Project Number: MQP-BIO-DSA-4696

C-REACTIVE PROTEIN & TRADITIONAL CARDIAC RISK FACTORS

A Major Qualifying Project Report

Submitted to the Faculty of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

in

Biology and Biotechnology

by

_____
Andrea Hafner
March 3, 2005

APPROVED:

_____	_____	_____
Yunsheng Ma, Ph.D., MPH	David Chiriboga, M.D.	David S. Adams, Ph.D.
Preventive and Behavioral Medicine	Family Medicine and Community Health	Biology and Biotechnology
UMass Medical School	UMass Medical School	WPI Project Advisor
Major Advisor	Co-Major Advisor	

_____
Ira Ockene, M.D.
Cardiovascular Medicine
UMass Medical School
Co-Major Advisor

ABSTRACT

This project examines longitudinal associations between serum C-reactive protein (CRP) levels, measured using a high-sensitivity assay, and established cardiovascular risk factors such as blood pressure and lipid levels, as well as resting heart rate, waist/hip ratio and daily hours of sleep. Unexpected negative associations were found with total cholesterol and triglyceride levels, contrary to what was expected. A significant positive association, not explained by physical activity or infection status, was found with resting heart rate. Further study is warranted.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would first like to thank Dr. Ira Ockene, primary investigator of the "Determinants of C-Reactive Protein" study, for allowing me to access the SEASONS study data for my project and for providing guidance in the interpretation of the data and the writing of the report. I would like to thank Dr. Yunsheng Ma for his support and guidance in data analysis and report writing, and for being willing to oversee the project from day to day; also Dr. David Chiriboga for his assistance in data interpretation and review of the written report. In addition, I would like to thank Dr. Wenjun Li for his willingness to explain statistical methods and their application to my project, Mr. Phil Merriam for reviewing report drafts and seeing to it that we received the CRP data, and Ms. Katherine Gendreau for helping me learn the Stata computer program and prepare datasets. Finally, I wish to thank Dr. Dave Adams of the WPI Department of Biology and Biotechnology for his role in initiating and advising this project, as well as providing feedback on drafts of the report.

BACKGROUND

CRP: General Information

C-reactive protein (CRP) is one of a class of proteins known as pentraxins, and the CRP molecule is made up of five identical subunits arranged in a ring shape (Mazer and Rabbani, 2004). See Figure 1 below for CRP structure.

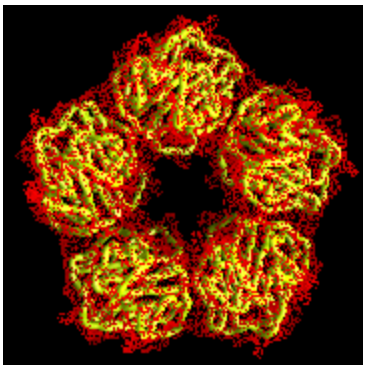


Figure 1: Molecular Structure of CRP (Greenhough and Shrive, 2004).

Each subunit has a weight of 23 kilodaltons (Ridker, 2003). It was discovered in 1930 and named "C-reactive protein" because it reacts with the C-polysaccharide on pneumococci (Gabay and Kushner, 1999). CRP is an acute-phase protein whose levels in serum are elevated by inflammation, infection and tissue injury (Koenig et al., 1999). During an acute inflammatory event such as an infection, serum CRP levels can increase by more than a hundred fold, and then return to baseline level within about two weeks, as seen in Figure 2 below (Gabay and Kushner, 1999).

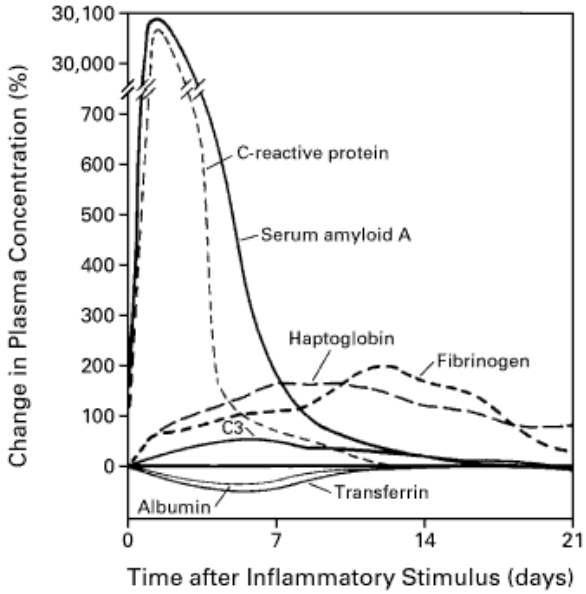


Figure 2. Characteristic Patterns of Change in Plasma Concentrations of Some Acute-Phase Proteins after a Moderate Inflammatory Stimulus. (Gabay and Kushner, 1999).

CRP is mainly produced in the liver but has recently been found to be produced by some cells in atherosclerotic plaque as well (Mazer and Rabbani, 2004). In the liver, CRP is secreted in response to stimulation by IL-6 and IL-1 and, in the case of a bacterial infection, its function is to opsonize bacteria for phagocytosis by macrophages (by binding to phosphocholine in the cell membranes) as well as activate the complement cascade to lyse the bacterial cells (Janeway and Travers, 1994). In addition, CRP binds cell debris resulting from tissue damage and therefore appears to play a role in the "clean-up" following such damage (Mazer and Rabbani, 2004).

Relationship between CRP and Cardiovascular Disease

It is now generally believed that inflammation plays a major role in the development and progression of atherosclerosis. Many observational studies have shown that higher levels of CRP are directly associated with increased risk of myocardial

infarction (MI) and other adverse cardiovascular events. This increased risk appears to be independent of other risk factors such as lipid levels (Ridker, 2003). In one study (Ridker et al., 1998), the combination of CRP levels with total cholesterol and HDL was found to predict first MI in initially healthy men, better than the lipid measures alone. This case-control study used data from the Physicians' Health Study, comparing baseline CRP and cholesterol levels in 245 subjects who were healthy at the beginning of the study, but later had MI's, with those from 372 controls who did not have MI's. Another study (Ridker et al., 2003) examined the relationship of CRP levels, the metabolic syndrome and cardiovascular events among 14, 719 participants in the Women's Health Study (the women were 45 years and older, and apparently healthy) and found that CRP levels added to the predictive value of the metabolic syndrome and its components for cardiovascular events. Previously, Ridker and colleagues used data from the Women's Health Study to suggest that CRP levels may in fact be better than LDL cholesterol levels for prediction of cardiovascular events, but that even greater predictive value is obtained by using both CRP and LDL measurements (Ridker et al., 2002). The MONICA Augsburg Cohort Study (Koenig et al., 1999) followed 936 healthy men ages 45 to 64 for eight years to determine the association between CRP levels and the prevalence of first coronary events. This study showed CRP levels and the incidence of cardiovascular events to be strongly correlated. It has also been observed that patients with unstable angina who also have high CRP levels are more likely to have additional adverse coronary events than unstable angina patients without elevated CRP (Mazer and Rabbani, 2004). Finally, a correlation has been found between elevated CRP levels in heart transplant patients and transplant-associated coronary artery disease (coronary

atherosclerosis in the transplanted heart), which is a major cause of transplant failure aside from that caused by rejection (Mazer and Rabbani, 2004).

Hypothesized Mechanisms for the Relationship between CRP and Cardiovascular Disease

Although it appears reasonably well established that CRP is a marker of cardiovascular risk, the specific reason for this relationship is not clear. It is known that atherosclerotic plaques in various stages of development contain CRP, and also often contain monocytes and macrophages in the same areas where the CRP is found (Mazer and Rabbani, 2004). CRP in the artery wall appears to enhance levels of monocyte chemoattractant protein-1 (MCP-1), which, as the name implies, attracts monocytes (which differentiate into macrophages) to the area, leading to their accumulation where they can contribute to plaque formation (Mazer and Rabbani, 2004). It has also been demonstrated that it may be the monomeric subunits of CRP, rather than the entire CRP pentamer, that are responsible for raising levels of MCP-1; this indicates that CRP must dissociate in order to contribute to inflammation in the endothelium (Khreiss et al., 2004). If this is the case, there could be mutations in the CRP gene that would cause the pentamer to dissociate more easily, and such mutations could potentially increase a person's risk of atherosclerosis. Experiments have also shown that endothelial cells treated with CRP *in vitro* decrease their production of nitric oxide (NO), which is an important mediator of vasodilation; one of the first pathological changes in an artery developing atherosclerosis is a decrease in the capacity for such vasodilation (Mazer and Rabbani, 2004). Another experiment (Zwaka et al., 2001) showed that as long as serum is present, macrophages *in vitro* take up, by phagocytosis, LDL that has been incubated

with CRP but do not phagocytize LDL alone or CRP alone. This suggests that CRP opsonizes LDL for macrophages, and that this may be a mechanism for the formation of foam cells during the development of atherosclerosis. Foam cells are formed when macrophages in the arterial wall take up modified lipoproteins and lipids (Willerson and Ridker, 2004); these cells play a major role in the development of atherosclerotic plaques. The findings of this experiment may also help to explain why CRP and LDL levels together give the greatest predictive value of future MI, as opposed to LDL alone or CRP alone. In addition, exposure to CRP appears to affect vascular smooth muscle cells, causing them to proliferate and move into the space under the endothelium, which could also contribute to arterial blockage (Mazer and Rabbani, 2004). All of the above findings suggest that CRP may be actively involved in causing atherosclerosis, as opposed to simply being a passive marker of cardiovascular disease risk.

CRP and Blood Lipid Profile

Findings of previous studies examining the association of CRP levels with cholesterol and triglyceride levels have been somewhat mixed—some have found relationships, while others have not, and still others have found correlations with some components of the lipid profile but not others. The most consistent finding was that CRP levels showed an inverse association with HDL cholesterol levels, while findings of correlation between CRP levels and LDL cholesterol levels were the least consistent. These findings are summarized below in Table 1. In all of these studies, however, the analysis of relationships between CRP and blood lipid profiles was cross-sectional. This means that for each subject/patient, measurements were only made at one time point

rather than at multiple time points within a given period, so comparisons can only be made between subjects, not within subjects. Longitudinal data, on the other hand, allow comparisons within subjects because measurements are taken at multiple time points for each patient. The present study uses longitudinal data, so it is possible to make comparisons both between and within subjects. In addition, in this project it was possible to control for many more possible confounders than in some of the previous studies.

