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A re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from the Minnesota Coronary Experiment (1968-1973)

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Abstract

Objective To re-examine the traditional diet-heart hypothesis through analysis of recovered data from the Minnesota Coronary Experiment (MCE), a trial designed to evaluate the effect of lowering serum cholesterol by replacing dietary saturated fat (SFA) with vegetable oil rich in linoleic acid (LA) on coronary heart disease and death.

Design Unpublished data from the MCE, a double-blinded, parallel-group, randomized controlled primary and secondary prevention dietary trial conducted in 1968-73, were recovered and analyzed according to hypotheses pre-specified by the original study investigators.

Setting One nursing home and six state mental hospitals in Minnesota, USA.

Participants 9,423 institutionalized women and men aged 20-97. Longitudinal serum cholesterol measurements were performed on 2,355 participants exposed to the study diets for 1 year or longer; autopsy files were recovered for 149 out of 295 subjects.

Interventions Serum cholesterol-lowering dietary intervention replacing SFA with LA (from corn oil and corn oil polyunsaturated margarine). Control participants consumed a diet high in SFA from animal fats, common margarines and shortenings.

Outcome measures All-cause mortality; coronary and aortic atherosclerosis; myocardial infarcts at autopsy.

Results The intervention group had significant reduction in serum cholesterol compared to the control group (mean change from baseline -13.8% v -1.0%; $p < 0.001$). There was a 22% higher risk of death for each 30 mg/dL reduction in serum cholesterol in covariate-adjusted Cox regression models (hazard ratio (HR) 1.22; 95% CI 1.14, 1.32; $p < 0.001$). In our analysis of the partial autopsy cohort, there was no evidence of benefit for coronary atherosclerosis (total coronary narrowing score (24.4 v. 22.3, $p = 0.06$) or myocardial infarcts (41% v. 22%, $p = 0.04$) in the intervention group compared to the control group. An updated meta-analysis of randomized controlled dietary interventions that lowered serum cholesterol by providing vegetable oils rich in LA in place of SFA showed no evidence of coronary (HR 1.12 (0.85 to 1.46)) or all-cause mortality benefit (death HR 1.07 (0.92 to 1.23)). In meta-regression, there was no association between cholesterol lowering and coronary or all-cause mortality.

Conclusions These findings do not support the traditional diet-heart hypothesis. Together with recovered Sydney Diet Heart Study data, these findings add to growing evidence that incomplete publication has contributed to an overestimation of the benefits, and underestimation of the potential risks, of replacing SFA with vegetable oils rich in LA.

Introduction

The traditional diet-heart hypothesis [1-3] predicts that the serum cholesterol-lowering effects of replacing saturated fat (SFA) with vegetable oils rich in linoleic acid (LA) will diminish deposition of cholesterol in the arterial wall [4 5], slow progression of atherosclerosis [6], reduce coronary heart disease (CHD) events and improve survival [7 8]. This diet-heart paradigm is supported by clinical trial evidence demonstrating diet-induced reductions in serum total cholesterol and low density lipoprotein (LDL) [9-13](**Fig. 1 Part A**), and robust epidemiological associations linking serum total cholesterol and LDL to CHD events and deaths (**Fig. 1 Part B**)[14]. Despite these compelling relationships, serum cholesterol lowering diets have never been causally demonstrated to significantly reduce CHD events or deaths in a randomized controlled trial (RCT)(**Fig. 1 Part C**).

Recovery of unpublished data can shift the evidence base

Only a handful of RCTs have ever causally tested the traditional diet-heart hypothesis. The results for two of these trials were not fully reported. Our recovery and 2013 publication of previously unpublished data from the Sydney Diet Heart Study (SDHS, 1966-73), belatedly demonstrated that substitution of an LA-rich vegetable oil for SFA significantly increased the risks of CHD and all-cause mortality, despite reducing serum cholesterol [15]. Inclusion of these SDHS findings shifted the balance of available evidence; updated meta-analyses of RCTs no longer provide support for the traditional diet heart hypothesis [15 16]. Here the recovery of unpublished data from another diet-heart trial, the Minnesota Coronary Experiment (MCE), provides a rare opportunity to evaluate the proposed causal link between LA rich vegetable oil diets and death (**Fig. 1 Part C**), and whether risks are mediated by changes in serum cholesterol (**Fig. 1 Part B**).

The Minnesota Coronary Experiment

The MCE, an RCT conducted from 1968 to 1973, was the largest (n=9,423) and perhaps the most rigorously executed dietary trial of cholesterol lowering by replacing SFA with vegetable oil rich in LA. With an average serum cholesterol reduction of >13%, double-blinded ascertainment of CHD events and deaths, and more than 2,300 participants consuming study diets for one year or longer, the MCE dataset provides an exceptional opportunity to evaluate the effects of lowering serum cholesterol by replacing SFA with LA-rich vegetable oil. The MCE is also the only such RCT to complete a post-mortem assessment of coronary, aortic and cerebrovascular atherosclerosis grade and infarct status, and the only RCT to test the clinical effects of increasing LA in large groups of women and older adults.

Despite the potential importance of this trial, critical pre-specified MCE analyses have not been published. We recovered MCE datasets stored on two 9-track magnetic tapes, numerous green-bar paper files, and paper autopsy folders, as well as trial documents including: original and supplementary grant proposals, data collection forms, FORTAN coding sheets, and partial trial results reported in a 1989 journal publication [17], 1975 conference proceedings [18-20], and a 1981 Master's Thesis containing Kaplan Meier Life Table and Cox Proportional Hazards analyses [8]. Recovery of these extensive MCE materials allowed us to validate the recovered datasets and to evaluate hypotheses that were proposed by the original study investigators.

The objectives of the present study are to: (1) characterize the serum cholesterol lowering effects of replacing SFA with high LA vegetable oil (corn oil) in the pre-specified MCE study populations; (2) assess the relationship between changes in serum cholesterol and risk of death; (3) evaluate the effect of the intervention on the degree of atherosclerosis and risk of infarct at autopsy; and (4) to identify additional missing MCE data files that should also be recovered and analyzed.

Methods

Study design and participants

The MCE was a double-blinded, parallel-group, randomized controlled dietary intervention trial, designed to evaluate the effects of increasing n-6 LA from corn oil in place of SFA for primary and secondary prevention of cardiovascular events and deaths, and for reducing the degree of coronary, aortic, and cerebrovascular atherosclerosis, and the number of myocardial infarcts and strokes at autopsy. It was conducted from 1968 to 1973 in the state of Minnesota, USA. Eligible participants were men and women aged ≥ 20 years admitted to either the Oak Terrace Nursing Home or one of six state mental hospitals (Anoka, Fergus Falls, Hastings, Moose Lake, Saint Peter, Willmar). The experiment lasted from 41 to 56 months, depending on the hospital. The project was approved by the clinical research committee of the University of Minnesota and by each of the collaborating hospitals. The experiment was funded by the United States Public Health Service and National Heart Institute through the R01 mechanism (HE 09686), with Ivan Frantz, Jr., MD as Principal Investigator and Ancel Keys, PhD as co-Principal Investigator. Part 1 of the web appendix, written by Robert Frantz, MD (co-author and youngest son of the deceased MCE principal investigator), provides a tribute to recognize the extraordinary efforts of his father in leading the MCE research team.

