NOAA TECHNICAL MEMORANDUM
NMFS-SEFSC-399

BIOMEDICAL TEST MATERIALS PROGRAM:
SYNOPSIS OF RESEARCHERS' ANNUAL REPORTS FOR 1993 and 1994

by

PATRICIA A. FAIR and JULIE D. CARTER

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southeastern Fisheries Science Center
Charleston Laboratory
219 Fort Johnson Road
Charleston, SC 29412-9110
March 1997

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U. S. DEPARTMENT OF COMMERCE
William Daley, Secretary

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
D. James Baker, Administrator

NATIONAL MARINE FISHERIES SERVICE
Rolland A. Schmitten, Assistant Administrator for Fisheries

March 1997

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This report should be cited as follows:

Fair, P.A., and J. Carter. 1997. Biomedical Test Materials Program: Synopsis of Researchers' Annual Reports for 1993 and 1994, National Marine Fisheries Service, Charleston, SC. NOAA Tech. Mem. NMFS-SEFSC-399, p 28.

Authors' affiliation: NOAA, NMFS, Charleston Laboratory, Charleston, SC 29412.

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INTRODUCTION

Overview of The Biomedical Test Materials Program

The Biomedical Test Material (BTM) Program is a cooperative program between the National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service (NMFS) and National Institutes of Health (NIH), National Institute of Alcohol, Drug Abuse, and Alcoholism (NIAAA). The BTM Program provides the technical development and production of research grade materials for the investigation of the effects of omega-3 fatty acids in human health. The BTM Program, in operation since 1987, has supported more than 220 research studies including 77 clinical projects using these materials. The majority of these are grantees from the National Institutes of Health. There have been studies performed on various animal species, *in vitro* studies, and human studies. Currently, 43 researchers hold an Investigative New Drug Number (IND) for conducting human studies using the test materials. Four human studies have been conducted in other countries. Thus far, 67 researchers have received test materials for studies not directly within the biomedical field. Many analytical laboratories also have received test materials for use as qualitative and quantitative standards for lipid analyses. These test materials, supplied by the NOAA/NMFS Charleston Laboratory, are being utilized by U.S. and foreign investigators for research on cardiovascular diseases, arthritis, diabetes, inflammatory and autoimmune diseases, kidney disorders, cancer, malaria, skin disorders and lipid metabolism. The BTM Program has helped to generate high quality research in this area leading to a better understanding of the health effects of omega-3 fatty acids and helped to set standards for the commercial sector.

The Charleston Laboratory, NOAA, thus has made a considerable investment in facilities, personnel, training and time in developing this program to purify marine oils into various products. The Memorandum of Understanding (MOU) with NIH provides cooperation with a leading laboratory at the NIH involved in fatty acid research and encourages a two-way transfer of expertise in this area. The BTM Program provides support for an undervalued industry (menhaden) by the production of biomedical test materials from menhaden oil that will lead to a better understanding of the role of the unique marine fatty acids (omega-3's) in nutrition and health. The program's goal is to help attain a conclusive research decision on the effects of omega-3 fatty acids based on objective evaluation of results and to further the design and conduct of studies to yield such results.

Establishment of the BTM Program

A conference in 1985 on "Health Effects of Polyunsaturated Fatty Acids in Seafoods" investigated the role of omega-3 fatty acids in modulation of human metabolism as well as their role in the prevention and treatment of a number of diseases. The conference had two objectives: 1.) to review research data on the health effects of polyunsaturated fatty acids (PUFAs) in seafoods and the impact on eicosanoid formation, thrombosis, inflammation, and the role of docosahexaenoic acid (DHA) in membrane function; and 2.) to develop a research agenda to determine the range of effects of PUFAs of seafood origin in the American diet. This conference concluded that additional basic information from biochemical and clinical studies was needed prior to formulating recommendations. A thorough examination of the problems associated with

meeting research objectives led to the conclusion that the lack of highly purified. quality-assured omega-3 PUFA test materials needed to conduct large-scale studies, combined with their high cost on commercial markets, were limiting factors in omega-3 research. Long-term, systematic studies using standardized, quality-controlled omega-3 test materials were lacking.

This need was realized within NOAA and a program was formed to meet this challenge. The establishment of an MOU between NOAA and NIH prepared the framework for providing a long-term, consistent supply of test materials to facilitate the evaluation of the role of omega-3 fatty acids in health and disease. The agencies agreed to cooperate on, and provide support to, research activities related to the biological mechanisms by which a seafood diet or the ingestion of fish oils may influence health. The NOAA/NMFS Charleston Laboratory is committed under this MOU to provide a long-term, consistent supply of quality-assured test materials. Since the availability of high quality test materials was viewed as a critical element, NMFS embraced the opportunity to fill this need and to give direction to research agendas. A commitment was made in 1985 for NOAA/NMFS to provide research test materials of known composition, free from contaminants, and in the quantity required for studies over their duration. Proper evaluation of lipid components found in seafood and fish oils require that specific, quality-controlled test materials be made available to researchers. A strategic plan was formulated utilizing internationally recognized academic, clinical and research managers to support research agendas relative to the biochemical action of omega-3 lipids. The NIH serves in an advisory capacity on research and technical issues.

Strategic elements of the BTM Program consisted of a controlled production facility, extensive quality assurance, packaging and distribution of omega-3 fatty acid test materials. The joint administration of the program was by a committee formed in recognition of the MOU. The Fish Oil Test Material Advisory Committee (FOTMAC) was structured to provide advice from a broad base of internationally known research scientists and managers from academic and government institutions, including individuals of international reputation and knowledge in the chemistry, biochemistry, and nutrition of omega-3 lipids, particularly in the conduct of human nutrition and disease. The FOTMAC is chaired currently by Dr. Norm Salem (NIAAA) and is composed of scientists representing the funding agencies (NIH), the research community, Department of Commerce (DOC), and the Food and Drug Administration (FDA). The FOTMAC provides scientific advice to the DOC regarding the types of materials needed by research scientists, shipping procedures for the materials, and additional quality control and production issues. In addition, a Fish Oil Test Materials Distribution Committee (FOTMDC) was formed to process the applications received from investigators. The FOTMDC, composed of NIH and other Federal scientists, advises the DOC of applicants who have fulfilled the application process, and makes recommendations regarding the distribution of requested materials. Applications are considered from researchers having funded, peer-reviewed research studies indicating a need for the requested materials.

