“To everything there is a season, 
and a time to every purpose under heaven”

1. Specific Aims

Despite recent advances in prevention and therapy, coronary heart disease (CHD) remains the single most common cause of mortality in the United States. An elevated blood cholesterol level is one of the most important risk factors for CHD (1). The National Cholesterol Education Program (NCEP) has recommended universal cholesterol screening for adults, and has generated algorithms for screening and therapy with specific cholesterol level cutpoints (2, 3). Yet it is well documented in the literature that there is significant seasonal variation of cholesterol levels, a factor which has important implications for screening and therapy but has not been taken into account in the NCEP guidelines. The etiology of this phenomenon has also been poorly delineated. The primary aim of this study is to definitively describe and delineate the causes of seasonal variation of blood lipid levels in the general population. Specifically we will:

1. Assess the magnitude and timing of the seasonal effect of blood lipid levels in both sexes and at different ages, and in the important lipoprotein subfractions (total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), total triglycerides (TG), apolipoproteins A, B, and lipoprotein (a));
2. Identify and quantitate the effects of the main factors determining the variation;
3. Assess the seasonal variation of other blood elements thought to play a role in the development of coronary heart disease, including hemostatic factors and antioxidants;
4. Explore the implications of the phenomenon of seasonal variation for public health policy.

The study population will be 600 randomly selected adult patients at the Fallon Clinic, a large health maintenance organization (HMO) in central Massachusetts. Patients will be followed for a one year period, during which time multiple measurements will be made of serum lipids and other biochemical factors (antioxidants, hemostatic factors), diet, physical activity, light exposure, and psychosocial variables.

2. Background and Significance

2.1 Introduction and importance of the proposed study

During the past half-century several small longitudinal and larger cross sectional studies have been published suggesting that cholesterol levels are higher in the fall and winter than in the spring and summer (4, 5, 6, 7, 8, 9). The most striking of these studies suggested that in areas of extreme seasonal climatic variation, such as Finland, there may be as much as a 100mg/dl seasonal variation in serum cholesterol levels (10). Recently Råstam and colleagues reported a cross-sectional study of seasonal variation in plasma cholesterol levels drawn as part of the screening process for the Minnesota Heart Health Program (11). Cholesterol levels were found to peak in January, with a statistically significant seasonal variation evident in men. In women there was a non-significant trend towards higher winter levels. Using the NCEP guideline for hyperlipidemia of >240 mg/dl, 25.4% of men were at or above this level in winter, whereas only 13.5% met this cutpoint in summer.

Gordon and colleagues also have pointed out that seasonal variation in cholesterol levels could be responsible for as much as a 30% difference in the number of patients labeled as hypercholesterolemic during the winter season versus the summer (12). However, seasonal variation in blood cholesterol level is not considered in the U.S. National Cholesterol Education Program guidelines, including the recent update to those guidelines (2, 3). It is also rarely taken into account in the normal clinical management of hyperlipidemia, although the summer/winter differences described above in the frequency of patients being labeled as having “high” cholesterol carry extraordinary cost implications, given the recommendation that all adults undergo cholesterol screening. The described seasonal variation would lead to a much lower percentage of patients reaching goal cholesterol levels if treatment were initiated in the summer and remeasurement carried out in the winter, than if the converse were true.
Our knowledge of the seasonal variation of specific lipoprotein cholesterol fractions and of triglyceride levels is inadequate. In addition, as will be described further below, the physiologic basis for the seasonal variation of blood cholesterol levels is essentially unknown, with no study able to attribute more than a minor portion of the variance to known factors. To date, no careful prospective study has been carried out that attempts to systematically collect and analyze all of the data necessary to study this phenomenon, and to lay the groundwork for intervention studies that would be aimed towards preventing the winter rise in cholesterol levels. Our proposal is intended to fill this void.

2.2. Background
Seasonal variation of a variety of behaviors has been extensively documented in animals, but seasonal effects in humans have been less well studied. Research utilizing population data has suggested that there are seasonal rhythms in human birth rates, suicide, and mortality. Of particular interest are apparent seasonal rhythms in cardiovascular morbidity and mortality, primarily sudden death and stroke. These variations have been related to changes in temperature, exposure to daylight, and various other mechanisms that lead to changes in blood coagulability. Seasonal affective disorder (SAD), involving a marked winter-time increase in self-reported depression, weight, and appetite, has received considerable recent attention, highlighting the importance of seasonal rhythms in human behavior. Spoont and colleagues have reported that variations in mood due to changes in seasons occur in the entire population, with SAD patients only representing the extreme right tail of the distribution.

2.2.1 Blood lipids
Seasonal plasma lipid and lipoprotein cycles were studied in a large cohort (~1,400) of hypercholesterolemic men followed for 7 years in the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT). Significant synchronous sinusoidal seasonal cycles, peaking in the first month of winter, were demonstrated for plasma levels of TC, LDL, and HDL, with mean seasonal changes (nadir to zenith) of 7.4, 6.4, and 0.8 mg/dl, respectively. There was an irregular but statistically significant seasonal pattern for plasma TG levels, with peak levels in the autumn. Seasonal variation in plasma TG levels were not synchronous with those of cholesterol levels. Averaged over the course of the study, TG levels were highest in August-September and lowest in April-May. Seasonal variations of about 0.06 mg/dl (or 1/6th of the amplitude of the variation) could be explained by differences in analytical procedures, reagents or instruments.

The seasonal LDL cycles were approximately synchronous at the 12 LRC centers, with zeniths ranging between December 19 (Washington) and January 16 (Oklahoma City). However the zenith’s amplitudes varied over a 2.2 fold range (2.05–4.46 mg/dl). There was a trend towards smaller amplitudes at more northern centers, a surprising result, in view of the greater seasonal changes in hours of daylight and ambient temperature. There was an inverse linear relationship between seasonal LDL (and TC) changes and seasonal changes in hours of daylight (but not temperature) which was statistically significant. There was considerable geographic heterogeneity: the seasonal LDL effect in Seattle resembled that observed in other northern centers, like Toronto and Minneapolis, rather than that observed in the California centers, which have similarly mild climates. Changes in BMI and diet predicted only 29% of the observed variation, although the investigators acknowledge the flaws in their dietary assessment tool, a single 24 hr dietary recall every six months. Physical activity was assessed too infrequently to be adequately evaluated for its contribution to seasonal variation in lipid levels. There was no statistical evidence of heterogeneity of TC, LDL, HDL, TG, or BMI among subgroups adjusted for age, height, cigarette smoking, physical activity, alcohol consumption or caloric consumption per unit of body weight. The investigators note that their data fail to establish a single explanation for the phenomenon.

In the study of Råstam and colleagues cross-sectional data are reported from the screening phase of the Minnesota Heart Health Program. In 3377 men and 3900 women the 95% confidence interval of the peak to trough distance in men was 5.8–13.8 mg/dl, corresponding to 2.6–6.3% of the average cholesterol level; corresponding figures for women were 2.0–9.3 mg/dl, or 1.0–4.6%. There was considerable monthly
Variation. Variation in BMI was found to explain only a small fraction of the seasonal variation. Other than BMI, no data were collected that would permit further elucidation of contributing factors.

Woodhouse and colleagues studied seasonal variation of serum lipids in a group of 96 volunteers aged 65-74 (24). In this elderly population, they noted seasonal effects at least as strong as those previously reported in younger individuals, with a mean seasonal difference for total cholesterol of 0.32 mmol/l (12.4 mgs/dl) (95% CI 0.12-0.19, p<0.0001).

Our own cross-sectional study of over 6000 individuals has also shown a peak in blood cholesterol levels in winter, with a mean 19.5 mgs/dl seasonal difference (25). (see section 3.1)

A study by Cucu and colleagues in Bucharest of a group of 4800 men aged forty to sixty at entry who participated in a 10-year multifactorial trial of coronary heart disease prevention showed spontaneous seasonal variations of serum cholesterol, also characterized by increases in winter and decreases in summer (9). Similar variation patterns have been reported in the Trømso Heart Study, though of a smaller magnitude, a mean of 271 mg/dl in February versus 262 mg/dl in July (6).

HDL levels were specifically studied in a thousand 40 year-old residents of the town of Leiden, The Netherlands. Mean levels (±SD) in women were 48.0 ± 11.0 mg/dl; in men 42.5 ± 10.7 mg/dl. In March mean levels in women were approximately 5 mg/dl lower than in June (p < .001); in men there was a similar difference between mean levels in April and June (P<0.002) (26).

A number of other studies have documented the phenomenon of seasonal variation of serum lipids, but all have been flawed by inadequate numbers, lack of longitudinal data, non-representative populations, or failure to collect adequate data with regard to potential mediating variables (5, 8, 27).

No adequate studies have looked at seasonal variation of apolipoproteins or lipoprotein(a). Such studies would permit differentiation of changes in the composition of the lipoprotein particle (i.e., cholesterol/protein ratio) vs. changes in the actual number of lipoprotein particles as they relate to seasonal variation of lipid levels.

2.2.2 Diet

Although intuitively appealing, the hypothesis that seasonal differences in diet largely explain the seasonal differences observed in serum lipids has not been adequately tested. Dietary changes observed in the LRC study accounted for only 10.5% of the difference in lipid values, as predicted by the Keys equation (7).

Dobson and colleagues reported that the daily administration of one gram of ascorbic acid (i.e., 16 times the RDA) abolished the winter rise in serum cholesterol levels in a group of 8 individuals (mean age 38); they also noted that in a group of 12 individuals the same dose of ascorbic acid produced a 16% fall in serum cholesterol levels within 2 months (28). They therefore suggest that the seasonal variation of cholesterol levels may be related to changing intake of ascorbic acid over the seasons.

De Castro investigated seasonal variations in the nutrient intakes and meal patterns of individuals in Atlanta, GA by paying 315 adults (121 males and 194 females) to maintain a complete 7-day diet diary, which included recording each meal’s start and finish time, as well as their subjective state of hunger on a seven point scale at the beginning and end of the meal (29). The subjects did not record over any major holiday. A marked seasonal rhythm of nutrient intake was observed with increased total caloric intake in the fall (mean 222 kcal/day difference between the total daily intake in the fall versus the spring, i.e., a difference of 14%), especially of carbohydrates. This increase was associated with an increase in meal size and a greater speed of eating. Over the entire fall season, there was an average of 20,000 kcal of additional intake, which if converted to fat would increase weight by over 2.5 kg. This increase in caloric intake could not be accounted for by increased thermoregulatory energy requirements in the fall because the winter, when thermoregulatory needs are greatest, did not have a comparable increase in intake. The subjects rated themselves hungrier at the end of the meal in the fall even though the larger meals resulted in a greater estimated amount of food in the
stomach. In the winter and spring there was a strong negative relationship between the amount eaten in the meal and self-rated hunger at the end of the meal. This correlation was absent during the fall. The results suggest that even with modern heating and lighting seasonal rhythmicity of food intake persists in humans and is a major influence on eating that may act by suppressing satiety mechanisms. The results also suggest that the food intake regulatory changes thought to be specific to seasonal affective disorder (SAD) – increased weight, appetite, and carbohydrate craving – are present in non-SAD sufferers.