MCE data recovery and validation

We obtained approval from the NIH Office of Human Research Protection (OHSRP #5743) and collaboration from Robert Frantz, MD to recover, analyze, and interpret de-identified MCE data and study-related materials stored on two 9-track magnetic tapes and an extensive collection of paper documents. Part 2 of the web appendix describes the methods used to recover these original MCE datasets; convert recovered data into a useable format; verify the accuracy of recovered data; and merge each of the recovered datasets into a master file. For further validation, each of the recovered MCE datasets were compared to each other and to data reported in the 1989 study publication [17], the 1981 Master's Thesis (S.K. Broste. Lifetable Analysis of the Minnesota Coronary Survey) [8], and the 1975 conference proceedings [18][19][20], as well as numerous other recovered MCE documents and data sources as described in Part 2 of the web appendix.

Overview of MCE objectives

According to the MCE R01 grant proposal entitled "Effect of a Dietary Change on Human Cardiovascular Disease", the project objective was to "provide evidence concerning the possibility of reducing the incidence of clinical manifestations of atherosclerosis by dietary modification"[7]. The MCE team planned to test whether the dietary intervention reduced CHD events and deaths in the

total population and in pre-specified subgroups of women, men, among ages \geq and $<$ 65, and among participants with and without established CHD (primary and secondary prevention), with a special emphasis on participants exposed to study diets for one year or longer. [7 21-23] (**Table 1**). The inclusion of women and older adults was considered a unique advantage of this RCT [7], since the clinical effects of serum cholesterol lowering diets in these populations were (and remain) largely unknown.

Extensive post-mortem data was collected to allow investigators to test the hypothesis that the serum cholesterol lowering diet would reduce coronary, aortic, and cerebrovascular atherosclerosis, as well as autopsy-confirmed myocardial infarcts and strokes [23].

Table 1. Full cohort and pre-specified subgroups of the Minnesota Coronary Experiment

(Sub)groups	Key Considerations
Full cohort	With >9,400 participants, the full MCE population was easily the largest of any randomized controlled diet-heart trial testing whether provision of vegetable oil rich in n-6 LA in place of SFA reduced risk of CHD and death. This single trial accounted for about 80% of all diet-heart participants with provision of vegetable oil rich in LA in place of SFA.
Women	The MCE is the only diet-heart trial to evaluate clinical effects in women after random assignment to either serum cholesterol lowering diet or control diet. Since CHD manifests later in women than men, the expanded age range of the MCE population was considered a unique opportunity to assess clinical efficacy of the diet in women.
Men	Men were considered a subgroup likely to establish benefit of the serum cholesterol lowering diet, due to higher incidence of CHD than women.
\geq age 65	The MCE is the larger of only two RCTs testing the clinical effects of a serum cholesterol-lowering diet in individuals older than 65. Analyses of this high-risk subgroup were planned but not reported.
$<$ age 65	Participants $<$ 65 were considered a population that was likely to see benefit from the serum cholesterol lowering diet.
Primary prevention (No EKG evidence of prior myocardial infarction)	The majority of MCE participants (about 95%) did not have EKG evidence of a current or prior myocardial infarction (no pathological Q wave) at randomization.
Secondary prevention (EKG evidence of prior myocardial infarction)	In the grant proposal, MCE investigators reported that the presence of a pathological Q wave on EKG upon entry into the pre-randomization observational phase was associated with an increased risk of a subsequent event by a factor of 2.6 [21]. Analyses of this high-risk subgroup were planned but not reported.
Participants consuming study diets for \geq 1 year	MCE investigators hypothesized that the effects of the serum cholesterol lowering diet would take substantial time to manifest. A special emphasis was placed on participants who were exposed to study diets for \geq 1 year, in terms of serum cholesterol measurements, sample size calculations, and subgroup analyses [21-23].
Autopsy cohort	MCE investigators hypothesized that participants randomized to the serum cholesterol lowering diet would have less advanced coronary, aortic and cerebrovascular atherosclerosis and fewer myocardial infarcts and strokes at autopsy.

[7 8 17-23] CHD, coronary heart disease; EKG, electrocardiogram.

MCE hypothesis and endpoints specified in funded proposal

Power and Sample size considerations

Based on epidemiological associations between serum cholesterol and CHD events in non-randomized cohorts, the MCE investigators applied the Cornfield equation [risk = k(serum cholesterol)ⁿ] to predict that between 2,490 and 11,645 participants would be required to “obtain a difference in 5 years significant at the 95% confidence level” with alpha=beta=0.05 [21]. Based on the

rate of strictly defined coronary deaths observed during the MCE observational phase, and the elimination of all participants in the hospital less than 1 year, the estimated duration of the experiment required to assess the efficacy of the intervention was 3.6 years (with $\alpha=\beta=0.05$) [22].

Partial results of the MCE were reported in three documents, a 1989 manuscript [17], three abstracts presented at the 1975 AHA Scientific Conference [18-20], and a 1981 Master's Thesis with Kaplan Meier Life table and Cox Proportional Hazards Analysis [8]. The 1989 publication and 1975 abstracts reported that the experimental diet effectively lowered serum cholesterol, but provided only a broad overview of the clinical outcomes without statistical comparisons, and did not report outcome data for two pre-specified high-risk subpopulations (the age ≥ 65 and secondary prevention cohorts). The 1989 publication and 1975 abstracts did not report the relationships between the degree of serum cholesterol lowering and the risk of CHD events and deaths. The 1981 Master's Thesis (S.K. Broste) provided Life table analyses and Cox proportional hazard models using MCE data [8], however to our knowledge this document has never been cited. We present principal results of this Thesis in **Figure 3** and provide the full Thesis in Part 3 of the web appendix. To our knowledge no MCE autopsy findings have ever been reported or published.

TIMELINE/SETTING

Pre-randomization phase

The RCT phase was preceded by a 33-month pre-randomization observational phase (2/1966 - 11/1968), during which the study team characterized the hospital populations, developed and refined procedures for baseline and follow-up visits, sick visits, blood collection, electrocardiograms, and post-mortem examination, as well as the data collection and management plans.

RCT phase

The experimental dietary intervention phase, which was initiated over a 15-month period according to hospital-specific diet start dates, lasted for a maximum of 56 months. The start dates and diet duration for each hospital are presented in Part 4 of the web appendix. Participants who were admitted to a given hospital after its respective diet phase was underway completed baseline risk assessment, electrocardiographic testing, and serum collection before starting the study diets.

STUDY DIETS

Pre-randomization hospital diet

Prior to randomization, each hospital's food production program was covered by free provision of surplus USDA food commodities (common margarine, shortening, skim milk, flour and rice) and Minnesota state funding [7]. The average baseline hospital diet provided 18.5 and 3.8% of calories as saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), respectively [18] (**Figure 2**). Based on the traditional distribution of PUFA species in US diets (about 90% of PUFAs are LA), this baseline hospital diet provided about 3.4% of calories as LA.

Participants admitted to an MCE hospital after its respective diet phase was underway had a brief period of exposure to the usual hospital diet before randomization to either the serum cholesterol lowering diet or the control diet.