Table 1: Summary of Studies Examining Relationship Between CRP and Lipid Profile

Reference	Study Design	Population Sample Size	Total chol.	LDL	HDL	Triglycerides
Choi et al., 2004	Cross-sectional	South Korean adults, separated into hypertensive (n=122) and normotensive (n=64); excluding those with overt cardiac disease, serum creatinine >150M or the presence of other systemic diseases	0	0	-	+
Cook et al., 2000	Cross sectional	699 children from 10 towns in England and Wales, ages 9-11 (364 boys and 335 girls)	0	0	-	0
Ford et al., 2004	Cross sectional	2205 US women 20 years or older, supposedly representative sample	+	NA	NA	+
Frohlich et al., 2000	Population -based cross-sectional	Adults ages 18-89 years in former West Germany, excluding those with acute illnesses; n=1703 total	+	NA	-	+
Laaksonen et al., 2004	Cross sectional analysis at baseline	680 middle-aged Finnish men	NA	+	-	+
Lambert et al., 2004	School-based cross-sectional survey	2224 children/adolescents ages 9, 13 and 16 in Québec, Canada	NA	NA	-	+
Mendall et al., 1996	Cross sectional	303 men aged 50-69 years in London, UK	+	+	-	+
Retterstol et al, 2003	Population -based twin study	Healthy Norwegian monozygotic twins (68 male pairs and 87 female pairs) ages 38-57 years	0	0	-	+

Saito et al., 2003	Cross-sectional	Japanese adults ages 30-79 who entered hospitalized health-check program (n=566 for CRP analysis)	0	NA	-	+
Schillaci et al., 2003	Cross sectional	135 newly diagnosed, never-treated hypertensive patients and 40 normotensive matched controls; no evidence of inflammation in the past month	+	+	NA	+
Strand-berg and Tilvis, 2000	Population-based cross-sectional	3 elderly cohorts aged 75, 80 and 85 years from Helsinki Ageing Study; n=455	not clear--highest TC group had lower CRP—possibly due to HDL	NA	-	0
Vikram et al., 2003	Cross sectional	377 healthy adolescents and young adults aged 14-25 years (331 male and 46 female) in urban North India, excluding smokers and anyone who had an acute illness in the past 6 months	0	0	0	0
Williams et al., 2004	Cross-sectional	822 men and women at the age of 26 years	+	NA	-	+
Wu et al., 2003	Cross sectional	835 children (410 boys and 435 girls) aged 12-16 years in Taiwan	0	0	-	+ (in boys)

* + denotes positive correlation; - denotes inverse correlation; 0 denotes lack of correlation, and NA indicates that the variable was not studied.

CRP and Blood Pressure

While many studies have found positive associations between CRP and blood pressure (especially systolic pressure), some have found no correlation. In the sleep-deprivation experiments (Meier-Ewert et al., 2004), a positive correlation was observed between CRP and both systolic and diastolic blood pressure. A number of observational studies, including two of hypertensive and normotensive adults in South Korea (Sung et al., 2003; Choi et al., 2004), as well as three others (Retterstol et al., 2003; Saito et al., 2003; Laaksonen et al., 2004) also found positive associations between CRP and both systolic and diastolic blood pressure. A study of elderly men and women without cardiovascular disease or diabetes found no correlation of CRP with either systolic or

diastolic blood pressure (Barbieri et al., 2003). Similar results were reported in a study of men ages 50-69 (Mendall et al., 1996), as well as a study of children ages 9-11 (Cook et al., 2000). Three studies found associations between CRP and systolic blood pressure, but no correlation with diastolic pressure. These include a study of US women ages 20 and over (Ford et al., 2004), a study of ischemic stroke patients (Di Napoli and Papa, 2003), and a study of hypertensive patients and matched normotensive controls (Schillaci et al., 2003). Another study found a relationship between CRP and systolic blood pressure, but this was no longer significant after adjustment for body mass index (BMI) (Lambert et al., 2004). One group of researchers found no relationship between CRP levels and diastolic blood pressure, but their findings with systolic blood pressure were unique and interesting. The correlation between CRP and systolic blood pressure was positive in women, but negative in men (Williams et al., 2004)—no other study had found a negative relationship between CRP and either blood pressure component. Such a correlation does not seem to make biological sense, considering the experimental evidence that CRP can lead to a decrease in nitric oxide production and thus a lack of vasodilation. These studies (with the exception of the sleep-deprivation experiments) were all cross-sectional in design; longitudinal data are lacking. Below is a table summarizing the findings.

Table 2: Summary of Studies Examining Relationship between CRP and Blood Pressure

Reference	Study Design	Population Sample Size	Systolic BP	Diastolic BP
Barbieri et al., 2003	Cross-sectional	Elderly, excluding those with diabetes, cardiovascular disease, and taking drugs that affect BP, glucose metabolism and IL activity	0	0

Choi et al., 2004	Cross-sectional	South Korean adults, separated into hypertensive (n=122) and normotensive (n=64); excluding those with overt cardiac disease, serum creatinine >150M or the presence of other systemic diseases	+	+
Cook et al., 2000	Cross sectional	699 children from 10 towns in England and Wales, ages 9-11 (364 boys and 335 girls)	0	0
Di Napoli and Papa, 2003	Cross sectional	535 first-ever ischemic stroke patients	+	0 when adjusted for other BP components
Ford et al., 2004	Cross sectional	2205 US women 20 years or older, supposedly representative sample	+	0
Laaksonen et al., 2004	Population-based cohort study	680 middle-aged Finnish men	+	+
Lambert et al., 2004	School-based cross-sectional survey	2224 children/adolescents ages 9, 13 and 16 in Québec, Canada	0 after adjustment for BMI	NA
Meier-Ewert et al., 2004	2 separate experiments	Healthy adults ages 22-38; 10 for each experiment (exp. 1 was all men; 2 was 6 men and 4 women)	+	+
Mendall et al., 1996	Cross sectional	303 men aged 50-69 years in London, UK	0	0
Retterstol et al., 2003	Population-based twin study	Healthy Norwegian monozygotic twins (68 male pairs and 87 female pairs) ages 38-57 years	+	+
Saito et al., 2003	Cross-sectional	Japanese adults ages 30-79 who entered hospitalized health-check program (n=566 for CRP analysis)	+	+
Schillaci et al., 2003	Cross sectional	135 newly diagnosed, never-treated hypertensive patients and 40 normotensive matched controls; no evidence of inflammation in the past month	+	0
Sung et al., 2003	Cross-sectional	South Korean adults without acute inflammatory disease; 4813 men and 3534 women; divided into hypertensive and non-hypertensive	+	+
Williams et al., 2004	Cross-sectional	822 men and women at the age of 26 years	- in men; + in women	0

* + denotes positive association; - denotes inverse association and 0 denotes no association. NA indicates that the variable was not studied.

CRP and Waist/Hip Ratio

Only a few studies have examined the association between CRP and waist-hip ratio, and there is agreement between them; however, they have all been cross-sectional

and therefore do not show what happens to CRP levels if there is a change in abdominal adiposity over time. Four studies found a positive correlation between waist-hip ratio and CRP levels (Rexrode et al., 2003; Vikram et al., 2003; Wu et al., 2003; Laaksonen et al., 2004). In addition, Ford et al. (2004) as well as Williams et al. (2004) found a positive correlation between CRP and waist circumference, but did not measure waist-hip ratio. This method raises the possibility of confounding due to differences in overall body size, which may result in larger or smaller waist circumferences but not necessarily different waist/hip ratios.

CRP and Sleep Deprivation

Only one study to date has examined the relationship of CRP levels to sleep deprivation (Meier-Ewert et al., 2004). In this experimental study, both acute total sleep deprivation and short-term partial sleep deprivation were examined, with 10 subjects in each experiment. CRP levels increased with sleep deprivation in both experiments, suggesting a causal relationship between sleep deprivation and higher CRP levels. See Figure 3 below. However, no previous studies have examined the relationship of sleep and CRP levels in a free-living population such as the one followed in the study that generated the database used in this project. This type of information may be useful in determining whether CRP levels appear to be affected by mild or moderate chronic sleep deprivation in "real life", though from an observational study it is not possible to infer causal associations.

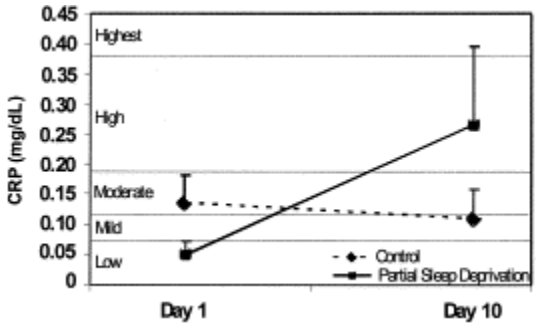


Figure 3: Effect of Partial Sleep Deprivation on CRP Levels. Data were log-transformed before analysis. For ease of interpretation, the mean values ± SEM were transformed from the log scale back to the usual scale (mg/dl) in subjects undergoing 10 consecutive days of short-term partial sleep deprivation (n = 4, **squares**) and control subjects (n = 5, **diamonds**). Significance of difference in change from baseline to day 10 between groups ($p < 0.08$ for interaction) by mixed-models analysis of variance on log-transformed data: the change from baseline to day 10 for the short-term partial sleep deprivation group was significant ($p < 0.05$), whereas the change from baseline to day 10 in the control subjects was not significant ($p = 0.72$). The **horizontal lines** indicate risk boundaries (mild to highest) of C-reactive protein (CRP) quintiles derived from analysis of >5,000 apparently healthy Americans. (Meier-Ewert et al., 2004)

CRP and Resting Heart Rate

A positive relationship between CRP levels and resting heart rate was found in a study of children in England and Wales (Cook et al., 2000). In the sleep deprivation study (Meier-Ewert et al., 2004), a positive correlation was also found between CRP and heart rate in the short-term partial sleep deprivation experiment; in the acute total sleep deprivation experiment both increased as well. However, this relationship has not previously been well studied, and the current project, using longitudinal data, yields additional information. It was also possible, to some degree, to control for the use of medications that can affect heart rate, such as beta blockers. In addition, the possibility was considered that any correlation could stem partly or wholly from deconditioning in people with higher resting heart rates (i.e. people may have higher resting heart rates because they are not well conditioned, and such deconditioning could potentially contribute to higher CRP levels), and in this study it was possible to control for physical

activity in order to better determine whether this could be a plausible explanation. Infection history could be another contributing factor, as heart rates tend to increase during infections; it was possible to control for this to some degree in this project.