2.2.3 Physical activity

Few studies have looked at the relationship between seasonal variation in physical activity and serum lipid levels. The Evans County study evaluated seasonal serum cholesterol change in black and white residents of the Georgia county (30). Seasonal variation was greater in males than in females, and was not seen in sedentary whites (too few blacks had sedentary occupations for analysis); they infer that seasonal variation in serum cholesterol levels may be related to seasonal changes in physical activity related to occupation. As in the study of Råstam and colleagues, seasonal variation of cholesterol was less striking in women; this finding also may be related to a lower level of occupation related physical activity in females.

In a Scottish study of leisure time activity among 7202 men and 9284 women, considerable seasonal variation was found in both indoor and outdoor activity (31). During the peak in July, 32% of respondents reported exercising for at least 20 minutes three or more times weekly, whereas the corresponding figure for the winter was only 23%. Older respondents and those who exercised at a higher level showed greater seasonal variation. No lipid data were collected. The authors point out that Scotland is at a high latitude, and consequently has large seasonal differences in hours of daylight, but the country’s maritime climate mitigates extreme temperature changes.

A study at the Mayo Clinic examined seasonal variation in physical activity in 65 healthy post-menopausal women. A physical activity score (PAS) was assessed on an ordinal scale from 0-18. This score was obtained monthly for two years. There was a strong (p<0.001) seasonal pattern in physical activity peaking in August, with a seasonal range of 2 PAS units. The corresponding low value occurred early in February. The average annual range in PAS due to seasonality was approximately 21% of the raw PAS mean score of 9.3 (calculated over all subjects readings) (32).

2.2.4 Body mass index

The LRC-CPPT found that BMI had an even stronger cyclic pattern than that observed for plasma cholesterol. An average 5 ft. 10 in. participant gained 0.7 lb per year and weighed 1.8 lb more in mid-winter than in mid-summer. The seasonal cycles of BMI were highly synchronous among LRC centers (zeniths between January 27 and February 21). The amplitude was not correlated with either seasonal daylight or temperature differences. Participants with large winter-summer weight differences tended also to show significantly larger seasonal changes in plasma levels of TC and LDL (7).

2.2.5 Behavioral data

2.2.5.1 Seasonal affective disorder

SAD is a condition characterized by fall and winter depressions, alternating with non-depressed periods in spring and summer. During their winter depression these patients usually report symptoms of fatigue and overeating, especially “carbohydrate craving”, weight gain, and oversleeping, together with the usual cognitive and affective symptoms of depression. Bright artificial light has been shown to reverse the symptoms of SAD, including carbohydrate craving (33).

Prevalence rates of SAD were estimated for four areas of the USA (Nashua NH, New York, NY, Montgomery County, MD, and Sarasota, FL, using the Seasonal Pattern Assessment Questionnaire (SPAQ) mailed (during winter 1987-88) to a sample population stratified by sex (34). Prevalence rates of winter SAD and subsyndromal SAD were found to be significantly higher at the more northern latitudes. The positive
correlation between latitude and prevalence of winter SAD applied predominantly to the age groups over 35. The prevalence rates of subsyndromal SAD was approximately 10% of the population in all sites except Sarasota, where it was only 2.6%.

Kräuchi and colleagues gave a Food/Drink Frequency Questionnaire (FDFQ) to female SAD patients and control subjects in each of the four seasons (35). SAD patients consumed carbohydrate-rich foods (starch and not sweets) more often than controls and also showed a seasonal rhythm with maximum values in winter and minimum values in summer. In contrast, protein-rich food intake was identical in both groups and did not show seasonal variation. Fiber-rich food intake was also increased in SAD patients. SAD patients ate more meals per day, both at breakfast and in the second half of the day, with a maximum in winter. The authors suggest that these symptoms may represent a "medial hypothalamus syndrome" involving alpha 2-noradrenergic and serotonergic mechanisms. Interestingly, the results of the eating pattern study by de Castro and colleagues suggest that the food intake regulatory changes thought to be specific to SAD – increased weight, appetite, and carbohydrate craving – are present in normal humans (29). This is in keeping with recent epidemiologic studies which have found that the behaviors that characterize SAD show seasonal variation in 92%-95% of the general population, suggesting that seasonal variation in behavior and mood is a continuous, dimensional variable extending throughout the general population, defined at the upper extreme by SAD (23).

In a study of 463 university students (60% female, age range 19-32), analysis of Inventory of Seasonal Variation (ISV) scores revealed that a winter pattern of seasonality was reported by over 95% of subjects, a pattern that was more pronounced in women than men, while a summer type of seasonality was reported by only 0.6% of the general population (23). The mean of the distribution of scores for the SAD group coincides with the 97th percentile line of the total university population, and the entire range of ISV scores for the SAD sample falls above the mean of the university sample’s distribution of ISV scores.

There is therefore substantial evidence that most human beings undergo some degree of seasonal change in both mood and eating pattern that, in the extreme, is characterized as “SAD”, and in its more widespread and near-universal form may be related to seasonal variation in blood levels of cholesterol and other lipids.

2.2.6 Seasonality and cardiovascular disease

2.2.6.1 Coronary heart disease

There is significant seasonal variation in coronary heart disease events, with death rates being at least 35% higher in the winter than in the summer. This seasonal variation has been well documented for the Northern Hemisphere, both in Europe and North America (19, 36). As with secular variations in mortality, the increase in winter mortality could be due either to an increase in incidence or in case fatality rates. The magnitude of the mortality excess is greater in the United Kingdom than in many other European countries. Examination of the data for Northern Ireland indicates that myocardial infarction, respiratory disease and stroke exhibit the greatest increases during winter. Excess deaths from these conditions are closely associated with low environmental temperature (18).

In the southern hemisphere a study in the North and South Islands of New Zealand found a major seasonal variation in coronary and cerebrovascular deaths in both sexes and both islands, with a zenith in June/July/August (winter) and a nadir in December/January/February (summer). After standardizing for age, coronary mortality rates (but not cerebrovascular mortality rates) were significantly higher in South Island than in North Island (37).

It is possible that the final common pathway in the causal chain is an increased risk of thrombosis. The Northwick Park Heart Study and the Framingham Study have shown that higher concentrations of fibrinogen, factor VII, and factor VIII are associated with increased risk of coronary heart disease (38, 39). There are increases in platelet and red cell counts and blood viscosity in response to mild blood cooling, as well as an increase in plasma cholesterol concentration (40). In a study of 100 subjects aged 75 and over, significant seasonal effects were found for fibrinogen, plasma viscosity, and HDL cholesterol (21). Plasma fibrinogen
concentrations showed the greatest seasonal change and were 23% higher in the coldest six months as compared to summer months. Fibrinogen was negatively related to core body temperature and all measures of environmental temperature. The seasonal variation in plasma fibrinogen concentration is large enough to increase the risk of both myocardial infarction and stroke in winter. As fibrinogen is an acute phase protein, with changes in concentration occurring in response to acute and chronic inflammation, minor respiratory infections occurring during the winter may be the mediator between temperature change and increased fibrinogen levels (21).

2.2.6.2 Blood pressure

The Medical Research Council’s treatment trial for mild hypertension demonstrated seasonal variation of blood pressure levels, with systolic and diastolic pressures approximately 7/3 mm Hg higher in winter than in summer. The seasonal variation in blood pressure was greater in older than in younger subjects and was significantly related to maximum and minimum daily air temperature, but not to rainfall (41). A seasonal influence on arterial blood pressure (with values during the winter higher by 2-10 mm Hg than in the summer) also has been shown in other studies (42, 43). In general these observations suggest that arterial blood pressure may be strongly influenced by environmental temperature.

In a pilot investigation of seasonal hemodynamic changes, 5 normotensive and 21 mildly hypertensive subjects in Buffalo, NY, were followed through the four seasons (44). In the upright position wintertime blood pressures increased by 3% (P = NS) over summer values whereas cardiac output and stroke volume decreased by 18% and 21%, respectively (P <.002 for each). Similarly, wintertime upright heart rate increased by 7% (P<.02 with larger parallel increases in systemic vascular resistance (+24%, P<.002) and plasma norepinephrine (+26%, P<.02). The supine values followed similar trends but the magnitude of changes was about 50% less than the corresponding upright values. These data suggest that in the northern US, wintertime vasoconstriction is related to increased sympathetic nervous activity and decreased cardiac output. When these reciprocal changes are proportional, blood pressure remains constant.

2.2.6.3 Stroke

In an Italian retrospective study of the onset of symptoms in 667 cases of stroke a significant circadian, circaseptan, and circannual rhythmicity was noted. Peaks occurred in the morning hours, on the weekend, and in winter (45). No sex difference was found. The cold temperature is associated with an increase in fibrinolytic activity and a decrease in levels of antithrombin III, an increase in blood viscosity, an increase in red blood cells and platelet count, an increase in arterial blood pressure, and an increase in sympathetic tone. A similar study in Japan of 311 cases of stroke found a significant seasonality in the incidence of all stroke (p < 0.01), of intracerebral hemorrhage (p < 0.05), and of cerebral infarction (p <0.01), whereas subarachnoid hemorrhage had no significant seasonal pattern (17).

Capon and colleagues, in a retrospectively studied sequential series of 236 patients with non-traumatic cerebral hemorrhage observed in Brussels over a period of 8 years, found marked seasonal variation in incidence, with the highest value (23%) observed in November-December and the lowest (10%) in July-August (20). Seasonal variations in incidence of cerebral hemorrhage were shown to be correlated not only with the inverse of ambient temperature, but also with the inverse of hours of sunshine and with ambient humidity. There were no differences between hypertensive and normotensive patients, suggesting that the seasonal variation in stroke incidence is not related to the influence of low ambient temperature on blood pressure. The relationship of stroke to such meteorological factors as hours of sunshine also was shown by Tsementzis and colleagues, who pointed out that the strong intercorrelation between meteorological variables makes the selection of the most important predictor variable difficult (46).

3. Preliminary Studies
Summary of Specific Aims of the WATCH, and Experience with Recruitment

WATCH is a randomized controlled trial designed to evaluate the effects of: 1) a nutrition intervention training program for primary care internists; and 2) a structured office practice environment for nutrition management on eating patterns (i.e. reduction of calories from saturated fat) and serum cholesterol of patients of both sexes in the upper quartile of the cholesterol distribution enrolled in the Fallon Clinic. The effectiveness of the interventions are being evaluated by a randomized controlled design in which 1500 patients are allocated by site to one of three conditions: 1) control; 2) physician nutrition counseling training; and 3) physician nutrition counseling training plus a structured office environment for nutrition management. Twelve sites have been randomized to the three conditions, and over forty physicians have been trained in techniques for dietary intervention. The trial is now in its third year, fifteen months into the recruitment phase. We are presently screening patients at the rate of 160 per week, utilizing a recruitment strategy that is closely integrated into the already-existent Fallon Clinic telemarketing system. A sophisticated computer program has been developed that provides weekly downloads of physicians’ schedules into the telemarketing computers; allows for automatic dialing; has codes that permit identification of previous refusers, ineligible patients, patients already screened or in the study, and numerous other items of interest; and screens out ineligible patients by age, medications, and ineligible disease states. This program was developed by a collaborative team from UMMC and the Fallon Clinic. We have had a 33% acceptance rate among patients called by telemarketing and invited for screening, and the final consent rate among patients who are screened as eligible and are invited to join the study by our trained site coordinators has been 92% (681/740). These results demonstrate our ability to access and recruit patients from the Fallon Clinic system with a very high rate of success.