Serum cholesterol lowering diet

The MCE experimental serum cholesterol-lowering diet was derived from the 'BC' diet of the institutional arm of the National Diet-Heart Feasibility Study at Faribault Hospital [7 17 18 22 24-26]. Liquid corn oil was used in place of the usual hospital cooking fats (including hydrogenated oils), and was also added to numerous food items (for example salad dressings, filled milk, filled cheeses, filled beef (lean ground beef with added oil)). Soft corn oil polyunsaturated margarine was used as a spread in place of butter. This intervention produced a mean reduction in dietary SFA by about 50% (from 18.5 to 9.2% of calories) and increased LA intake by more than 280% (from about 3.4 to 13.2 % of calories) [17 18] (**Figure 2**). Hospital-specific fatty acid compositions based on a 3-week food supply in 1971 are shown in Part 5 of the web appendix. There was substantial between hospital variability in study diets, with SFA ranging from 8.0-12.3% and LA ranging from about 11.3-16.5% of calories. However, SFA was markedly reduced, and LA was markedly increased, in each hospital. Based on the average dietary changes, the Keys equation predicts that the MCE intervention diet would produce a marked reduction in serum cholesterol (**Figure 2 and Table 3**).

Control diet

The Control MCE diet was patterned after the 'D' diet of the National Diet Heart Study. It was designed to appear very similar to the experimental diet. Notably, free surplus USDA food commodities including common margarines and shortenings were key components of the Control diet, making the daily per participant allocation from the state of Minnesota adequate to cover the full costs [17 18 24]. Since common margarines and shortenings of this period were rich sources of industrially produced trans fatty acids (TFA) [27-29], the Control diet contained substantial quantities of TFA. Compared to the pre-randomization hospital diet, the Control diet did not change SFA intake,

but did substantially increase LA intake (by about 38%, from 3.4 to 4.7% of calories). Based on this increase in dietary LA alone, the Keys Equation predicts that the Control diet would reduce average serum cholesterol levels compared to baseline (**Figure 2 and Table 3**). However, this reduction is predicted to be modest compared to the intervention group.

RANDOMIZATION/MASKING

MCE study investigators and colleagues were concerned that critical design limitations, including incomplete masking and randomization of hospitals rather than individuals [30], cast serious doubt on the reliability of previous diet-heart trial conclusions [7 30]. To address these limitations and to ensure that the MCE conclusions would “carry conviction” [22], the MCE team randomly assigned individual participants and employed a rigorous double-blinding paradigm.

Randomization

The original hospital inpatient population was randomized according to a stratified randomization scheme utilizing 512 cells on the basis of eight variables (age, gender, length of stay in the hospital, weight, blood pressure, diabetes, cigarette smoking, and electrocardiogram evidence of a previous myocardial infarction). When new subjects were admitted after the hospital randomization date, the stratified randomization scheme utilized four cells, according to age and gender [17].

Masking

Participants were masked to group assignment. Study foods were designed to appear very similar in both groups. Both diets were served in a single line. Each study participant received his or her group-specific food tray based on a unique computer-generated code number, which was designed to be incomprehensible to the participants, but easily interpreted by the food servers [17]. Twenty-one labels were printed out for each participant each week. Labels remaining on the sheet were used to record missed meals, which were transferred to a "Port-o-Punch card" for later assessment of the correlation between adherence (defined as the percentage of meals received) and cholesterol response. The principal investigator, other study physicians, assistants, nurses, nutritionists, laboratory technicians, pathologists, and all other study staff were masked to group assignment.

ASSESSMENT OF CLINICAL OUTCOMES AND INTERMEDIATE ENDPOINTS

Data Management

Fifteen MCE forms were devised for recording the data from the hospitals and laboratories (Part 6 of the web appendix). The data collected on these forms and the adherence data collected on the Port-

o-Punch cards were transferred to magnetic tapes for later analysis (Part 2 of the web appendix).

Baseline assessment and routine follow-up assessments

Upon study entry and at 6-month intervals thereafter throughout both the pre-randomization and RCT phases, a project technician carried out a brief evaluation of the subject's risk status [7], fasting serum was sealed under nitrogen and stored at -20°C, and an electrocardiogram was obtained.

Intermediate endpoints: serum cholesterol and triglyceride assays

Serum cholesterol and triglyceride assays were performed according to the standard protocol of the Lipid Research Clinics [17 31] in a laboratory standardized and monitored by the Center for Disease Control (Atlanta, GA).

MCE investigators hypothesized that the clinical effects of serum cholesterol lowering would take substantial time to manifest [7 17 21 22]. In order to avoid doing analyses “on patients who remained in the hospital for too short a time to contribute significantly to the results” [17], they did not analyze serum from the full cohort. A special emphasis was placed on the subgroup of participants exposed to either of the study diets for one year or longer [7 17 21-23]. Cholesterol and triglyceride measurements from this ≥ 1 year serum cholesterol cohort served as a basis for a Cox proportional hazards analysis relating changes in intermediate endpoints to risk of CHD death and all-cause mortality, published as a Master's Thesis in gray literature in 1981 (see **Figure 3** and Part 3 of the web appendix) [8]. We recovered serum cholesterol data for 2,355 subjects who were exposed to study diets for ≥ 1 year. The primary aim of the present analysis is to evaluate the relationship between changes in serum cholesterol and risk of death in this ≥ 1 year serum cholesterol cohort.

Electrocardiogram assessment

Electrocardiograms were read by two independent reviewers according to the Minnesota Code [32]. Discrepancies were resolved by consultation between the two readers [17].

Evaluation of clinical events and deaths

MCE investigators categorized fatal and non-fatal events into ten categories (Part 7 of the web appendix). A conservative approach was used to attribute the cause of death to CHD. The MCE team noted “reluctance to classify a death in this category unless objective evidence is at hand”, and did not hesitate “to code a death as pneumonia, if no underlying basis for pneumonia could clearly be

established". Close attention was therefore directed to the overall death rate since "atherosclerosis may contribute to many deaths in which no actual fresh myocardial infarct or coronary occlusion has occurred" [7], and deaths attributed to pneumonia or other causes could be related ultimately to coronary events [22]. We recovered all-cause mortality data (documented on MCE Form 05 (Part 6 of the web appendix)), but did not recover non-fatal events or CHD deaths. However, data on CHD deaths in relation to intermediate endpoints existed, and was reported in the 1981 Master's Thesis.

Postmortem examination of heart, aorta and brain

Detailed autopsy data analysis plans were included in the MCE grant proposal, supplements and FORTRAN coding sheets [7 21-23], and a 9-track magnetic tape file with full autopsy data (MCE Tape #380, Part 8 of web appendix) was known to exist. However, to our knowledge, no autopsy results have ever been published or reported. According to the 1989 publication, 57.1% (corresponding to 295 of the 517 reported deaths) of participants who died during the experimental dietary intervention phase had an autopsy performed. Hearts, aortas, and brains were sent to the University of Minnesota for blinded grading by University pathologists. We recovered heart and aorta autopsy data for 149 out of these 295 completed autopsies. The remaining autopsy data remain unaccounted for.

Degree of coronary atherosclerosis and number of myocardial infarctions

The degree of coronary atherosclerosis and mapping of myocardial infarcts were evaluated by the multiple cross-section technique as described by Spiekerman, Brandenburg et al [22]. At each of 16 coronary vessel sites, vessel narrowing was scored on a 4-point scale based on the percentage closure of the coronary lumen with 1, 2, 3, and 4 signifying <25%, 25-50%, 50-75%, and 100%, respectively. These data were recorded on MCE Form Number 8 (Part 6 of the web appendix). Scores at each site were summed to calculate a total coronary atherosclerosis score (range 16-64). Subendocardial and transmural infarcts were identified after cutting the ventricles into transverse sections, and recorded on MCE Form Number 9 (Part 6 of the web appendix).

