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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TABLE OF CONTENTS

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.

A C K N O W L E D G E M E N T S

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L I S T O F T A B L E S

L I S T O F A P P E N D I C E S

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