Background on SEASONS study

The Seasonal Variation of Blood Cholesterol Levels (SEASONS) study was a prospective study intended to examine seasonal changes in blood lipid levels and relevant covariates (Merriam et al., 1999). Longitudinal data were collected from a cohort of healthy adults in Central Massachusetts from 1994 through 1998, including lipid levels, dietary data, physical activity and many other measures. In addition, blood samples from most participants were stored and analyzed later for CRP levels; these data were used in this project, along with the data previously gathered. The SEASONS study was based at the University of Massachusetts Medical School.

PROJECT PURPOSE

The purpose of the "Determinants of C-Reactive Protein" study at the University of Massachusetts Medical School was to more clearly define the factors that affect C-reactive protein levels in healthy adults by analyzing longitudinal data collected during the SEASONS study (described briefly in Background and in more depth in Methods section) in combination with measurements of CRP levels, using a high-sensitivity assay, in stored blood samples from 617 SEASONS study participants. These factors include body mass index (BMI), dietary factors, physical activity, and psychological factors such as depression.

The purpose of my specific part of the study was to examine the longitudinal and cross-sectional relationships between CRP levels and the following factors: blood lipid levels, blood pressure, resting heart rate, waist/hip ratio, and daily hours of sleep. The completeness of the data set allowed adjustment for possible confounders such as BMI, physical activity, and dietary factors, as well as infection history and medication use. Although other studies had looked at the relation between CRP and some of these factors, they were all cross-sectional in design (with the exception of the experimental sleep-deprivation study) and therefore provided no information about within-subject effects. In addition, there was disagreement on these relationships in some of the previously published studies, likely due in part to differences in study populations. Special attention was paid to potential correlations between serum CRP levels and resting heart rate since this relationship had not been previously well studied.

METHODS

SEASONS study and database description

The Seasonal Variation of Blood Cholesterol (SEASONS) study was designed to describe seasonal changes in blood lipids in the general adult population and to attempt to explain possible causes of such changes. There were 641 healthy adults in the study cohort. They ranged in age from 20 to 70 years, were predominantly white and both genders were about equally represented (Merriam et al., 1999). Subjects were required to be literate in English and not be on any lipid-lowering drugs or on a diet to lose weight or lower lipids. In addition, subjects could not be included if they had any potentially fatal illness, any condition that could cause elevations in lipid levels, a history of cancer (except for skin cancers other than melanoma) less than 5 years ago, or any psychiatric or other problems that could interfere with their completing the study (Merriam et al., 1999).

Major factors examined in the SEASONS study included blood lipid levels, dietary intake, physical activity (sleep information was collected along with this), light exposure, psychosocial factors and anthropometric measurements. Psychosocial factors included anxiety and depression. In addition, information was collected on subjects' use of medications at baseline and their infection history each quarter. Anthropometric measures used were height, weight, body mass index (BMI), waist circumference, hip circumference and waist/hip ratio (Merriam et al., 1999).

Data were collected using a variety of methods. Questionnaires were used to gather demographic and health information at baseline, as well as to assess psychosocial factors such as depression and anxiety (Merriam et al., 1999). At baseline, participants were also asked to list any medications they were taking. Subjects completed the

depression and anxiety questionnaires five times; once at baseline and then every 13 weeks. At the same 13-week intervals, clinic visits were conducted in which subjects were weighed and asked questions about their recent health history, including infection history. Before each visit, subjects were asked to use a home blood pressure monitor to measure their blood pressure and pulse, then bring this information to the appointment. This was done before 9:30 in the morning, after sitting for 5 minutes but after the subjects had been awake for a half hour or more. Telephone interviews were used to collect dietary intake information around the time of each clinic visit; subjects were asked to recall what and how much they ate over the past 24 hours, as well as provide information on their physical activity during the same period; this was called a 24-hour recall, or 24HR. Glycemic index (GI), a measurement of carbohydrate quality, was determined from the 24HRs using published tables (Foster-Powell et al., 2002; Brand-Miller et al., 2003). Glycemic load (GL) (GI of a food x the amount of carbohydrate eaten)/ 100) was also calculated, as has been previously reported (Ma et al., 2005). The 24-hour physical activity data collection methodology was adapted from methodology developed for a seven-day recall of physical activity (Sallis et al., 1985), and was administered together with the diet recall as mentioned above. Methods described by Ainsworth and colleagues (Ainsworth et al., 2000) were used to estimate total daily energy expenditure in metabolic equivalent task hours (MET-hour) based on the reported time spent at each activity type and intensity level (Matthews et al., 2000; Matthews et al., 2001; Matthews et al., 2001). This methodology has been validated (Matthews et al., 2000). Waist/hip ratio was measured at the first and last visits. For lipid measurements, 12-hour-fasting venous blood samples were drawn around the time of each clinic visit. The samples were drawn

between 7:00 and 10:00 am to avoid any confounding due to circadian fluctuations in lipid levels, and to make 12-hour fasting tests feasible. Levels of total cholesterol, HDL, and triglycerides were tested; assays were performed in a Centers for Disease Control standardized laboratory (Merriam et al., 1999). LDL cholesterol levels were calculated using the standard Friedewald formula (see below) if triglyceride levels were less than 400 mg/dl.

Friedewald formula for calculating LDL cholesterol levels: total cholesterol = HDL + LDL + 0.2(triglycerides); all measurements are in mg/dl.

Sample storage conditions and CRP assay

At the time of collection, serum aliquots were placed in individual tubes for analysis and were frozen to -80 degrees Celsius. Aliquots not used for the original lipid analyses or other tests during the original study remained frozen at this temperature until they were needed for the CRP analysis. Because the SEASONS study began at the end of 1994, some of these samples were in frozen storage for nearly ten years. However, this does not appear to pose a problem for the accuracy of the measurements. A study of extended storage showed no significant changes in CRP concentrations in plasma and serum samples that were frozen at -70 degrees Celsius for over 20 years (Ledue and Rifai, 2003); this indicates that CRP is very stable during long periods of storage and therefore the fact that these samples were stored for up to ten years does not diminish the accuracy of the results.

To determine CRP levels, a high-sensitivity immunoturbidimetric assay was carried out. The equipment used included a Hitachi 917 analyzer from Roche Diagnostics in Indianapolis, IN. Reagents and calibrators were supplied by Denka Seiken

in Niigata, Japan. In this assay, an anti-CRP antibody, which has been sensitized to latex particles, binds CRP in the sample and causes agglutination. Light scattering is increased as a result of the agglutination, and this scattering is measured by a spectrophotometer. The change in light scattering is directly proportional to the CRP concentration of the sample; the assay can detect CRP concentrations as low as 0.03 mg/l. All blood analyses for CRP were carried out in the laboratory of Dr. Nader Rifai at Children's Hospital, Boston.

Data analysis

The statistics software package used for all analyses was Stata 8 SE for Windows (Stata Corporation; College Station, TX). This package can be used to perform a wide variety of statistical tests, as well as plotting regression lines and other results, and also allows new variables to be easily generated from existing variables in the data set. The main functions used were descriptive statistics and plots, regression analyses (bivariate and stepwise/multivariate), and longitudinal regression.

In addition to the general exclusion criteria for the SEASONS study (discussed under "SEASONS study and database description"), the original plan for this project called for specific exclusion criteria due to factors that would be unlikely to affect lipid levels and other measurements examined in the original study but could affect CRP results and/or other variables being examined in the CRP project. However, this was not feasible due to the way the relevant information was collected. For example, subjects were going to be excluded from the entire analysis if they were taking any anti-inflammatory medications, as these are known to lower CRP levels. Subjects who were taking beta blockers would have been excluded from the analyses that include heart rate

and blood pressure, as beta blockers lower these and may also lower CRP levels; the concern was that this could lead to confounding. Finally, subjects who had recently had an infection or other inflammatory process were going to be excluded. The problem lies in the fact that when the SEASONS study was originally conducted, there was no plan for the CRP study; the idea for this study came later. Therefore, data on medication use were only collected at baseline rather than at each clinic visit; using this information would have required the assumption that all patients who were taking medication at baseline continued taking it throughout the study, and vice versa. In addition, the only information collected on infections or other illnesses was a section in the quarterly questionnaire asking if the patient had had a cold, flu, allergy or other illness within the past three months. This is not a very useful measurement of current infection status, as CRP levels return to normal within two weeks after an illness (Gabay and Kushner, 1999), and therefore it was decided not to use this information or the medication data to exclude patients from the analysis. Instead, the longitudinal analysis simply controlled for infection history and beta-blocker use (did not control for anti-inflammatory medications, as these are often taken on a short-term basis).

Statistical methods

Introduction

Descriptive statistics, including mean, standard deviation and percentiles, were first tabulated for all CRP values in the entire dataset. Each patient in the study had up to five clinic visits, which included blood draws. Some patients had more than one CRP measurement for a given clinic visit, due to having more than one stored vial of blood

available from that visit for analysis. When this occurred, all values for that time point were averaged to arrive at the overall CRP value for that visit. The distribution of the number of CRP measurements per patient was then determined, to see how many had all five measurements and how many had fewer. Patients with at least one measurement were included in the initial cross-sectional analyses used for building the model, but excluded from the longitudinal analyses. For the cross-sectional analysis, the mean of all CRP values was computed for each patient in order to have a single average value per patient. A histogram was then made of these mean CRP values, and the distribution was highly positively skewed; natural-log transformation made the distribution sufficiently normal to permit the use of linear regression analysis (see figures 4 and 5 in Results section). Mean values were computed for each patient for all other variables in the analysis as well (lipids, BMI, dietary intakes etc.). It was necessary to create a separate dataset for the cross-sectional analyses, with the mean values of each variable, in order to avoid having multiple measurements in the data set for each patient.