An oversight board consisting of study personnel (one of whom is a physician on the Fallon clinic staff) and administrative staff from the Fallon Clinic meets monthly to anticipate and deal with problems and facilitate the course of the study. Laboratory personnel have been trained to assist with patient recruitment, and the Fallon Clinic laboratories have participated in a lipid standardization program with the laboratories of Dr. Robert Nicolosi at the University of Massachusetts-Lowell (one of the two fully CDC standardized laboratories in the state), where study lipid profiles are analyzed. The Fallon Clinic will be the recruitment site for the presently proposed project, and Dr. Nicolosi’s laboratory is the proposed site for analysis of lipids and antioxidant vitamins.

Seasonal Variation of Cholesterol in the WATCH

As of January 1993 we had screened 3894 adult HMO (Fallon Clinic) subscribers living in Worcester County, MA utilizing CDC standardized finger stick (Cholestech Corp; Haywood, CA) screening cholesterol measurements. A striking seasonal shift of the cholesterol distribution to lower values in summer as compared to winter was noted (25). National Cholesterol Education Project (NCEP) standards were applied against the values for the two lowest months (July, August = “summer”) and the two highest months (January, February = “winter”).

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A mean 19.5 mgs/dl difference was noted between “winter” & “summer” months. Using the NCEP cutpoint of 240 mgs/dl, 27.4% of pts would be classified as having an “undesirable” cholesterol level in the winter, as compared to only 11.2% of pts in the summer. These seasonal differences are similar to those described by...
Råstam and colleagues for males, and although not shown here, were greater in our female population than was found in their study (11).

Since that time we have continued to collect data, now complete through August of 1993 (n=6270). The combined data for the 20 month period 1/92 – 8/93 is presented below in figure 1 and table 2.

Figure 1: Cholesterol level by month

![Cholesterol level by month graph]

Data for Jan-July represent two consecutive years; August-December are 1992 only

Table 2: Screening cholesterols by month

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Validation Study of a Dietary Recall Tool

Dr. Hebert is a Co-PI for WATCH. He, in collaboration with Dr. Ockene and other WATCH investigators, developed a seven-day dietary recall (7DDR) instrument for the purpose of improving on presently available tools to assess dietary fat intake. A validation study was carried out in the HMO population in October-November 1991. Its purpose was to test the utility of the 7DDR in estimating participants’ intake of fat and a variety of other nutrients. Forty-two participants filled out a baseline 7DDR using food models to aid recall. Over the succeeding three weeks they were telephoned on seven random days, representing each of the seven days of the week, and a 24 hour recall (24HR) was obtained. In week five the 7DDR was again administered to the participants. The seven 24HRs represented all seven days of the week and were conducted on days randomly selected over the three-week study period. For both administering the 24HR and computing nutrient scores, we used the Nutrient Data System (NDS) of the Nutritional Coordinating Center (NCC) at the University of Minnesota (47). Based on the NDS data base and the proportional intake of foods indicated in the cumulative 24HRs, a nutrient database was constructed for the 7DDR. Results indicate a higher level of agreement between the 7DDR and 7 random days of 24HRs than seen in prior studies. For both total calories and fat the Pearson correlation was .72, and for alcohol it was .84. Study results indicated much lower levels of concordance (i.e., r<.40) for most micronutrients. This finding coupled with the variance data on intermethod comparisons (see 3.2 below) has caused us to select the multiple 24HR as the assessment method of choice in this study.
3.2. Diet and NK Activity Trials

The research team has considerable experience in conducting studies that require repeat measures of dietary intake using multiple dietary assessment instruments. We conducted an intervention trial to test the hypothesis that linoleic acid suppresses natural killer (NK) cell activity (48). In this study we had each study participant complete a four-day food diary before the double crossover study began and once again after each one of the arms of the clinical trial was completed. In addition, we had them complete a food frequency questionnaire and we conducted telephone-administered 24 HRs on up to 18 randomly selected days over the nine-month study period. The study showed that we could achieve excellent compliance with intensive monitoring methods (also that we could intervene to cause fat intakes of about 23% of total calories in healthy volunteers). Study results showed that there was a large and significant increase in NK activity with decreasing overall fat intake (about .79 absolute % increase in killing for each 1% decrease in total fat calories) (48). Other results show that the 24HRs have the lowest overall variance (total error) of any of the assessment methods we used (49, 50). This is consistent with results of inter-method comparisons in the Framingham Study which indicated that the 24HR had the lowest total variance of all methods being compared. (51). Exercise data from the 24HR also proved to be useful in classifying individuals as to physical activity. The relevant reprint from that study is included in appendix G. Also included in that article is a description of statistical methodology used for those longitudinal data that may be appropriate to this study.

3.3. Studies of Breast Cancer Prognosis.

In an NCI-funded study that enrolled 472 early stage cancer patients at Memorial Sloan Kettering Cancer Center from late 1982 until late 1984, we found that dietary fat and fiber were related to tumor characteristics indicative of prognosis, including tumor size and extent of axillary node involvement (52). We also have found in followup data from this cohort that obesity (indicated by the seventy-fifth percentile of weight-adjusted-for-height from the cohort population) is associated with disease or death, especially in those women with earliest stage disease (53). This paper demonstrates our ability to relate dietary data to biological endpoints of interest.

3.4. Dietary Assessment

In addition to his role in dietary assessment in the WATCH study (see 3.1.), Dr. Hebert is the Chair of the Nutrition Assessment Working Group of the NCI-funded worksite cancer risk factor intervention study (Working Well). He has overseen development of assessment instruments that have now been used with over 25,000 study participants in nine states. His experience with a wide array of studies ranging from cross-sectional surveys, to case control and followup studies, to clinical trials, has provided him with extensive experience using the complete range of dietary assessment techniques.

3.5. Epidemiologic and Statistical Issues in Large-Scale Multidisciplinary Trials

Our entire team has wide-ranging experience working with large, complex datasets. Dr. Hebert in particular has as his main substantive area of expertise the measurement and statistical issues related to dietary and nutritional factors, which pertain to both psychosocial factors (where intercorrelation, reliability, and criterion/construct validity are major issues) and immunologic factors (where random analytic and biologic variability are major issues).
3.6. Biological Nutrient Assessment:

Drs. Hebert and Nicolosi are currently analyzing data from a study investigating biochemical markers of nutrients thought to affect cancer risk. Study subjects were 50 people in three nutritionally distinct groups: Cambodian immigrants and Hispanics (living in Lowell, MA), Macrobiotics, and "nutritionally average" Americans. Nutrients included the carotenoids, tocopherol, fatty acids, and other lipids. Each person kept a seven-day diet diary and then presented for blood drawing and to complete a short lifestyle questionnaire.

In addition to the specific aim of describing dietary nutrient-biochemical relationships, this study has enabled us to examine inter-group differences in nutrient exposures. Results of descriptive analyses of these data are shown below. These indicate enormous intergroup differences especially in carotenoid levels.

Table 3. Descriptive Statistics

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<tr>
<th></th>
<th>Macrobiotics (n=21)</th>
<th>Cambodian/Hispanic (n=10)</th>
<th>Omnivores (n=19)</th>
<th>P-Value of Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>AT (ug/ml)</td>
<td>10.66</td>
<td>2.39</td>
<td>8.00</td>
<td>2.00</td>
</tr>
<tr>
<td>BC (ug/ml)</td>
<td>1.55</td>
<td>0.58</td>
<td>0.17</td>
<td>0.11</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>149.95</td>
<td>19.71</td>
<td>194.50</td>
<td>30.31</td>
</tr>
</tbody>
</table>

General linear models based on these data indicate that around 36% (model $R^2 = .358$) of variability in blood BC levels are explained by dietary BC and 60% is explained by a combination of fat intake, body mass and blood cholesterol level. These results were obtained after accounting for direct and passive smoke and time spent in traffic. For blood AT, only 3% of variability was explained by dietary intake whereas about 35% was explained by study factors including tocopherol intake, body mass, total fat intake, and blood cholesterol level. These results indicate that dietary factors, while helping to predict blood levels of the nutrients, may explain only a modest portion of total variability. They also point out the necessity to collect data on important covariates.

4. Experimental Design and Methods

Introduction

To date, despite a number of studies describing the phenomenon of seasonal variation of cholesterol levels and discussing the implications of this phenomenon, there has been no study that has followed a cohort of individuals and obtained serial individual data on both lipid levels and those factors thought to be possibly causally related. The proposed study will fill this need, and is designed to produce a rich dataset that will provide the best-possible assessment of diet, physical activity and a number of other important parameters.

4.1 Overall study plan

A cohort of individuals recruited from the patient population of the Fallon clinic will be followed prospectively for one year. Baseline and serial measures taken quarterly for one year will be carried out. The quarterly interval was chosen because: of our experience in the WATCH which shows a gradual change in cholesterol levels across the seasons; the relatively short period of time required (~6 weeks) for cholesterol levels to respond to environmental stimuli,(54, 55). and the importance of the “off seasons” (i.e., fall and spring) as correlates of health-related behavior change (29). These serial measures will include: serum lipids and lipoproteins; platelets and hemostatic factors; weight, waist-hip ratio, height, BMI and blood pressure;
dietary intake; physical activity; light exposure; and psychologic factors. Meteorologic data also will be collected. Data will be collected by written questionnaire and by the use of computer-assisted (evening) telephone 24 hour recalls (CATI), which will be used for the collection of dietary, physical activity and individual light-exposure data.

4.2 Hypotheses:

1. There is seasonal variability in blood cholesterol levels.
2. Seasonal variation of cholesterol will be paralleled by seasonal variations in LDL and HDL.
   2a. Secondary hypothesis: Triglycerides and apolipoproteins, including lipoprotein (a), also will show seasonal variation.
3. Seasonal variation of cholesterol will be largely (i.e., >50%, $R^2=.7$) but not entirely, explained by changes in diet and physical activity. The few studies not finding a close association between these factors and seasonal variation of cholesterol were cross-sectional and flawed by use of inadequate data collection tools, especially for diet.
4. Seasonal variation also will be found in factors related to hemostasis, including fibrinogen, PAI-1 and factor VII; and the magnitude of these variations will parallel the magnitude of seasonal variation of cholesterol.
5. Seasonal variation of cholesterol will parallel in magnitude seasonal variation in physiologic factors, including weight, BMI, blood pressure, and heart rate.
6. Seasonal variation in biochemical factors (antioxidants, vitamins and hemostatic factors) will parallel in magnitude seasonal variation in psychological factors, including mood, energy, and hours of sleep. After controlling for diet and physical activity these factors will explain a significant portion of total variability (i.e., around 25%).
7. The magnitude of seasonal variation of cholesterol will parallel the magnitude of exposure of individuals to seasonal change: the less the exposure of individuals to seasonal change (e.g., variation in indoor temperature; time spent out-of-doors; use of air conditioning at home, work, and in transportation; work in offices with or without windows) the less the magnitude of variation of cholesterol and of physiologic factors. Within this analysis, the most important determinant factor will be exposure to natural light and changing length of day light. The total effect of these individual exposures (not controlling for other factors) will be to explain >50% of total variation in blood cholesterol and in mood disturbances.
8. Seasonal variation in blood cholesterol levels, when applied to the NCEP cutpoints, will result in significant variation in the percentage of individuals meeting these cutpoints.

