

SUMMARY PROGRESS REPORTS

A) Summary Page

1. Research Grant Ho. HE 7410
Feasibility Study - Diet and Heart Disease (closed study at Faribault, Minnesota.
2. Principle Investigator - Ivan D. Frantz, Jr., M.D.
Co-Principle Investigators - Ancel Keys, Ph.D., and Edward John Engberg, M.D.
3. Name of sponsoring institution -
The University of Minnesota
4. Period covered by the report -
June 1, 1952 through November 1, 1963.
5. Date of preparation of the report -
November 22, 1963
6. Summary Statement -

Work on *the* project has progressed in strict accordance with the protocol as agreed upon by the Executive Committee of the National Diet Heart Study. The following steps have been accomplished;

- (1) Physical examinations, electrocardiograms, and chest x-rays were completed on all men in the Faribault State Hospital considered potentially eligible for the study.
- (2) Serum for cholesterol determination was sent to the Communicable Disease Center in Atlanta on all of these potential subjects on three occasions at least two weeks apart.
- (3) Red cells and adipose tissue specimens were collected from all for gas-liquid chromatography,
- (4) All needed personnel were hired as listed in the application for renewal.
- (5) The kitchens in the Hickory Cottage were remodeled.
- (6) A bus route for the transportation for the subjects at mealtime was established.
- (7) Setting up of the routine for gas-liquid chromatography of our specimens as well as those from Chicago and from the open study in the Twin Cities has been completed. Routine analysis of samples is progressing.
- (8) Feeding of 233 subjects was begun on March 18. Final exclusions and randomization into the four dietary groups were completed and the feeding of the four experimental diets was begun on April 16, 1963.

B) Detailed Report

This project has been carried out according to a common protocol agreed upon by the Executive Committee, modified slightly to meet the special requirements of the closed study with prior approval of the Executive Committee. The original protocol as adapted to the Faribault Center is included as Attachment A. Work at Faribault got underway earlier than in the open centers, and this report covers six and one-half months on the experimental diets.

It was the opinion of the investigators, Central Staff, and Executive Committee in meetings on November 3-9, 1963, that progress to date at Faribault, as in the open center has been encouraging. It was the opinion that the closed study has made and will continue to make a definite contribution, and that the protocol should be continued unaltered through the full twelve months on experimental diet for each participant. This is a textual report; detailed information is available in the attachments and tables prepared by the Statistical Center, Attachment D, and by Dr. Eldon Rice, Director of Food Production and Distribution, Attachment C.

Organizations Major decisions have been made by the "Executive Committee (Attachment D). Operational decisions have been made by the principle investigator. Coordination of the operation with the open centers has been effected by a Central Staff consisting of Dr. Bragdon (Executive Director), Dr. Green (Executive Secretary), Dr. Rice (Director of Food Production and Distribution), and Mr. Cornfield and Mr. Schachter, (Statistical Center). Services of the Statistical Center and of Dr. Green have been contributed by the NHI.

Recruitment: Recruitment was initiated late in 1962 by the perusal of the hospital record of every male patient in the Faribault State School and Hospital in the age range of 40-59 years. Two hundred seventy-eight patients appeared to be eligible for the study in the sense that no definite reason for exclusion could be gleaned from the hospital records. As a result of the initial examinations including physical examination, electrocardiogram, urinalysis, and chest x-ray, 93 subjects failed to meet the strict criteria for inclusion. With the approval of the Executive Committee 36 of these persons were included despite the abnormalities noted in order to bring the number as close as possible to the agreed upon value. The abnormality in these subjects was either left axis deviation by electrocardiography or weight in excess of 40% above the desirable level. These subjects may be handled separately in the final evaluation of the study. Recruitment at Faribault was somewhat disappointing, for an institution with 3000 patients. It must be remembered that all of these patients are mental defectives, and there are an unusually large number of children. Recruitment might be expected to be more favorable in other types of mental institutions.