Sample description

Baseline sample characteristics, as well as relevant clinical, physiological, psychological and lifestyle characteristics, were described as mean (SD) for continuous variables, and as n (%) for categorical variables.

Cross-sectional model building

Exploratory graphical techniques (locally weighted regression) were used to determine the bivariate relationship of natural-log-transformed CRP with all possible predictors considered. Associations were found to be approximately linear. Therefore, linear regression was used for all analyses. The first step was to perform crude bivariate

regression analyses with natural-log-transformed CRP (mean value) as the dependent variable and each possible covariate or variable of interest as the independent variable. This allowed determination of factors that were related to CRP levels if nothing else was controlled for. These crude analyses were carried out for the overall study group as well as stratified by BMI classification (separating normal, overweight and obese), since BMI is a known strong predictor of CRP levels and there was a reasonable sample size in each category. Any variables, other than the variables of interest, for which the p value was less than or equal to 0.20 in the crude analysis were then put into a stepwise regression (multiple independent variables) with log-transformed CRP again as the dependent variable. Any of these variables that remained in the model after the stepwise regression (p value less than or equal to 0.20) were put into the final model for CRP. This model was then used for the longitudinal analysis. These steps (from crude bivariate analysis to model building) were performed for the overall study group, and again with patients stratified by BMI category. In the BMI stratified analysis, BMI was still treated as a covariate within each category, to control for residual effects.

Longitudinal analysis

Linear mixed models were used for longitudinal analysis, making the distinction between cross-sectional (between-subject) and longitudinal (change over time within subjects) effects of the risk factors of interest and other predictors. The "cross-sectional effect" refers to the average value of a given variable (for example, LDL level) for a particular subject, while "longitudinal effect" refers to the difference from this average at a given time point (Ma et al., 2005). The longitudinal analysis included only subjects with two or more CRP measurements. Subject and time point (quarter) were treated as

random effects, while the cross-sectional and longitudinal effects of the various predictors were considered fixed effects. Both unadjusted and adjusted analyses were performed, for the overall study group and stratified by BMI classification as in the cross-sectional model building. The adjusted analysis controlled for covariates found to be related to CRP levels in the original cross-sectional model building. The results of this adjusted analysis are considered to be the final results.

RESULTS

Demographic information

Six hundred and seventeen subjects (96% of the SEASONS subjects) with at least one CRP measurement were included in the SEASONS CRP data analysis. Both sexes were equally represented in this cohort. The patients in the study were predominantly white, married and employed full time. See table 3 below for detailed demographic information.

Table 3: Demographics of SEASONS CRP study cohort (n = 617)

		n (%) or mean (SD)
Age (years)		47.33 (12.32)
Gender:	Male	318 (51.54%)
	Female	299 (48.46%)
Race:	White	510 (85%)
	Non-white	90 (15%)
Education:	High school or less	154 (25.08%)
	Some college or associate's degree	227 (36.97%)
	Bachelor's degree or more	233 (37.95%)
Employment:	Full-time	416 (67.64%)
	Part-time	91 (14.80%)
	Unemployed or retired	108 (17.56%)
Marital status:	Married or living with partner	470 (76.42%)
	Not living with a partner	145 (23.58%)

Baseline characteristics of study cohort

The majority of patients in the study were overweight or obese, with the average BMI of 27.28 in the "overweight" range. Of the 602 subjects for whom smoking status information was available, 82% were non-smokers. The average total cholesterol level of 218 mg/dl was in the borderline-high range, although average LDL, HDL and triglycerides were all within the acceptable range. Average systolic and diastolic blood

pressures were also within normal range. The average CRP level of 2.43 mg/l falls within American Heart Association criteria for ”average” cardiac risk (Pearson et al., 2003).

Table 4: Baseline clinical, physiological, psychological and lifestyle characteristics of SEASONS CRP study cohort (n = 617)

	Mean (SD)	**Median**	**Range**
BMI (kg/m ^2)	27.28 (5.42)	26.69	16.83-56.70
Waist/hip ratio	0.859 (0.092)	0.862	0.657-1.20
Total cholesterol (mg/dl)	218 (43.72)	213	75-419
LDL cholesterol (mg/dl)	143 (38.70)	140	18-331
HDL cholesterol (mg/dl)	47 (12.75)	46	22-108
Triglycerides (mg/dl)	140 (118.17)	113.5	26-1534
Systolic blood pressure (mm Hg)	120 (18.80)	118	76-224
Diastolic blood pressure (mm Hg)	77 (11.26)	76	51-119
Resting heart rate (beats/min)	69 (11.73)	68	37-113
CRP (mg/l)	2.43 (5.12)	1.10	0.02-62.79
Leisure physical activity (MET-hrs/day)	1.99 (3.09)	0.833	0-26.00
Daily hours of sleep	7.12 (1.13)	7.17	2.83-11.00
Beck depression score	6.26 (5.57)	5	0-28
Beck anxiety score	4.40 (5.84)	3	0-49
Daily caloric intake (kcal)	1951 (673)	1840	769-5813
Daily % of calories from fat	31.38 (7.35)	31.40	7.78-58.19
Daily % of calories from sat. fat	11.22 (3.42)	11.13	2.10-21.56
Daily % of calories from carbohydrate	51.51 (8.86)	51.46	26.89-79.56
Daily % of calories from protein	15.97 (3.46)	15.64	6.38-29.25
Daily dietary glycemic index	83.43 (7.06)	83.65	52.94-104.59
Daily dietary glycemic load	196.11 (82.30)	183.21	40.19-1084.81
Daily total fiber intake (g)	15.90 (6.97)	14.48	3.25-59.04
Daily soluble fiber intake (g)	5.73 (2.42)	5.27	1.06-16.42
Daily water intake (g)	1874.99 (680.22)	1785.48	196.82-5371.92
Daily alcohol intake (g)	7.68 (15.67)	0.177	0-148.78

Distribution of CRP levels

Subjects' mean CRP levels (including subjects with only one measurement) showed a non-Gaussian distribution; they were heavily skewed to the right (figure 4); $p < 0.001$ for Shapiro-Francia normality test, where a p value less than 0.05 indicates a non-Gaussian distribution. Natural-log transformation normalized the CRP data (figure 5). When the Shapiro-Francia test was performed on the log-transformed data, the p value was 0.61, indicating a normal (Gaussian) distribution.

Figure 4: Histogram of patients' mean CRP levels. Note that the vast majority are under 10 mg/l.

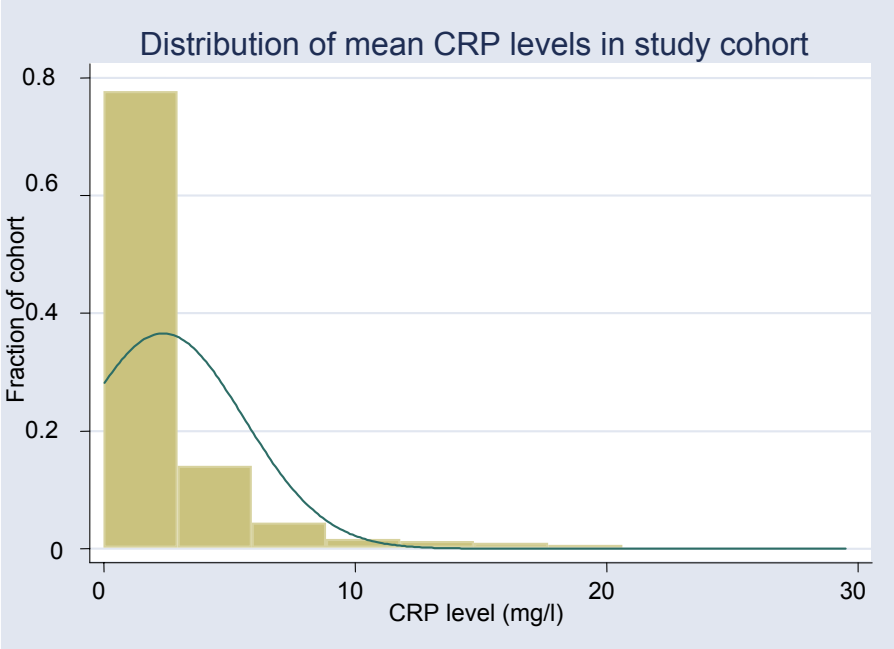
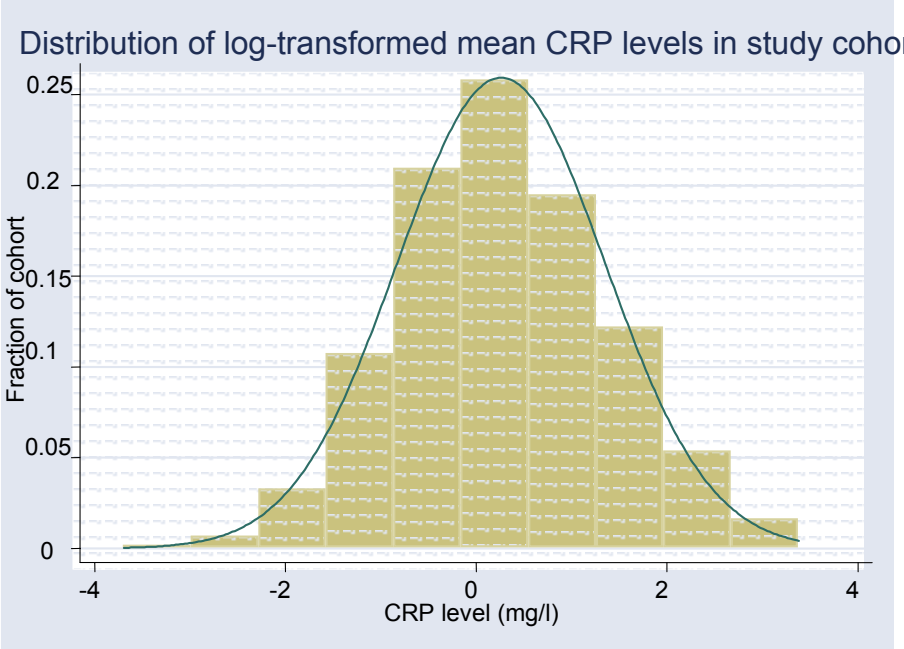


Figure 5: Histogram of natural-log transformed mean CRP levels. Note that this is a reasonable approximation of a normal distribution.



