INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700  800/521-0600
ACUTE PHASE REACTANTS PRIOR TO DIAGNOSIS
OF CANCER OR MYOCARDIAL INFARCTION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

James L. Fisher, Jr., M.S.

*****

The Ohio State University
1999

Dissertation Committee:

Dr. Judith Schwartzbaum, Advisor

Dr. Randall Harris

Dr. Jean Snook

Dr. Gary Stoner

Approved by

Judith Schwartzbaum
Advisor
School of Public Health
Division of Epidemiology and Biostatistics
ABSTRACT

The purpose of these investigations was to evaluate hypotheses concerning associations between the acute phase response (APR), an adaptive response to cellular injury during which hepatocyte protein synthesis is altered, and preclinical cancer and myocardial infarction (MI). The goal of the first investigation (Chapter 2) was to determine how long prior to cancer or MI diagnoses alterations in serum albumin, transferrin (assessed as iron binding capacity [IBC]), and serum iron occur. Age-adjusted statistically significant decreases in serum albumin, IBC, and serum iron prior to both cancer and MI diagnoses are found. Men not diagnosed with either cancer or MI have initial IBC and serum iron levels significantly lower than men developing cancer and MI, and show significant increases in IBC and serum iron during the eight-year study period. Results from the second investigation (Chapter 3) indicate that routinely-measured acute phase reactants are altered at least three years prior to diagnoses of two smoking-related cancers: lung and bladder cancer, although results vary by sex. For example, among males, risk of bladder cancer is 8.24 times greater (95 percent confidence interval [CI]: 3.39 - 20.04), and risk for lung cancer is 2.95 times greater (95 percent CI: 1.90 - 4.56) in men with WBCC in the upper quartile compared to men in remaining quartiles. Results from the third investigation (Chapter 4) indicate that serum micronutrients are altered during the APR. Statistically significant inverse associations between the APR and the following micronutrients are found: serum iron, selenium, vitamin C, vitamin A,
α-carotene, β-carotene, and lycopene. Finally, results of the last investigation (Chapter 5) indicate that urinary albumin levels greater than 100 μg/dl are found associated with the APR independent of serum albumin (prevalence odds ratio = 1.80, 95 percent CI: 1.19 - 2.70), and urinary albumin excretion is associated with increased risk of prostate (RR = 1.88, 95 percent CI: 0.98 - 3.56), lymphatic/hematopoietic tissue (RR = 2.84, 95 percent CI: 1.31 - 6.17), and uterine (RR = 2.48, 95 percent CI: 0.78 - 7.95) cancers. The effects of the APR occur early in disease processes and are more wide-ranging than previously believed.
Dedicated to my parents
ACKNOWLEDGMENTS

I wish to thank my advisor, Dr. Judith Schwartzbaum, for her guidance on this and all research I have been involved with. I thank Dr. Schwartzbaum for encouraging me, for exciting me about research, for sharing her knowledge and creativity, for always making time to think about and answer my latest question, and for making my experience as a graduate student everything I had hoped for.

I also wish to thank Dr. Jean Snook for her exceptional teaching, for the many stimulating and helpful discussions about this project and others, and for being so kind to me. I was fortunate to have met and been taught by Dr. Randall Harris. I wish to thank Dr. Harris for his thoughtful teaching of epidemiology and cancer epidemiology. I am also very grateful to Dr. Gary Stoner for meeting with me individually to teach me about cancer chemoprevention.

I wish to thank Dr. Ramzi Naahas for his explanation of the SAS mixed procedure, Ms. Lynn Mitchell for her help with statistical analyses, and Dr. J.R. Wilkins III for providing me with funding during the last year of my doctoral studies.

In addition, I wish to thank my parents, James and Sue Fisher, as well as Kim, Joey and Staci Farfsing, for their support, their interest, and their good wishes. I would also like to thank Amy Martin, Tracy Miller, Holly Burgess, Lauren Meredith, and Dr. J. Mac Crawford for their support.
VITA

September 15, 1966..................................................Born - Cincinnati, Ohio

June, 1988.............................................................B.A.