The construction of the NMFS pilot production facility began in 1986 and was operational in September 1987. Concurrently, an extensive quality assurance program and methods for handling, storage, packaging and distribution of these labile products were developed. In 1988, production of omega-3 ethyl ester concentrate was begun. The NMFS Charleston Laboratory was inspected by the FDA and certified as an approved manufacturer of products derived from

fish oils. In addition, during 1989 extensive support information for human studies, in the form of a Drug Master File (DMF), was prepared and filed with the FDA. The DMF provides the information needed by researchers when applying for an investigational new drug (IND) number to conduct human research studies. In 1990, production of purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) allowed the use of these highly valuable materials in research studies. The test materials are distributed on a gratis basis to researchers upon the recommendation of the FOTMDC. With the availability of consistent quality fish oil test materials, researchers are able to reach a new level of confidence in their data. The availability of materials for the duration of the study allows the design and conduct of long-term projects. The NMFS provides technical information on diet preparation, methodology, product stability, handling, and storage. It is anticipated that these actions help to set the pace for the production and quality assurance of caliber fish oils in the commercial sector.

Production

Key elements in production are a consistent supply of single-source raw material (menhaden oil) for processing, optimized operating parameters, use of a nitrogen environment during processing, and handling and storage techniques designed to protect materials.

Quality Assurance

Each lot of material is thoroughly analyzed to assure composition, quality and safety. Some products may require up to 29 separate analyses to assure that the product meets specifications. Analyses are provided for placebo materials procured by researchers from other sources to assure that antioxidant levels are balanced with BTMs obtained from the program. In addition, long-term stability testing is also performed.

Distribution

Test materials are available for the duration of the research project. This allows researchers to design and execute long-term projects with confidence in their supply. The materials are provided in the quantity required with packaging and dosage delivery customized according to need. Bulk-packaging is designed for optimal use and storage of the material. Soft-gel capsules are matched with placebos for double-blind clinical studies.

Technical Information

A number of technical services and information are afforded to researchers receiving test materials. This includes information on experimental diet preparation, methods for balancing antioxidants, product delivery form, product stability, recommended storage and handling procedures, analytical procedures, use of placebos in double-blind studies, export requirements for drugs, and assistance in obtaining IND approvals. Preparation of the Drug Master File was instrumental in expediting the approval of the use of omega-3 fatty acids in human clinical studies. Manuals on Analytical Methods for the Quality Assurance of Fish Oil, Production Methods/Safety and Distribution were produced by the DOC and are available to investigators.

Test Materials Available

- Vacuum-deodorized menhaden oil in bulk and soft-gel capsules
- omega-3 ethyl ester concentrate, prepared from winterized Atlantic Menhaden (*Brevoortia tyrannus*) oil, in either bulk or soft-gel capsules
- Encapsulated commercial preparations of corn, olive, and safflower oils
- Encapsulated and bulk preparations of ethyl esters of corn, olive, and safflower oils
- Encapsulated and bulk preparations of purified EPA and DHA ethyl esters (>95% purity) prepared from menhaden oil.

The following is a list of researchers who received Biomedical Test Materials during 1993 and 1994 and responded to the report request. These researchers are grouped according to their research area. The BTM Program appreciates the responses and updates on research progress from studies using these materials.

ARTHRITIS/INFLAMMATION

Dr. William R. Henderson
University of Washington

Title: Omega-3 Supplementation

Objective: Massive influx of neutrophils into the lungs of patients with cystic fibrosis (CF) and release of inflammatory mediators may contribute to the destruction of airway tissue in this disease. Dietary supplementation with fish oils high in omega-3 fatty acids may lead to the release of less potent mediators and reduce the magnitude of both neutrophil influx and resulting pulmonary inflammation. Prior to initiating a long-term efficacy trial of omega-3 fatty acid supplementation to decrease pulmonary inflammation in patients with CF, the FDA requested that we perform a pharmacokinetic study to determine the minimal dose of fish oil capsules required for absorption in an effort to minimize drug toxicity.

Findings: Four patient volunteers received 6 one-gram capsules of the ethyl ester concentrate of fish oil supplementation daily for 3 weeks. The ratio of the omega-3 fatty acid eicosapentaenoic acid (EPA) to the omega-6 fatty acid arachidonic acid (AA), i.e., the EPA/AA ratio was comparable to that achieved in our previous study (Henderson, W.R. Jr et al, J Pediatr 124:400-8, 1994) by patients with CF on 8 one-gram capsules daily for 3 weeks. In patients with CF, comparable absorption was documented at the 6 capsules/day dose (mean EPA/AA = 0.77) compared to the 8 capsules/day dose (mean EPA/AA = 0.72). We are currently repeating the pharmacokinetic study in subjects with CF (n=8 to be enrolled) with the fish oil dose reduced to 4 capsules/day for three weeks. One of the four subjects with CF on 6 capsules fish oil/day had an increase in SGPT to 131 IU/L at 3 weeks compared to a baseline level of 46 IU/L. Three weeks after stopping the capsules, the subject's level returned to baseline with an SGPT of 52 IU/L. There were no changes in other liver function tests in this individual; no significant changes in any liver function tests were observed in the other three individuals after 3 weeks of supplementation with 6 capsules of fish oil daily.

Dr. Joel Kremer
Albany Medical College

Title: Determination of the Clinical and Immunological Effects of Ibuprofen and Fish Oil in Patients with Active Rheumatoid Arthritis

Objective: To determine 1.) if patients with rheumatoid arthritis (RA) can discontinue non-steroidal anti-inflammatory drugs (NSAID's) while on fish oil and 2.) the clinical efficacy of a high dose of fish oil supplementation in RA patients.

Findings: Patients consuming fish oil dietary supplements exhibit many clinical improvements from baseline, including tender joints, and these improvements are associated with significant decreases from baseline in interleukin-1. Patients on fish oil do better 8 weeks after discontinuation of NSAID's than those on corn oil. Some patients on fish oil are able to discontinue NSAID's without experiencing a flare in their disease. Dietary intervention can alter clincial manifestations and cytokine profile of patients with RA.

Dr. James N. Moore
University of Georgia, College of Veterinary Medicine

Title: Use of Intravenous Omega-3 Fatty Acids in Equine Enotoxemia

Objectives: 1.) To determine the effects of short-term intravenous administration of omega-3 and omega-6 fatty acid enriched lipid emulsions on the fatty acid composition of equine monocyte membrane phospholipids and the inflammatory response of these cells to endotoxin *in vitro*. 2.) To compare the effects of omega-3 and omega-6 fatty acid enriched lipid emulsions on the response of horses challenged *in vivo* with endotoxin. 3.) To evaluate a potential mechanism by which omega-3 fatty acids exert anti-inflammatory effects. Specifically we wish to examine the effect of omega-3 and omega-6 fatty acids on the activity of two isoforms of phospholipase A_2 in endotoxin-stimulated equine monocytes.

Findings: The omega-6: omega-3 ratio in equine monocyte membrane phospholipids can be altered by short term intravenous infusion of a 20% menhaden oil emulsion. The lipid emulsion was infused at a dose of 1 gm/kg body weight over 6 hours. Changes in the omega-6: omega-3 rate of monocyte phospholipids were detected immediately after the end of the infusion. These changes persisted for at least 6 days after the end of the infusion.