Food Procurement: Fair different diets were to be tested in a double blind manner, Except for lean meats and a few specialty items such as potato chips, this meant formulating four different products of each food item. The four diets were designed to offer the following characteristics:

- Diet B: 30% of calories from fat; P:S* ratio of 1,5; cholesterol 350-450mg/day
- Diet C: 40% of calories from fat; P:S ratio of 2.0; cholesterol 350-450mg/day
- Diet D: 40% of calories from fat; P:S ratio of 0.4; cholesterol 650-750mg/day
- Diet E: 40% of calories from fat; P:S ratio of 4,4; cholesterol 150-200mg/day

Dr. Seth T. Shaw, formerly of Safeway Stores, was obtained in a consultant capacity to make the initial contacts with food producers. He was highly successful in obtaining their cooperation. For reasons of quality control it was considered desirable to have each item produced by only one source and to be distributed nation-wide. Among our producers are well-known firms (see Attachment C). On October 1, 1962, Dr. Eldon Rice, a food technologist, was granted 9 months' leave of absence by his employer to work with the food producers over the details of production and the logistics of supply and distribution. The four varieties of each item were to be as similar as possible in taste and physical characteristics. This desideratum could be attained to only a limited extent.

In addition to the specifications supplied by the producers a contract was arranged with Woodson-Tenent Co. of Memphis, Tennessee, to analyze our D-H foods. These analyses include moisture, ash, protein, fat, cholesterol, and GLC analysis of fatty acids. A tremendous amount of work has gone into producing a Food Code Book. This book lists by code number all imaginable foods, both Diet-Heart products and those available on the open market. A practical serving size is also included. When coding a 7-day Food Record the Nutritionist enters the code number after each item and also the number of servings. These data are transferred to punch cards and fed into a Minneapolis-Honeywell 800 computer, which gives out the information to be found in Unit 12 of Attachment B.

Food Center; Storage of food presented no problem because of available space in the hospital's walk-in freezers, refrigerators, and root cellars. A number of items have been provided from the regular hospital menu without cost to the study. These include such articles as cereals, vegetables, fruits, and skim milk. Special meats and baked goods and other special products used by the participants in the open studies have also been used in the closed study. The hospital has offered excellent cooperation in the delivery of raw food-stuffs to the diet kitchen. The hospital has contributed 63¢ per subject per day towards the cost of food, which is the cost of feeding the patients not in the study.

Mixed Diet Period: A four week period during which the participants all ate foods _____. From the four different diets was included, as in the open study.

Randomization: During the fourth week on the "mixed diet" the participants were randomly assigned to one of the four experimental diets. This was done at the Statistical Center and the information transmitted only to the Nutritionist. The participants were first ranked as to their serum cholesterol and the array divided equally into two groups. Each of the resultant groups was then similarly divided into two sub-groups according to their diastolic blood pressure. The process was repeated using relative weight as the variable. A final subdivision was made depending on whether the participants smoked cigarettes. This gave 16 groups or strata and within each of them participants were assigned to dietary groups on a restricted random basis. The medium cholesterol at Faribault was 206 mg.%, a value definitely lower than in any of the open centers. Smoking, on the other hand, was much more common among the Faribault participants, 62% of whom smoked.

Serum Cholesterol Determinations: At each visit blood is drawn for serum cholesterol determination. A sample of serum is placed in each of two tubes, frozen, airmailed in dry ice to the Heart Disease Control Laboratory at the Communicable Disease Center USPHS, in Atlanta. Duplicate analyses are performed by the Abell-Kendall method with no more than one week elapsing between determinations. If the duplicates differ by more than 9 mgs. per 100 ml. they are repeated. Current experience has shown that about 13% of these determinations must be repeated. An independent check on this laboratory has been established in the following manner. Each center obtained one or two pools of serum, put aliquots into tubes, and froze them. The pools were given fictitious names and numbers. At time intervals corresponding with clinic visits samples are sent to Atlanta. The average standard deviation of the sets of data is 3.37 mg/100 ml. There has been no drift over time (see Unit 7, Attachment B).

Characteristics of Participants: All of the participants at Faribault have abnormal low intelligence quotients. A number of paraplegics are included, as well as cretina Mongols and eunuchs.

Routine for Handling the Diets and the Subjects: It was necessary to remodel a food serving space in the Hickory Dormitory to facilitate the preparation of the four diets. All the cooking is done in this kitchen. The participants eat in four different dining rooms and those not living within easy walking distance are transported to their dining rooms by bus. Psychiatric aids accompany the subjects, assist them in the serving line and make certain that each participant receives the proper tray. The psychiatric aids also keep a record of attendance at meals and they record plate waste.