Psychology/Mathematics,
University of Cincinnati

1989 - 1990.......................................................Graduate Teaching

Associate, University of
Cincinnati

1988 - 1994..........................................................Counselor,

Talbert House,
Mental Health Services
Northwest,
Valley Mental Health
Services, Cincinnati, Ohio

1995 - present....................................................Graduate Research

Associate,
The Ohio State University

1996.................................................................M.S. Preventive Medicine,
The Ohio State University

PUBLICATIONS

Wilkins JR 3rd. Engelhardt HL. Rublaitus SM. Crawford JM. Fisher JL. Bean TL.
Prevalence of chronic respiratory symptoms among Ohio cash grain farmers. Amer J of

Schwartzbaum JA. Fisher JL. Goodman J. Octaviano D. Cornwell D. Hypotheses
concerning roles of dietary energy, cured meat, and serum tocopherols in adult glioma
FIELD OF STUDY

Major Field: Public Health
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>v</td>
</tr>
<tr>
<td>Vita</td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiii</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 The Acute Phase Response</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Albumin</td>
<td>9</td>
</tr>
<tr>
<td>1.3 C-reactive Protein</td>
<td>17</td>
</tr>
<tr>
<td>1.4 Research Questions</td>
<td>21</td>
</tr>
<tr>
<td>2. Serum Transferrin, Albumin, and Iron Concentrations Decrease</td>
<td>24</td>
</tr>
<tr>
<td>Prior to Cancer and Myocardial Infarction</td>
<td></td>
</tr>
<tr>
<td>2.0 Abstract</td>
<td>24</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>26</td>
</tr>
<tr>
<td>2.2 Methods</td>
<td>30</td>
</tr>
<tr>
<td>2.3 Results</td>
<td>37</td>
</tr>
<tr>
<td>2.4 Discussion</td>
<td>49</td>
</tr>
</tbody>
</table>
3. Concentrations of Acute Phase Reactants Are Altered At Least Three Years Prior to Lung and Bladder Cancer Diagnoses................................. 55
   3.0 Abstract................................................................................. 55
   3.1 Introduction............................................................................ 57
   3.2 Methods.................................................................................. 60
   3.3 Results.................................................................................... 63
   3.4 Discussion.............................................................................. 67

4. Serum Micronutrient Concentrations Are Decreased During the Acute Phase Response................................................................. 72
   4.0 Abstract.................................................................................... 72
   4.1 Introduction............................................................................... 74
   4.2 Methods................................................................................... 76
   4.3 Results..................................................................................... 79
   4.4 Discussion............................................................................... 87

5. Urinary Albumin is Associated with the Acute Phase Response and Marks an Increase in Uterine, Lymphatic, and Prostate Cancer Risks Independent of Serum Albumin......................................................... 91
   5.0 Abstract.................................................................................... 91
   5.1 Introduction............................................................................... 93
   5.2 Methods................................................................................... 96
   5.3 Results..................................................................................... 99
   5.4 Discussion............................................................................... 105

6. Summary and Suggested Future Research.............................................. 108

List of References.................................................................................. 119
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Distribution of cancer by site among 245 Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) and follow-up study participants</td>
<td>41</td>
</tr>
<tr>
<td>2.2</td>
<td>Pre-diagnostic means of characteristics of three groups of study participants first diagnosed with cancer, myocardial infarction, and neither cancer nor any cardiovascular event throughout the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) and follow-up</td>
<td>42</td>
</tr>
<tr>
<td>2.3</td>
<td>Age-adjusted estimates of slopes reflecting the change in serum albumin (g/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT)</td>
<td>46</td>
</tr>
<tr>
<td>2.4</td>
<td>Age-adjusted estimates of slopes reflecting the change in iron binding capacity (ug/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT)</td>
<td>47</td>
</tr>
</tbody>
</table>
2.5 Age-adjusted estimates of slopes reflecting the change in serum iron (g/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) ........................................................................................................ 48

3.1 Adjusted risk ratios (RR) for lung cancer from acute phase reactants among 155 incident lung cancer cases and 12,534 non-case National Health and Nutrition Examination Survey (NHANES) I study participants............................... 65

3.2 Adjusted risk ratios (RR) for bladder cancer from acute phase reactants among 41 incident bladder cancer cases and 12,534 non-case National Health and Nutrition Examination Survey (NHANES I) study participants............................... 66

4.1 Demographic and potentially confounding characteristics of National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than 3.5 mg/dl) - evidence of an acute phase response........................................ 82

4.2 Known acute phase reactants in National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than 3.5 mg/dl) - evidence of an acute phase response........................................ 83