Degree of aortic atherosclerosis

The degree of aortic atherosclerosis was graded from one to seven according to the technique devised by the Committee on Grading of Lesions of the Council of Atherosclerosis of the American Heart Association [22], and recorded on Form 10 (Part 6 of the web appendix). Each aorta was graded independently by two observers without knowledge of age, gender, diagnosis, hospital of origin, or diet group. An aortic atherosclerosis score variable was created by taking the average of

the two measures for each individual.

Degree of cerebrovascular atherosclerosis, and number of ischemic and hemorrhagic strokes

While records were recovered for coronary and aortic atherosclerosis and myocardial infarcts, records for cerebrovascular (Circle of Willis) atherosclerosis and arteriosclerosis, and autopsy-confirmed strokes were not recovered. The degree of atherosclerosis and arteriosclerosis in 16 locations in the Circle of Willis vasculature and smaller vessels of the brain supplying the midbrain, pons, medulla, and cerebellum was graded according to recommendations of the World Federation of Neurology and the National Institute of Neurological Diseases and Blindness, United States Public Health Service [22], and recorded on Forms 12 and 13 (Part 6 of the web appendix). Scores at each site were summed to calculate total cerebrovascular atherosclerosis and arteriosclerosis scores. These data were expected to determine “whether atherosclerosis of the cerebral arteries, as well as the coronaries, is subject to dietary influence.” [7] Brains were also examined for evidence of ischemic and hemorrhagic strokes at each of these 16 sites [22].

2015 DATA ANALYSIS

Overview

The central hypothesis of the MCE investigators was that the dietary intervention would reduce serum cholesterol, which in turn would lead to reductions in atherosclerotic progression, CHD events and deaths. Our recovery of unpublished MCE data and extensive study-related materials (Part 2 of the web appendix) allowed us to evaluate specific components of this hypothesis that were proposed by the original study investigators but were not published. All analyses were carried out with Stata version 13.1.

Association between changes in serum cholesterol and the risk of death

Our analyses utilized longitudinal data for the 2,355 participants in the ≥ 1 year serum cholesterol cohort. First, to determine the effect of the dietary intervention on serum cholesterol, we analyzed group differences in serum cholesterol over time using a generalized estimating equation (GEE) model with time x group interaction. Second, we examined the effect of serum cholesterol on death (conditional on being in the study for at least one year) using Cox proportional hazard models adjusting for clustering within hospital. We present crude models, models adjusted for relevant variables including age, gender, blood pressure, BMI, and adherence to diet (percent of missed meals), and sensitivity analyses further adjusting for time-varying changes in BMI and systolic blood pressure. All models were tested for effect modification by diet group.

In addition, we provide a crude visual representation of the cholesterol-death association by graphing the distribution of change in total serum cholesterol (using the average of pre-randomization measurements and the average of post-randomization measurements for each participant) along with the number and percentage of deaths, and followed by plots of age-adjusted logistic regression models.

Atherosclerotic progression and number of autopsy-confirmed myocardial infarcts

Since only 149 of the original 295 autopsy files were recovered, our analysis of the effects of the serum cholesterol-lowering diet on atherosclerotic progression and number of autopsy-confirmed myocardial infarcts should be considered provisional until the complete autopsy data can be recovered. The MCE investigators hypothesized that replacing SFA with LA-rich oil would reduce atherosclerosis and myocardial infarcts observed at autopsy. We calculated incidence rate ratios for total infarcts according to diet group. We used linear regressions (adjusting for clustering within hospital) to examine whether diet group assignment or changes in serum cholesterol were associated with coronary and aortic atherosclerosis. We examined the effect of serum cholesterol on risk of infarcts using Cox proportional hazard models adjusting for clustering within hospital. All models were tested for effect modification by diet group.

Updated meta-analysis of serum cholesterol lowering randomized controlled trials replacing SFA with LA rich vegetable oils

To put the MCE findings into context, we updated our prior systematic review and meta-analysis evaluating the clinical effects of replacing SFA with vegetable oils rich in LA [15 33], to include recovered serum cholesterol lowering data reported separately for men and women. To test the hypothesis that dietary interventions replacing SFA with LA-rich vegetable oils can reduce the risk of CHD death and all-cause mortality, we calculated pooled risk estimates (hazard ratios and 95% confidence intervals) for each endpoint. The traditional diet-heart hypothesis predicts that serum cholesterol-lowering is the critical mechanism underlying the proposed beneficial effects of the intervention. We therefore used meta-regression to evaluate whether the diet-induced changes in serum cholesterol observed in RCTs were related to risk of death from CHD and all causes.

Inclusion criteria for main analysis and sensitivity analyses

The main analysis included all serum cholesterol-lowering diet-heart trials that (a) randomly assigned individual participants, (b) provided vegetable oil rich in LA (e.g. corn, safflower, cottonseed, soybean oils), and (c) were not confounded by the addition of n-3 EPA and DHA. The sensitivity analyses included diet-heart trials that provided large quantities of fish oil and seafood (major sources of EPA and DHA), or provided advice only without provision of study oil rich in LA.

Detailed statistical methods along with descriptions and dietary compositions, study population and study design characteristics, other key considerations of each trial, publication bias assessment, heterogeneity analyses, pooled risk estimates, and sensitivity analyses are provided in Part 9 of the web appendix.

RESULTS SECTION

Baseline demographics and clinical characteristics

Characteristics of the full MCE population reported in the 1981 Masters Thesis (n=9,423) are shown in Part 3 of the web appendix. Characteristics of the ≥ 1 year serum cholesterol cohort (n=2,355) are presented in **Table 2**. The intervention and control groups were well balanced at baseline, with no detectable differences in any of the recovered variables. The age ranged from 20 to 97 years, with mean age 52 years. Slightly more than half were women, 25% were 65 years of age or older. Average BMI was 24.5 kg/m² and average serum cholesterol was 208 mg/dL. Mean follow-up for participants in this cohort was 2.9 years (median = 3.1 years).

Table 2: Characteristics of the ≥ 1 Year Serum Cholesterol Cohort (n=2,355)

	Intervention		Control		T-Test p-value
	Mean (SD)	n	Mean (SD)	n	
Age at randomization, years	51.5 (18.4)	1178	52.1 (18.2)	1174	0.446
Systolic blood pressure, mmHg	123 (19.7)	1175	124 (19.3)	1170	0.158
Diastolic blood pressure, mmHg	76.6 (11.8)	1175	76.5 (11.8)	1170	0.919
Serum Cholesterol, mg/dL	208 (44.1)	1179	208 (43.1)	1176	0.943
Serum Triglycerides, mg/dL	117 (62.4)	1176	116 (55.3)	1175	0.918
BMI	24.6 (4.78)	1166	24.5 (4.74)	1164	0.552
% Male	53.9	1179	50.9	1176	0.144
Diet exposure in days	1063 (371)	1179	1055 (377)	1176	0.601
% Missed meals ¹	9.61 (12.9)	1179	9.44 (12.5)	1176	0.744

¹Average percentage of missed meals throughout the full study period

Did the MCE dietary intervention effectively reduce serum cholesterol?

MCE investigators hypothesized that replacing SFA with vegetable oil rich in LA would reduce serum cholesterol in a manner consistent with the Keys equation [34] (**Table 2**). As predicted, the dietary intervention effectively reduced serum cholesterol in the ≥ 1 year serum cholesterol cohort (mean change -31.2 mg/dL, (SD 30.6 mg/dL); -13.8% (SD 13.0%) p<0.001), and in each of the pre-specified subgroups (all p's<0.01).

