THE EFFECT OF EGG YOLK CHOLESTEROL ON SERUM LIPID PATTERNS AND SERUM CHOLESTEROL SPECIFIC ACTIVITY OF HEALTHY YOUNG MEN

A Thesis

Presented to

THE FACULTY OF GRADUATE STUDIES

University of Manitoba

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

DEPARTMENT OF FOODS AND NUTRITION

by

Marsha Lynn Morris

July, 1977

THE EFFECT OF EGG YOLK CHOLESTEROL ON SERUM LIPID PATTERNS AND SERUM CHOLESTEROL SPECIFIC ACTIVITY OF HEALTHY YOUNG MEN

BY

MARSHA LYNN MORRIS

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TABLE OF CONTENTS

ABSTRACT

A metabolic study was undertaken to examine the response of serum lipids and serum cholesterol specific activity in healthy young men to cholesterol intakes of 113 to 1819 per day. Five diets, a 0-Egg, 1-Egg, 2-Egg, 3-Egg and 6-Egg diet were designed to differ in the content of egg yolk cholesterol only. A mixture of fats, formulated to simulate the fatty acid composition of the average Canadian diet, provided three-quarters of the calories from fat in each of the experimental diets. This mixture was prepared from lard, tallow, corn oil, butter and hydrogenated soybean oil. The remaining one-quarter of the calories from fat was provided by egg yolk lipid, simulated yolk fat prepared by combining Palm oil and hydrogenated soybean oil in a 2:1 mixture, or a combination of these depending upon the number of whole eggs in the diet. Using these fat mixtures ensured that fat provided 40% of the calories in the 3000 kcal diets and that the fatty acid composition of the five diets was similar.

The subjects were 10 young men 18-29 years of age. They resided in their own homes and maintained their usual activities throughout the study, but were required to eat their meals in the Home Economics facility on the university campus. Approximately 25 days prior to commencing the study, subjects were infused with 50 uc. of tritium labelled cholesterol. The 42 day metabolic trial was divided into 2 three-week diet periods. Two subjects were randomly assigned to each of the 5 diets for the first three weeks.

At the end of this diet period subjects were reassigned to different diets. This Incomplete Latin Square Design provided 4 replicate observations for each diet. Fasting venous blood samples were taken at the beginning of the study and at weekly intervals thereafter.

The level of cholesterol in the diet had a significant effect ($P<0.05$) on serum cholesterol levels. The relationship between dietary cholesterol and serum cholesterol was linear ($P<0.01$) and highly correlated ($r^2 = 0.90$). The relationship between the number of whole eggs consumed and serum cholesterol concentration is defined by the equation: serum cholesterol (mg/dl) = 147.7 + 11.9 (the number of whole eggs per day). Thus, for each additional egg in the diet (300 mg cholesterol) serum cholesterol increased by approximately 12 mg/dl. The effect of dietary cholesterol on serum phospholipids and triglycerides was not significant. The pattern of response in serum phospholipids was similar to that of serum cholesterol butthe magnitude of response was more variable. With an increase in egg consumption the proportion of cholesteryl arachidonate in the cholesteryl esters increased slightly, less than 5%, but consistently and this appeared to be primarily at the expense of cholesteryl linoleate. The proportion of saturated, monounsaturated and polyunsaturated fatty acids of cholesteryl esters was not altered by a change in egg consumption.

There was no significant change in the slope of the plasma specific activity - time curve for any subject when the level of cholesterol in the diet was changed. However, for several of the

subjects the change in serum cholesterol, consequent to a change in dietary cholesterol intake, was accompanied by a small change in the slope of the plasma specific activity - time curve. These changes are likely to be a result of an increase or decrease in absorption of cholesterol and thus an increase or decrease in the dilution of the plasma label, respectively. The transient nature of these changes suggests that in the human compensation for an increase or decrease in absorption of cholesterol occurs rapidly.

ACKNOWLEDGEMENTS

The author wishes to express sincere thanks to her advisors, Dr. Bruce McDonald and Dr. Vivian Bruce of the Department of Foods and Nutrition, University of Manitoba for their advice and guidance in the direction of this research and in the preparation of this manuscript.

Special thanks are also extended to Ms. Marie Fuller for her part in this research project and to Mr. John Bate, Mr. Leigh Campbell, Mr. Earl Cooper, Mr. Bruce Derraugh, Mr. Ron Everard, Mr. Darrell Grymonpre, Mr. Baldur Hafstedt, Mr. John Markowski, Mr. Harry Muc, and Mr. Barry Silver for their active participation as subjects in this metabolic study.

Gratitude is also expressed to Dr. Bruce Johnson of the Department of Statistics for his advice on the statistical methods employed in this study, Ms. Marilyn Latta and Ms. Stacy Johnson for their technical assistance throughout the course of the study.