4.3 Univariate mean dietary intakes in National Health and Nutrition Examination Survey (NHANES) III study participants with (n = 45) and without (n = 6,718) considerable levels of C-reactive protein (greater than 3.5 mg/dl) - evidence of an acute phase response........................................ 84
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4</td>
<td>Univariate mean levels of serum micronutrients in National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than 3.5 mg/dl) - evidence of an acute phase response</td>
</tr>
<tr>
<td>4.5</td>
<td>Adjusted prevalence odds ratios for high c-reactive protein levels (greater than 3.5 mg/dl) from serum micronutrients among apparently healthy National Health and Nutrition Examination Survey (NHANES) III study participants</td>
</tr>
<tr>
<td>5.1</td>
<td>Adjusted odds ratios (OR) for the acute phase response from the stratification of serum albumin and urinary albumin among National Health and Nutrition Examination Survey (NHANES) III study participants</td>
</tr>
<tr>
<td>5.2</td>
<td>Number of National Health and Nutrition Examination Survey (NHANES) I study participants developing cancers after sequential exclusions for baseline malignant tumors (1), and incident benign tumors and absence of measurement or reporting of selected acute phase reactants (2)</td>
</tr>
<tr>
<td>5.3</td>
<td>Adjusted risk ratios (RR) for cancers by site of neoplasm from significant albumin in urine among incident cancer cases and 12,534 non-case National Health and Nutrition Examination Survey (NHANES) I study participants</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.1</td>
<td>Mean serum albumin by number of years in trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)</td>
</tr>
<tr>
<td>2.2</td>
<td>Mean iron binding capacity by number of years in trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)</td>
</tr>
<tr>
<td>2.3</td>
<td>Mean serum iron by number of years in trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)</td>
</tr>
</tbody>
</table>
CHAPTER 1

Introduction

The Acute Phase Response

The acute phase response (also referred to as the acute inflammatory response or the non-immune inflammatory response, hereafter referred to as the APR) is an adaptive response to cellular injury, infection, or inflammation (Kushner, 1982; Cruickshank et al., 1989; Mackiewicz, 1997). During an APR tissue adjacent to the injury becomes inflamed (Kushner 1982; Elmquist et al., 1997). In the absence of chronic disease, infection or inflammation, the APR is short-lived - lasting no more than a few weeks (Mackiewicz, 1997; Biro et al., 1998; Feelders et al., 1998). However, in individuals with cancer and/or chronic inflammation, the APR itself can become chronic. Mackiewicz (1997) states that:

"The acute phase response represents the substitution of new set-points for the homeostatic mechanisms that normally maintain stability of the internal environment during good health. In the face of tissue injury and infection, defense mechanisms take priority over optimal homeostatic states. The acute phase response may be transient, returning to normal with recovery, or can be persistent in chronic disease, paradoxically forming a chronic acute-phase response."

Most research on the APR in humans concerns the short-lived APR, usually focusing on the APR response to a transient assault, such as surgery. Relatively little is known about the long-lasting APR, or about the conversion from the short-lived APR to the chronic type.
Results from several investigations have suggested that, during the long-lasting APR, iron metabolism is considerably altered causing the condition known as the anemia of chronic disease (ACD) - a condition thought to (at least partially) result from increased production of serum ferritin (a protein for storage of serum iron) (Konijn, 1994; Means, 1995). ACD has been witnessed in individuals with an APR as demonstrated by decreased serum iron, decreased serum transferrin, and decreased survival time of red blood cells (Cash and Sears, 1989; Feelders et al., 1998; Means, 1995). Another difference between the short-lived APR and the long-lasting type is that the regulatory mechanisms differ, although this difference is, thus far, poorly characterized (Biro et al., 1998).

The short-lived APR is thought to be initiated, regulated, coordinated, and terminated by a complex activated cytokine cascade (Baumann and Gauldie, 1994; Feelders et al., 1998). Two primary changes are associated with the APR. First, the temperature set-point in the hypothalamus (febrile response) is altered (Moshage, 1997). Three cytokines released from injured tissue - interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-6 (IL-6) - appear to regulate the febrile response (Elmquist et al., 1997; Mackiewicz, 1997). Production of IL-1 and TNF-alpha by activated macrophages and monocytes appear to initiate the cascade (Baumann and Gauldie, 1994; Mackiewicz, 1997; Feelders et al., 1998; Gabay and Kushner, 1999). Second, the pattern of biosynthesis of certain proteins by the liver - acute phase (response) proteins (APPs - proteins whose concentrations are altered by at least 25 percent in response to inflammation) - is changed (Elmquist et al., 1997; Mackiewicz,
1997). This altered pattern of protein synthesis is complex in its regulation, although most findings suggest that IL-6 regulates the expression of many, if not most, hepatic genes of APP’s (Le and Vilcek, 1989; Baumann and Gauldie, 1994).