4.3 Study population

4.3.1 Inclusion and exclusion criteria:

4.3.1.1 Inclusion criteria

1. Fallon Clinic subscribers who attend the Plantation Street site, and are residents of Worcester County, MA.
2. Ages 20 to 70 years old.
3. Literate in English.
4. Willing to provide frequent responses to various study assessments many of which are conducted over the telephone.

4.3.1.2 Exclusion criteria:

1. An inability or unwillingness to give informed consent.
2. Is on specific pharmacologic therapy to lower lipid levels (e.g., resins, fibric acid derivatives, HMG-CoA reductase inhibitors, nicotinic acid), or is taking a drug known to affect lipids (e.g., oral contraceptives, post-menopausal hormone-replacement therapy, retinol).
3. Has a secondary cause of hyperlipidemia (e.g., hypothyroidism, pregnancy).
4. Has a serious disorder which is likely to be fatal within five years (e.g., metastatic cancer, chronic renal failure, congestive heart failure).
5. Has or has had any cancer within the past five years, other than non-melanoma skin cancer, as all are known to alter lipids because of their role in modifying the LDL receptor (56, 57, 58).
5. Plans to move out of the site area within the study period.
6. Has a psychiatric illness which limits ability to participate.
7. Has no telephone.
8. Has a known condition that might limit participation (e.g., alcoholism).
9. Is currently on a lipid-lowering or weight-control diet or plans to begin one during the course of the study.
4.4 Study site

The Fallon Community Health Plan is a state licensed and federally qualified group model health maintenance organization (HMO) operating in Central Massachusetts since 1977. Total current membership as of 1992 is about 173,000. Approximately 40,397 people between the ages of 20 and 70 (19,274m; 21,123f) are followed at the Plantation Street site, which is the largest of the Fallon Clinic sites and the one closest to UMMC, at a distance of one-quarter mile. The Fallon Clinic does not collect specific ethnic or racial information about its members, but it is believed that the clinic population is representative of the population of Worcester county as a whole: approximately 4% Hispanic; 2% non-Hispanic, non-white.

The UMMC investigators and FCHP have an excellent working relationship and presently collaborate on an NHLBI-funded clinical trial for dietary intervention to lower cholesterol levels in men and women, aged 20-65 years (WATCH - see section 3.1). In that study, we are presently screening 160 patients a week and 92 percent of eligible patients accept participation in the study. Recruitment for WATCH will end December, 1993. Through this study we have been very successful in developing a smooth and efficient system to manage and recruit patients into large projects at the FCHP.

4.5 Study Organization and management

The Principal Investigator, Dr. Ira S. Ockene, will have ultimate responsibility for all study activities. He will coordinate the activities of the three subcontracts and the other investigators, and will ensure that the protocol is completely and accurately implemented. Dr. Ockene will oversee recruitment activities and supervise Mr. Phil Merriam, the project coordinator, and Ms. Donahue, the Study Recruitment, Screening and Followup coordinator (RSFC). He will hold meetings with all study investigators and staff: these will be held weekly during the first 6 months of the project and bi-weekly during the second 6 months. In the subsequent years of the study the meetings will be monthly. When circumstances require them, additional meetings will be held. Dr. Ockene will have overall responsibility for outcome manuscript preparation and presentations.

The Co-Principal Investigator, Dr. James Hebert, will oversee all collection and management of study-related data. He will directly supervise the masters level statistician; the research assistant, whose role includes some data entry; and the programmer, who will be responsible for setting up the tracking system and data entry screens in the first year and one half of the study. Dr. Hebert also will collaborate closely with Dr. Stanek, the project statistician, on matters related to study design and data analysis. As nutritional epidemiologist on the project, he also will work closely with Sarah Ellis, Senior Nutritionist, to ensure the timely, accurate, and precise collection of dietary data. He will also collaborate with Drs. Well, Harmatz, and Freedson to ensure that data on light exposure, psychological state and traits, and physical activity are collected in an efficient manner and have reasonable psychometric properties.

Other investigators including Drs. Well (cognitive scientist with extensive experience in psychometric analyses), Harmatz (psychologist whose focus here will be on issues related to psychological factors), Freedson (exercise physiologist) and Nicolosi (lipid biochemist) have contributed and will continue to contribute to measurement methodology and study design issues. They will also be intimately involved in the analysis of data and interpretation of results. Dr. Stanek will provide ongoing statistical design and analysis support for this large and complex dataset.

The project coordinator, Phil Merriam, will be responsible for ensuring the smooth flow of all study-related data. He will directly supervise the tracking system and ensure that schedules are provided for all necessary patient followup, and will assure the orderly flow of samples and data to and from the subcontractors. He will communicate very closely with Deirdre Donahue, the Recruitment/Screening/Followup Coordinator (RSFC). He will be assisted in these tasks by the research assistant whose responsibilities will include collection of data, transmission of data, and maintaining all study correspondence in secure facilities.
Deirdre Donahue, the Recruitment/Screening/Followup Coordinator (RSFC) at the Fallon Clinic, will be responsible for all activities related to recruitment and screening, of participants in the trial, and also will participate in participant followup. She will monitor the progress of recruitment, coordinating and implementing the different activities involved. She will assure scheduling of initial visits and the timely followup of missed or canceled appointments, and she will supervise the telemarketing team which will be responsible for making the initial telephone contact. She also will serve as the interface with the Fallon Clinic clinical laboratories and with the computing/scheduling system, which has excellent scheduling/tracking capabilities (see 3.1 for our experience with this system in the WATCH study). As a Fallon employee, Ms. Donahue will serve as a link with Fallon Clinic clinical and support staff. She has had extensive experience in working in UMMC/Fallon Clinic collaborations through the WATCH study (see 3.1).

Dr. Saperia will be the P.I. of the Fallon Clinic subcontract, and will oversee all of the participant-related activities at that site. He will coordinate the efforts of the RSFC, the telemarketing system, the clinical laboratories, and the Fallon Clinic administration. He will also participate in analysis of the data and writing of manuscripts.

Sarah Ellis, the chief research nutritionist at UMMC, will coordinate all of the activities relating to dietary assessment, and will supervise the registered dieticians who will be conducting the telephone administered 24HRs. She will work under the direct supervision of Dr. Hebert.

The telemarketing team at FCHP will be responsible for scheduling all initial study visits. They will report directly to the Recruitment/Screening/Followup Coordinator.

4.6 Phases of the investigation and time line

4.6.1 Phase I - Planning and development (3 months)
Phase I, comprising the first 3 months of our investigation, will allow time for the hiring and training of personnel and the further refinement and pretesting of the tools to be used in the investigation. In particular, this will focus on the 24HR recall telephone assessment of light exposure and physical activity, and the newly developed written light exposure assessment instrument. We will also calibrate the Actillume™ for estimating of energy expended in physical activity during this phase (see 4.7.1.5). Audiotaping of all training sessions and interviewing skills of staff will occur during this phase. These tapes will be rated and data derived from this process will be used in assessing reliability (see 4.10.) This will be repeated for all staff hired after the run-in phase. Reliability and validity of all previously untested assessments will be established. Discussion of techniques to test reliability and validity are included in Section 4.9.

4.6.1.1. Process Objective:
By the end of Phase I development and pre-testing of all recruitment and evaluation materials will be completed; reliability and validity of the assessments will be established; the Actillume™ will be calibrated for physical activity; and all staff members will be in place and trained.

4.6.2 Phase II - Recruitment and followup (2 years)
Recruitment of the 600 subjects will take place over a period of two years, at a rate of 75 participants per quarter. This will allow time for the orderly entry of participants into the trial and for the collection of the large amounts of followup data. Given the one year followup time, data will be collected over a three-year period, allowing us to look at seasonal variability over three full cycles. (Table 4)

We will recruit a random sample of Fallon Clinic members who receive their care at the Plantation Street site. Recruitment will be blocked by sex and age (in decades, 20-70). Each quarter a list of 500 potential participants will be identified from the Fallon computing system, sorted according to age and sex, put into a random sequence within strata, (using the UNIFORM function in SAS (59)) and provided to the telemarketing group. Patients contacted from this list will be identified in the Fallon Clinic scheduling system by a specific field identifier, so that in future quarters they will not be recontacted. Telemarketing personnel will explain the purpose and overall design of the project, and will obtain verbal consent for the first three 24HRs. At the initial 24HR, the interviewer (an RD) will further explain the project, and obtain
inclusion/exclusion information. If the individual meets criteria for inclusion, the three baseline 24HRs (diet, physical activity and light exposure) will be obtained as detailed below and in section 4.7.1.3, and a subsequent appointment will be scheduled for the initial study visit. The three 24HRs will always include at least one weekend day, and will include two separate days of the week, randomly selected within a six week window prior to the blood test appointment. At the initial study visit the research assistant will explain the study in detail, and obtain full written consent. Initial physical measurements will be made, baseline questionnaires provided together with a postage-paid envelope, and baseline bloods will be obtained. A randomized subset of 10 individuals/quarter will be given activity monitors to wear for a seven-day period. These individuals will have the activity monitoring repeated each quarter.

An incentive of $100 will be provided to each participant, to be distributed as $25 at each of the four quarterly followup contacts. No incentive will be provided at baseline. The contact time and repeated tests involved in this study justify such an effort to promote participant retention.

At three-month intervals the battery of studies will be repeated. Thus there will be five sets of measurements for each individual: baseline and quarterly x 4 to the one-year point.

Process objective: Six hundred participants will be recruited into the study over a two-year period, and will have baseline and quarterly assessment made on schedule. Systems will be in place for orderly recruitment, study testing and followup.

4.6.3 Phase III - Continuation of followup (1 year)

During Phase III there will be no further recruitment, but the 300 participants recruited in the prior year will continue to be followed to each of their one-year points.

Process objective: Followup will be completed for three hundred participants recruited in the preceeding year.

4.6.4 Phase IV - Close-out and data analysis (9 months)

This study will generate a number of very large and complex datasets. We are requesting nine months of funding for analysis and completion of manuscripts. However, we recognize that the work to be generated from this study will extend beyond the end of the funding period.

Process objective: The major analyses and endpoint manuscripts will be completed for the study during Phase IV.