The author also acknowledges the Canadian Egg Marketing Agency and the National Research Council of Canada for financial support.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

APPENDIX TABLE

INTRODUCTION

Death as a consequence of a compromised circulation to the heart, brain, kidneys and limbs is the most prominent cause of mortality operating in affluent societies today (Kannel, 1974). There is much evidence to suggest that modification of our life-style by modern technology - which has replaced manpower with machines thus diminishing the need for physical exercise while at the same time providing a surfiet of rich food and drink - has contributed to the increased toll by cardiovascular disease. That atherosclerosis is the leading cause of death in North America and that alteration of our environment may be promoting it demands some kind of action.

Atherosclerosis is a disease characterized by the accumulation of lipid substances in the walls of large blood vessels (Gresham, 1972). The disease is manifest as myocardial infarction or angina pectoris when the coronary arteries are involved and as stroke when the cerebral arteries are involved. It is a disease that evolves under the influence of multiple contributors and to date no single essential factor, without which the disease fails to occur, has been identified. While the general tendency to deposit lipid in the arterial walls is largely determined by the level of blood lipids and blood pressure, dynamics of blood flow, arterial calibre and the integrity of the vascular intima also influence whether and where atheromata will form (Altshule, 1974).

These parameters are under the influence of genetic, cultural and environmental factors. Of the cultural and environmental variables known to increase the risk of atherosclerosis many are under the control

of the individual; dietary habits, sedentary living habits, smoking habits and emotional stress. Genetic influences regarded as predisposing factors in atherosclerosis are generally not perceived as responsive to preventative intervention; racial influences, sex differences, familial history of vascular disease and diabetes mellitus.

The concept of multiple continuous interrelated variables has confounded delineation of the degree to which each of these contributes to the atherosclerotic process. Abnormalities considered atherogenic are more a matter of degree than kind - blood lipids, blood pressure and glucose tolerance. These biological factors are graded characteristics of normal body constituents, continuously distributed within the population with no discernible critical values separating diseased fron non-diseased individuals. As well, the complex tissue histology and haemodynamics influencing the evolution of atherosclerotic lesions, the inability at present to accurately assess the presence and extent of the disease in living persons and the lack of a satisfactory experimental animal model for study of the disease process have further complicated efforts to identify and isolate these predisposing factors.

Although cardiovascular disease has been correlated with a number of risk factors the one that stands out more than any other in clinical tests, animal experimentation and epidemiological observations is the association of coronary disease with blood lipid elevations. All serum lipids have been incriminated in atherogenesis: cholesterol, triglycerides, phospholipids and fatty acids as well as the associated lipid-protein structures which exist for lipid transport.

A disproportionate amount of coronary heart disease in the

general population develops among individuals with serum cholesterol values greater than 250 mg/ dl. (Dawber, 1962; Kannel, 1971; Schrimshaw and Gunzman, 1968). And, hypercholesterolemia appears to be a factor common to all forms of coronary heart disease, hence interest has focused on serum cholesterol. Epidemiological studies such as the Framingham Enquiry and the National Diet Heart Study have shown that diseases associated with hypercholesterolemia are also associated with premature atherosclerosis and that the risk of developing coronary heart disease is proportional to the elevation of blood cholesterol (Gordon, 1971; National Diet Heart Study Research Group, 1968). Countries with high average serum cholesterol values among their inhabitants report high coronary death rates, those with low values report lower rates (Epstein, 1965; Carlson and Bottiger, 1972). This association between blood cholesterol and atherogenesis has also been substantiated by clinical tests and animal experimentation: persons with inborn errors of cholesterol metabolism exhibit precocious development of atherosclerotic disease (Kannel, 1974); atherosclerotic deposits have abnormally high cholesterol concentrations and concentrations as high as 500% of those in normal aorta have been reported in fatty streaks of human aorta (Insull and Bartsch, 1966); movement of cholesterol from blood into atherosclerotic deposits has been demonstrated by Jagannathan et al (1974); inducing hypercholesterolemia in experimental animals produces atherosclerotic deposits and studies with dogs, fowl and primates indicate that these deposits can be made to regress by lowering serum cholesterol (Wissler et al, 1968; Armstrong et al, 1970).