Table 3: Predicted & observed serum cholesterol changes in the intervention & control groups

	Observed Dietary changes*		Serum cholesterol changes	
	LA (% Change)	SFA (% Change)	Predicted based on the Keys equation**	Observed in the MCE (n=2355)***
Intervention diet	+288	-51	-18.1%	-13.8% (SD 13.0%) p<0.001
Control diet	+38	-1	-1.1%	-1.0% (SD 14.5%) p<0.001

[17-20 26]

*Changes from the baseline hospital diet were calculated from 1975 abstract, with LA estimated by multiplying total PUFA by 0.9.

** $\Delta\text{Chol}=1.3(2\Delta\text{S}-\Delta\text{P})$ where S and P are the percent of calories from Saturated and Polyunsaturated fatty acids, respectively

***percent change in serum cholesterol calculated for each individual in the ≥ 1 year cohort.

P-values are from paired t-test testing within person change in serum cholesterol.

LA, linoleic acid; SFA, saturated fat.

Among the intervention group, higher adherence (fewer missed meals) was associated with a more pronounced reduction in serum cholesterol ($p < 0.001$). Participants who missed $\leq 2\%$ of their meals achieved serum cholesterol reduction of -18.0% , which is nearly identical to that predicted by the Keys equation (**Table 3** and Part 10 of the web appendix).

The control diet, which increased dietary LA by 38% but did not alter SFA, produced a modest but statistically significant reduction in serum cholesterol compared to baseline (-5 mg/dL (SD 30 mg/dl); -1.0% (SD 14.5%); $p < 0.001$) (**Figure 2 and Table 3.**). Higher adherence to the control diet was also associated with greater reduction in serum cholesterol ($p = 0.004$).

Was the dietary intervention successful at reducing the number of deaths?

MCE investigators hypothesized that randomization to the intervention group would reduce the risk of death. However, published MCE data provide no evidence of mortality benefit in the full population, or in any pre-specified MCE subgroup. A survival analysis of the full MCE population that was presented in the 1989 manuscript [17] showed that “the small difference in the two life tables is in an unfavorable direction”. Crude risk ratios for all-cause mortality calculated from data published in the 1989 manuscript show no indication of benefit in the total cohort, men, or women (**Table 4**).

The intervention and control groups in the full MCE population had similar risk of cardiovascular events. The authors of the 1989 manuscript provided a lifetable of cardiovascular events and noted that “the similarity of the two curves is striking” [17]. The original study investigators noted that there were slightly fewer events and deaths in intervention vs. control group among men < 50 years old who were in the study ≥ 2 years. However, this was not a pre-specified comparison and the difference was not statistically significant.

Table 4. Risk Ratios for all-cause mortality calculated from 1989 MCE publication data (n=9,057)

	RR	95% CI	P-value	Intervention		Control	
				n	deaths	N	Deaths
Full cohort	1.08	(0.91-1.28)	0.40	4541	269	4516	248
<i>Subgroups</i>							
Men	1.03	(0.83-1.28)	0.78	2197	158	2196	153
Women	1.16	(0.88-1.52)	0.30	2344	111	2320	95
≥1 year on diet ¹	1.21	(0.92-1.59)	0.17	1178	114	1174	94

¹The number of participants with ≥1 year of diet exposure was not reported in the 1989 publication. Risk ratio for this ≥1 year cohort calculated using deaths reported in the 1989 publication and the numbers of randomized participants recovered from Tape 2 data.

Kaplan Meier Life Table graphs of cumulative mortality from the 1981 Masters Thesis are shown in

Figure 3. Based on these Life Tables, the thesis author noted that "obviously, the findings of the [MCE] with regard to total mortality do not exhibit evidence of a beneficial effect of the experimental diet" [8]. Moreover, the Life Table for the ≥65 cohort (**Fig. 3E**) suggests the possibility of increased risk of death for the intervention group compared to controls. The thesis author noted that "the excess mortality in the diet group seems to have been confined primarily to patients 65 or older" [8].

However, data are not available to determine the statistical significance of this finding.

The MCE investigators anticipated that any beneficial effects of the serum cholesterol-lowering diet would take substantial time to manifest, and placed a special emphasis on the cohort of subjects with ≥1 year of diet exposure [7]. However, according to the 1989 publication [17], there were slightly more deaths in the treatment group (114) than the control group (94) among participants exposed to study diets for 1 year or longer (**Table 4**). This lack of mortality benefit with long-term diet exposure is contrary to the pre-trial expectation that the mortality benefit of the experimental diet would take time to accrue. Recovered data from our ≥1 year serum cholesterol cohort is consistent with this lack of benefit from long-term diet exposure. Crude incidence rate ratios show no indication of benefit in any subgroup (Part 11 of web appendix), and like the Thesis Life Tables, suggest the possibility of increased risk of death among participants aged ≥65. The potential for delayed onset of increased risk is consistent with the Thesis statement that "the [cumulative mortality] graph shows rather similar mortality for the two groups up to about 400 days, and rather remarkable divergence thereafter."(**Fig. 3E**).

Thus, collective data from the 1989 publication and 1981 Master's Thesis provide no evidence for mortality benefit, and suggest the possibility of increased risk of death in older adults. This lack of benefit, despite a significant reduction in serum cholesterol, does not provide support for the traditional diet-heart hypothesis.

Was the reduction in serum cholesterol related to lower risk of death?

The traditional diet heart hypothesis predicts that participants with greater reduction in serum cholesterol would have lower risk of death (**Fig. 1, line B**). However, MCE participants with greater reduction in serum cholesterol had higher, rather than lower, risk of death. We provide two ways of examining these results. **Figure 4** provides a visual representation using average change in serum cholesterol; **Table 5** provides hazard ratios (HR) for crude, adjusted and sensitivity models using time-varying cholesterol.

The average change in cholesterol in the intervention, control, and combined groups were, in mg/dL, -31 (SD = 31), -5 (SD = 30), and -18 (SD= 33), respectively (**Fig. 4, row 1**). The number and proportion of deaths increased as serum cholesterol decreased (**Fig. 4, rows 2 & 3**). The probability of death increased as serum cholesterol decreased, in logistic models adjusted for age and baseline serum cholesterol (**Fig. 4, row 4**). The observed higher risk of death with decreasing serum cholesterol was significant in each group, and did not differ between groups (likelihood ratio test p-value =0.67).

In our survival analysis (**Table 5**), there was also a robust association between decreasing serum cholesterol and increased risk of death, and this association did not differ between the intervention and control group (p-values for serum cholesterol x intervention interaction were all >0.16). Among both groups combined, a 30 mg/dL decrease in serum cholesterol was associated with 22% higher risk of death from any cause (HR 1.22; 95% CI 1.14, 1.32) based on a Cox model adjusted for baseline serum cholesterol, age, gender, adherence to diet, BMI, and systolic blood pressure.