CARDIOVASCULAR

Dr. Lawrence E. Boerboom
Medical College of Wisconsin

Title: Prevention of Vein Graft Atherosclerosis with Fish Oil

Objective: This investigation was designed to test the hypothesis that dietary fish oil supplementation prevents the development of atherosclerosis in vein bypass grafts in monkeys fed an atherogenic diet. Another objective was to determine whether fish oil acted synergistically with aspirin in this regard. Four treatment groups were studied: 1.) fish oil, 2.) fish oil + aspirin, 3.) olive oil, and 4.) olive oil + aspirin.

Findings: Bleeding time was significantly reduced in all other groups as compared with the group treated with olive oil alone ($p<0.05$). Despite this apparent effect on platelet function, cholesterol levels in vein bypass grafts removed four years after implantation were similar for all treatment groups.

Dr. Myung S. Chi
Lincoln University

Title: Effects of omega-3 Fatty Acids on Hypertension and Physiological Parameters

Objective: 1.) To determine the lowest level of dietary omega-3 fatty acids provided by fish oil, at which blood pressure in atteniated in reduced renal mass and Dahl Salt-sensitive rats. 2.) To assess metabolic interactions between total amount of dietary fat and omega-3 fatty acids and the effects of such interactions on blood pressure. 3.) To investigate mechanisms by which dietary omega-3 fatty acids alter blood pressure including the production of endogenous eicosanoids and alterations in function of the adrenergic system in hypertensive animals given omega-3 fatty acids. 4.) To study plasma and tissue fatty acid profiles and plasma lipoprotein cholesterol in hypertensive animals fed dietary omega-3 fatty acids. These parameters are known risk factors for atherothrombotic disorders.

Findings: Feeding fish oil lowered systolic blood pressure in Dahl salt-sensitive and salt-resistant rats. However, dietary fish oil did not affect the sympathetic nervous system activity. In a previous study with reduced renal mass-salt hypertensive rats, we observed reduced urinary excretion of norepinephrine and also reduced norepinephrine turnover rate in the heart and kidney. The explanation for the difference between two studies is not obvious, however, it could be due to the differences between the models.

Dr. Anne M. Gillis
University of Calgary

Title: Pharmacodynamics of Antiarrhythmic Drugs: Influence of Dietary Fat

Objective: To evaluate the influence of dietary fat on the pharmacodynamics of antiarrhythmic drugs. In the past year, we have completed a study comparing the effects of I_{to} and I_K(ATP) blockers on action potential duration in intact hearts during hypoxia. The data are being analyzed now and we are just beginning GC analysis of fatty acid content in myocardium from rabbits treated with lard, menhaden oil or safflower oil.

Findings: Data are presently being analyzed.

Dr. Richard J. Head
CSIRO Division of Human Nutrition

Title: Antihypertensive Properties of Fish Oil

Objective: To characterize the antihypertensive properties of fish oil with particular emphasis upon neuroeffector function and vascular relaxation.

Findings: 1.) We have demonstrated that dietary fish oils lower blood pressure in a genetic animal model of hypertension and 2.) This decrease in blood pressure is associated with a decrease in the contractility of blood vessels to agents such as noradrenaline and sympathetic nerve stimulation and 3.) Dietary fish oils restore the impaired relaxation of blood vessels associated with hypertension in genetically hypertensive animals.

Drs. Edward Lakatta and Salvator Pepe
NIH/National Institute on Aging/Gerontology Research Center

Title: Effect of omega-3 Fatty Acids on Myocardial Membrane Fatty Acids, Cardiac Function and Aging.

Objective: To investigate the consequences of dietary modulation of cardiac membrane omega-3 fatty acids on cardiac function, metabolism and aging.

Findings: 1.) We have demonstrated an age-associated increase in ventricular membrane arachidonic acid (omega-6 PUFA) and decrease in docosahexaenoic acid (omega-3 PUFA). Dietary modulation of omega-3/omega-6 PUFA (i.e. increased consumption of omega-3 PUFA) resulted in increased omega-3 PUFA content in cardiac and isolated mitochondria membranes. 2.) Using an experimental protocol which stresses cell membrane ion homeostasis, increase in omega-3 PUFA incorporation into cardiac membranes resulted in an attenuation of age-associated calcium ion intolerance in isolated cardiac myocytes. 3.) Increased omega-3 PUFA intake resulted in a reduction of

the age associated increase in mitochondrial State IV (-ADP) pyruvate and palmitoyl carnitine oxidation rates and activation of pyruvate dehydrogenase. 4. A significant reduction in blood pressure was measured in omega-3 PUFA fed rats, particularly in 24 month rats. These measures were associated with reduced sarcoplasmic reticulum calcium ion concentration in arterial smooth muscle cells.

Dr. Cheryl Lovelady
University of North Carolina at Greensboro

Title: The Role of Nutrition and Exercise as Interventions in Hyperlipidemia and Cardiovascular Disease in African-American Women

Objective: The objective of this study was to determine the effect of omega-3 fatty acid (omega-3 PUFA) supplementation on aerobic exercise for 10 weeks on plasma lipoprotein(a) [Lp(a)]; triglycerides (TG); total (TC), low density lipoprotein (LDL-C), and high density lipoprotein (HDL-C) cholesterol concentrations in premenopausal African-American women with Lp(a) levels greater than 20 mg/dl. Initial concentrations of the other plasma lipids were within normal limits. The supervised exercise program consisted of walking 5d/wk for 45min/d at 60% of max heart rate. Fish oil capsules provided a total of 3.9 g omega-3 PUFAs/d. Dietary intake of all subjects remained constant during the study.

Findings: There was no effect of omega-3 PUFAs or exercise on Lp(a) levels. TG decreased from 77 to 62, 84 to 73 and 74 to 70 mg/dl in the fish oil, exercise and control groups, respectively. However, this decrease was not significant. TC, LDL-C and HDL-C did not change significantly in any group. Exercising subjects lost significantly more weight (2.7 vs0.2 and 0.1 kg) and improved in cardiovascular fitness (12.6 vs -4.2 and -0.3%) significantly more than the control or fish oil subjects, respectively. The covariates age and initial Lp(a) levels accounted for the greatest variance in changes in Lp(a) ($p=0.03$, $r^2=0.34$). There was no effect of initial weight or weight loss on changes in plasma lipids among subjects. In conclusion, omega-3 PUFAs or exercise did not alter plasma lipids in African-American women.

Dr. Lawrence Rudel
Bowman Gray School of Medicine
Wake Forest University

Title: Low-Density Lipoprotein Metabolism in Atherosclerosis

Objective: Our studies are designed to determine the effects of individual dietary fats on the lipoprotein cholesterol metabolism related to atherosclerosis in two species of monkeys. We are testing the hypothesis that dietary polyunsaturated fatty acids reduce the extent of coronary atherosclerosis through their effects to modify the composition and decrease the concentration of plasma low density lipoproteins. We have done studies in the past documenting that fish oil, when fed together with cholesterol, leads to reduced

atherosclerosis in vervet (African green) monkeys. One of the questions we have been repeatedly asked is, how does fish oil compared to other dietary fats, affect lipoprotein cholesterol metabolism when fed without cholesterol? We have begun new groups of vervet monkeys and have entered them in a protocol in which we are doing further studies on how different dietary fats affect cholesterol metabolism and atherosclerosis. In these groups, we have the opportunity to examine the effects on lipoprotein metabolism of dietary omega-3 fatty acids in the presence of low amounts of dietary cholesterol.