Although the most intense focus has been directed toward serum

cholesterol and its relationship to atherosclerosis several other blood lipids have also undergone scrutiny in this regard. Turpeinen et al (1968) have stated that the risk for coronary disease can be shown to rise in relation to the serum phospholipid level. The significance of this is difficult to establish as phospholipid levels have been found to parallel those of serum cholesterol (Connor et al, 1961; Erickson et al, 1964; and Anderson et al, 1976). Insull and Bartsch (1966) have also reported phospholipid values in atherosclerotic deposits which are 150% of those in adjacent normal intimae. Since 1959 when Albrink and Mann suggested that elevated serum triglycerides might be related to the pathogenesis of heart disease several investigators (Brown, 1959; Albrink et al, 1961; Allard and Goulet, 1967; and Albrink, 1973) have demonstrated a firm relationship between elevated serum triglycerides and coronary heart disease. Carlson and Bottiger (1972) and Allard and Goulet (1967) have reported that coelevations of serum triglycerides and cholesterol are more critical in producing morbidity than an increase in either lipid component alone. And, Ho et al (1974) has observed that serum phospholipid and triglyceride levels of US whites are linearly related to serum cholesterol levels even in the presence of elevations in serum cholesterol.

Results of numerous controlled clinical trials have established that dietary lipids have an effect on serum lipid patterns in man. Diets high in saturated fats, containing high proportions of C12:0, C14:0 and C16:0 fatty acids have been shown to elevate serum cholesterol (Grande et al, 1972; Cobden, 1975). Diets containing high proportions of poly-unsaturated fats are generally conceived of as having a cholesterol

depressing effect (Sodhi et al, 1967; Nestel et al, 1975). Studies have also shown that dietary cholesterol has a cholesterol elevating effect (Mattson et al, 1972; Anderson et al, 1976). The changes induced in serum cholesterol by a change in dietary lipids are generally paralleled by changes in the serum phospholipid concentration (Connor et al, 1964; Grande et al, 1965; Cobden, 1975). It has also been suggested that the serum triglyceride concentration is responsive to changes in dietary fat and dietary cholesterol intake (Connor et al, 1964; Grande et al, 1972) and that the serum cholesteryl esters, phospholipid fatty acid esters and triglyceride fatty acid esters can be altered by changing the fat component of the diet (Ahrens, 1957; Nestel et al, 1965).

To counteract the present incidence and mortality from atherosclerosis in North America, the diet should be modified to minimize elevations of serum lipids. Epidemiological studies have shown that populations who consume diets low in fat, such as those which characterize tribal African and rural Japanese populations, have a much lower incidence of atherosclerotic disease (Wynder et al, 1972). Metabolic studies have indicated that dietary alterations should include, besides a decrease in total fat, replacement of animal fats with vegetable oils in order to increase the proportion of polyunsaturated fatty acids and to decrease the proportion of saturated fatty acids and cholesterol in the diet. But generally these diets tend to be less acceptable to the North American population at large.

Many of the early studies which implicated various fats in the diet as being atherogenic were conducted with institutionalized subjects who were fed formula diets or with subjects with manifest hyper-

lipidemia and/or atherosclerosis. It now seems appropriate to investigate the effects of these dietary fats on serum lipid patterns in healthy free living individuals in order to determine which dietary alterations are truly beneficial with respect to serum lipid levels. At the same time it is imperative that it be determined by what mechanism the dietary alteration effects these changes in serum lipids. That is, it is unacceptable if a decrease in serum lipids is brought about by deposition of lipid in tissues as it is likely that localized accretion of lipid in arterial walls is critical in the development of atherosclerosis. With these facts resolved it will be possible to make realistic recommendations with respect to preventative measures that will be beneficial to the North American society at large.

A very important food, in terms of the Canadian economy as well as the nutrient needs of Canadians, which contains a high level of dietary cholesterol is the egg. Since the general recommendation to Canadians is to decrease their egg consumption to 3 eggs per week the effect of egg yolk cholesterol on serum lipids of healthy free living individuals needs to be better defined to justify these practices. The present study investigated the effect of egg yolk cholesterol on serum lipids in healthy young men consuming a mixed diet and the mechanism whereby dietary cholesterol effects a change in serum cholesterol levels.

OBJECTIVES

The primary objective of this research was to investigate the effect of dietary cholesterol from egg yolk on cholesterol metabolism of healthy young men. Five levels of cholesterol intake were achieved by incorporating 0, 1, 2, 3 and 6 whole eggs into diets consisting of natural foods in which 40% of calories were supplied by egg yolk lipid and a mixture of fats similar to those in the Canadian diet. The diets resembled the average Canadian diet with respect to proportion of calories from fat, protein and carbohydrate and fatty acid composition but provided five levels of dietary cholesterol (113, 441, 781, 1058 and 1819 mg/day). The effects on cholesterol metabolism were assessed by determining serum cholesterol levels and changes in the specific activity of serum cholesterol. Secondary objectives included examination of the response of serum triglycerides, serum phospholipids and fatty acid patterns of serum cholesteryl esters to egg cholesterol.