4.7 Measurements

4.7.1 Participant measurements

A summary of participant measurements is presented in table 3 below

<table>
<thead>
<tr>
<th>Table 4: Workload: Patient-Related Activities*</th>
</tr>
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<tbody>
<tr>
<td>Phase I</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Year 1</td>
</tr>
<tr>
<td>Quarter</td>
</tr>
<tr>
<td>Participants recruited</td>
</tr>
<tr>
<td>24 HRs**</td>
</tr>
<tr>
<td>Questionnaires</td>
</tr>
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</table>
**Activity/light Monitors***

<table>
<thead>
<tr>
<th>Activity/light Monitors***</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
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<th>50</th>
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<th>30</th>
<th>20</th>
<th>10</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Draws</td>
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<td>225</td>
<td>300</td>
<td>375</td>
<td>375</td>
<td>375</td>
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<td>300</td>
<td>225</td>
<td>150</td>
<td>75</td>
<td>3000</td>
</tr>
</tbody>
</table>

*The largest number of participants who will be followed in any one quarter will be 375, reflecting a recruitment of 75 participants/quarter and a one-year followup.

**At peak workload, there will be approximately 94 24HRs per week, at an average time of 30 minutes each, for the combined diet/physical activity/light exposure 24HRs.

***A total of 80 participants (13%) will have the physical activity data collected by questionnaire and 24HRs validated with the use of activity monitors, as described in section 4.7.1.4.

### 4.7.1.1 Baseline questionnaire

A baseline questionnaire will be used to assess participant demographic characteristics, brief health history, smoking, weight loss history, and occupation. It will be based on the baseline questionnaire developed for the WATCH study (see appendix F) but will not include most of that instrument questions on diet.

### 4.7.1.2 Blood pressure and anthropomorphic measurements:

Height, weight, body mass index (BMI), waist/hip ratio, pulse and blood pressure will be obtained at baseline; all but height will be remeasured every 3 months through the one-year followup point. Blood pressure will be measured in a standardized manner, in the right arm after 15 minutes of quiet sitting. Blood draws will take place following these measurements to avoid blood pressure increases related to the stress of the venipuncture.

### 4.7.1.3 Blood Studies

All participants will be scheduled to have bloods drawn at baseline and quarterly in the Plantation Street laboratory, which is in the same building as the clinical services. Studies will be done within a three-week window on either side of the individual's quarter-year date. Blood will be collected from the antecubital vein of 12-hour fasting individuals into four 10 ml tubes:

- one tube with EDTA for determination of hemoglobin, white blood cell count, and platelet count,
- one tube with EDTA for plasma lipids (plasma, total cholesterol, HDL-C, Triglycerides, LDL-C, apolipoproteins)
- one tube with EDTA for antioxidant assay (alpha-tocopherol, beta-carotene),
- one tube with sodium citrate for fibrinogen, factor VII and PAI-I. (to be analyzed by the laboratory of Dr. Russell Tracy at the University of Vermont).

CBCs and platelet counts will be processed immediately in the laboratory and the reports obtained daily for entry into the participants' database. For the lipid and antioxidant studies, plasma will be harvested by low-speed centrifugation at 4 degrees centigrade, aliquoted into individual tubes as appropriate and quickly frozen to -70c and maintained at this temperature indefinitely. Bloods for antioxidant vitamin determinations will be protected from light and air by immediate wrapping in aluminum foil. Blood for determination of fibrinogen, factor VII and PAI-I will be spun at 3000 g for 10 minutes. Because it is cold-sensitive, the sample for factor VII activity will be spun at room temperature, and then frozen. All aliquots to be frozen will be stored in cryovials with screw-top caps to exclude air. Assays will be carried out in Dr. Robert Nicolosi’s laboratory at the University of Massachusetts at Lowell (lipids, lipoproteins, antioxidants) and in the laboratory of Dr. Russell Tracy at the University of Vermont (fibrinogen, factor VII, PAI-I). Because there is known circadian variation in a number of these substances, and because participants will be fasting, all blood draws will occur between 7AM and 9AM.

On a weekly basis bloods will be packed in dry ice and shipped via overnight service (Dr. Tracy) or courier (Dr. Nicolosi) to the offsite laboratories.

### 4.7.1.3.1 Lipid measures

Blood obtained by venipuncture will be tested for total cholesterol, HDL, LDL, and triglycerides. In a random subset of 120 individuals, we also will measure apolipoproteins B and A1 and Lp(a) levels. All of these
measurements will be made in the Centers for Disease Control (CDC)-standardized laboratory of Dr. Robert Nicolosi at the University of Massachusetts at Lowell. A random sample of 5% will be re-tested for quality control. Total cholesterol (60), and triglycerides (61), will be measured by enzymatic methods using a Beckman System 700 autoanalyzer. HDL will be measured in the supernatant after heparin manganese precipitation of apo B-containing lipoproteins (62). These methods have met the criteria of the CDC-NHLBI Lipid Standardization Program (63).

Apolipoproteins A1 and B are measured in serum samples with an immunoturbidimetric technique using commercially available kits (Atlantic Antibodies, Scarborough, Maine). Essentially, goat antibodies specific for apolipoprotein A1 or B purified from human HDL or LDL respectively are incubated with the sample and the apolipoprotein standards. The multivalent apolipoproteins in the sample react with the apolipoprotein antibodies to form a lattice of antibody-antigen complexes. This lattice of immunocomplexes in solution scatters light and the change in absorbance (?A) is measured over a 5 minute time period at 340 nm using an Abbott VP analyzer. The concentrations of the apolipoproteins in the serum samples and standards is proportional to the ?A.

Lp(a) concentration is determined with the use of a Macra™ Lp(a) diagnostic kit. Plasma is incubated in microtiter test wells containing monoclonal anti-Lp(a) antibody, followed by incubation with a polyclonal anti-Lp(a) antibody conjugated with horseradish peroxidase. Unbound enzyme conjugate is removed, leaving the Lp(a) monoclonal/polyclonal antibody complex bound to the test well. The plate is then incubated with substrate (hydrogen peroxide) and chromogen (o-phenylenediamine). Color development is stopped by 2 N sulfuric acid and the absorbance read at 492 nm in a microtiter plate reader. The absorbance is directly proportional to the concentration of Lp(a).
4.7.1.3.2. Determinations of alpha-tocopherol and beta-carotene:
Beta carotene (BC) and alpha-tocopherol (AT) will be determined by high performance liquid chromatography with photodiode array detection. This allows simultaneous determinations of each component, thus enhancing precision. Also, monitoring each component at selected wavelengths yields maximum absorption signals for enhanced sensitivity and minimum interferences from other coeluting components. The technique we will use is a National Institute of Standards and Technology (NIST) approved method (64).

4.7.1.3.3 Hemostasis measures
Blood will be obtained for measurement of hemoglobin, platelet count, fibrinogen, factor VII, and PAI-1. Hemoglobin will be used to assess hemoconcentration. Fibrinogen, factor VII and PAI-I have all been correlated with an increased risk of CHD events (38).

The fibrinogen measurement is based on the Dade method using the clotting of citrated plasma after the addition of 100 NIH units/mL of thrombin (65). Fibrinogen, a soluble plasma protein, is converted to fibrin, an insoluble polymer of fibrinogen, in the presence of thrombin. Fibrinogen concentration determines the reaction rate using high concentrations of thrombin and relatively low concentrations of fibrinogen. The thrombin clotting time versus fibrinogen concentration is linear when plotted on log-log paper. Therefore, the longer the clotting time, the less the concentration of fibrinogen. The University of Vermont will run this assay on the State ST4 instrument which determines an optical endpoint for fibrin clot information. Fibrinogen standardization utilizes the College of American Pathologists standard and the Dade Data-Fi standard. The interassay coefficient of variation (CV) is approximately 3-4%.

Factor VII(a) is activated by complexation with tissue factor in the presence of calcium ions and lipid, and then activates Factor X to Xa in vivo. Factor Xa then activates Factor II (prothrombin) in the presence of Factor Va, calcium, and a phospholipid surface to form thrombin that cleaves fibrinogen to fibrin. In vitro, the clotting time when plasma is added to a specific factor-deficient system compared to the clotting time when a normal plasma (or another standard source of the factor) is added to the same system determines the quantitative analysis of plasmas for specific factors. The University of Vermont will determine Factor VII activity using a General Diagnostics Coag-A-Mate X2 that has a photo-optical clot detection endpoint. Factor VII is standardized to the World Health Organization Factor VII international standard. The interassay CV is approximately 6-7%.

The Plasminogen Activator Inhibitor-1 (PAI-1) assay is a two-site ELISA using a set of monoclonal antibodies provided to us by Drs. Paul Declerck and Desiré Collen of the University of Leuven, Belgium (66). This assay is specific for free, uncomplexed PAI-1 and has an interassay CV of 6-10%, recovery between 95 and 107%, and sensitivity to at least 2 ng/mL. The normal range observed is 24+/−9 ng/mL.

4.7.1.4 Dietary intake
Because of its overall variance and reduced probability of bias (49, 50, 51) in relation to other assessment methods we will use 24 HRs as the dietary assessment method in this study. Dietary intake will be assessed by evening telephone interview every 3 months. Three 24HRs will be conducted on randomly selected days representing two week days and one weekend day during each trimester. All three 24HRs will be administered within the 6-week period prior to the participant’s quarterly clinic visit and will be performed by RDs who will have been trained to collect dietary data using our interview system and to query respondents on physical activity and light exposure.

All dietary data for the 24HRs will be entered and analyzed using the Nutrition Data System (NDS 2.3) (see 4.7.1.4.1 below).

Dietary data that we collect can be used to compare levels of 93 nutrients. Those that we will focus on will include total caloric intake, percentage of calories as total fat, and all major contributors to saturated, mono- and polyunsaturated fat, as well as dietary fiber intake. Currently available information indicates a
predominant effect of lipid components in determining blood cholesterol levels (55, 67). Because of their role in lipid oxidation we also will analyze data on dietary antioxidants, including beta-carotene, ascorbic acid and alpha-tocopherol. The three days that we will use to characterize diet are sufficient for dietary macronutrients (68, 69, 70). For the micronutrients this number of days represents a reasonable number to describe intake within a season (68, 71). However, we do note that this shorter time frame has not been the concern of most studies aimed at dietary assessment. It is widely thought that 15 days of 24HRs in a year characterize dietary intake of most individuals for most nutrients (69, 70).

4.7.1.4.1 The Nutrition Data System
For analysis of 24HRs (diet) we will use the University of Minnesota Nutrition Coordinating Center's (UM-NCC) Nutrition Data Systems software. This software accomplishes a number of data management tasks in addition to streamlining data entry. Essentially, it checks for errors in range and simple logic and prompts for corrections. At the end of the interview it produces an analytic data file.

We currently use this system in the WATCH study, and have used it for our validation study of our main study assessment method in WATCH (see 3.1). The UM-NCC Nutrition Data System consists of data entry software, analysis software and comprehensive food product and nutrient databases including many exotic foods that may be eaten by persons in this study. The UM-NCC was developed in 1974 to support nutrient analyses for randomized intervention trials, and has since developed into the United States' leading resource for nutrient database and analysis systems for scientific research. The recent addition of data entry software that prompts coders for information in English makes the Nutrition Data System relatively easy to use.