Findings: We have almost completed the *in vivo* measurements of LDL receptor function in the cynomolgus monkeys fed saturated, omega-6 polyunsaturated, monounsaturated or omega-3 polyunsaturated fat for a period of three years. We have also completed the termination study protocol on three fourths of the monkeys and will complete them by May of 1995. At termination, we carry out liver perfusion studies, measuring the rates of hepatic secretion of cholesterol and cholesteryl ester in addition to apoB, apoE, and VLDL and LDL particle secretion. We have completed cholesterol absorption measurements in each of the animals. We have found that the different types of dietary fatty acids did not induce major differences in intestinal cholesterol absorption. The only statistically significant difference was that the animals fed saturated fat had the highest percentage cholesterol absorption, 72.8%, a value significantly higher than the 60.9% for the omega-6 polyunsaturated fat group. Plasma lipoprotein endpoints have been measured in the four dietary fat groups of cynomolgus monkeys. Perhaps the most surprising findings were that the omega-3 polyunsaturated fat fed cynomolgus monkeys had the highest total and LDL cholesterol concentration of all the dietary fat groups. This clearly contrasts with what we saw in African green monkeys. The data have also shown that the monounsaturated fat group had the largest LDL particles with the highest cholesteryl oleate content. The data suggest the possibility that, in cynomolgus monkeys, neither fish oil nor monounsaturated fat will be particularly protective against coronary artery atherosclerosis. The effect of fish oil was to protect against atherosclerosis development in African green monkeys, and this was apparently due to beneficial effects on hepatic metabolism of lipoprotein cholesterol. The capacity for fish oil to exert the same effects on the livers of cynomolgus monkeys seems to be quite different although we do not understand why. We hope that our studies on isolated liver perfusion will provide some clues. It is our hope that these species differences in our experimental primate models will provide insights that can be applicable to man, where individual differences in response to fish oil have also been described.

DIABETES

Dr. Theresa M. Bianco
Oregon State University College of Pharmacy

Title: Effects of Fish Oils on Insulin Sensitivity in Glucose Intolerance

Objective: To assess the effects of marine fish oils on insulin sensitivity in patients with impaired glucose tolerance and hypertriglyceridemia.

Findings: Due to difficulties with validation of the method for determining insulin sensitivity this protocol has not yet been initiated.

HEMATOLOGY

Dr. Janice Bright
University of Tennessee College of Veterinary Medicine

Title: Effects of omega-3 Fatty Acid Supplementation and Arachidonic Acid Reduction on Feline Platelet Aggregation

Objective: The goal of this research was to determine whether feeding cats a diet high in omega-3 fatty acid content and low in arachidonic acid content would result in reduced platelet thromboxane production, impaired *in vitro* platelet function parameters, and prolonged mucosal bleeding time.

Findings: Data obtained from this research indicate that feline platelet function can be safely altered by combining dietary arachidonic acid restriction with dietary enrichment of omega-3 fatty acids. Normal research cats fed the experimental diet for 10 weeks had a prolongation of mucosal bleeding time (mean increase of 87 ± 16 s, $p = 0.0015$) and a reduction of platelet thromboxane production (mean decrease of $1.32 \pm 0.4 \times 10^{-6}$ pg/platelet, $p = 0.018$) compared to control (baseline) values. These results suggest a potential beneficial effect of the experimental diet in cats prone to arterial thromboembolism.

Dr. James R. Eckman
Emory University School of Medicine

Title: Comprehensive Sickle Cell Program Project 4: Thrombotic Changes with Pain Episodes; Prevention by Omega-3 Fatty Acids

Objectives: A. To assess thrombogenic activity in patients with sickle cell disease B. To intervene with the prothrombotic state by long-term treatment with dietary omega-3 fatty acids.

Findings: Thirteen patients have been enrolled and studied at baseline. Ten have been studied at 1 month, nine at 3 months, and three at 6 months following treatment. No data are yet available as to the clinical effect of the treatment on the frequency and severity of pain episodes. Overall data comparing the hemostatic profile (evidence for *in vivo* thrombogenic activity) at baseline versus on treatment show decreased platelet activation and platelet and RBC membrane procoagulant activity following therapy. Circulating plasma markers for thrombotic activity including D-dimers, plasmin-antiplasmin and thrombin-antithrombin complexes also decreased. Additional data obtained showed: 1.) higher content of omega-3 fatty acids in some plasma samples thus, confirming patients' compliance, and 2.) lower content of arachidonic acid in some platelet samples, a change that may confer decreased *in vivo* reactivity.

IMMUNOLOGY

Dr. Karen Campbell
University of Illinois

Title: The Effects of Dietary omega-3 vs. omega-6 Fatty Acids on *in vitro* Neutrophil Leukotriene B4 Generation

Objective: To determine the effects of diet high in omega-3 fatty acids on neutrophil generation of LTB4, and on IL-8-induced inflammation in the skin of dogs, relative to a diet equivalently high in omega-6 fatty acids.

Findings: At this time we are still collecting data.

Dr. Robert S. Chapkin
Texas A&M University

Title: Dietary Significance of Gamma-linolenic Acid

Objective: To determine how dietary fatty acids influence the ability of macrophages to modulate smooth muscle cell (SMC) DNA synthesis *in vitro*.

Findings: Macrophages isolated from animals supplemented with dietary oils containing gamma-linolenic acid reduced SMC DNA synthesis in a cyclooxygenase-dependent manner and therefore may favorably modulate the atherogenic process.

Title: Non-invasive Detection of Colonic Cellular Markers: Modulation by Diet and Carcinogen

Objective: To determine the molecular mechanisms by which dietary fats modulate colonic epithelial cell proliferation and malignant transformation.

Findings: Dietary fat had a significant effect on the composition of rat fecal diacylglycerol (DAG). There was a significant interaction between fat and carcinogen on DAG omega-3 fatty acid composition, which was elevated with carcinogen/fish oil treatment. Since the production of fecal DAG, an activator of protein kinase C, may alter colonic cell proliferation, our data offers insight into a mechanism by which diet may modify the risk of colon cancer development.

Drs. Robert S. Chapkin and McMurray
Texas A&M University

Title: Dietary Significance of Gamma-linolenic Acid; Nutritional Deficiencies and Tuberculosis Vaccine Efficacy

Objective: To determine the mechanisms of action of dietary EPA and DHA on T-lymphocyte proliferation and the inflammatory process.