Food Records: A record is kept of all foods served for purposes of evaluation of foods consumed. The plate waste on selected participants in each dietary group is taken into account.

Drop-outs After Randomization: As of November 1 there had been a total of 9 drop-outs; Three participants were discharged from the State School. Six patients were dropped due to indefinite hospitalization. Three patients had expired.

Adherence: It is believed that adherence has been very nearly perfect except for periods when the participants are away on vacation or are confined to the hospital. A complete record of these periods of non-adherence is being kept. The fatty acid composition of the red blood cells is being determined during the control visit and at 12-, 24- and 48 weeks after beginning the experimental diets. Fatty acid analyses of the adipose tissue during the control period and at the end of the study will also be carried out. Serum triglyceride levels are being determined.

The Double Blind Features At Faribault the double blind feature has operated in a slightly different manner than in the open centers. In the latter, the warehouseman who has no contact with the subjects, has full knowledge of the code, while the Nutritionists, because of their close relations with the subjects, must be kept blind. At Faribault, the Chief Nutritionist, Mrs. Ashman, is serving the function of the warehouseman in this respect. Mrs. Ashman's relationship to the subjects is such that her knowledge of the code can have no effect on the experiment. It is believed that the subjects have remained completely blind, and it is certain that the investigators have not broken the code.

Changes That Have Occurred: The changes in blood cholesterol are summarized in the tables of Units 5 and 6 of Attachment B. It is noteworthy that the largest average change in blood cholesterol seen in any center appeared during the D-4 visit at Faribault, It is also apparent that the largest difference between the experimental diet and the control diet has occurred at Faribault. As in the open centers, remarkably large drops in cholesterol occurred following the mixed diet period, and an unexpectedly long time elapsed before the cholesterol returned to base-line levels in the participants on the D diet. Thus, the reduction amounted to 6.7% even after 12 weeks on the experimental diet. After 20 weeks, however, the decrease on the D diet was only 1.0%. The basis for this protracted fall is obscure, and the discovery of an explanation for it represents one of the important unsolved problems. It is of interest that the group at Faribault at 20 weeks showed some tendency for the drops of cholesterol not to be maintained at the minimum level. The reason for this gradual

rise towards the control level is not certain, but the possibility exists that the cause was a small change in the diet made at the request of the Central Staff to try to hold the dietary cholesterol nearer to the design value. The nature of this change was to include in the Faribault diets more of the special baked goods and more milk concentrate. The basis for this change was the belief that these items contained more cholesterol than the locally procured items being used initially. Later and better information revealed that such was not the case. Furthermore, the special baked goods did not meet specifications with respect to P:S ratio. It has therefore been decided to go back to the original dietary formulas, and it will be of interest to see whether the cholesterols fall again. It is possible that the fluctuations noted are not statistically significant. Another point of interest is that the D diet, which, according to predictions, should have produced a much lower cholesterol than the R and C diets, failed to do so. The differences noted are not as great as would have been expected. It is possible that this result is caused by the unusually low initial cholesterol levels at Faribault. It is of considerable interest that the cholesterol reductions obtained compared very favorably with those in the open centers, in spite of the fact that the initial levels were so low.

Other Items: A subcommittee concerned with the preparation of tables has met on several occasions. "Mock-ups" of over 40 tables have been prepared. Much more work in this field, however, remains to be done,

An Initial Report of this study appeared in the Journal of the American Medical Association in the issue of July 13, 1963, a reprint of which is attached to this report.

The investigators and the Executive Committee, in their meetings of 8-9 November 1963, after carefully reviewing the experience and data available at that time, concurred that the study was proceeding better than they had anticipated.

Attachment A

Protocol for a Feasibility Study of the Effect of Diet on the Incidence of Manifestations of Atherosclerosis in a Closed Population.