The majority of patients had at least four of the five possible CRP measurements. 74 patients had only one measurement. See table 5 below for a summary of measurement frequency.

Table 5: Number of CRP measurements (out of a possible 5) for patients in study

Measurements	Frequency (number of patients)	Percentage of study group	Cumulative percentage
5	227	35.4	35.4
4	129	20.1	55.5
3	108	16.9	72.4
2	79	12.3	84.7
1	74	11.5	96.2
0	24	3.7	99.9

*Did not add to exactly 100% due to rounding.

Crude bivariate analyses

When linear regression was carried out between natural-log transformed mean CRP levels and individual physiological and lifestyle factors, many of the factors were found to be significantly related to CRP levels. This was true both for the entire study

group and for the groups stratified by BMI classification (normal, overweight and obese). There were significant associations with many of the variables of interest (lipids, blood pressure, waist/hip ratio, and heart rate) in the entire group and in some of the BMI groups; however, no association was seen with daily sleep time in any group. See tables 6 and 7 below for detailed results of these analyses; table 6 is for the study group as a whole and table 7 is stratified by BMI classification. Note that these analyses included patients with only one CRP measurement.

Table 6: Summary of preliminary crude bivariate analyses for entire group (n = 617): linear regression of CRP (average value for each patient) with other factors

Variable	**Regression Coefficient (β)**	**SE**	**P value**
Age	0.015	0.004	*< 0.001*
Gender: *Male*	--	--	--
Female	-0.006	0.088	0.947
Marital status: *Married or living with partner*	-0.073	0.104	0.481
Not married or living with partner	--	--	--
Education: *High school or less*	--	--	--
Associate's degree or some college	-0.313	0.112	*0.005*
Bachelor's degree or higher	-0.527	0.111	*< 0.005*
Employment: *Full time*	--	--	--
Part time	0.111	0.124	0.372
Unemployed or retired	0.353	0.116	*0.002*
Race: *White*	0.110	0.124	0.378
Non-white	--	--	--
Smoking status: *Non-smoker*	--	--	--
Smoker	0.386	0.115	*0.001*
BMI	0.095	0.007	*<0 .001*
Leisure physical activity (MET-hrs/day)	-0.100	0.022	*<0 .001*
Caloric intake (kcal)	-0.0001	0.0001	0.541
% of calories from fat	0.028	0.007	*<0 .001*
% of calories from sat. fat	0.056	0.015	*<0 .001*
% of calories from non-sat. fat	0.041	0.012	*0.001*
% of calories from carb.	-0.025	0.006	*<0 .001*
% of calories from protein	0.027	0.016	0.098
Alcohol intake (g)	0.002	0.003	0.514

Total fiber intake (g)	-0.027	0.008	*<0 .001*
Water intake (g)	0.0001	0.0001	0.532
Soluble fiber intake (g)	-0.062	0.022	*0.005*
Daily GI	0.013	0.009	0.139
Daily GL	-0.002	0.001	*0.016*
Daily hrs of sleep	-0.016	0.050	0.749
Beck anxiety score	0.034	0.008	*<0 .001*
Beck depression score	0.035	0.008	*<0 .001*
Total cholesterol (mg/dl)	0.004	0.001	*<0 .001*
LDL cholesterol (mg/dl)	0.004	0.001	*0.001*
HDL cholesterol (mg/dl)	-0.020	0.004	*<0 .001*
Triglycerides (mg/dl)	0.002	0.0004	*<0 .001*
Systolic BP(mm Hg)	0.018	0.003	*<0 .001*
Diastolic BP (mm Hg)	0.031	0.005	*<0 .001*
Heart rate (bpm)	0.020	0.004	*<0 .001*
Waist/hip ratio	2.490	0.473	*< 0.001*

P values in *italics* indicate significance; association was taken to be statistically significant if p <= 0.05. -- indicates reference group for categorical variable.

Table 7: Bivariate regression analyses of the relation of log-transformed CRP with other factors, stratified by BMI category

	Normal BMI (n = 227)		**Overweight (n = 243)**		**Obese (n = 147)**	
	Regression coefficient (SE)	p	Regression coefficient (SE)	p	Regression coefficient (SE)	p
Demographic factors:						
Age	.023 (.005)	*<0.001*	.010 (.005)	*0.043*	.0006 (.007)	0.924
Gender: *Male*	--	--	--	--	--	--
Female	-.110 (.134)	0.412	.128 (.128)	0.319	.471 (.157)	*0.003*
Marital status: *Married or living with partner*	-.046 (.148)	0.753	-.314 (.159)	*0.049*	.119 (.185)	0.522
Not married or living with partner	--	--	--	--	--	--
Education: *High school or less*	--	--	--	--	--	--
Associate's degree or some college	-.346 (.181)	0.057	-.131 (.169)	0.437	-.292 (.183)	0.113
Bachelor's degree or higher	-.376 (.169)	*0.027*	-.236 (.171)	0.169	-.417 (.206)	*0.044*

Employment: *Full time*	--	--	--	--	--	--
Part time	.198 (.177)	0.266	.102 (.184)	0.580	.159 (.240)	0.508
Unemployed or retired	.341 (.188)	0.071	.385 (.164)	*0.02*	.068 (.200)	0.733
Race: *White*	-.035 (.176)	0.842	.230 (.189)	0.223	.045 (.226)	0.843
Non-white	--	--	--	--	--	--
Physiological factors:						
BMI	.090 (.037)	*0.017*	.074 (.045)	0.100	.069 (.016)	*<0.001*
Waist/hip ratio	1.650 (.771)	*0.033*	.338 (.803)	0.674	-1.44 (.923)	0.120
Total cholesterol (mg/dl)	.005 (.002)	*0.007*	.002 (.002)	0.199	-.002 (.002)	0.399
LDL cholesterol (mg/dl)	.005 (.002)	*0.006*	.002 (.002)	0.338	-.002 (.003)	0.374
HDL cholesterol (mg/dl)	-.014 (.005)	*0.007*	-.005 (.006)	0.417	-.0001 (.008)	0.989
Triglycerides (mg/dl)	.002 (.001)	*0.004*	.001 (.001)	0.194	-.0003 (.0005)	0.588
Systolic blood pressure (mm Hg)	.012 (.004)	*0.006*	.004 (.004)	0.398	.005 (.006)	0.349
Diastolic blood pressure (mm Hg)	.023 (.008)	*0.002*	.004 (.007)	0.535	.003 (.011)	0.811
Resting heart rate (bpm)	.014 (.006)	*0.026*	.023 (.007)	*0.001*	.021 (.008)	*0.010*
Lifestyle factors:						
Leisure physical activity (MET-hrs/day)	-0.067 (.030)	*0.027*	-0.060 (.033)	0.066	-0.089 (.053)	0.097
Caloric intake (kcal)	-.000025 (.0001)	0.823	-.0000378 (.0001)	0.730	-.0003 (.0001)	0.070
% calories from non-saturated fat	.024 (.018)	0.191	.012 (.017)	0.476	.021 (.024)	0.390
% calories from saturated fat	.029 (.023)	0.207	.023 (.022)	0.288	.032 (.032)	0.331
% calories from carbohydrate	-.022 (.008)	*0.011*	-.001 (.009)	0.906	-.015 (.012)	0.220
% calories from protein	.022 (.025)	0.365	-.006 (.023)	0.781	.027 (.032)	0.395
Total fiber intake (g)	-.020 (.010)	*0.043*	-.010 (.012)	0.399	-.034 (.016)	*0.038*
Water intake (g)	.0001 (.0001)	0.555	-.00005 (.0001)	0.626	.0001 (.0001)	0.663

Alcohol intake (g)	.007 (.004)	0.095	-.003 (.005)	0.563	.001 (.006)	0.867
Daily glycemic index (GI)	.006 (.013)	0.657	.013 (.013)	0.344	.018 (.015)	0.229
Daily glycemic load (GL)	-.002 (.001)	0.111	-.0003 (.001)	0.744	-.003 (.001)	*0.034*
Daily hours of sleep	.030 (.072)	0.678	.002 (.073)	0.981	.015 (.091)	0.874
Smoking status: *smoker*	.357 (.161)	*0.028*	.687 (.164)	*<0.001*	.256 (.230)	0.268
Non-smoker	--	--	--	--	--	--
Psychological factors:						
Beck anxiety score	.021 (.014)	0.135	.025 (.015)	0.098	.026 (.011)	*0.025*
Beck depression score	.013 (.012)	0.277	.022 (.012)	0.069	.040 (.012)	*0.001*

-- indicates reference group for categorical variable; *italics* indicate significance as in table 6.

Longitudinal analyses

Overall group

The following factors were in the predictive model for CRP levels in the overall cohort: age, BMI, dietary protein percentage, Beck depression score, leisure physical activity and smoking status. The analysis also controlled for recent infection history, as infection greatly increases CRP levels. In analyses dealing with heart rate or blood pressure, adjustment was also made for beta blocker use, as beta blockers obviously affect heart rate and blood pressure and appear also to decrease CRP levels (Jenkins et al., 2002). Cross-sectionally, diastolic blood pressure, heart rate and daily hours of sleep were positively associated with CRP levels in the adjusted analysis. Longitudinally, total cholesterol and triglycerides showed an inverse association with CRP levels, and resting heart rate showed a positive association with CRP levels. See table 8 for a summary. Unadjusted analyses are presented for comparison.