Findings: Dietary EPA and DHA suppressed T-cell proliferation by 70 and 75%, respectively. Suppressed proliferation was preceded by reduced interleukin-2 levels and ceramide mass. The effect of EPA and DHA may be mediated by the reduced formation of ceramide, a bioactive mediator which regulates T-cell IL-2 production.

Dr. Gabriel Fernandes
University of Texas Health Science Center

Title: Influence of Diet on Regulation, Autoimmunity and Aging

Objective: The objective of this proposal is to dissect out the role of source and level of dietary lipids (omega-6 and omega-3), and calorie restriction (CR) on immunological and molecular mechanisms involved in causation and prevention of the autoimmune phenomenon in NZBxNZW F1 female mice, an animal model for human systemic lupus erythematosus (SLE). Also the impact of supplementing antioxidants to the diets on survival and the mechanisms involved in the amelioration of immune complex-mediated glomerulonephritis will be studied. Given the increased consumption of omega-6 lipids in the U.S., these studies are of particular relevance. By extrapolation from our animal studies, increased consumption of omega-6 lipids appears to increase the incidence of malignancies and autoimmune disorders, whereas omega-3 lipids may reduce the autoimmune phenomenon.

Findings: Moderate CR and/or supplementation with omega-3 lipids (fish oil vs. omega-6 (corn oil)) significantly extended the life span of NZBxNZW F1 mice from ~11 months to more than 2.5 years by ameliorating the immune complex-mediated glomerulonephritis. These improvements in CR animals are accompanied by preservation of naive T lymphocytes, decreased production of inflammatory cytokines and increased activity and expression of antioxidant enzymes in immune as well as in target tissues.

NEPHROLOGY

Dr. James V. Donadio
Director, Mayo Nephrology Collaborative Group

Title: Effects of omega-3 Fatty Acids in IgA Nephropathy

Objective: In a multi-center, placebo-controlled, randomized trial we tested the efficacy of fish oil in patients with IgA nephropathy who had persistent proteinuria. The daily dose of fish oil was 12 g; the placebo was a similar dose of olive oil. Serum creatinine concentrations, elevated in 68% of the patients at baseline, and creatinine clearance were measured for 2 years. The primary end point was an increase of 50% or more in the serum creatinine concentration at the end of the study.

Findings: Fifty-five patients were assigned to receive fish oil, and 51 to receive placebo. According to Kaplan-Meier estimation, 3 patients (6 percent) in the fish-oil group and 14 (33 percent) in the placebo group had increases of 50 percent or more in their serum creatinine concentrations during treatment ($P = 0.002$). The annual median changes in the serum creatinine concentrations were 0.03 mg per deciliter (2.7 μmol per liter) in the fish-oil group and 0.14 mg per deciliter (12.4 μmol per liter) in the placebo group. Poteinuria was slightly reduced and hypertension was controlled to a comparable degree in both groups. The cumulative percentage of patients who died or had end-stage renal disease was 40 percent in the placebo group after four years and 10 percent in the fish-oil group ($P = 0.006$). No patient discontinued fish-oil treatment because of adverse effects. See New England Journal of Medicine 331:1194-1199 (November 3), 1994.

Drs. Jerry McCauley and Mario Magnone
University of Pittsburgh

Title: A Placebo Controlled Trial to Determine the Effect of Fish Oil Supplementation on Renal Function in Transplant Recipients

Objective: To find out if fish oil supplementation improves renal function in transplantation patients receiving either cyclosporin or FK-506.

Findings: This study was terminated by the principal investigator due to both low patients enrollment and lack of personnel.

LIPID METABOLISM

Dr. Michael L. Booker
Boston University of Medicine

Title: Dietary Triglycerides and Plasma-Biliary Lipid Balance

Objective: The purpose of this project is to study the effects of various types of dietary fat on levels of plasma and biliary lipids, as well as on the activities of hepatic enzymes of cholesterol metabolism and the levels of the corresponding mRNAs. This research should help to elucidate the relationship between lipids in the plasma and biliary compartments and to clarify the central role of the liver in cholesterol homeostasis.

Findings: In preliminary studies, it has been demonstrated that at the levels of dietary fat and cholesterol used in this project, there are no significant differences in intestinal cholesterol absorption among the various dietary groups in the prairie dog model (i.e. safflower, olive, menhaden, and coconut oils). Therefore, no corrections will be required in the levels of exogenous cholesterol contained in the test diets as the main body of the studies is conducted.

Dr. George Bray
Pennington Biomedical Research Center

Title: Dietary Fat, Food Intake, Energy Expenditure and Body Composition

Objective: The objective of this research was to evaluate the oxidation and metabolism rates of four 13C methyl-end labeled fatty acids with cis isomer configuration: linolenic, linoleic, oleic (unsaturated fatty acids) and stearic (saturated fatty acid), concurrent with chronic dietary supplementation with fish oil.

Findings: The study was conducted with four subjects on the specified diets with fish oil supplementation (12 g n-3 fatty acids/day) for 6 weeks. The results indicate that the dose of dietary omega-3 fatty acids, rather than the omega-3/omega-6 ratio, is the determining factor in altering lipid and thrombotic endpoints in humans.

Dr. William E. Connor
Oregon Health Sciences University

Title: Essentiality of Dietary Omega-3 Fatty Acids

Objective: Repletion of omega-3 deficient monkeys with DHA ethyl ester

Findings: Previously we have added fish oil containing EPA in the repletion diets of omega-3 deficient monkeys. In this study we added only a purified source of DHA and studied the changes in the molecular species which occurred. Deficient monkeys given

the DHA ethyl ester in the diet recovered to a near normal molecular species composition in the retina. Replacement was asymmetric and functionally incomplete. It may be that repletion at the age of 10 months is too late to assure completely adequate biochemistry and function. Note that the ERG's still remained abnormal. We plan to administer DHA at 3-4 months of age in the deficiency state and then note the recovery.

Dr. Ronenn Roubenoff
USDA Human Nutrition Research Center on Aging
Tufts University

Title: Doctoral Program in Human Nutrition and Metabolism

Objectives: This research is examining the hypothesis that the weight loss observed in dogs with congestive heart failure (CHF) is caused by cytokine-mediated loss of lean body mass, and that this loss can be mitigated by the administration of omega-3 fatty acids. The specific aims that address this hypothesis are presented below: 1.) To characterize body composition of dogs with CHF and to compare this to normal dogs. Short term changes in body composition in dogs with CHF will also be determined 2.) To determine the production of tumor necrosis factor and interleukin-1 in dogs with CHF compared to healthy control dogs. 3.) To determine the effect of fish oil administration as a therapeutic intervention for the muscle wasting (cachexia) that is associated with canine CHF.

Findings: One third of the projected cases have been enrolled. Complete data are not yet available on these subjects, but preliminary data show that dogs receiving fish oil tend to become less cachectic compared to dogs in the placebo group. There was also a trend for dogs receiving fish oil to show an improvement in heart function. As more subjects complete the study, we will see if these trends continue.