The UM-NCC database contains over 16,000 foods and 5,000 brand name products and corresponding values for 93 nutrients. The combination of these foods and the analysis software allows over 150,000 food variants, differing in preparation methods and ingredients. The database is purposefully broad, including culturally unique foods. The database is updated at least annually to reflect new analytic data, new foods, and changes in composition of manufactured foods (47). We have contributed data on an assortment of foods to the NDS from our on-going studies.

The system is especially well-suited to open-ended assessment techniques such as 24HRs (diet) that demand matches for a wide array of specific food items. We also have used the UM-NCC system to analyze diet in terms of servings of certain food groups. This has allowed us to focus on categories of foods such as cruciferous vegetables.

4.7.1.5 Physical activity assessment
Data collection for physical activity patterns will be conducted using three types of assessment: a self-administered 7-day activity questionnaire; telephone interview 24HR; and a computerized activity monitor, the Actillume™ (Ambulatory Monitoring, Inc, Ardsley, NY).

The assessment of physical activity presents a number of difficulties. People are active in different ways, at various times, in different settings, and for varied reasons. Activity patterns differ both between and within individuals. Activities may differ from day to day, weekday to weekend, week to week, and season to season (72). Even within an activity, considerable variation in frequency, duration, and intensity may exist from one time to another. As a result, a number of methods to assess physical activity have been developed, with questionnaire/survey methods being most common. In addition, the recent development of motion sensor technology now provides the capability of recording and storing activity data for extended periods of time. Our group has considerable experience with the use of such devices (73).

The self-administered questionnaire to be used in this study will be the 7-day activity recall instrument developed for community surveys as part of the Five-City Project (see appendix A for a copy of this instrument) (72, 74). Although intended to be interviewer-administered, this questionnaire has been previously modified for self-administration (75). and we will adapt it in a similar manner. The final form of this questionnaire will be tested...
against the Actillume™ derived data (corresponding to criterion validity) and responses from the 24HR physical activity telephone interviews (concurrent reliability).

The physical activity information obtained during the 24HRs will be derived from a standardized question format in which the day’s activities are gone over with the participant and directly entered on the computer. We will provide a wide range of responses that will be available to the interviewer for participant prompting. This will take the same form as the response prompts for the NDS, except many fewer choices (under several hundred activities) will be needed. These items will be taken from the compendium of physical activities recently developed by Ainsworth and colleagues (76). In addition to activity type, the duration of the activity, and an estimate of relative exertion (to others involved in the same activity) also will be recorded. Total time needed to collect these data will be about 3 minutes.

In order to validate the above instruments, daily physical activity also will be assessed objectively in a subset of 80 randomly selected participants (10 per recruitment quarter) using the Actillume™ device, an instrument that incorporates both light and activity detectors. (see section 4.7.1.6. for a discussion of this device's light-detecting capabilities). The Actillume™ monitors acceleration and detects both quantity and intensity of movement. The monitor is approximately the size of a large wristwatch and can be worn on the wrist, but may also be worn on the waist. As we are most interested in large body movements that result in caloric expenditure, we will place the device on the non-dominant hip (see below for discussion of substudy to evaluate such placement) and place the accessory light sensor on the participant’s lapel or similar location (see 4.7.1.6.) The Actillume™ is capable of storing units of integrated acceleration for extended periods of time, and in this study will be utilized to record 7-day periods corresponding to the sampling period evaluated with the 7 day physical activity recall questionnaire and occurring within one week of each laboratory blood work session. Subjects will be given the device one week prior to their clinic visit and instructed how to attach and remove the monitor. Using the provided interface and software, the date, subject’s name, ID, height, weight, gender, and age will be programmed into the device along with the sampling interval which will be set at 5 minutes. This sampling interval will provide an integrated acceleration measurement that will be stored in the monitor’s memory register for each 5 minute period of data collection. At the end of the 7 day assessment period, the device will be returned and data will be downloaded into the PC for subsequent analysis. The data will be stored as an ASCII file (units are counts). The mean of the 7 days will be used to represent physical activity.

In Phase I of the study we will carry out a substudy to: 1) develop a predictive equation to estimate energy expenditure (kcal/min) from the activity count output, and 2) identify the best location for wearing the Actillume™ (wrist vs. hip). Twenty subjects (10m, 10f) with ages corresponding to those of the study sample will have energy expenditure (EE) (kcal/min) and activity counts (cts/min) obtained under steady-rate conditions for the following activities: walking, jogging/running, cycling, stairclimbing, gardening, knee bends, reading, and typing. EE will be calculated from the caloric equivalent of the steady-rate VO₂ and the respiratory exchange ratio using the portable Teem 100 (Aerosport, Inc., Ann Arbor, MI) metabolic measurement system. EE will be assessed for 10 minutes for each activity. One Actillume™ device will be worn on the non-dominant hip and one will be worn on the non-dominant wrist for all testing sessions. These data will provide a wide spectrum of energy expenditure values so that appropriate predictive equations for caloric expenditure can be developed, and so that the optimal site for placement of the device can be determined.

The device is known to be inaccurate in physical activity situations where there is little motion at the placement site, such as during bicycle riding, and also cannot be worn in the water. The use of the questionnaire and 24hr recall methodologies will allow us to correct for these situations. Subjects will be instructed to remove the device when bathing or swimming, and to record the time of each removal, the activity being done, and the time when the device is reattached. The motion sensor technology should be seen as adding an objective level of validation to our activity measurement methodology; it is not the primary assessment tool. Validation studies with this system have shown a significant correlation (r=.73) with direct
measurement of oxygen uptake and heart rate (r=.71) (p<.0001), and a very high test-retest reliability for
twelve activities (r=.98) (77). In this study the unit will be initialized as described in section 4.7.1.4 above, and
an interval of 15 minutes used for light exposure sampling (different sampling times can be set for the activity
and light-assessment functions).

Procedure. Prior to the individual's visit for physiologic assessments, subjects will be given the questionnaires and
asked to complete them at home within a three day period around the time of blood drawing. Participants
receiving the Actillume™ will be instructed to respond for the same seven day period as that in which the
Actillume™ monitoring will occur.

Data Analysis. Data coming from each of the sources, i.e., interviewer prompted questioning via the 24HR and
the questionnaires, will be used separately in analyses as we have done previously with dietary data (48). Inter-
method comparisons are subsumed under the category of reliability and validity testing (see section 4.10).
Because the various physical activity assessment instruments produce different types of measurements (ratio and
ordinal), non-parametric correlational methods (e.g. Spearman Rank Order) will be used to estimate the degree of
association between instruments. Where data meet assumptions of parametric tests we will use linear regression
to assess agreement.

4.7.1.6 Light exposure

We are devoting considerable resources in this study to the measurement of individual light exposure, in
addition to more global measures such as length of day and meteorologic data. We believe that this is
important, as the literature as reviewed in section 2 fails to clearly establish the nature of those factors
responsible for seasonal variation of blood cholesterol levels, and changing exposure to light is responsible for
many seasonal phenomena in both plants and animals. The work of de Castro and colleagues cited earlier is
also suggestive that seasonal dietary change in humans may be related to changing light exposure in the fall
and spring (78).

Light exposure will be measured in two ways: a questionnaire and a 24HR instrument. In addition, we will
validate these methodologies in a subset of 80 participants by the use of a device that accurately measures
light exposure (Actillume™, Ambulatory Monitoring, Inc, Ardsley, NY; see section 4.7.1.5 above for a full
description of this device and its use in activity assessment).

We have developed a comprehensive questionnaire for assessing light exposure for this project. This
instrument is designed to provide measures of exposure to different kinds and intensities of light, including
direct and indirect daylight as well as artificial lighting. The instrument asks about typical light exposure
during the current time of year and distinguishes between work and non-work periods. The instrument also
assesses preferences for types of lighting and asks about consistency of exposure; for example, whether there
have been recent events (such as vacations) that may have altered patterns of light exposure significantly. The
questionnaire will be administered at baseline and at the quarterly followup points.

The information obtained during the 24HRs will be derived from a standardized question format in which the
day’s activities are reviewed with the participant and directly entered on the computer. Collection of
information about light exposure will be coordinated with the portion of the recall interview dealing with
physical activity. The day will be disaggregated into parts and the subject asked to recall information about
outdoor and indoor exposure to natural lighting as well as exposure to artificial lighting.

Light exposure validation:
The Actillume™ device accurately measures both activity and light exposure. It contains two calibrated
photovoltaic transducers, one on the device itself and the second attached via a wire and intended to be
remotely mounted (as for example, on a lapel or collar). The system has been shown to have a linear response
over the entire range of light exposure from 0.1–200,000 lux (79). Because the light exposure apparatus
simply responds to electromagnetic stimuli, it does not pose the kind of requirement for validation and

- 21 -
reliability testing that he physical activity portion does. As such, it actually represents an excellent validator of participants self-respect of light exposure.

The data collection methods will be further developed and refined in the run-in period. The validity of the light-exposure questionnaire will be assessed in a group of volunteers by comparing the estimates of light exposure it provides with those obtained from (1) the Actillume™ device worn for the week prior to the questionnaire being filled out and (2) in-depth interviews with the same sample of people. The test-retest reliability of the instrument will also be measured. This includes test-retest of questionnaire responses, inter-method reliability testing, and validation testing, as noted above.

4.7.1.7 Psychosocial measures

**Beck Depression Inventory (80):**
This instrument is a well-recognized standard for the assessment of depression. It has been used widely, including studies carried out by colleagues in the Division of Preventive and Behavioral Medicine here at the Univ. of Massachusetts Medical Center (81). It has well established psychometric properties, including high internal consistency (alpha = 0.86). In studies looking at validity, i.e. comparing responses with clinical assessments, the Pearson correlation coefficients are approximately .66. Time to complete: 3 minutes.

**Beck Anxiety Inventory (82):**
Developed by Aaron T. Beck and colleagues, this 21-item self-report inventory is a convenient measure for assessing anxiety levels. Results correlate highly with the Anxiety subscale of the SCL-90-R, but are not redundant. It has high internal consistency (alpha=0.92) and test-retest reliability over one week (r=0.75) as well as good concurrent and discriminant validity. It was constructed to avoid confounding with depression. Colleagues in the Division of Preventive and Behavioral Medicine used it as a primary outcome measure to assess the role of meditation in anxiety and panic disorder (81). We have chosen to use this instrument because of the possible close association of the seasonal effects on anxiety and depression. Time to complete: 3 minutes.

**The Seasonal Pattern Assessment Questionnaire (34, 83)**
The SPAQ was developed as an instrument for retrospectively evaluating the degree of seasonal variation in mood and behavior among patients entering studies of SAD and photo-therapy. The questionnaire elicits information on times of the year when subjects feel worst and feel best, the extent to which seasonal change is a problem, and the degree of seasonal change experienced across six parameters of mood and behavior: sleeping, eating, weight gain, socializing, energy level and mood.

**The Inventory of Seasonal Variation Questionnaire (23)**
The Inventory of Seasonal Variation (ISV) was developed as a dimensional measure of seasonal variation in mood and behavior. It produces a broad, finely graded distribution of seasonality scores. The ISV has been demonstrated to have high internal consistency (α = .88) and test-retest reliability (intraclass correlation = .85). Although the SPAQ is the standard testing instrument used in the field, the ISV is more comprehensive in its exploration of seasonal variations in mood and behavior.