Table 8: Association of lipids, BP, heart rate, WHR and daily sleep with natural-log transformed CRP levels, overall cohort

	Unadjusted				Adjusted*			
	Cross-sectional		Longitudinal		Cross-sectional		Longitudinal	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
Total cholesterol	0.005 (.001)	< *0.001*	-0.003 (0.001)	*0.002*	0.0003 (0.001)	0.751	-0.004 (0.001)	*0.001*
LDL	0.004 (0.001)	< *0.001*	-0.002 (0.001)	0.191	-0.0003 (0.001)	0.760	-0.002 (0.001)	0.082
HDL	-0.021 (0.003)	< *0.001*	-0.003 (0.003)	0.406	-0.003 (0.003)	0.335	-0.003 (0.004)	0.445
Triglycerides	0.002 (0.0003)	< *0.001*	-0.001 (0.0003)	*0.001*	0.0005 (0.0003)	0.175	-0.001 (0.0003)	*0.003*
Systolic BP	0.022 (0.003)	< *0.001*	0.002 (0.002)	0.446	0.005 (0.003)	0.078	0.001 (0.002)	0.716
Diastolic BP	0.044 (0.005)	< *0.001*	0.003 (0.003)	0.266	0.011 (0.005)	*0.029*	0.002 (0.003)	0.484
Heart rate	0.023 (0.004)	< *0.001*	0.012 (0.002)	< *0.001*	0.018 (0.004)	< *0.001*	0.013 (0.003)	< *0.001*
Waist/hip ratio	2.995 (0.446)	< *0.001*	1.440 (0.987)	0.145	0.234 (0.444)	0.597	1.152 (1.043)	0.269
Daily sleep (hours)	0.047 (0.050)	0.344	-0.014 (0.022)	0.508	0.105 (0.044)	*0.018*	0.002 (0.023)	0.923

*Adjusted for age, BMI, dietary protein percentage, Beck depression score, leisure physical activity, smoking status, infection history and (for analyses dealing with heart rate and BP) beta blocker use.

A longitudinal analysis was also performed in which the group was stratified by gender (data not shown), to assess any differences between men and women in these associations. From this analysis, it was found that the inverse longitudinal association of triglyceride levels with CRP levels was only present in men, while only the women showed a significant positive cross-sectional association between triglycerides and CRP. The positive cross-sectional association between diastolic blood pressure and CRP levels was found in the men and not the women. Associations of CRP levels with total, HDL and LDL cholesterol, systolic blood pressure, resting heart rate and hours of sleep were similar between men and women. In women, there was a strong positive cross-sectional

association between waist/hip ratio and CRP levels; no such association was found in men.

Normal BMI

For subjects with normal BMI, the predictive model for CRP included age, BMI, dietary carbohydrate and non-saturated fat percentage, Beck anxiety score, leisure physical activity and smoking status. Adjustment was also made for infection history and beta blocker use as in the analysis for the overall group. Cross-sectionally, daily hours of sleep were positively associated with CRP levels. Longitudinally, resting heart rate showed a positive association with CRP levels. In addition, there was a trend toward a positive cross-sectional association of WHR with CRP levels. See table 9 for a summary. This group included, in addition to subjects with normal BMI, 6 underweight subjects (BMI < 18.5). However, this is a small percentage of the group and therefore should not affect the results.

Table 9: Association of lipids, BP, heart rate, WHR and daily sleep with natural-log transformed CRP levels, normal BMI subgroup

	Unadjusted				**Adjusted***			
	Cross-sectional		**Longitudinal**		**Cross-sectional**		**Longitudinal**	
	β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Total cholesterol	0.005 (0.001)	*0.001*	-0.004 (0.002)	*0.050*	-0.002 (0.002)	0.374	-0.003 (0.002)	0.177
LDL	0.006 (0.002)	< *0.001*	-0.003 (0.002)	0.191	-0.001 (0.002)	0.467	-0.002 (0.002)	0.442
HDL	-0.012 (0.005)	*0.010*	-0.005 (0.006)	0.355	-0.008 (0.005)	0.093	-0.002 (0.006)	0.728
Triglycerides	0.002 (0.001)	*0.004*	-0.001 (0.001)	0.467	0.001 (0.001)	0.286	-0.001 (0.001)	0.301
Systolic BP	0.014 (0.004)	< *0.001*	0.003 (0.004)	0.539	0.004 (0.005)	0.361	0.001 (0.004)	0.817
Diastolic BP	0.022 (0.007)	*0.002*	0.006 (0.006)	0.311	0.005 (0.008)	0.530	0.007 (0.005)	0.250
Heart rate	0.011 (0.006)	*0.047*	0.012 (0.005)	*0.007*	0.007 (0.006)	0.239	0.012 (0.005)	*0.013*
Waist/hip	2.542	<	1.490	0.525	1.307	0.076	2.184	0.352

ratio	(0.611)	*0.001*	(2.344)		(0.736)		(2.347)	
Daily sleep (hours)	0.086 (0.064)	0.181	-0.029 (0.043)	0.504	0.123 (0.062)	*0.045*	-0.006 (0.043)	0.882

*Adjusted for age, BMI, dietary carbohydrate and non-saturated fat percentage, Beck anxiety score, leisure physical activity, smoking status, infection history and (for analyses dealing with heart rate and BP) beta blocker use.

Overweight

In the subgroup of overweight subjects, the predictive model for CRP included age, Beck depression score, smoking status and alcohol drinking status; adjustment was also made for infection history and beta blocker use as in the other analyses. Cross-sectionally, heart rate and diastolic blood pressure showed a positive association with CRP levels. Longitudinally, total cholesterol and triglycerides showed an inverse association with CRP levels; resting heart rate showed a positive association with CRP levels. See table 10 for a summary.

Table 10: Association of lipids, BP, heart rate, WHR and daily sleep with natural-log transformed CRP levels, overweight subgroup

	Unadjusted				**Adjusted***			
	Cross-sectional		**Longitudinal**		**Cross-sectional**		**Longitudinal**	
	β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Total cholesterol	0.002 (0.001)	0.172	-0.001 (0.0004)	0.100	0.001 (0.002)	0.631	-0.004 (0.002)	*0.036*
LDL	0.001 (0.002)	0.440	0.001 (0.002)	0.746	-0.0003 (0.002)	0.876	-0.001 (0.002)	0.690
HDL	-0.005 (0.006)	0.354	-0.001 (0.006)	0.891	-0.003 (0.006)	0.573	-0.002 (0.007)	0.756
Triglycerides	0.001 (0.001)	0.121	-0.001 (0.0004)	*0.005*	0.001 (0.001)	0.231	-0.001 (0.0005)	*0.009*
Systolic BP	0.008 (0.005)	0.065	0.003 (0.003)	0.274	0.005 (0.005)	0.362	0.003 (0.003)	0.438
Diastolic BP	0.025 (0.008)	*0.001*	0.003 (0.005)	0.476	0.023 (0.008)	*0.006*	0.002 (0.005)	0.747
Heart rate	0.027 (0.006)	< *0.001*	0.019 (0.004)	< *0.001*	0.029 (0.007)	< *0.001*	0.021 (0.004)	< *0.001*
Waist/hip ratio	0.635 (0.788)	0.421	-0.584 (1.243)	0.638	0.328 (0.833)	0.693	-0.033 (1.239)	0.979
Daily sleep	0.032	0.676	-0.031	0.402	-0.005	0.955	-0.027	0.477

(hours)	(0.077)		(0.037)		(0.083)		(0.038)	

*Adjusted for age, Beck depression score, smoking status, alcohol drinking status, infection history and (for analyses dealing with heart rate and BP) beta blocker use.

Obese

In the obese subgroup, the predictive model for CRP included BMI, dietary glycemic load and Beck depression score; adjustment was also made for infection history and beta blocker use. No significant associations were found in the adjusted analysis. However, there was a trend toward an inverse longitudinal association between total cholesterol and CRP levels, and toward a positive cross-sectional association of CRP levels with systolic blood pressure and daily hours of sleep. See table 11.

Table 11: Association of lipids, BP, heart rate, WHR and daily sleep with natural-log transformed CRP levels, obese subgroup

	Unadjusted				**Adjusted***			
	Cross-sectional		**Longitudinal**		**Cross-sectional**		**Longitudinal**	
	β (SE)	**p**	**β (SE)**	**p**	**β (SE)**	**p**	**β (SE)**	**p**
Total cholesterol	-0.002 (0.002)	0.336	-0.005 (0.002)	*0.005*	-0.0002 (0.002)	0.932	-0.003 (0.002)	0.073
LDL	-0.003 (0.002)	0.234	-0.004 (0.002)	*0.037*	-0.001 (0.002)	0.767	-0.002 (0.002)	0.280
HDL	-0.0003 (0.007)	0.967	0.002 (0.006)	0.745	-0.003 (0.007)	0.681	0.001 (0.006)	0.927
Triglycerides	-0.0002 (0.0004)	0.649	-0.0005 (0.0003)	0.108	-0.00004 (0.0005)	0.923	-0.001 (0.0004)	0.133
Systolic BP	0.008 (0.005)	0.109	-0.004 (0.003)	0.193	0.009 (0.005)	0.063	-0.003 (0.003)	0.272
Diastolic BP	0.009 (0.010)	0.382	-0.002 (0.004)	0.710	-0.002 (0.011)	0.882	0.002 (0.004)	0.718
Heart rate	0.029 (0.008)	*0.001*	-0.00001 (0.004)	0.998	0.015 (0.008)	0.065	0.004 (0.004)	0.254
Waist/hip ratio	-0.935 (0.813)	0.250	2.171 (1.705)	0.203	-0.125 (0.819)	0.879	1.761 (1.691)	0.298
Daily sleep (hours)	0.143 (0.087)	0.101	-0.006 (0.030)	0.832	0.143 (0.083)	0.083	0.035 (0.031)	0.263

*Adjusted for BMI, dietary glycemic load, Beck depression score, infection history and (for analyses dealing with heart rate and BP) beta blocker use.

DISCUSSION

Cross-sectional results for the association between CRP levels and traditional cardiovascular risk factors (lipids, blood pressure and resting heart rate), as well as waist/hip ratio, were generally consistent with findings from previous studies. Some longitudinal results, however, were unexpected; others were somewhat expected but the biological mechanism has not been elucidated. Further study is needed to examine these associations.