Dr. Donna Ryan
Pennington Biomedical Research Center

Title: Determination of a Minimum Level of Dietary omega-3 Fatty Acids to Reduce the Risk of Coronary Heart Disease

Objective: The objective of this research was to determine whether it is the absolute amount of fish oil or the dietary omega-3/omega-6 ratio that determines the efficacy of fish oil to reduce certain risk factors for cardiovascular disease in humans.

Findings: The results indicate that the dose of dietary omega-3 fatty acids, rather than the omega-3/omega-6 ratio, is the determining factor in altering lipid and thrombotic endpoints in humans.

Dr. Rosemary Wander
Oregon State University

Title: Interaction of Vitamin E and Fish Oil on Lipid Peroxidation in Older Women

Objective: The overall goal is to evaluate through impact on *in vivo* lipid peroxidation the sufficiency of vitamin E intake when fish oil supplements are consumed. The first objective was to measure urinary and plasma indices of lipid peroxidation in postmenopausal women both using (+HRT) and not using (-HRT) hormone replacement therapy when fish oil is ingested at four supplemental levels of vitamin E. Since no standardized technique for measuring lipid peroxidation currently exists, the second objective will be to measure indices of lipid peroxidation by several methods. In plasma, lipid peroxides as thiobarbituric acid reactive substances (TBARS) and as the specific malondialdehyde thiobarbituric acid (MDA-TBA) adduct, vitamin E, and oxidatively susceptibility of low density lipoprotein will be measured; in urine, TBARS and MDA-TBA adduct.

Findings: The plasma fatty acid profile contained significantly higher levels of omega-3 fatty acids after each supplementation period. The plasma fatty acid profile contained significantly higher levels of omega-3 fatty acids after each supplementation but the change was more marked at the lowest dose. Only small increases, eliminated by α-tocopherol (α-T), were seen in plasma thiobarbituric acid reactive substances after fish oil. The urinary excretion of TBARS and MDA-TBA adduct increased about 20% after the fish oil supplement. This increase was not returned to the pre-fish oil value by even the highest dose of α-T. Fish oil did not change total cholesterol or LDL cholesterol, increased high density lipoprotein cholesterol, and decreased triglycerides in both -HRT and +HRT; +HRT women had a less atherogenic lipid profile; and α-T produced minimal changes in these variables. Lag time of production of conjugated dienes was shorter after fish oil and only marginally lengthened by the highest dose of α-T. Propagation rate of conjugate dienes was decreased after fish oil, minimally influenced by α-T, and faster in the -HRT women. The plasma fatty acid profile contained significantly higher levels of omega-3 fatty acids after each supplementation period. The vitamin E content of plasma increased significantly at each level of supplementation but the change was more as thiobarbituric (TBA)-MDA adduct, increased about 20% after the fish oil supplement. This increase was not returned to the prefish oil value by even the highest dose of α-T.

Dr. Jay Whelan
University of Tennessee

Title: The Role of Dietary Arachidonic Acid As An Antagonist to omega-3 Polyunsaturated Fatty Acids: the Effects of Lipid and Metabolism, and Platelet Activity

Objectives: To determine the effect of dietary arachidonic acid (an omega-6 polyunsaturated fatty acid) on lipid and eicosanoid metabolism, and platelet reactivity; and to determine whether dietary arachidonic acid abrogates the beneficial effects

associated with the consumption of omega-3 polyunsaturated fatty acids found primarily in marine and fish oils.

Findings: 1.) Dietary omega-3 PUFA are potent antagonists to tissue arachidonic acid (AA) and metabolites of the AA cascade. However, when AA is supplemented to diets containing omega-3 PUFA any inhibitory effects on the production of AA derived eicosanoids observed with omega-3 PUFA were completely eliminated. These results clearly demonstrate that dietary AA is a more potent antagonist to omega-3 PUFA than omega-3 PUFA is towards AA.
2.) Eicosanoid production, in general, is increased when AA is added to the diet. When AA and EPA are concommitantly added to the diet in equivalent amounts, AA completely eliminates any effects EPA has on AA and its metabolites.
3.) Dietary AA appears to increase circulating triglyceride levels.
4.) Even though adding AA to the diet increases TXA_2 production 5-10 fold in platelets stimulated with ADP or collagen, dietary AA does not alter platelet aggregation.

MALARIA

Dr. Orville Levander
U.S. Department of Agriculture Human Nutrition Center

Title: Role of Vitamin E and Selenium in Human Health Promotion

Objective: To determine the effect of dietary oxidative stress induced by feeding fish oil to vitamin E-deficient mice on the course of infection, specifically malaria and coxsackievirus.

Findings: Such dietary oxidative stress protects against cerebral malaria in a mouse model of the disease. This form of malaria is particularly lethal accounting for thousands of deaths each year in the tropics. However, such dietary oxidative stress also exacerbates the heart damage caused by the coxsackievirus in mice.

OTHER

Dr. Ephrain Yavin
The Weizmann Insitute

Title: A Rapid and Safe Method of Fetal Brain DHA Enrichment

Objective: Rapid and safe enrichment of rat fetal brain lipids with DHA, by intraamniotic administration of ethyl docosahexaenoate.

Findings: Administration of ethyl docosahexaenoate by the intraamniotic route to 17-3 and 19-day rat fetuses proved to be feasible and safe. Examination of the fetal brains and livers 72 h and 24 h later (at day 20 of gestation), showed that most lipid fractions were

enriched with DHA, as compared to ethyl oleate-injected fetuses. Furthermore, changes in the phospholipid amounts of both the fetal brain and liver were observed following ethyl docosahexaenoate administration.

Dr. Clemens von Schacky
University of Munich

Title: Omega-3 Fatty Acids and Gene Expression

Objective: As outlined in more detail in the application, we try to answer the following questions a) Does the reduction of PDGF-specific mRNA levels in unstimulated human mononuclear cells brought about by ingestion of omega-3 fatty acids that we observed previously persist after stimulation of mononuclear cells by adhesion, and is it reflected by reduced levels of the respective proteins? B) Are the effects we observed previously specific for omega-3 fatty acids, or can they also be observed after ingestion of omega-6 or n-9 fatty acids in comparable amounts?

Findings: The study is ongoing and volunteer participation should be completed in April 1995. Therefore, as yet, there are no findings.

NEOPLASTIC PROCESSES

Dr. George L. Blackburn
New England Deaconess Hospital

Title: Mucosal Proliferation and Fish Oil in Colorectal Cancer

Objective: The original proposal specific aims included: 1.) to test the hypothesis that fish oil supplementation decreases the proliferation potential of colonic crypt cells in individuals with Duke's A and B-I colon cancer, 2.) to determine the safety and tolerance of 12-month fish oil supplementation amongst patients with Duke's A and B-1 colorectal cancer and 3.) to develop flow cytometry methods for assessment of cytoplasmic and nuclear changes in colonic crypt cells.