4.7.2. Climatologic variables

National Oceanic and Atmospheric Administration (NOAA) data for Worcester will be used to collect measurements for daily rainfall and snowfall, high- low- and mean temperatures, and degree-days (a measure combining temperature and wind velocity), as well as percent of cloud cover. Cloud cover is tabulated hourly to the nearest ten percent. Hours of daylight will be obtained from the same source. As cloud cover does not measure light intensity, we will also directly measure the intensity of sunshine using the Nimbus Solar Radiation Instrument (Sensor Instruments, Concord, NH) (see appendix E for a copy of a NOAA report and appendix C for documentation for the Nimbus instrument). This instrument measures and records either instantaneous power (in W/sq. m.) or its integral, energy (in KW-hr/sq. m.), and has a 35 day hourly memory. The record of the instantaneous hourly value represents a snapshot of the energy being received at the end of
the hour. When the time of the day and year are taken into account the reading can be made relative to the amount of sunshine possible at that time. The integrating version records the energy received during the entire hour. Thus, this record is proportional to the average energy received during that hour and can be summed for the day.

As each individual in the study will have a different quarter-year interval based on date of entry into the study, daily data will be used to construct quarter-year means for each individual. Essentially, this entails the aggregating of all daily data for the interval defined by the collection of the two blood samples. In order to provide the study with expertise with regard to the collection and analysis of climatologic data we will utilize the consultant services of Dr. Robert Lautzenheiser of the New England Climatic Center. Dr. Lautzenheiser is a well-known expert in this field who will assist us in understanding a complex set of data.

4.8 Training the RD Interviewers

Our training of the telephone interviewers who will be RDs will be based mainly on our experience in training interviewers for the WATCH study and its validation of the 7DDR (see 3.1.). The methods are based partly on the recommendations of the University of Minnesota Nutrition Coordinating Center and our considerable experience in training interviewers. The 24HR is the primary interview technique learned and used by RD nutritionists. Our experience in the WATCH validation study as well as our work with other studies using multiple 24 HRs with more than one interviewer is that the interviewer effect in ANOVAs produces a $\beta = 0$, indicating no variability by interviewer (84, 85). Methods for the non-dietary measures (i.e., light exposure and physical activity) will require additional training. We will follow training procedures as outlined by Dillman (1978) (86). Special attention will be paid to teaching strategies for avoiding interviewer and respondent bias. Interviewers will be instructed to not react to what the person says, practice a neutral attitude, and deal with the subjects' anxieties without influencing answers. In addition, interviewers will be taught to be aware and document times when respondents seem to be answering based on motives other than those implied by the question (e.g., fear of looking stupid). Finally, all interviewers will be trained to ask questions in a consistent manner and to interpret recorded responses in a standard fashion, verbatim whenever possible.

RD interviewers will be trained based on the following components as described by Dillman (1978),(86)

1) Matters relating to telephone technology - This includes how to obtain information on new or changed numbers when necessary, if for example a recording informs them that a number has been changed. It also encompasses strategies for using telephone answering machines to increase study efficiency. A few moments of instruction and a sheet on how to dial various types of calls has been found to increase interviewer efficiency markedly – especially in the early stages of a survey (86)

2) Answering Respondent Questions – Interviewers will be given sufficient background on the survey objectives and likely concerns of respondents. This will include a description of the steps in the survey process that occur before the interviewing and what happens after the interview is completed. A list of anticipated questions also will be reviewed with each interviewer and appropriate responses to respondents' questions will be rehearsed.

3) Completing the Call Record – Interviewers will be trained to keep a “call record” which will assure efficient use of the interviewers' and respondents' time, and avoid duplication of work. It represents the work log generated by the participant tracking system. Each line of the log corresponds to a single participant to be called along with responses regarding items such as: disconnected phones, refusals, completed interviews, call back later tonight, call back tomorrow morning, messages left on answering machines, and date and times of repeated no answers.

4) Administering the Questionnaire – Interviewers will participate in four types of practice interviews before actually calling respondents. This will enable the trainers and interviewers to give feedback regarding: 1) adjustment of voice inflection and speed; 2) learning to key in the response to a question while mentally
preparing to state the next question; and 3) gaining skill at politely asking respondents to slow down so that there is time to pull down response screens. A “rule book” of 1-2 page instructions also will be developed to acquaint the interviewers with pre- and post-interview procedures, as well as with the actual interview schedule. This also will provide interviewers with a reference for appropriate responses to difficult situations.

Following are the four practice interviews to be conducted: The first interview will be one in which the interviewer observes only. The second, will involve a trained interviewer interviewing another trainee. During the third practice interview, the trainee interviews a supervisor who acts the role of a very difficult to interview respondent. The fourth interview will be actual pretests of the questionnaire with a sample of persons from the same population as the study sample itself.

4.9. Data Coordination And Management:

Data will be obtained from a number of different sources including: self-administered questionnaires; 24HR conducted by telephone; demographic data; physical examination (weight, BP, BMI, waist-hip ratio); results from the lipid, antioxidant, and hemostasis determinations; participant tracking logs; and physical activity and light monitors. The greatest volume of data will be obtained by telephone interview. Work Logs schedules for the telephone interviews will be prepared directly from the participant tracking system by the Research Assistant under the supervision of the Project Coordinator. The tracking system will use a SQL-based programming language. In the past we have used D-base (WATCH and COMMIT), Paradox, and R-base for this purpose. In this study we will use Paradox because of its strong relational capabilities.

The telephone interviews will be conducted exclusively by the RDs who also will ask questions on light exposure and physical activity. The computer-assisted telephone interview system that will be used in this study obviates two costly and potentially error-prone data conversion steps by enabling an expert interviewer to enter data directly into a computer software system that is programmed to flag illogical and out-of-range values and prompt for correction. As an added quality control measure, the Research Nutritionist Sarah Ellis will listen in on a random sample of 1% of all calls. Because of our excellent record of training interviewers we anticipate that accuracy will not be a problem. If an interviewer is found to deviate from the protocol the interview will be repeated and the interviewer will be warned. If it happens a second time the interviewer will be terminated. In addition to this procedure, we regularly conduct univariate analyses on dietary data (e.g., dietary fat) and we carefully monitor interviewer-bias (it should be 0) and total variance (it should be equal to about 9% CF and should not vary much across interviewers).

As most of the calls will be originated in the evenings and off-site, the computers on which the interview systems are loaded will be connected to UMMC by modem. After an interview is completed it becomes part of the analytic database by uploading to the main file server in our LAN. The log file is then incorporated into the tracking dataset that flags for specific data management tasks such as listing persons not-at-home who need a callback. For short-term tasks, the schedule worked out by project staff and incorporated into the tracking system becomes a workplan because the staff know roughly when to expect materials to convert into data or requests for additional data and when to expect each portion of the analytic database.

Though quantitatively much less important than telephone-derived data, we will need to collect and manage data from the other sources mentioned above. Self-administered questionnaires will be entered by the research assistant using double-entry programs written in Turbo Pascal by the programmer. Laboratory data can be uploaded directly into our analytic database via modem or by internet (as we currently are capable of doing). Data from the participant tracking system can easily be exported to the analytic database using a number of simple transfer utilities. Data from the activity/light exposure monitors will be downloaded to our PC using the interface and software provided with the devices. The software provides for easy exportation of data to database, spreadsheet and statistics programs in ASCII format with control of delimiters, time and date format, and other parameters. “Macros” permit automating of the downloading process.
Several of the forms that we will use will be entered via optically scannable questionnaires. This eliminates two expensive and error-prone data transfer steps. We currently use a National Computer Systems OPSCAN 5 Model 30 scanner capable of reading barcodes, ink as well as pencil marks, and two-sided copy. It is equipped with a transport printer that automatically prints the status of the questionnaire once it is read, codes it, and creates an analytic data file. It uses SCANTOOL’s, a data management software package that links the OPSCAN machine to our databases and provides data checking and validation algorithms.

As for the WATCH study, the Project Coordinator will backup the entire tracking and data systems on schedules (the shortest interval being twice daily) that virtually eliminate any material loss of information and provide periodic archiving of all databases. All analytic data files will be backed up by the Masters Level Statistician/Data Manager. All original forms will be stored at UMCC in locked file cabinets.

4.10. Reliability And Validity Testing

Most study instruments were chosen because they previously have been validated and they will produce results both interpretable to a wide audience and directly comparable to results obtained from other studies. Because even widely used instruments require testing when used under new conditions, we will test the reliability and validity of questionnaire and interview items. Reliability of methods is relatively easier to check than validity, which requires data from a source independent of the primary collection method. Reliability will be established by re-asking of original respondents identical questions. This can be accomplished by inserting duplicate questions on a different version of the questionnaire. Construct validity will be tested by comparing observed responses with expectations based on known organic relationships between an environmental stimulus (e.g., known meteorologic changes during a well-defined, short time interval) and a predicted response (e.g., reported exposure to sunlight or time spent outdoors). To a limited extent, criterion validity can be assessed (for example, by comparing questionnaire responses to data obtained using electronic devices to measure physical activity). For the most part we recognize, however, that criterion validity will not be a reasonable standard by which to judge the utility of self-reported data.

Psychosocial and dietary data raise some acute problems in validation. Fortunately, the dietary measures that are most prone to such problems are those that will be collected utilizing the services of an expert interviewer (the RD). Most psychosocial assessments have been widely used (such as the Beck inventories).

Specific methods of concordance testing will be used to assess reliability. These include variants of the general linear model for scalar and continuous variables approximately meeting assumptions of linear regression (see 4.11.). For ordinal predictors, we can use techniques such as Spearman rank correlation and, for dichotomous predictors, the Kappa statistic (87). Interview drift will be analyzed using similar statistical methods. For both inter-rater reliability and interviewer drift analyses, we will rely on audiotapes as we have in previous work in both the SR&RP and in our other behavioral research.

4.11. Statistical Power And Sample Size

We plan on selecting a stratified simple random sample of subjects for enrollment in the study based on 5 10-year age groupings for males and females. A total of 600 subjects will be enrolled, with anticipated followup rate of 90% for one year. Under these assumptions, a total of 540 subjects will complete the study, with approximately 270 subjects per sex group. We anticipate a seasonal variation of 13 mg/dl for cholesterol, with an assumed standard deviation of 40 mg/dl based on the WATCH study data. Using these assumptions, we will have in excess of 90% power to detect a seasonal difference in cholesterol at these levels for each sex group, based on a two-sided test > 0.05. The power calculation for this sample size is based on a cross-sectional design, assuming there is minimal tracking of serum cholesterol levels for subjects over time. We anticipate that such tracking will be present, which will enhance the power of the proposed study. The extent of reduction in the variance within subjects relative to the between subject variance component is at this time unknown.
The major hypotheses of interest in this study focus on specific effect of diet and other factors such as light exposure and physical activity on blood lipid values. Because so little is known about the true nature of the variability, as well as its cause(s), our estimates of sample size will make reasonable assumptions about the overall size of the seasonal variability and the fraction of the total variability we ascribe to the various factors described below. Although we expect interval (i.e., quarterly) differences to be smaller than the overall seasonal shift, we also expect that the repeated measures available to us through this design would tend to increase statistical power.