Clinically, the results of changes in temperature and protein biosynthesis are profound. Symptoms associated with the APR include: fever, somnolence, anorexia, increased synthesis of some endocrine hormones (such as thyroxin), decreased erythropoiesis (red blood cell production), alterations in plasma cation concentrations, inhibition of bone formation, negative nitrogen balance (most likely a result of decreased protein synthesis by skeletal muscle), and alterations in lipid metabolism (Elmquist et al., 1997; Mackiewicz, 1997; Moshage, 1997). It has been difficult to determine whether many of these APR effects occur as the primary result of the APR, as the secondary result of altered serum protein levels, or in association with disease state(s).

Among individuals with cancer, the prolonged production of APR-regulating proinflammatory cytokines may cause or be associated with cancer cachexia. Approximately 50 percent of cancer patients demonstrate symptoms of cachexia, a syndrome characterized by anorexia, wasting, weight loss, weakness, fatigue, a loss of adipose tissue and skeletal muscle mass, and an elevation in basal metabolic rate and increased energy expenditure (Toomey et al., 1995; Puccio and Nathanson, 1997; Tisdale, 1997; Mulligan and Bloch, 1998). Cachexia is not reversible by nutritional supplementation (Tisdale, 1997), or increased caloric intake (Puccio and Nathanson, 1997). Unlike weight loss due to starvation, weight loss in cancer is the result of both muscle and fat loss, and the process is characterized by increased catabolism of skeletal
muscle and simultaneous alterations in protein synthesis. IL-6, TNF-alpha, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), and interferon gamma (INF-gamma) are involved in the cachexia-associated loss of skeletal muscle (Moldawer and Copeland, 1997; Tisdale, 1997; Matthys et al., 1998).

Much of the research on APP's concerns the switch in protein synthesis away from peripheral tissues and toward the liver, and the resulting change in metabolism that may be responsible for cancer cachexia (Geiger et al., 1988; Falconer et al., 1994; Mackiewicz, 1997). The processes mediating cancer cachexia are complex. In short, the bulk of the research has been guided by searches for cytokines governing APR-induced cachexia and the pharmaceuticals that may disrupt the process (Tracey et al., 1988; Strassmann et al., 1989; Falconer et al., 1994; Mackiewicz, 1997; Seymour et al., 1997). Pharmacologic intervention using cytokine-specific antagonists or inhibitors for cachexia may be capable of reversing the wasting process (Moldawer and Copeland, 1997; Matthys et al., 1998). However, clinical trials demonstrate that the regulation of cachexia may be too poorly understood to attempt clinical trials of single agents that are specific inhibitors of cytokines. For example, pentoxifylline was found to inhibit TNF-alpha, but failed as a therapeutic intervention for cachexia (Haslett, 1998). It is likely that, as stated by Matthys et al. (1998) “cachexia can rarely, if ever, be attributed to one single cytokine but rather to a set of cytokines that work in concert in cachexia.”

However, nonsteroidal antiinflammatory drugs (NSAID's) and fish oils rich in n-3 polyunsaturated fatty acids appear to impact some of the cytokines governing cachexia. n-3 polyunsaturated fatty acids contain eicosapentaenoic acid (EPA) and docosahexanoic
acid and have been found to down-regulate interleukin-1 (IL-1) and TNF-alpha in vitro (Endres et al., 1991). n-3 fatty acid-supplemented healthy individuals have been found to have lower concentrations of positive acute phase proteins (Emst et al., 1991; Meydani et al., 1993). Wigmore et al. (1997) found that fish oil supplementation significantly reduced C-reactive protein levels in individuals with cancer of the pancreas. Tashiro et al. (1998) found that EPA supplementation to postoperative esophageal cancer patients reduced IL-6 production and improved cell-mediated immunity. Several nonsteroidal antiinflammatory drugs (NSAID’s) have also been found to mediate APR-associated cytokines, perhaps because cytokine effects are mediated via the cyclo-oxygenase pathway, and NSAID’s inhibit cyclo-oxygenase (Seibert et al., 1995). The author speculates that fish intake and NSAID use may decrease cancer risk and prolong cancer survival time by down-regulating the APR.

Though the study of APPs has a long history, an understanding of all affected proteins and their actions remains incomplete (Mackiewicz, 1997). Moreover, even less is known about APR effects on non-protein plasma components. However, there is evidence suggesting changes in trace element concentrations (iron, zinc, and copper) during, or as a result of, the APR (Fraser et al., 1989; Thurnham, 1997; Sattar et al., 1997). Similar findings have been reported for serum antioxidants (Louw et al., 1992; Talwar et al., 1997).