Findings: The research to date have confirmed the rationale and provided supporting evidence for the study. This includes both the role of a low fat, high omega-3/omega-6 fatty acid diet and use of current biomarkers of flow cytometry. We have completed the report on the NCI Workshop on Hereditary Colon Cancer (in press JNCI, 1995); this provides support for the priority of this research proposal competing renewal. The U.S. DHHS gives highest priority to research in cancer control science and cancer prevention. Specific requests for research include efficacy of potential chemopreventive agents and identification of biologic and biochemical markers of dietary exposure. This priority was also the consensus of the NCI Workshop on Hereditary Colon Cancer. If high-risk individuals are to be identified, then a non-toxic long-term use chemopreventive agent is needed. Omega-3 fatty acids contained in fish oil is a candidate agent.

Dr. William T. Cave
The University of Rochester Medical Center

Title: "The Role of IGF's in the Dietary Lipid Regulation of Breast Cancer"

Objective: The overall objective of this study is to improve our understanding of the lipid dependent biochemical processes involved in breast cancer development, in order to develop more effective diet based cancer prevention strategies. The specific aims of the project are: 1.) To determine and compare the effects of qualitatively different (omega-3 vs omega-6) high fat (20% w/w), and low fat (5% w/w) PUFA diets individually, and in blends on IGF-II and IGFBP gene expression, and IGF-I binding capacity. 2.) To correlate the alterations in IGF-II and IGFBP expression, and IGF-I binding capacity with changes in tumor development and membrane lipid profile. Our laboratory has previously shown that both quantitative and qualitative alterations in dietary lipid can significantly influence the development of n-methyl nitrosourea (NMU) induced mammary tumors, and others have indicated that they have significant effects on the development of the transplantable R3230 AC mammary tumor. Recent experiments in other laboratories have indicated that receptors for the insulin like growth factor IGF-1 are present in both these mammary tumor models. Therefore, this proposal is designed to explore to what extent, if any, dietary lipid manipulations may influence the expression of these IGF receptors and their binding proteins in these two different mammary tumor models. If such changes are found, it will help improve our understanding of how nutritional and enocrine factors may interact to regulate the development of breast cancer, and provide a basis for further experimental investigation.

Findings: We will be analyzing the tumor tissues for IGF related data over the next several months. Our results, to date have shown the anticipated affects of the different PUFAs on mammary tumor growth (i.e. that the mammary tumors grow fastest in the high corn oil diet group, and slower in the high fish oil and low corn oil groups), although the addition of the antioxidants to these oils did seem to modify the tumor response somewhat.

Dr. Amiram Raz
Tel Aviv University

Title: A Novel Paradigm for Rapid and Substantial Exchange of Arachidonate for EPA and Its Effect on Tumor Proliferation

Objective: We employ a mouse tumor model in which C57BL mice are treated with melanoma or lung carcinoma cells. The mice are injected with tumor cells in the foot pad and develop a solid tumor in 25 - 30 days. Removal of the primary tumor leads to appearance of metastatic lung tumors in 25 - 30 days. The objectives are: 1.) to contrast the effects of dietary pretreatment with corn oil vs fish oil on induction of primary tumors or metastatic tumors. 2.) to determine the effects of individual omega-3 acids and of dietary antioxidant (Vit. E) deprivation or addition on primary and metastatic tumors' growth.

Findings: In initial experiments, using a novel dietary paradigm that we developed for exchanging AA with omega-3 acids, we produced drastic (>90%) inhibition of appearance of B16 melanoma lung tumors development. We are proceeding with the experiments according to the objective outlined above.

Dr. Henry Thompson
AMC Cancer Research Center

Title: Omega-3 Fatty Acids and Mammary Cancer

Objectives: 1.) To determine whether net *in vivo* oxidative events (lipid peroxidation and oxidation of deoxyguanosine) are increased in rats fed menhaden oil relative to rats fed corn oil or a more saturated fat. 2.) To determine if *in vivo* oxidative events if detected (Objective 1) can be blocked by feeding increased levels of antioxidant. 3.) To determine whether fish oil protects against mammary tumorigenesis in rats administered MNU.

Findings: Urinary malondialdehyde (MDA), a marker of *in vivo* lipid peroxidation, is significantly higher for rats fed a diet containing 24.6% fat as 17.9% menhaden oil:6.7% corn oil, with marginal vitamin E, than for rats fed a 24.6% fat diet with 21.2 palm oil:3.4% corn oil (formulated to contain the same concentration of linoleic acid as the fish oil diet), adequate with respect to vitamin E. Thus, with the more unsaturated, less protected diet, there was greater MDA in the urine and, presumably, more lipid peroxidation. Comparing rats fed 17.9% menhaden oil: 6.7% corn oil diets with either 1.) marginal, 2.) adequate (by calculation from the number of double bonds), or 3.) 10 times adequate vitamin E, we found urinary MDA to be the lowest for the adequate group and highest for the "10x" group, suggesting that antioxidant status affects *in vivo* lipid peroxidation and that vitamin E can serve as an antioxidant or as a prooxidant, depending on the amount provided. Rats fed a diet with 17.9% menhaden: 6.7% corn oil (adequate vitamin E) had more mammary cancers/rat than did rats fed a diet containing 21.2% palm oil: 3.4% corn oil and the same as rats fed 24.6% corn oil. No protection against mammary carcinogenesis by fish oil was observed.

LIPID PEROXIDATION DISORDERS

Dr. Manuela Martinez
Hospital Materno-Infantil
Paseo Valle De Hebron, S/N

Title: Treatment of Peroxisomal Patients with DHA

Objectives: Patients with Zellweger syndrome and other related peroxisomal disorders have a profound deficiency of docosahexaenoic acid (22:6 omega-3, DHA). Therefore, the objective of the research study was double: First, to determine whether or not the DHA deficiency of peroxisomal-disorder patients can be corrected by exogenous

administration of DHA ethyl ester, and if such a correction is accompanied by some kind of clinical improvement; Second, to study the biochemical changes of these patients under DHA therapy.

Findings: The most important findings of the study are the following:
1.) DHA deficiency can be corrected, at least in blood, with an oral treatment of DHA ethyl ester.
2.)Normalization of the blood DHA levels is accompanied by a clear clinical improvement in most patients, especially of visual function.
3.)Blood plasmalogens, in particular the 18:0DMA/18:0 ratio, increase in parallel to the increase in blood DHA levels. In plasma, the very long chain fatty acids (VLCFA), 26:0 and 26:1 and the two ratios 26:0/22:0 and 26:1/22:0 decrease during DHA therapy, despite the fact that the VLCFA intake in the diet is higher.

Dr. Hugo W. Moser
John Hopkins University
Kennedy-Krieger Institute

Title: DHA Therapy of Peroxisomal Disorders

Objective: To determine whether oral administration of DHA can improve or slow the deterioration of visual and neurological function in patients with peroxisomal diseases such as the Zellweger syndrome, neonatal adrenoleukodystrophy or Infantile Refsum disease.