Table 5. Hazard ratios for death from any cause as a function of serum cholesterol in ≥ 1 year serum cholesterol cohort (n=2,355)¹

	Intervention			Control			Both Groups		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
<i>All Subjects</i>	<i>n = 1179</i>			<i>n = 1176</i>			<i>n = 2355</i>		
Crude ²	1.45	1.27, 1.66	<0.001	1.44	1.17, 1.76	<0.001	1.39	1.23, 1.58	<0.001
Adjusted ³	1.22	1.04, 1.44	0.016	1.28	1.09, 1.51	0.003	1.22	1.14, 1.32	<0.001
Sensitivity ⁴	1.20	1.03, 1.41	0.023	1.32	1.11, 1.57	0.002	1.24	1.15, 1.33	<0.001
<i>Subjects <65 years old</i>	<i>n = 886</i>			<i>n = 874</i>			<i>n = 1760</i>		
Crude ²	1.02	0.60, 1.73	0.936	1.17	0.82, 1.69	0.389	1.07	0.93, 1.23	0.368
Adjusted ³	0.92	0.61, 1.37	0.680	1.12	0.79, 1.57	0.525	1.01	0.88, 1.16	0.924
Sensitivity ⁴	0.94	0.58, 1.53	0.816	1.16	0.79, 1.70	0.448	1.02	0.91, 1.16	0.703
<i>Subjects ≥ 65 years old</i>	<i>n = 293</i>			<i>n = 302</i>			<i>n = 595</i>		
Crude ²	1.55	1.39, 1.71	<0.001	1.53	1.24, 1.88	<0.001	1.50	1.31, 1.71	<0.001
Adjusted ³	1.42	1.22, 1.67	<0.001	1.39	1.10, 1.76	0.006	1.35	1.18, 1.54	<0.001
Sensitivity ⁴	1.36	1.17, 1.58	<0.001	1.43	1.14, 1.78	0.002	1.35	1.19, 1.53	<0.001

¹Cox regressions for death as a function of time-varying serum cholesterol. All estimates represent the HR for each 30 unit decrease in serum cholesterol.

All models account for clustering within hospital.

²Model adjusted for baseline serum cholesterol

³The "Adjusted" model was adjusted for baseline serum cholesterol, age, BMI, gender, adherence to the diet, and systolic blood pressure (SBP).

⁴The "Sensitivity" model is further adjusted for time-varying percent change from baseline for BMI and SBP.

The higher risk of death associated with decreased serum cholesterol seems to be driven by the ≥ 65 year-old subgroup. Among participants who were greater than 65 years old at baseline, a 30 mg/dL decrease in serum cholesterol was associated with 35% higher risk of death (HR 1.35; 95% CI 1.18, 1.54), whereas among subjects younger than 65 years of age at baseline there was no relationship between the change in serum cholesterol and death (HR 1.01; 95% CI 0.88, 1.16).

Sensitivity analysis to account for frailty

To explore the possibility that frailty (which is associated with both low cholesterol and death [35 36]) could confound these results, we did a sensitivity analysis adjusting our Cox models (**table 5**) for two known markers of frailty (changes in body weight and changes in systolic blood pressure) [35-37]. These adjustments did not materially change the effect estimates, which remained statistically significant in both groups.

This finding that greater serum cholesterol reduction was associated with higher, rather than lower, risk of death in the MCE does not provide support for the traditional diet-heart hypothesis.

Serum cholesterol lowering and risk of death in the 1981 Thesis

Despite the use of different methods, findings from our analyses are consistent with the findings reported in the 1981 Thesis (see Part 3 of the web appendix). For example, both analyses found that serum cholesterol changes were impressive, were positively associated with higher compliance with the diet, and that overall the cholesterol reductions and assignment to the diet group did not translate into a mortality benefit either for death from any cause or for CHD mortality. In the case of older patients, the diet appears like it might have had an unfavorable effect on mortality. Moreover, the Thesis HR estimates from Cox regression associated with a 30-point reduction in serum cholesterol are similar to those reported in the present manuscript.

Provisional autopsy findings

Characteristics of the partially recovered autopsy cohort are shown in Part 12 of the web appendix; the intervention and control groups were well balanced at baseline, with no significant differences evident for any demographic, clinical or laboratory variables. Mean age was 69.5 years, 36% were women, and the median follow-up was 298 days (316 days for intervention group, 217 days for control group). Baseline serum cholesterol was 210mg/dL. The mean change in serum cholesterol during follow up was -17.9% in the intervention group and -1.3% in the control group.

MCE investigators hypothesized that intervention group participants would have less advanced atherosclerosis and fewer autopsy-confirmed myocardial infarcts. In the autopsy cohort, however, intervention group participants had a signal toward higher coronary narrowing scores (24.41 v. 22.31), and higher risk of having at least one myocardial infarct (IRR 1.90; 95% CI 1.01, 3.72) (**Table 6**). These findings should be interpreted with caution due to partial recovery of autopsy files.

We found no evidence of association between changes in serum cholesterol and risk of infarcts, coronary atherosclerosis, or aortic atherosclerosis in covariate-adjusted models (Part 13 of the web appendix).

Table 6. Diet group assignment and the risk of infarcts and degree of atherosclerosis in the 2015 autopsy sample¹

	Intervention		Control		Intervention vs. Control			
	N	Mean	n	Mean	n	Coef. ²	95% CI	P-value
Coronary atherosclerosis score	75	24.41	74	22.31	149	2.10	(-0.12, 4.33)	0.064
Aortic atherosclerosis score	73	4.79	70	4.56	143	0.24	(-0.16, 0.64)	0.241

	N	Number %	n	Number %	n	IRR ³	95% CI	P-value
	Myocardial Infarcts ⁴	76	31 40.8%	73	16 21.9%	149	1.90	(1.01, 3.72)

¹149 of 295 completed autopsies were recovered. Therefore, this analysis should be considered provisional until the complete autopsy data can be recovered.

²Coefficients based on regression of atherosclerosis score as a function of diet group assignment

³Incidence rate ratio; ⁴number and percent of subjects with at least one infarct.

Does the available body of RCT evidence support the traditional diet heart hypothesis?

The updated meta-analysis of RCTs that lowered serum cholesterol by providing vegetable oils rich in LA in place of saturated fats showed no evidence of coronary or all-cause mortality benefit. This was true in both the main analysis (pooled HR 1.12 and 1.07), and in numerous sensitivity analyses (HR ≥ 1.0), see Part 9 of the web appendix. In meta-regression, there was no clear association between changes in serum cholesterol and coronary or all-cause mortality either in the main analysis or sensitivity analyses. Detailed results including publication bias assessment, pooled risk estimates, heterogeneity analyses, and sensitivity analyses are provided in Part 9 of the web appendix.

Discussion

The traditional diet-heart hypothesis predicts that replacing SFA with vegetable oil rich in LA will reduce CHD events and deaths by lowering serum cholesterol. Many studies have yielded results consistent with pieces of this hypothesis. However, the clinical benefits of these serum cholesterol-lowering diets have never been causally demonstrated in a RCT and thus remain uncertain. We recovered previously unpublished data from two landmark RCTs that were designed to provide causal evidence to support the diet-heart hypothesis. In a prior publication, we reported that the Sydney Diet Heart Study intervention group had increased risk of death from CHD and all causes, despite a significant reduction in serum cholesterol [15].

The present analysis of MCE data examines missing links along the proposed causal chain of events linking diet to serum cholesterol and to clinical outcomes. The MCE intervention effectively reduced serum cholesterol in all pre-specified subgroups; however there was no clinical benefit in any group. Paradoxically, MCE participants who had greater reduction in serum cholesterol had higher, rather than lower, risk of death. In addition, the MCE intervention group did not have less atherosclerosis or fewer infarcts at autopsy. Pooled analyses of diet-heart RCTs including previously unpublished SDHS and MCE data showed no indication of benefit, and no clear association between cholesterol lowering and death from CHD or all-causes. Thus, collective findings from RCTs provide no support for the central diet-heart tenet that the serum cholesterol lowering effects of replacing SFA with LA translate to clinical CHD risk reduction.