The first recognition of what is now referred to as the APR can be traced back to the ancient Greeks, who recognized that the blood of sick individuals formed four layers prior to clotting (Mackiewicz, 1997). Results from studies early this century first
suggested that the blood of individuals with inflammatory diseases had a more rapid erythrocyte sedimentation rate (ESR) and, later, that the rapidity was due to elevated plasma fibrinogen - an APP, and immunoglobulins (Mackiewicz, 1997). In 1930, C-reactive protein (CRP) was identified in sera of individuals with pneumococcal pneumonia and acute phase was first used to describe sera containing CRP (Mackiewicz, 1997). CRP was originally named for its binding of the C-polysaccharide of Pneumococcus. Since that time, many more proteins have been found to be associated with the APR including alpha-1 acid glycoprotein, alpha-1 protease inhibitor, antithrombin III, ceruloplasmin, and haptoglobin. This discussion will primarily focus on a prototypic APP - CRP, and the most ubiquitous APP - serum albumin.

To classify APP's there are three systems (Mackiewicz, 1997; Moshage, 1997). (Because there is great interspecies variability [Mackiewicz, 1997], the proceeding discussion applies only to humans.) First, based on the direction of change in concentration, APPs can be positive or negative. Negative APPs are decreased in concentration during the APR, apparently to allow an increase in the capacity of the liver to synthesize positive APPs. Progressively more hepatocytes are recruited to synthesize positive APPs (Macintyre et al., 1982; Cruickshank et al., 1989; Moshage, 1997). CRP is a positive APP with a concentration increase of up to 1000-fold and albumin is a negative APP with a concentration decrease of up to 50 percent (Cruickshank et al., 1989; Mackiewicz, 1997). Second, based on concentration kinetics, APPs can be either first- or second-line proteins. Both CRP and serum albumin are first-line APPs. The changes in their concentrations reach peaks one to three days after stimulation of the APR and return
to normal relatively quickly (Louw et al., 1992; Mackiewicz, 1997). Little is known about
the concentrations of CRP and serum albumin during a prolonged APR. Among negative
APP’s (prealbumin, albumin, and transferrin) because of differences in half-lives, the
drop in prealbumin is seen first, while the concentration of albumin may take longer to
drop significantly.

Third, and less understood, based on the cytokine group responsible for inducing
gene expression, type I APPs are induced by TNF-alpha and, synergistically, by IL-6,
while type II APPs are induced only by IL-6 (Baumann and Gauldie, 1994; Mackiewicz,
1997). CRP is a type I APP, but albumin is unclassified because its induction is less
understood (Baumann and Gauldie, 1994; Mackiewicz, 1997). However, Seymour et al.
(1997) reported that IL-6 is inversely correlated with serum albumin ($r = -0.43, p =
0.0003$) among 65 individuals with Hodgkins disease, and Yanagawa et al. (1995)
reported that serum albumin is lower in lung cancer patients with detectable serum IL-6
levels than in both those without detectable serum IL-6 levels and patients with benign
lung diseases. Several investigations have shown that administration of IL-6 can result in
alterations of most acute-phase reactants (Banks et al., 1995; Schuler et al., 1996).
However, another study suggested that concentrations of IL-6 in cancer patients were
correlated only with CRP and fibrinogen, and not with other acute phase reactants (Gallo
et al., 1995). Walther et al. (1998) found that IL-6 was associated not only with decreased
serum albumin ($p = 0.034$), but also with fever - a characteristic of the APR ($p = 0.051$).
Albumin

Albumin, the most abundant protein in plasma, constitutes approximately 60 percent of the protein in plasma. Approximately forty percent of albumin is present in plasma and 60 percent is extravascular (Rothschild, 1988). Albumin is formed, principally, in the liver at a rate of 12 grams per day. Albumin has three primary functions. First and most importantly, because of its high concentration in plasma and low molecular mass (69-70 kilodaltons), albumin maintains much of the colloid osmotic pressure of plasma (Hastings and Wolf, 1992; Wagner and DAmelio, 1993; De Gaudio, 1995). Therefore, albumin is an important regulator of the exchange of water between plasma and interstitial compartments. Albumin is too large to pass through capillary membrane filtration and small enough to be abundant, as opposed to formed elements which are too large and/or few in number to exert an effect on colloid osmotic pressure (Hastings and Wolf, 1992; Wagner and DAmelio, 1993). Water is pulled through capillary walls into tissue spaces until the pull of albumin molecules causes it to stop. Therefore, a lower concentration of albumin in plasma causes an increase in the flow of water out of plasma (Hastings and Wolf, 1992; Wagner and DAmelio, 1993). This increase in tissue fluid can result in edema and is apparent during inflammation. Second, perhaps because of its ability to fluctuate between several isomeric forms in aqueous solution, albumin serves as a carrier and distributor protein for various dissimilar ligands including vitamins, free fatty acids, amino acids (especially tryptophan and cysteine), some steroid hormones (such as cortisol when its specific binding globulin is saturated), bilirubin, metals (copper, calcium, and zinc), some plasma tryptophan, and
pharmaceuticals, including aspirin and penicillin (Rothschild, 1988; Doweiko and Nonpleggi, 1991). Third, albumin serves as a precursor to many tissue proteins (Doweiko and Nonpleggi, 1991; Rothschild, 1988). In addition to these functions, transthyretin (also referred to as prealbumin), included in the fractional determination of plasma albumin, acts with albumin and thyroxin-binding protein to transport thyroxin and iodothyronins (Doweiko and Nonpleggi, 1991).