Findings: We have administered the DHA to five patients. It was tolerated well. The plasma and red blood cell DHA levels increased in all patients. It is of interest that an increase in plasmalogen levels was observed in one patient. So far, we have not observed clear changes in chemical status.

OTHERS RECEIVING MATERIALS - NO REPORT SUBMITTED

Drs. Guy Chisolm and Kimberly C. Irwin
Cleveland Clinic Foundation
Toxicity of Oxidized LDL *in Vitro* and *in Vivo*

Dr. Daniel H. Hwang
Pennington Biomedical Research Center
Dietary Fat, Food Intake, Energy Expenditure and Body Composition

Dr. Howard Mueller
University of Texas Health Science Center
Effects of Omega-3 Fatty Acids on Platelet Activating Factor Synthesis and the Attenuation of Coronary Thrombosis

Dr. Dwight Robinson
Massachusetts General Hospital
Neutrophil Membrane and Cellular Basis of Hypersensitivity Diseases in Humans

Dr. Steven M. Schwarz
New York Medical College
Omega-3 Fatty Acids in the Treatment of Cystic Fibrosis

Drs. Edward Weiner & Robert Nicolosi
University of Lowell
Dietary Fat/Cholesterol and Low Density Lipoproteins - Effect of Dietary Fat on LDL Heterogeneity

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PARTICIPANTS

Dr. Theresa M. Bianco
Oregon State University College of Pharmacy
Portland VAMC-Mailcode 119P
3710 SW US Veterans Hospital Road
Portland, OR 97201

Dr. George L. Blackburn
New England Deaconess Hospital
185 Pilgrim Road
Boston, MA 02215

Dr. Lawrence E. Boerboom
Medical College of Wisconsin
Department of Cardiac Thoracic Surgery
8701 Watertown Plank Road - Lab
Milwaukee, WI 53226

Dr. Michael L. Booker
Boston University of Medicine
80 East Concord Street
Boston, MA 02118

Dr. George Bray
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808

Dr. Janice Bright
University of Tennessee College of Veterinary Medicine
Department of Internal Medicine and Cardiology
P.O. Box 1071
Knoxville, TN 37901-1071

Dr. Karen Campbell
University of Illinois
Department of Veterinary Clinical Medicine
1008 W. Hazelwood Drive
Urbana, IL 61801

Dr. William T. Cave
University of Rochester Medical School
Box 693 (Endocrine Unit)
Rochester, NY 14642

Drs. Robert S. Chapkin & David N. McMurray
Texas A&M University
Dept. of Animal Science & Medical Microbiology/Immunology
College Station, TX 77843

Dr. Myung S. Chi
Lincoln University
Human Nutrition Research Program
Jefferson City, MO 65109

Drs. Guy Chisolm and Kimberly C. Irwin
Cleveland Clinic Foundation
NC-10
9500 Euclid Avenue
Cleveland, OH 44195

Dr. William Connor
Oregon Health Sciences University
3181 Sam Jackson Park Road
Portland, OR 97201

Dr. James V. Donadio
Director, Mayo Nephrology Collaborative Group
1724 Mayo Building
Rochester, MN 55905

Dr. James R. Eckman, M.D.
Emory University School of Medicine
69 Butler Street, S.E.
Atlanta, GA 30303

Dr. Gabriel Fernandes
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, TX 78284

Dr. Anne M. Gillis
University of Calgary
3330 Hospital Drive NW
Calgary, Alberta, Canada T2N 4N1

Dr. Richard J. Head
CSIRO Division of Human Nutrition
P.O. Box 10041 Gouger Street
Adelaide, SA 5001, AUSTRALIA

Dr. William R. Henderson
University of Washington
Department of Medicine
SJ-10
Seattle, WA 98195

Dr. Daniel H. Hwang
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808-4124

Dr. Joel Kremer
Albany Medical College
A-100
47 New Scotland Avenue
Albany, NY 12208

Dr. Edward Lakatta
NIH/National Institute on
Aging/Gerontology Research Center
4940 Eastern Avenue
Baltimore, MD 21224

Dr. Orville A. Levander
U.S. Department of Agriculture Human
Nutrition Center
Building 307, Room220
Beltsville, MD 20705-2350

Dr. Cheryl Lovelady
University of North Carolina at Greensboro
Department of Food, Nutrition and Food
Service Management
318 Stone Building
1000 Spring Garden Street
Greensboro, NC 27412-5001

Dr. Manuela Martinez
Hospital Materno-Infantil
Paseo Valle De Hebron. S/N
Barcelona, SPAIN 08035

Drs. Jerry McCauley and Mario Magnone
University of Pittsburgh
Falk Clinic, 5-C
3601 5th Avenue
Pittsburgh, PA 15213

Dr. James N. Moore
University of Georgia, College of Veterinary
Medicine
Large Animal Medicine
Athens, GA 30602-7385

Dr. Hugo W. Moser
John Hopkins University
Kennedy-Krieger Institute
707 North Broadway
Baltimore, MD 21205

Dr. Howard Mueller
University of Texas Health Science Center
Division of Cardiology
6431 Fannin Street
Houston, TX 77030

Dr. Amiram Raz
Tel Aviv University
Department of Biochemistry
Tel Aviv, Israel 69978

Dr. Dwight Robinson
Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114

Dr. Ronenn Roubenoff, MD, MHS
USDA Human Nutrition Research Center on Aging
Tufts University
711 Washington Street
Boston, MA 02111

Dr. Lawrence L. Rudel
Bowman Gray School of Medicine, Wake Forest University
Medical Center Boulevard
Winston-Salem, NC 27157

Dr. Donna Ryan
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808

Dr. Steven M. Schwarz
New York Medical College
Vosburgh Pavilion, Room 322
Valhalla, NY 10595

Dr. Rolf J. Sebaldt
McMaster University
Room 2F11, McMaster University Medical Centre
Box 2000
Hamilton, ON L8N 3Z5

Dr. Henry Thompson
AMC Cancer Research Center
1600 Pierce Street, Diamond Building
Lakewood, CO 80214

Dr. Clemens von Schacky
University of Munich
Medizinische Klinik, Klinikum Innenstadt
Ziemssenstr. 1
Munichs, FRG 80336

Dr. Rosemary C. Wander
Oregon State University
Department of Nutrition and Food Management
Milam Hall 108
Corvallis, OR 97331

Drs. Edward Weiner & Robert Nicolosi
University of Lowell
Department of Clinical Sciences
Weed Hall
Lowell MA 01854

Dr. Jay Whelan
University of Tennessee
Department of Nutrition
229 Jesse Harris Bldg.
Knoxville, TN 37996-1900

Dr. Ephrain Yavin
The Weizmann Insitute
Department of Neurobiology
Rehovoth, Israel 76100