Limited and inconsistent evidence from non-randomized studies

While the RCT is the only study design that can demonstrate a cause and effect relationship, observational cohort studies can be used to investigate longer-term exposures than are typically feasible in RCTs [38]. However, limitations of observational studies (for example, healthy consumer bias) can sometimes distort [38-40], or even reverse [41-43], true associations. Self-reported intake of foods that are often high in LA was associated with lower CHD risk in two large prospective observational cohorts of U.S. health professionals [44 45], and in one pooled analysis of several cohorts [46]. However, other large prospective observational studies [47], and another pooled analysis [16] found no association. Similar inconsistent associations between circulating LA and CHD risk have been reported [48 49], with pooled analysis showing no association [16]. Ecological associations between LA intake and CHD have also been cited to support the diet-heart hypothesis [50]. However, these associations are subject to important confounders and are wholly dependent on the timeframe selected (see Part 14 of the web appendix). Thus, the conclusions that can be drawn

from non-randomized studies on this topic are limited. Together with the lack of support from RCTs (after SDHS and MCE data recovery), the totality of evidence no longer provides a sound basis to support the traditional diet-heart hypothesis.

Why didn't serum cholesterol reduction translate to clinical improvement in diet-heart RCTs?

A plausible explanation for the seemingly paradoxical results of the MCE and SDHS is that LA-rich vegetable oil was the agent used to lower serum cholesterol. As the major vehicle for delivery of cholesterol to vascular tissues, LDL is often considered a causal mediator of CHD [51]. Since replacing SFA with LA specifically reduces LDL (without affecting HDL [9]) it is tempting to assume that such dietary changes will automatically translate to CHD risk reduction. Critically, however, consumption of vegetable oils rich in LA produces a wide range of biochemical consequences, including qualitative changes in lipoprotein particle oxidation that could plausibly increase CHD risk (reviewed in [15] and [52]). Hence the clinical effects of replacing SFA with vegetable oils may reflect the net impact of reducing LDL concentrations while increasing its susceptibility to oxidation. Moreover LDL concentrations are influenced by many factors, such as delivery of LDL to blood vessels and other tissues, as well as hepatic clearance of native and oxidized LDL particles [53-56]. Therefore, LDL lowering can represent widely different biochemical phenomena. This broader understanding could help explain why some agents that lower LDL have been shown to reduce CHD risk [57 58] while others have no clear effect [59], and still others may actually increase CHD risk [60 61]. The collective data from diet-heart RCTs suggests that reducing serum cholesterol by replacing SFA with vegetable oils rich in LA has no clear effect or may increase CHD risk.

High LA intakes from vegetable oils are a recent and atypical nutritional phenomenon

In order to interpret research on LA one needs to consider both the food sources and the amounts of LA consumed. Individuals eating only minimally processed whole foods—as everyone did until about 100 years ago—would have consumed about 2-3% of calories from LA [62 63](**Fig. 6**). By contrast, among industrialized populations today, the majority of LA intake is derived from highly concentrated vegetable oils, in which the fatty acids are separated from the fiber, protein, and micronutrients that are naturally present in vegetables and seeds [64](Part 15 of the web appendix). Because these highly concentrated sources of LA are used widely as cooking and frying oils and added to many processed and packaged food items, the LA content of modern industrialized diets is much higher than natural diets (**Fig. 6**). For example, mean LA intake in the U.S. of about 17 grams per day (7% of calories)[65] is much higher than the approximately 6 grams of daily LA provided by natural food diets without added vegetable oils. If these concentrated sources of LA are considered to be dietary

supplements, then on average, Americans ingest the equivalent of 11 one-gram LA capsules per day above and beyond intake from natural foods.

The impacts of high LA intake extend beyond serum cholesterol lowering

Alterations in dietary LA could affect the risk of developing many diseases by altering the production and actions of bioactive, non-cholesterol lipid mediators (**Fig. 7**). For example, oxidized derivatives of LA that are regulated by diet [68-72] have been implicated in the pathogenesis of CHD (**Fig. 7B**)(reviewed in [15]), chronic pain [73-75], and steatohepatitis [76-78](**Fig. 7C**). While the biochemical and clinical consequences of high LA intakes are incompletely understood, there is a possibility for unintended harm. These potential risks highlight the importance of ensuring that the full evidence base from RCTs is available for consideration by scientists, policymakers and the public.

Historical context for the publication of SDHS and MCE findings

With today's recognition of publication bias and requirements for trial registration and timely publication upon completion of registered trials, the omission of key results of these two trials from the literature may seem difficult to understand. In the case of the MCE, the crude study results were clearly at odds with prevailing beliefs. One can speculate that the investigators and sponsors would have wanted to distinguish between a failed theory and a failed trial before publication. While robustly designed and carefully executed, the MCE had several unique features that complicated analysis and could have biased results. The MCE investigators may have been concerned that heavy censoring or the complicated health and social histories of study subjects could have impacted results. In addition, the methods of adjusting survival time analyses for covariates were just emerging, and statistical software packages were not widely available, even at the time the Thesis was written. Failure to measure cholesterol levels for subjects who left the hospital before one year could have introduced bias and would have reduced power for some analyses, and the heavy censoring might have further contributed to the possibility of Type II errors. There would have been little or no scientific or clinical trial literature at the time to support findings that were so contrary to prevailing beliefs and public policy. And finally, it is possible that medical journal reviewers would not have accepted study results for the reasons cited above.

Whatever the explanation for key MCE data not being published, there is growing recognition that incomplete publication of negative or inconclusive results can contribute to skewed research priorities

and public health initiatives [79-81]. Recovery of unpublished data can alter the balance of evidence, and in some instances can lead to reversal of established policy or clinical practice positions [79]. **Figure 8** provides an historical context for the completion and publication of the MCE and SDHS results in relation to key U.S. policy events over the past half-century. It is interesting to speculate whether complete publication of the results of these two trials might have altered key policy decisions (for example the McGovern Report (1977) [82] and National Cholesterol Education Program (1984-5) [51]), or contributed to a shift in research priorities. It remains to be seen what impact our recovery and publication of SDHS and MCE findings will have on future policy decisions and research directions.

Implications for recovery of additional MCE data files

MCE data files that remain missing could provide further insights into the diet-heart hypothesis (see part 8 of the web appendix). For example, there is a suggestion that high LA intake could adversely affect populations who are known to have increased LA oxidation, including smokers, heavy drinkers, and older adults (Reviewed in [15]). Recovery of missing data would allow us to determine if the high LA diet had deleterious effects in the MCE subgroups that are susceptible to LA oxidation (≥ 65 years of age, established CHD, smokers).

The partial recovery of 149 MCE heart and aorta autopsy files provides an intriguing clue that the intervention might have had unfavorable effects. However, since 146 heart and aorta files and the full cohort of 295 autopsied brains remain missing, these provisional findings are inadequate to draw conclusions. Since it is highly unlikely that a diet-heart trial of the size and scope of the MCE will ever be conducted again, it is essential that these missing autopsy files are recovered and analyzed as specified in the 1967 MCE grant application [7 21-23].