Normal serum albumin values range from 3.5 to 5 grams per deciliter. Changes in serum albumin concentrations can result from at least three factors. First, tobacco smoking decreases serum albumin (Das, 1985). Second, because albumin maintains osmotic pressure, albumin is sensitive to hydration (Hastings and Wolf, 1992; Wagner and DAmelio, 1993). A dehydrated individual will present a low serum albumin level, but the level returns to normal quickly upon hydration (Hastings and Wolf, 1992; Wagner and DAmelio, 1993). Third, results from most investigations have suggested that age is inversely associated with serum albumin in both males and females (Keating et al., 1969; Leto et al., 1970; Greenblatt, 1979; Pickart, 1983; Campion et al., 1988; Salive et al., 1992). A meta-analysis of 12 studies revealed that median serum albumin levels declined nine to 12 percent after age 30, for males and females combined (Dybkaer et al., 1981). In 4,115 individuals over age 70, the distribution of serum albumin for both males and females was found to be reasonably represented by a bell-shaped curve and the inverse association with age in this group was maintained after adjustment for age-related diseases (Salive et al., 1992). Salive et al. (1992) reported that, for a study population of individuals over age 70 years, the following factors increased risk for hypoalbuminemia
(albumin less than 3.5 grams per deciliter): elevated serum creatinine, anemia, prior hip fracture, recent cancer, current institutionalization, at least two physical limitations in daily activity, smoking at least 20 cigarettes per day, age over 80 years, and female sex.

Many diseases and health states have also been associated with changes in the concentration of serum albumin. For example, Herrmann et al. (1992) reported that low serum albumin increases adjusted odds for hospital stays of at least 10 days from the following health conditions: cancer, chronic obstructive pulmonary disease (COPD), diabetes, congestive heart failure (CHD), myocardial infarction (MI), stroke, mental illnesses, and cholecystectomy. Routinely-measured APR-induced concentration changes, such as that which may occur in the concentration of serum albumin, have been found to provide reasonably high sensitivity and specificity for cancer screening. For example, Pejovic et al. (1997) found that serum albumin and alpha 1-antitrypsin, a positive acute phase protein, have relatively high accuracy as a screening tool for kidney cancer.

It is speculated that changes in serum albumin result from either decreased albumin synthesis, increased albumin transvascular loss, or increased degradation of albumin (Rothschild, 1988; Doweiko and Nompleggi, 1991). Decreased synthesis may occur as a result of liver disease, dietary energy deficiency, or dietary protein deficiency (Rothschild, 1988; Doweiko and Nompleggi, 1991). However, regarding dietary factors, there is little evidence that anything but severe nutritional deficiency results in decreased serum albumin. Theoretically, in protein malnutrition, there is too little available protein for the liver to synthesize albumin (Doweiko and Nompleggi, 1991). Increased loss of albumin may occur as a result of kidney diseases characterized by increased albumin in
urine (nephrotic syndrome) (Kaysen and al Bander, 1990; Kaysen, 1993). Diseases of the large intestine may cause a loss of plasma into lumen and, therefore, a loss of albumin in stool (Rothschild, 1988; Doweiko and Nompleggi, 1991). In addition, severe burns result in protein loss from affected areas (Lewis, 1980). With regard to cancer, increased breakdown of albumin within the lysosomal system of the tumors (and not other tissues) of sarcoma-bearing mice has been shown to, at least in part, explain lower serum albumin levels (Andersson et al., 1991). It has been theorized that increases in both the tumor size and the tumor lysosomal uptake of albumin explain this increased breakdown. (Andersson et al., 1991). (Hypotheses concerning the mechanisms governing albumin loss are discussed further in Chapter 2 and Chapter 5.)