We will have \( >99\% \) power to detect the effect of a factor that accounts for \( >50\% \) of the total seasonal variability in cholesterol levels if it is equal to what we observed in WATCH. Following the Keys equation we expect that if diet accounts for \( 50\% \) of the cholesterol change (6.5 mg/dl) it corresponds to a change in percent of calories as fat of approximately \( 4.5\% \) (55). If it is responsible for only \( 25\% \) of the change (\( \sim 3.3 \) mg/dl) the corresponding change in percent of calories as fat is \( \sim 2.3\% \). This assumes proportionally equal changes across all dietary lipid categories. We will have over 80\% power to detect such an effect (i.e., explaining 25\% of total variability). To maintain a reasonable level of statistical power we will model age and sex as covariates in the models and not use them as stratification variables. For hypotheses that focus on residual variability (i.e., after accounting for other covariates - see hypotheses 5 and 6) we will have \( >80\% \) power to detect a factor accounting for 25\% of total variability.

Power calculations for hypotheses related to seasonal variation in biochemical factors are based on 120 subjects who will have completed the longitudinal study. Assuming a seasonal change in AT is equal the difference observed between Macrobiotica and Cambodian/Hispanice (i.e., \( 2 \) ug/ml), with standard deviation \( 2 \) ug/ml, there will be 90\% power to detect the difference based on a two sided test with \( \alpha = 0.05 \). Much greater power (99\%) will be present to detect a seasonal difference in BC of \( 1.4 \) ug/ml based on a standard deviation of \( 0.4 \) ug/dl. Each of these Power calculations is conservative, since gains in precision are expected as a result of the longitudinal design.

4.12.  Statistical Analyses:

Once data are as complete as possible and illogical and out-of-range responses corrected, we will employ standard methods of exploratory data analysis to detect departures from the assumptions of the multivariable models we will use to achieve our stated specific aims (88). These analyses will also focus on describing differences in the distributions of variables that are measured in two ways (i.e., the light exposure and physical activity data from the questionnaires and the telephone interviews).

Hypotheses to be tested in this study fall into two main categories: those related to the overall seasonal variability in the data; and other hypotheses that focus on explaining the overall change.

We will model both the 24 HR-derived data and the questionnaire-derived data on physical activity and light exposure. Results will be reported on both. However, we will consider the parameter with the lowest overall variance to yield the definitive study analysis. In support of the definitive analysis, we will examine correlations between continuous factors as noted above and the outcome measure, carry out t-tests and one-way ANOVAs for categorical predictors, and examine associations among predictors by means of cross-tabulation and analysis of variance. The definitive analyses will test the hypotheses listed in section 4.2. These relate changes in dietary, physical activity and psychosocial data to specific changes in cholesterol levels.

We will check that crucial measures have approximately normal distributions (Kolmogorov-Smirnov test and Shapiro test in SAS with appropriate adjustment for sample size) and equal variance across categories in the analysis (Bartlett's test). Similarly, we will test for equal correlation’s within repeated measures (the sphericity assumption) and, if needed, explore other correlation structures (BMDP 5V).

Much of the published information on seasonal pulse focuses on population changes in incidence or mortality data (89, 90, 91, 92). Our concern in this study is to describe seasonal pulse in levels of cholesterol across individuals. This information will be directly useful for hypothesis testing as well as to provide guidance for
fitting terms in the regression models used for measuring specific effects of the independent variables we are measuring. We also will conduct analyses to relate season variation in cholesterol levels directly to seasonal change in fat intake, total caloric intake, and energy expenditure. For this study we will use the method described by Gordon et al (7). This fits both sine and cosine terms to the data. Heterogeneity of seasonal cycles by subject characteristics (e.g. age, sex, and baseline cholesterol level) will be tested by multivariable ANOVA of the seasonal vector using PROC GLM in SAS (59). The Hotelling-Lawley Trace statistic will be used to test for homogeneity. The procedure will be repeated for dietary fat intake and total energy expenditure and the correlation coefficients relating the cycles of fat intake and caloric expenditure with cholesterol levels will be computed.

Initially, we anticipate estimating a common phase parameter by fitting mixed models with fixed overall sine and cosine parameters, and including in addition random individual subject parameters corresponding to subject specific deviations for the sine and cosine. For hypotheses 2-7 we will use repeated measures, multivariable analyses that will mix random and fixed effects (the mixed model) (93). We will use PROC MIXED to carry out this task. Patterns in missing data will be examined prior to this model fitting, to identify possible selection or biasing issues by comparing subjects with missing data with complete data subjects. Our model fitting using SAS PROC MIXED will enable subjects with some missing data to be included in the analysis, assuming a 1 year period. Variables for dietary intake, physical activity, and light will be added as time dependent covariates in modeling. To the extent that season in the northeast is associated with well-defined meteorologic differences, we expect to be able to account for a large portion of seasonal variability using data that themselves define seasonality. In our models we will use the equivalent of the Type I model where effects are modeled sequentially, and not orthogonally, and fit the meteorologic data list. We will test for homogeneity of effect across stratification variables (i.e., age and sex). Where effects do not vary by stratification variable, each such variable will be entered as a dummy variable in the final analysis.

Once the overall phase has been established in the base model, additional models will be fit that allow amplitude to vary for each subject, but keep the phase constant. Because the estimated amplitude and phase are formed as a ration estimate from estimated coefficients of the sine and cosine, fixing the phase will force a particular combination of sine and consine coefficients, which will result in a single parameter being included in the model (representing amplitude). The goal of the subsequent model fitting is to determine factors that are associated with this parameter for amplitude. We will accomplish this goal by including time dependent covariates in this model corresponding to measures of exercise, diet, and light.

It is possible that subjects with particularly high or low average cholesterol values, or subjects defined by some other variable (young, old, etc.) will exhibit different patterns in seasonality. As a second step in the analysis, we will include additional fixed effects in the mixed original mixed model that characterize subjects by such variables, with the intent of testing for differences in phase between subject groupings. The choice of variables that will be used at this point in the analysis will be jointly determined by the study team. Since this analysis is complex, graphic illustration of the modeling will be used extensively to illustrate differences in phase between subject groupings. Once differences in phase are identified, additional models will be constructed that fix the phase for the sub-groups, but allow amplitude to change between individuals in the sub-groups. Similar procedures will then be followed to identify other variables that contribute to explaining differences in amplitude.

The analysis plan outlined above will be conducted in a similar manner for other variables that are anticipated to have a seasonal variation. In each case, we plan on beginning with simple descriptive models (with a period of 1 year), and augment these models to estimate phase and amplitude, and the contribution of other variables to explanation of phase and amplitude.

Cost implications (hypothesis 8) will be addressed by evaluating the seasonal variation in the percentage of participants, both overall and in subsets by age and sex, who meet or fail to meet NCEP cutpoints (200 and 240 mgs/dl) for blood cholesterol levels. We will also evaluate the hypothetical effectiveness of treatment
(e.g., a dietary or pharmacologic approach that would give an assumed reduction in cholesterol level) and subsequent re-evaluation at various times of the year, and the public health implications thereof. For these analyses, we will also use methods developed by Urban to assess cost-effectiveness (94).
4.13 Potential weaknesses of this study

There are several areas of potential weakness in our study design:

1. The HMO population that we are using reflects the population of central Massachusetts, and as such is largely white and middle-class. Thus we will not have sufficient power to look separately at race, ethnicity, cultural, or socio-economic effects. However, this is a population with which we have considerable experience, and can anticipate relatively trouble-free recruitment and follow up.

2. Our study is restricted to participants living in one relatively restricted geographic area of the country. While this is appropriate in an initial study of this type, future studies should include sites at various latitudes and altitudes, and of various climatic conditions (e.g., coastal, inland, arid, humid).

3. In our desire to take fullest advantage of our study design we are looking at a considerable number of variables, creating a complex dataset. We believe that we have the expertise and experience to deal with this complexity. On the other hand, it is not possible to evaluate all possible variables in a biological phenomenon as complex as seasonal variation, and it may be that we have overlooked a variable or variables of importance.

4.14 Race, Gender, And Issues Of Generalizability

The study will enroll men and women in equal numbers. We expect that our study population will reflect the racial mix of Worcester County (about 94% white, non-hispanic and the remainder hispanic, black and asian). We are excluding individuals who cannot speak English because virtually all of our study instruments are developed for use in English-speaking populations and are calibrated for use in those populations. We do, however, encourage recruitment of Spanish/English bilingual subjects and we have staff with experience in working with those groups. As noted above we may not be able to freely generalize our findings to other latitudes, geographic conditions, or populations of differing racial/ethnic/socioeconomic/cultural makeup, but our findings will nonetheless be widely applicable, and there is no a priori reason to doubt that similar seasonal variation exists in other populations.

5. Human Subjects:

1. Active subjects will be males and females who are members of the Fallon Community Health Plan and receive their care at the Plantation Street site, the largest of the Fallon sites. We expect that these subjects will represent roughly the ethnic and racial composition of Worcester County, Massachusetts, which is overwhelmingly white. The rationale for selecting these study groups comes from the excellent experience of the WATCH, as described in 3.1.

2. Data will be obtained from three general sources: questionnaires, body measurements, and biological specimens. This study will also abstract some medical record data to confirm subject eligibility and exposure status. All other data will be collected exclusively for research purposes. Questionnaires will be used to gather data on demographic variables, lifestyle factors, psychosocial factors, health history, smoking, and diet. Simple anthropometric measurements will be taken. Blood will be drawn for analysis of lipids, hemostatic factors and antioxidant vitamins.

3. Consent will be sought after determining study eligibility. Verbal consent first will be obtained over the phone for the first three 24 hour recalls. Written consent will be obtained prior to the first blood draw.

4. There is a slight risk of hematoma from blood drawing.

5. Obtaining of specimens will be done under proper clinical conditions in the clinical laboratory of the Fallon Community Health Plan at the Plantation Street site. Medical staff, including numerous physicians and a research nurse, are equipped to deal with any medical emergency. All data will remain confidential through coded computer files. All tests will be performed by technicians blinded to the identities of individual study participants. Participants will be instructed to contact the Project Coordinator, research nurse, study
physicians, or the P.I. if they feel they are suffering in any way by participating. Additionally, they will be contacted quarterly by study staff who will query them on their progress in the study.

6. There is no direct benefit to participants. At the end of the study, each individual will be given a brief summary of results and access to his or her study data.
6. **Vertebrate Animals** - Not Applicable

7. **Consultants/Collaborators** - None

8. **Consortium/Contractual Arrangements:**

Subcontracts to the Fallon Clinic, UMass Amherst and UMass Lowell are included. The Fallon Clinic will provide the participant population, and all recruitment and followup physical blood measures will take place there. UMass Amherst will provide expertise in psychology, exercise physiology and statistics. Lipid and antioxidant studies will be performed at UMass Lowell.

9. **LITERATURE CITED:**