It is recognized that the ultimate objective of this cooperative study is the demonstration of the effect of dietary alterations on the incidence of atherosclerotic cardiovascular disease in the free-living population. Use of institutionalized subjects carries the disadvantage that the conclusions drawn may not be directly applicable to the general population, either because of the peculiar characteristics of the institutional inmate, or because the experimental conditions cannot be reproduced outside the institution. On the other hand, the following advantages are apparent:

- 1) Diet can be more rigidly controlled - non-adherence will be a less serious factor.
- 2) A more nearly ideal diet can be enforced.
- 3) Drop-outs will be less numerous. Most of the drop-outs which do occur will - be caused by factors unrelated to the experiment, will affect experimental and control groups alike, and will presumably be less likely to introduce bias into the results.
- 4) A statistically valid conclusion can probably be reached with a smaller number of subjects.

Because of these advantages, and because the feasibility of the experiment in the free-living population under present conditions is still in doubt, we believe that a preliminary study in an institution is desirable.

The Institution Chosen for the Study

Perusal of the populations of institutions of various types has led us to the conclusion that mental hospitals offer the best prospect for this research. The inmates of prisons are too young, and the turnover is too great. The population of individual monasteries is so small that an excessive number of separate units would be required. We proposed that the feasibility study be carried out in the state hospital at Faribault, Minnesota, an institution with a current total population of 3,179 patients, 1,762 of whom are males.

All of the patients at Faribault fall into the category of mental deficiency. We believe that patients with other types of mental illness could also be used, if this study were later to be expanded, but mental defectives are especially suitable. Their physiology is perhaps more nearly like that of the normal individual than is that of the schizophrenic. More importantly, the institution is under little pressure to discharge them home.

Faribault now cares for male patients of an age suitable for this study as follows:

<u>Age</u>	<u>Number of Men</u>
40-44	131
45-54	215
55-59	84

As discussed below, we have divided our experimental subjects into four groups of equal size. The subjects were divided into cells and assigned to the four groups at random, in much the same fashion as in the open study.

Diets

Final decision as to the nature of the diets to be used was made by the Executive Committee. The groups are as follows:

Group B. 30% of calories from fat. P:S ratio 1.5.

Group C. 40% of calories from fat. P:S ratio 2.0.

Group D. 40% of calories from fat. P:S ratio 0.4.

Group E. 40% of calories from fat. P:S ratio 4.4.

It is a source of concern that, on the basis of the best evidence now at hand, none of the diets to be used in the open study is likely to produce an average lowering of blood cholesterol much in excess of 15%. Considerable effort will be directed at making our E diet palatable. The object during the feasibility study will be to determine how closely the "ideal diet" can be approached without undue loss of cooperation from the subjects. A major feature of this diet will be that it should be acceptable to the general population under the more favorable conditions which may exist in the future. These would include full cooperation of the food industry, and solid scientific evidence of the effect of adherence on life expectancy.

If a large-scale closed study is organized (at a later date), it will be desirable to continue these same four groups, in order to obtain information concerning the degree of dietary change needed to obtain a satisfactory lowering in the incidence of coronary heart disease. If it should become apparent, however, that a marginal number of subjects will be available, I would prefer to omit Groups B and C. As I see it, the primary question to be answered by these studies is, "Can the incidence of coronary heart disease be significantly lowered by dietary manipulation?" If this question can be answered clearly in the affirmative, the finer details of the degree of dietary change needed can be worked out later. Group E appears to offer the greatest probability of providing this clear affirmative answer. If, on the other hand, the answer should be negative, it is highly unlikely that any of the other proposed diets would be successful, and they need not be tried.

Experimental Plan

The experimental plan is similar to that agreed upon for the free-living population, with modifications appropriate to the type of subject. The most important modifications are:

- 1) "Pep talks" by dieticians and physicians are unnecessary.
- 2) Evaluation of results must be entirely on the basis of objective evidence, primarily electrocardiography, transaminase in the case of acute events, and autopsy.

Eleven contacts are planned with each subject. The schedule is as follows:

Initial- Complete physical examination, urinalysis, electrocardiogram, chest film, cholesterol, red cells and biopsy of adipose tissue for analysis of fatty acids by gas-liquid chromatography.

March 18-22, 1963. Begin mixed diet period.

April 1-9. Two weeks on mixed diet. Weight, blood pressure, cholesterol.

April 15-23. Four weeks on mixed diet. Weight, blood pressure, cholesterol.

April 16-18. Begin experimental diets.

April 29-May 7. Two weeks on experimental diets. Weight, blood pressure, cholesterol.