The findings that serum albumin levels are lower in individuals with cancer and that higher serum albumin affords longer survival from cancer have been reported by many investigators. Serum albumin has been found to predict survival from non-small cell lung cancer (NSCLC) (Espinosa et al., 1995; Hespanhol et al., 1995), small-cell lung cancer (SCLC) (Maestu et al., 1997), colorectal cancer (Elder et al., 1986; Fountzilas et al., 1996), breast cancer (Coates et al., 1990; Yamasaki et al., 1992), ovarian cancer (Parker et al., 1994; Warwick et al., 1995), metastatic malignant melanoma (Sirott et al., 1993), renal cell carcinoma (Citterio et al., 1997), pancreatic cancer (Falconer et al., 1994), hilar cholangiocarcinoma (Su et al., 1996), hepatocellular carcinoma (Kouroumalis et al., 1997), lymphoma (Bastion et al., 1997), glioblastoma multiforme (Schwartzbaum et al., In Press, 1999) and, in general, advanced cancer (Maltoni et al., 1997). However, it is possible that any tumor (benign or malignant) may induce changes
in serum albumin. For example, rats injected with a chemical to produce benign cystic
tumors were found to have significantly lower serum albumin levels after five days, and
up to 21 days, than rats not injected, and, therefore, without tumors. (Kawashima et al.,
1989).

Explanations for the finding that serum albumin is decreased in individuals with
various neoplasms follow:

1. The effect occurs pre-disease or concurrent with disease and is a result of
insufficient dietary protein intake among individuals with cancer (Phillips et al., 1989).
There is little evidence to support this hypothesis. Merritt et al. (1985) report little
difference in both the protein and energy intake between pediatric cancer patients with
low and normal serum albumin. Cruickshank et al. (1989) report only a slightly higher
serum albumin among undernourished gastric and colorectal cancer patients electing
surgery compared to patients with standard body weight and triceps skinfold thickness. A
meta-analysis concerning the effect of total parenteral nutrition (TPN) on serum albumin
among cancer patients revealed that the serum albumin of patients receiving TPN was no
different than that of controls (Karlawish et al., 1994). Similarly, other studies suggest
that serum albumin responds slowly to (even protein-specific) nutritional status (Reeds
and Laditan, 1976; Russell et al., 1983; Donaldson et al., 1981; Fleck et al., 1985; Soeters
et al., 1990). However, the results of Lyoumi et al. (1998) suggest that, in rats, severe
dietary protein restriction is capable of inducing IL-6, as well as alpha-2 macroglobulin -
a positive APP. It may be that only severe dietary protein malnutrition is capable of
inducing the APR, and, consequently, a decreased concentration of serum albumin.
2. The finding may be a result of several transport and distribution functions of albumin. First, the concentration of circulating carcinogens or the levels of androgenic steroids that control tumor growth may be altered by serum albumin (Williams et al., 1990). Albumin also transports many pharmaceuticals and, therefore, low serum albumin may increase the free interaction of these drugs with non-blood tissues. When serum albumin is low the concentration of unbound pharmaceuticals is increased and this, mediated by individual clearance ability, may impact cancer risk if the pharmaceuticals are mutagens, teratogens, and/or carcinogens to tissues they were (perhaps) unintended to interact with (Kock-Weser and Sellers, 1976; Herrmann et al., 1992).

3. The effect may be a result of interactions between albumin and platelets. Serum albumin has been found to be associated with a lower platelet count (Janisch et al., 1994; Moutel et al., 1997). Albumin inhibits the promoting effect of free fatty acids on both platelet aggregation and thrombosis (Hoak et al., 1966), and provides protective coats to platelets (Rossi, 1972). Moutel et al. (1997) reported that serum albumin concentrations were higher and platelet counts were lower among pharyngeal cancer patients surviving longer compared to those with shorter survival. Similar survival results were reported by Janisch et al. (1994) for a relatively large (n=349) group of individuals with advanced or metastatic cancers at several sites. However, another study revealed that there was no significant difference in blood platelet counts between lung cancer patients and those with benign lung disease (Yanagawa et al., 1995).

4. Because both ischemia (Shearman et al., 1986) and malignancy (Fleck et al., 1985) are associated with increased vascular permeability, the associations between serum
albumin and these conditions may be a result of disease-related albumin leakage from vasculature (Gosling et al., 1990). (Vascular permeability is more thoroughly discussed in Chapter 2.)