Association of CRP with lipid levels

Interestingly, CRP levels showed an inverse longitudinal association with total cholesterol and triglyceride levels in the overall cohort, accounted for by the association present in men, and in the overweight subgroup. There was a trend toward a similar association for total cholesterol in the obese subgroup. This is the opposite of what was expected in light of the unadjusted cross-sectional associations, which agreed with the findings of previous (cross-sectional) studies. In addition, triglyceride levels are known to increase during infection and inflammation, a situation where CRP levels obviously increase (Khovidhunkit et al., 2000). From this, one would have expected a positive longitudinal association between triglyceride and CRP levels, but this is not what was found. LDL cholesterol levels, on the other hand, are known to decrease during infection and inflammation (Khovidhunkit et al., 2000), so this may help explain the inverse longitudinal association seen with total cholesterol levels, although it is also possible that the inverse relation with triglycerides is, at least in part, responsible for the inverse relation with total cholesterol, since the triglyceride level is included in the total cholesterol level. There was, in fact, a trend toward an inverse longitudinal association

between LDL cholesterol and CRP levels in the overall study cohort, consistent with a decrease in LDL levels in response to inflammation. HDL levels have also been found to decrease during inflammation (Khovidhunkit et al., 2000), but no significant association between HDL and CRP levels was seen in this study. Therefore, changes in HDL levels would not be responsible for the inverse association that was seen between total cholesterol and CRP levels. In summary, there is some support in the literature for an inverse longitudinal association between total cholesterol and CRP levels, but the same cannot be said for triglycerides. There also appears to be no biologically plausible explanation for the inverse association of CRP and triglycerides. Further investigation of this relationship and its biological mechanism is needed.

Association of CRP with blood pressure

There were no longitudinal associations found between either systolic or diastolic blood pressure and CRP levels. However, there was a positive cross-sectional relationship between diastolic blood pressure and CRP levels in the overall study cohort and in the overweight subgroup, and there was a trend toward a positive cross-sectional association of CRP levels with systolic blood pressure in the overall cohort and the obese subgroup. These findings differ somewhat from those of previous studies, which often found no association of CRP levels with diastolic blood pressure and if they did, always found a significant association with systolic blood pressure as well. One possible reason for the difference is that the previous studies did not have such extensive dietary and other lifestyle data and therefore did not adjust for some of the factors for which adjustment was made in the present study.

Association of CRP with resting heart rate

By far the most consistent association found in this study was the positive relationship between resting heart rate and CRP levels. This was found, cross-sectionally and/or longitudinally, in every analysis except that of the obese subgroup. The association is unlikely to be explained by differences in physical activity levels or by infection status, as these were among the factors for which adjustment was made in the statistical analysis. Although physical activity was not in the model for the overweight or obese subgroups, an analysis was performed in which it was forced in to determine whether it changed the association between CRP levels and heart rate. The association did not substantially change, suggesting that it is not due to physical activity differences. One possible explanation is that elevated resting heart rate and increased CRP levels are associated simply due to both being indicators of suboptimal health, but it is not possible to conclusively establish this from the present study, since it was performed in a "healthy" population without known coronary heart disease or other chronic illness. It is also possible that there is some hormonal or other factor that tends to increase both CRP and resting heart rate and that this is responsible for the association; however, there is no way to determine this from these results.

Association of CRP with waist/hip ratio

No significant association was found between waist/hip ratio and CRP levels, in the overall cohort or in the BMI stratified analysis, when adjustment was made for other factors affecting CRP levels; that is, WHR was not found to be independently associated with CRP. However, there was a positive cross-sectional association seen in women. There was also a trend toward a positive association in subjects with normal BMI. One

possible explanation for the general lack of association seen between WHR and CRP is the fact that in most subjects, WHR changed very little over the course of the study. In other words, an association could exist but subjects' changes in WHR in this study were of insufficient magnitude for it to be apparent. However, this does not account for the association that was seen in the women—there was no significant difference between men's and women's changes in WHR over the course of the study.

Association of CRP with average daily sleep

There was a positive cross-sectional association between average daily sleep time and CRP levels in the overall cohort and in the subgroup with normal BMI; there was a trend toward such an association in the obese subgroup. No longitudinal relationship was found. This is very different from the association that would be expected in light of the experimental study by Meier-Ewert et al. (2004), which found CRP levels to increase with sleep deprivation. However, that study examined the effects of severe sleep deprivation over a short period and under controlled conditions, as opposed to observing the effects of naturally-occurring fluctuations in sleep patterns in a free-living environment. The positive cross-sectional association could reflect the fact that increased CRP is a sign of a problem somewhere in the body, and more sleep is needed in this situation for the body to repair itself. However, it is not possible to make a definite conclusion such as this from an observational study.

Strengths of study

The SEASONS data set contains measurements of many variables that could affect CRP levels and other cardiovascular risk factors. This allowed careful examination of possible confounders, as well as adjustment for factors found to be significantly related

to CRP levels in the cohort. In addition, the data set contains up to five measurements from each subject, over a one-year period. This yielded new information, as longitudinal studies had not previously been conducted on the association of CRP levels and traditional cardiovascular risk factors.

Limitations of study

One major limitation of this study is the lack of detailed data on infection and inflammation history. When the original SEASONS study was conducted, there were no plans for the present study on the relation of other factors to CRP levels. Consequently, the only information gathered on infection/inflammation history was whether a subject had had a cold, flu, allergy or other illness within the three months preceding the clinic visit, and whether any illness was severe enough to warrant missing work or being hospitalized. There is no specific information as to when during that time the illness occurred, and it is likely that subjects would have had difficulty recalling a minor illness closer to the beginning of the three months. The other major limitation is that information on medication use was only collected at baseline and one year, not at each clinic visit. This makes it difficult to reliably control for medication use, as it is quite possible for a subject to have been taking a medication at the beginning of the study but not later on; many medications are taken on a short-term basis. Therefore, it was not possible to control for the use of anti-inflammatory medications etc. which could affect CRP levels.

Future studies

To further explore the inverse longitudinal association between triglyceride and CRP levels, it would be useful to conduct an intervention study of patients with elevated

triglyceride and CRP levels, in which they are given treatment to lower their triglycerides and the changes in CRP levels are also monitored. This would help to show whether lowering triglyceride levels somehow causes CRP levels to increase, or whether the association seen in this study was likely due to some other reason. A similar study could be done to examine the similar association found between CRP and total cholesterol levels, although care would need to be taken not to use a treatment that is known to lower both cholesterol and CRP levels, such as the statin drugs, which appear to lower CRP levels independent of LDL lowering (Ridker et al., 2005). Another area that warrants further study is the positive association of resting heart rate and CRP levels, both cross-sectionally and longitudinally.

BIBLIOGRAPHY

Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR, Jr., Schmitz KH, Emplaincourt PO, Jacobs DR, Jr. and Leon AS (2000). Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 32: S498-504.

Barbieri M, Ferrucci L, Corsi AM, Macchi C, Lauretani F, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C and Paolisso G (2003). Is chronic inflammation a determinant of blood pressure in the elderly? *American Journal of Hypertension* 16: 537-543.

Brand-Miller J, Wolever TM, Foster-Powell K and Colagiuri S (2003). The New Glucose Revolution: The Authoritative Guide to the Glycemic Index--the Dietary Solution for Lifelong Health. New York, NY, Marlowe & Company.

Choi H, Cho DH, Shin HH and Park JB (2004). Association of high sensitivity C-reactive protein with coronary heart disease prediction, but not with carotid atherosclerosis, in patients with hypertension. *Circ J* 68: 297-303.

Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, Miller GJ and Strachan DP (2000). C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. *Atherosclerosis* 149: 139-150.

Di Napoli M and Papa F (2003). Association between blood pressure and C-reactive protein levels in acute ischemic stroke. *Hypertension* 42: 1117-1123.

Ford ES, Giles WH, Mokdad AH and Myers GL (2004). Distribution and correlates of C-reactive protein concentrations among adult US women. *Clinical Chemistry* 50: 574-581.

Foster-Powell K, Holt SH and Brand-Miller JC (2002). International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr* 76: 5-56.

Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeing H, Muche R, Brenner H, and Koenig W (2000) Association Between C-Reactive Protein and Features of the Metabolic Syndrome. *Diabetes Care* 23:1835-1839.

Gabay C and Kushner I (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* 340: 448-454.

Greenhough TJ and Shrive AK (2004) Keele University. Image of CRP structure; accessed on 10/7/2004 from: http://www.keele.ac.uk/depts/bi/research/comb2/px/highlights.html.

Janeway CA, Travers P (1994) Immuno-Biology: The Immune System in Health and Disease. Garland Publishing, New York.

Jenkins NP, Keevil BG, Hutchinson IV and Brooks NH (2002). Beta-blockers are associated with lower C-reactive protein concentrations in patients with coronary artery disease. *American Journal of Medicine* 112: 269-274.

Khovidhunkit W, Memon RA, Feingold KR and Grunfeld C (2000). Infection and inflammation-induced proatherogenic changes of lipoproteins. *Journal of Infectious Diseases* 181 Suppl 3: S462-472.

Khreiss T, Jozsef L, Potempa LA and Filep JG (2004). Conformational rearrangement in C-reactive protein is required for proinflammatory actions on human endothelial cells. *Circulation* 109: 2016-2022.

Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL and Pepys MB (1999). C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 99: 237-242.

Laaksonen DE, Niskanen L, Nyyssonen K, Punnonen K, Tuomainen TP, Valkonen VP, Salonen R and Salonen JT (2004). C-reactive protein and the development of the metabolic syndrome and diabetes in middle-aged men. *Diabetologia* 47: 1403-1410.

Lambert M, Delvin EE, Paradis G, O'Loughlin J, Hanley JA and Levy E (2004). C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. *Clinical Chemistry* 50: 1762-1768.

Ledue TB and Rifai N (2003). Preanalytic and analytic sources of variations in C-reactive protein measurement: implications for cardiovascular disease risk assessment. *Clinical Chemistry* 49: 1258-1271.