Strengths & Limitations

The MCE had several exceptional features and strengths. For example, the MCE is by far the largest RCT to test the central diet heart tenet that lowering serum cholesterol by replacing SFA with LA rich vegetable oil will translate to clinical CHD risk reduction. The MCE is also the only RCT to test the clinical effects of increasing LA in large cohorts of women and individuals aged ≥ 65 , and the only such RCT to complete a post-mortem assessment of coronary, aortic and cerebrovascular atherosclerosis grade and infarct status.

Key strengths of the MCE, especially when compared to other studies of the diet-heart hypothesis, are that participants were randomly assigned to the diets and all meals were provided. Because participants were randomly assigned to the intervention or control diet, we know that changes in LA and SFA were due to the intervention itself. Thus, MCE effectively addressed the problem of healthy consumer bias that confounds many observational studies [38]. Also, the effect of replacing SFA with LA is not a statistical estimation, but an actual dietary change in each participant. Moreover, many studies investigating the effects of nutrients on CHD risk use less precise methods (for example, food frequency questionnaires [38-40]) to approximate dietary intakes. By contrast, the MCE used chemical analyses of the specific foods that were served at each hospital to more accurately gauge nutrient intakes.

The MCE also had several important limitations in study design, generalizability, and perhaps statistical power. To reduce the cost of the study, blood samples were analyzed for all randomized participants in the study at least one year, thus our analysis of serum cholesterol and death is limited to this group. LDL (and HDL) subfractions, which are more closely linked to CHD risk than total serum cholesterol, were not assessed. Importantly, however, numerous RCTs have shown that replacing SFA with vegetable oil rich in LA leads to predictable reductions in LDL without altering HDL [9]. Thus, the serum cholesterol lowering effects of the MCE diets were likely specific to LDL.

Because the trans fatty acid (TFA) contents of MCE study diets are not available, one could speculate that the lack of benefit in the MCE intervention group was due to increased consumption of TFA. Indeed, in addition to liquid corn oil the MCE intervention diet also contained a serum cholesterol-lowering soft corn oil polyunsaturated margarine, which likely contained some TFA. However, the MCE PI (Ivan Frantz) and Co-PI (Ancel Keys) were well aware of the cholesterol-raising effects of TFA prior to initiating the MCE [83]. Moreover, Frantz and Keys previously devised the diets used in the institutional arm of the National Diet Heart Feasibility Study (NDHS), which achieved the greatest reductions in serum cholesterol of all NDHS study sites [2 3 24 84-88]. Hence, it is highly likely that this experienced MCE team selected products containing as little TFA as possible in order to maximize the degree of cholesterol lowering achieved. Perhaps more importantly, it is clear from the MCE grant proposal that common margarines and shortenings (major sources of TFA) were important components of the baseline hospital diets and the control diet (but not the intervention diet). Thus, confounding by dietary TFA is an exceedingly unlikely explanation for the lack of benefit of the MCE intervention diet.

Another potential limitation in the interpretation was incomplete data collection and recovery. For example, in the SDHS the increased mortality in high LA group was most evident in smokers and heavy drinkers. Without additional recovery MCE data we are not able to determine if the effects of the high-LA diet varied by smoking status, pre-existing CHD, psychiatric history, or medication use.

Limitations in generalizability

The MCE intervention diet contained almost twice as much LA as the average American diet. Only a small percentage of the U.S. population currently consumes LA in amounts that overlap the MCE intervention diet (**Fig. 6**). Since this high-LA diet produced a maximal reduction in serum cholesterol, it was ideal for testing the diet-heart tenet that serum cholesterol is the critical mediator linking diet to CHD. However, one cannot necessarily extrapolate findings to lower LA intakes.

The decision to conduct the MCE in mental hospitals and nursing homes reduced the number of missed meals and maximized the degree of serum cholesterol-lowering achieved. These hospitals also provided broader age and gender distributions than other diet-heart RCTs. However, the results are not necessarily generalizable to populations without mental illnesses or living outside of nursing homes.

Since the MCE, SDHS and other diet-heart RCTs used concentrated vegetable oils, the results should not be generalized to nuts or other natural, unprocessed food sources of LA.

Limitations in statistical power

The possibility of a type II error cannot be ruled out; in other words, the lack of benefit from replacing SFA with LA may be a false negative due to insufficient statistical power or inadequate duration of diet exposure. However, contrary to pre-trial expectations several of the associations reported here are in the opposite direction, and seemed to worsen with long-term diet exposure. Thus it is unlikely that increases in MCE sample size or a longer follow-up would have yielded results supporting the diet-heart hypothesis.

Meta-analysis limitations

Limitations of our updated meta-analysis include the small number of RCTs that have tested the diet-heart hypothesis, the differences in design and population characteristics of each trial, and general limitations of meta-analyses such as publication bias (reviewed in Part 9 of the web appendix).

Nevertheless, this meta-analysis, which is the first to include both the recovered serum cholesterol changes and clinical endpoint data from the SDHS and MCE men and women, is the most complete

meta-analysis to date. Moreover, the robustness of meta-analysis results—with HRs ≥ 1.0 in all sensitivity analyses—shows that the lack of evidence for benefit is not likely due to selection bias.

The Big Picture

The kilograms of molecules that we eat every day as foods play key structural roles and act as substrates, which enter into and regulate numerous highly-leveraged biochemical pathways [75 89-93]. Thus, although the traditional diet-heart hypothesis did not unfold as predicted, the foods that we eat likely play critical roles in the pathogenesis of many diseases. However, given the extraordinary complexity of biological systems and the many limitations of our research methods, current understanding of the biochemical and clinical effects of foods are rudimentary.

The history of the traditional diet-heart hypothesis suggests that the field of nutrition could be improved by: (a) not overemphasizing intermediate biomarkers; (b) cautious interpretation of non-randomized studies; and (c) ensuring timely and complete publication of all RCTs. Given the limitations of our current evidence base, the best approach may be one of humility, highlighting limitations of current knowledge and setting a high bar for advising intakes beyond what can be provided by natural diets.

Summary and Conclusion

As the SDHS before it, the MCE data recovery has filled a critical gap in the published literature archive. MCE findings add to growing evidence that incomplete publication has contributed to overestimation of the benefits, and underestimation of the potential risks, of replacing SFA with vegetable oils rich in LA.

Footnotes

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Contributors: CER designed and directed the project; located, managed, and validated the recovered data and materials, performed the literature review, and was the main writer of the manuscript. SFMH located, managed and validated the recovered data, and assisted in the literature review and in writing and revising the manuscript. John Svee (Data Conversion Resource) completed the recovery of 9-track tape data and the optical character recognition of greenbar data, with conversion to modern spreadsheet format, in collaboration with CER and SFMH. DZ conducted the statistical analyses in collaboration with CMS, and was a main writer of the manuscript. KRF conducted the meta-analyses, in collaboration with SFMH, CER, JMD, and CMS. KRF, JMD and CMS contributed to the interpretation of study results and the writing and revision of the manuscript. SB wrote the 1981 Master's Thesis, provided insights into trial design, data analyses and incomplete publication, and contributed to the writing and revision of the manuscript. RF located recovered data, wrote the tribute to Ivan Frantz and the MCE research team, and revised the manuscript. AR validated the recovered data and revised the manuscript. JRH directed the project and contributed to writing and revision of the manuscript. All authors contributed to analyses or interpretation of results and to the intellectual content of the manuscript. CER is the guarantor.

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