May 27-June 4. Six weeks on experimental diets. Weight, blood pressure, cholesterol.

July 8-July 16. Twelve weeks on experimental diets. Weight, blood pressure, cholesterol. Red cells for analysis of fatty acids.

Sept. 2-10. Twenty weeks on experimental diets. Weight, blood pressure, cholesterol.

Oct. 23-Nov. 5. Twenty-eight weeks on experimental diets. Weight, blood pressure, cholesterol. Red cells for analysis of fatty acids.

Dec. 23-31. Thirty-six weeks on experimental diets. Weight, blood pressure, cholesterol,

Feb. 17-25. Forty-four weeks on experimental diets* Weight, blood pressure cholesterol. Red cells for analysis of fatty acids.

April 13-21. Fifty-two weeks on experimental diets. Weight, blood pressure, cholesterol, electrocardiogram. Red cells and biopsy of adipose tissue for analysis of fatty acids,

After the first four weeks one technician will be able to collect the blood samples, determine the serum cholesterol concentrations, and record and mount the electrocardiograms. During the initial phase, personnel were borrowed from other projects. The electrocardiograms will be read by electrocardiographers from University of Minnesota Hospitals. Criteria will coincide as closely as possible with those to be used in the open study.

Ancillary Experiments

Our original protocol included some measurements of cholesterol excretion and degradation in subjects maintained on the various diets. The plan was to administer cholesterol-C¹⁴ intravenously, separate the fecal excretion products into bile acid and unsaponifiable fractions, and determine the amount of cholesterol eliminated by correlation of the activity in the two fractions with the specific activity of the serum cholesterol. In the time which has elapsed since the protocol was written, we have completed, in collaboration with Dr. Keys' laboratory, measurements of this type on five normal human subjects. The conclusion is fairly clear that polyunsaturated fats cause increased excretion of both bile acids and neutral sterols. I am inclined to believe that we do not need to carry out more analyses of this type. We should not move on to an investigation of cholesterol synthesis in subjects on the different diets.

Since cholesterol excretion appears to be increased by polyunsaturated fats in the diet, cholesterol synthesis is probably increased, also. Otherwise, a steady state could not be maintained. Although various attempts have been made to study cholesterol synthesis in human subjects, the methodology used is open to question. Mevalonic acid is a poor precursor for the measurement of cholesterol synthesis in vivo. Most of any given dose can be expected to be converted to cholesterol, regardless of the circumstances. The time required for the labeled cholesterol to appear in the serum is more a function of the time of mixing of the various pools rather than of the rate of synthesis. Acetate is also subject to criticism as a precursor. The amount converted to cholesterol depends on the rate of activation, the amount being directed into other pathways, the availability of endogenous acetyl coenzyme A, and perhaps other factors, all unrelated to the actual rate of synthesis. We propose to approach this problem by the use of tritiated water. About half of the hydrogens of cholesterol come from water, and in this case the specific activity of the immediate precursor is known. The use of this tracer may also be criticized, but we believe that some experiments of this type will lead to valuable data. The subjects in the diet project will be ideal for the purpose. Their diets will be under rigid control, and their hospitalization is already provided for. Subjects showing both good and poor responses to diet will undoubtedly be available. Initial tests can be done during the pre-diet period, and then repeated after a prolonged period on the diet, when the tritium from the first test will have fallen to a low level. Comparison with other tracers will be made. The feasibility of this type of experiment is enhanced by recent improvements in counting efficiency.

Preliminary to the initiation of experiments on cholesterol synthesis with tritiated water, we felt that it would be necessary to check the constancy of the ratio of hydrogen to carbon derived from acetate. If this ratio is constant under various dietary conditions, we may assume with reasonable certainty that the number of hydrogens derived from water is also constant, since all of the hydrogens in cholesterol appear to be now in the process of counting samples from eight of our subjects at Faribault who were given doubly labeled sodium acetate (two subjects from each dietary group). These men are being handled like the experimental subjects, but had been officially excluded from the main study for various reasons. The double blind design was not compromised,

We also expect to do rather extensive work on the gas-liquid chromatography of the fatty acids of serum, red cells, and adipose tissue, as a test of the usefulness of this criterion of dietary adherence. Comparison of the subjects in the closed and open studies will be of great interest.