Ma Y, Olendzki B, Chiriboga D, Hebert JR, Li Y, Li W, Campbell M, Gendreau K and Ockene IS (2005). Association between Dietary Carbohydrates and Body Weight. *American Journal of Epidemiology* 161: 359-367.

Matthews CE, Freedson P, Hebert J, Stanek E, Ockene I and Merriam P (2000). Comparison of physical activity assessment methods in the Seasonal Variation of Blood Cholesterol Levels Study. *Med Sci Sports Exerc* 32: 976-984.

Matthews CE, Freedson PS, Stanek EJ, Hebert JR, Merriam PA, Rosal MC, Ebbeling CB and Ockene IS (2001). Seasonal Variation of Household, Occupational, and leisure-time Physical Activity: Longitudinal Analyses from the Seasonal Variation of Cholesterol Study. *Am J Epidemiol* 153: 172-183.

Matthews CE, Hebert JR, Freedson PS, Stanek EJ, 3rd, Merriam PA, Ebbeling CB and Ockene IS (2001). Sources of variance in daily physical activity levels in the seasonal variation of blood cholesterol study. *Am J Epidemiol* 153: 987-995.

Mazer SP and Rabbani LE (2004). Evidence for C-reactive protein's role in (CRP) vascular disease: atherothrombosis, immuno-regulation and CRP. *Journal of Thrombosis and Thrombolysis* 17: 95-105.

Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF and Mullington JM (2004). Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *Journal of the American College of Cardiology* 43: 678-683.

Mendall MA, Patel P, Ballam L, Strachan D and Northfield TC (1996). C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ* 312: 1061-1065.

Merriam PA, Ockene IS, Hebert JR, Rosal MC and Matthews CE (1999). Seasonal variation of blood cholesterol levels: study methodology. *Journal of Biological Rhythms* 14: 330-339.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Jr., Taubert K, Tracy RP and Vinicor F (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare

professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 107: 499-511.
Retterstol L, Eikvar L and Berg K (2003). A twin study of C-Reactive Protein compared to other risk factors for coronary heart disease. *Atherosclerosis* 169: 279-282.
Rexrode KM, Pradhan A, Manson JE, Buring JE and Ridker PM (2003). Relationship of total and abdominal adiposity with CRP and IL-6 in women. *Annals of Epidemiology* 13: 674-682.
Ridker PM, Glynn RJ and Hennekens CH (1998). C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* 97: 2007-2011.
Ridker PM, Rifai N, Rose L, Buring JE and Cook NR (2002). Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. *New England Journal of Medicine* 347: 1557-1565.
Ridker PM (2003). Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 107: 363-369.
Ridker PM, Buring JE, Cook NR and Rifai N (2003). C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation* 107: 391-397.
Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA and Braunwald E (2005). C-reactive protein levels and outcomes after statin therapy. *New England Journal of Medicine* 352: 20-28.
Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M and Matsuoka H (2003). Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis* 167: 73-79.
Sallis J, Haskell W, Wood P, Fortmann S, Rodgers T, Blair S and Paffenbarger R (1985). Physical activity assessment methodology in the Five-City Project. *Am J Epidemiol* 121: 91-106.
Schillaci G, Pirro M, Gemelli F, Pasqualini L, Vaudo G, Marchesi S, Siepi D, Bagaglia F and Mannarino E (2003). Increased C-reactive protein concentrations in never-treated hypertension: the role of systolic and pulse pressures. *Journal of Hypertension* 21: 1841-1846.
Strandberg TE and Tilvis R (2000) C-Reactive Protein, Cardiovascular Risk Factors, and Mortality in a Prospective Study in the Elderly. *Arterioscler Thromb Vasc Biol.* 20:1057-1060.
Sung KC, Suh JY, Kim BS, Kang JH, Kim H, Lee MH, Park JR and Kim SW (2003). High sensitivity C-reactive protein as an independent risk factor for essential hypertension. *American Journal of Hypertension* 16: 429-433.
Vikram NK, Misra A, Dwivedi M, Sharma R, Pandey RM, Luthra K, Chatterjee A, Dhingra V, Jailkhani BL, Talwar KK and Guleria R (2003). Correlations of C-reactive protein levels with anthropometric profile, percentage of body fat and lipids in healthy adolescents and young adults in urban North India. *Atherosclerosis* 168: 305-313.
Willerson JT and Ridker PM (2004). Inflammation as a cardiovascular risk factor. *Circulation* 109: II2-10.
Williams MJ, Williams SM, Milne BJ, Hancox RJ and Poulton R (2004). Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral

contraceptive use in young adults. *International Journal of Obesity and Related Metabolic Disorders* 28: 998-1003.

Wu DM, Chu NF, Shen MH and Chang JB (2003). Plasma C-reactive protein levels and their relationship to anthropometric and lipid characteristics among children. *Journal of Clinical Epidemiology* 56: 94-100.

Zwaka TP, Hombach V and Torzewski J (2001). C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis. *Circulation* 103: 1194-1197.

APPENDIX A: STATA CODE SAMPLES

Cross-sectional model building, overall study cohort stratified by gender

```
use "C:\Documents and Settings\hafnera\My Documents\Andrea MQP\x-sectionalcrpdataset_withinf.dta", clear

**Men**

xi: regress logmeancrp i.smoking_status if gender == 0
xi: regress logmeancrp i.drinker if gender == 0
xi: regress logmeancrp i.employed if gender == 0
xi: regress logmeancrp i.marital if gender == 0
xi: regress logmeancrp i.ever_inf if gender == 0
xi: regress logmeancrp i.race2 if gender == 0
xi: regress logmeancrp i.education if gender == 0
xi: regress logmeancrp i.SAdis if gender == 0
xi: regress logmeancrp i.betablockers if gender == 0
regress logmeancrp meanleisuremets if gender == 0
regress logmeancrp meankcals if gender == 0
regress logmeancrp meancarbpcnt if gender == 0
regress logmeancrp meanprotpcnt if gender == 0
regress logmeancrp meanfiber if gender == 0
regress logmeancrp meanwater if gender == 0
regress logmeancrp meansfpcnt if gender == 0
regress logmeancrp nsfatpcntavg if gender == 0
regress logmeancrp dailyGlwbavg if gender == 0
regress logmeancrp meananx if gender == 0
regress logmeancrp meandep if gender == 0
regress logmeancrp age if gender == 0
regress logmeancrp bmiavg if gender == 0
regress logmeancrp tcavg if gender == 0
regress logmeancrp ldlavg if gender == 0
regress logmeancrp hdlavg if gender == 0
regress logmeancrp tgavg if gender == 0
regress logmeancrp sbpavg if gender == 0
regress logmeancrp dbpavg if gender == 0
regress logmeancrp hravg if gender == 0
regress logmeancrp whravg if gender == 0

xi: sw regress logmeancrp i.smoking_status i.education meanleisuremets meancarbpcnt meanfiber meansfpcnt nsfatpcntavg dailyGlwbavg meandep age bmiavg if gender == 0, pr(0.20)


**Women**

xi: regress logmeancrp i.smoking_status if gender == 1
xi: regress logmeancrp i.drinker if gender == 1
xi: regress logmeancrp i.employed if gender == 1
xi: regress logmeancrp i.marital if gender == 1
xi: regress logmeancrp i.ever_inf if gender == 1
xi: regress logmeancrp i.race2 if gender == 1
xi: regress logmeancrp i.education if gender == 1
xi: regress logmeancrp i.SAdis if gender == 1
```

```
xi: regress logmeancrp i.betablockers if gender == 1
regress logmeancrp meanleisuremets if gender == 1
regress logmeancrp meankcals if gender == 1
regress logmeancrp meancarbpcnt if gender == 1
regress logmeancrp meanprotpcnt if gender == 1
regress logmeancrp meanfiber if gender == 1
regress logmeancrp meanwater if gender == 1
regress logmeancrp meansfpcnt if gender == 1
regress logmeancrp nsfatpcntavg if gender == 1
regress logmeancrp dailyGlwbavg if gender == 1
regress logmeancrp meananx if gender == 1
regress logmeancrp meandep if gender == 1
regress logmeancrp age if gender == 1
regress logmeancrp bmiavg if gender == 1
regress logmeancrp tcavg if gender == 1
regress logmeancrp ldlavg if gender == 1
regress logmeancrp hdlavg if gender == 1
regress logmeancrp tgavg if gender == 1
regress logmeancrp sbpavg if gender == 1
regress logmeancrp dbpavg if gender == 1
regress logmeancrp hravg if gender == 1
regress logmeancrp whravg if gender == 1

xi: sw regress logmeancrp i.smoking_status i.education meanleisuremets meankcals
meancarbpcnt meanprotpcnt meanfiber meansfpcnt meandep age bmiavg if gender == 1,
pr(0.20)
```

Example of longitudinal analysis using model built by cross-sectional process

```
use "C:\Documents and Settings\hafnera\My Documents\Andrea MQP\longitudinal-with-
infection.dta", clear

**Generating mean and residual values for each covariate and variable of interest**

foreach y in logcrp BMI sports_metsavg carbohydrate_percentavg protein_percentavg
dietary_fiberavg satfat_percentavg nsatfat_percentavg energy_caloriesavg sleephrsavg
beck_d beck_a tc ldl hdl tg Systolic_BP Diastolic_BP heartrate dailyGl_whitebreadQ
dailyGL_whitebreadQ drinks whr {
egen `y'_mean = mean(`y'), by(id)
gen `y'_res = `y' - `y'_mean
}

tsset, clear

**Example of longitudinal analysis, using model built by cross-sectional process**

#delimit;

xi: xtgee logcrp age
BMI_mean BMI_res
protein_percentavg_mean protein_percentavg_res
beck_d_mean beck_d_res
sports_metsavg_mean sports_metsavg_res
ldl_mean ldl_res    **or other variable of interest**
```

i.smoking_status
i.infe_infla, **also beta blocker use, for analyses with HR or BP**
i(id) t(quarter) corr(ar1) force;

****repeat for each gender separately and BMI groups (normal, overweight, obese) using appropriate model****