lipoprotein showed no change in amount of CHOL carried, regardless of increases in SCHOL however, all showed increased binding

activity. These studies concentrated on individual responses rather than statistical analysis of group responses and the results are not conclusive. It appears that larger CHOL intakes and perhaps longer test periods were required to produce specific changes in lipoprotein fractions.

The mean triglyceride levels were significantly different; the mean level was higher at the end of period A than at the end of period B. The addition of eggs did not appear to have an effect on plasma triglycerides. Triglycerides have been found to be varied and less consistent in response to dietary CHOL than plasma cholesterol (Keys et al, 1965; Hegsted et al, 1965). Change in carbohydrate is thought to have more effect on triglycerides (Grande et al, 1965). High carbohydrate diets, for example, one in which carbohydrate comprises over 75% of the total calories, cause an increase in plasma triglyceride concentration. However, in this experiment, the carbohydrate content of period B was slightly higher than period A. It was noted from comparison of food intakes, that a greater amount of sucrose, as snack foods, was consumed in period A than in period B. Evidence suggests that sucrose causes greater hypertriglyceridemia than does

starch (Albrink, 1973). This may provide an explanation for the higher triglyceride levels in period A. Overall, these lipid levels were higher on the self-selected diet than during the constant diet.

Conclusion

The consumption of 3 eggs daily by young adult males did not have an effect on total PCHOL, LDL-CHOL, or HDL-CHOL. The outcome was similar whether the eggs were added to a self-selected diet or included as part of the total lipid in a constant diet. The self-selected diets of the subjects contained an average of 246 mg CHOL daily on the basis of four 24-hour recalls. The addition of 3 eggs did not increase the CHOL intakes in excess of 800 mg daily, which is considered to be the upper limit in the Canadian diet. Plasma triglycerides decreased during the 21-day period when the subjects consumed the constant diet. It is postulated that this decrease may be partially attributed to a decrease in the intake of sucrose. This study showed that an estimation of the nutrient intakes for self-selected diets results in intakes which may deviate markedly from mean intakes for population groups.

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Appendix Table 1

Analysis of Variance: Plasma Total Cholesterol

Source of Variance	df	SS	MS	F-Value	p
Treatment	3	291.35	97.12	1.01	n.s.
Block	7	14870.72	2124.39	22.09	0.01
Error	21	2019.40	96.16		

Appendix Table 2

Analysis of Variance: Plasma Low Density Lipoprotein Cholesterol

Source of Variance	df	SS	MS	F-Value	p
Treatment	3	273.60	91.20	.65	n.s.
Block	7	16617.72	2373.96	16.96	0.01
Error	21	2940.16	140.00		

Appendix Table 3

Analysis of Variance: Plasma High Density Lipoprotein Cholesterol

Source of Variance	df	SS	MS	F-Value	p
Treatment	3	14.50	11.50	.40	n.s.
Block	7	1027.00	146.71	5.11	0.01
Error	21	603.50	28.74		

Appendix Table 4

Analysis of Variance: Plasma Triglycerides

Source of Variance	df	SS	MS	F-Value	p
Treatment	3	6404.50	2134.83	7.31	.01
Block	7	6586.50	940.93	3.22	.05
Error	21	6135.00	292.14		

Appendix Table 5

Mean Nutrient Values and S.D. for Dietary Recalls During Period A, and Calculated Nutrient Composition of Diet B

Diet	Energy	Protein	Fat	Carbo-hydrate	Calcium	Phos-phorus	Iron	Sodium	Potas-sium	Vitamin A Value	Thiamin	Ribo-flavin	Niacin	Ascorbic Acid	Chol-esterol
	kcal	g	g	g	mg	mg	mg	mg	mg	IU	mg	mg	mg	mg	mg
A	2752	115.1	126.5	294.3	1237.8	1964.4	33.8	3148.9	3295	15890	3.73	2.79	25.07	96	1245
	±378	±15.4	±26.2	±56.3	±357.7	±403.9	±29.2	±577.7	±890	±2035	±3.42	±.71	±8.58	±75	±86
B	2881	122	117	335	1612.0	2269.6	15.5			9941	1.64	2.93	46.67	152	1275