5. The findings are due to other serum proteins - globulins. Darne et al. (1990) reported that the previously strong association between serum albumin and mortality retreated after adjustment for serum globulin. Similar results were reported by Sweetnam and Yarnell (1990). However, in both of these studies, albumin maintained at least a marginally statistically significant association, though the associations with globulin were stronger. The theory that globulins are more important is also supported by the finding that alpha 2-globulin, itself, is a risk factor for cardiovascular disease (Ducimetiere et al., 1976). (It should be noted that serum globulin levels are often calculated by subtracting serum albumin from total serum protein. Low serum albumin infers high globulin, and vice versa. Because of this, Phillips et al. [1989] measured globulins directly and reported results suggesting that serum albumin was the more important protein regarding mortality.)

6. Because albumin may function in iron acquisition and excess iron has been found to increase cancer risk (Selby and Friedman, 1988; Knekt et al., 1994; Wurzelmann et al., 1996), low serum albumin may result in increased iron availability to tumor cells and potential tumor cells (Stevens et al., 1986).

7. Albumin exhibits antioxidant activity (Soriani et al., 1994; Karten et al., 1997). Halliwell (1988) speculated that a relatively small fraction of albumin may scavenge free radicals and, in the process, be destroyed, and that albumin is so abundant that this loss is
insignificant to the functions of albumin. Additionally, the cysteine residue of serum albumin has been found to scavenge carbon-centered free radicals (Soriani et al., 1994). However, the results of Butcher et al. (1995), in a study of 19 individuals with alcohol-related liver disease, suggested that concentrations of serum albumin were not correlated with free radical concentration.

8. Low serum albumin may lead to decreased synthesis of conjugated linoleic acid (CLA) from linoleic acid because albumin is required for this conversion (Stevens et al., 1990). Supporting this line of thought, desBordes (1995) suggested that the anti-cancer effects (growth inhibition and stimulation) of C18 unsaturated fatty acids on cancer cells are influenced, at least in part, by the ratio of the albumin to fatty acid concentrations.

9. Perhaps the most studied and most complex explanation is that lower serum albumin levels result from (and are markers for) the APR. There are many findings supporting this hypothesis. For example, Merritt et al. (1984) found that lower serum albumin was strongly associated with high temperature (the febrile response, an occurrence of the APR) among pediatric oncology patients.

It is feasible that each of these factors plays some role. The theories are not mutually exclusive.
C-reactive Protein

CPR is considered an ideal marker for the induction of the APR (Mackiewicz, 1997). Because it is easy and inexpensive to measure, CRP is used as a nonspecific biomarker of tissue damage and infection (Goransson et al., 1996). CRP functions as an opsonin for bacteria, parasites and immune complexes. It binds pathogens and damaged cells interacting with effectors to initiate their elimination (often via uptake by phagocytes) (Volanakis, 1982; Mackiewicz, 1997). CRP also activates the classical pathway of the complement, inhibits platelet-activating factor, thus inhibiting platelet aggregation, and mediates phagocyte function (Tebo and Mortensen, 1990; Mackiewicz, 1997).

With respect to cancer, CRP levels are associated with immunologic functioning (Goransson et al., 1996). The quick responsiveness of CRP to the APR (CRP appears in serum 4-6 hours after initiation of the APR) in addition to its wide range in concentration, have led to the use of CRP levels to monitor the severity of inflammation and the APR. Albumin responds less quickly, and its concentration is more restricted and, therefore, decreases in serum albumin are more easily overlooked as markers of APR induction or severity. Like albumin, tobacco smoking has been found to alter the concentration of CRP in the direction associated with the APR (Das, 1985). However, the association between age and CRP has been, largely, neglected. Sattar et al. (1997) reported no significant difference in age between apparently healthy controls, NSCLC patients with high CRP, and NSCLC patients with low CRP.
CRP levels are increased in individuals with cancer (Mackiewicz, 1997; Talwar et al., 1997). Investigators have attributed this to an inflammatory response elicited by the tumor. However, CRP may also be a specific biomarker of malignant (versus benign) tumors. In a study of 261 individuals with cancer, preoperative CRP levels were found to be significantly elevated in those with malignant (versus benign) tumors (Goransson et al., 1996). In the same study, the CRP levels in colorectal cancer cases increased with increasing tumor burden, using Duke's classification to determine tumor burden (Goransson et al., 1996). Other studies have suggested that CRP levels are lower in breast cancer (compared to other cancers) because breast cancers represent smaller tumor burdens (Hillyard et al., 1982; Robertson et al., 1991). Both CRP and albumin were found to increase and decrease, respectively, according to postoperative clinical outcome/treatment (Goransson et al., 1996). That is, controls and individuals receiving radical operations had lower preoperative CRP levels and higher preoperative albumin levels than those receiving non-radical operations who, in turn, had lower preoperative CRP levels and higher preoperative albumin levels than those with a palliative treatment plan (Goransson et al., 1996). Stamatiadis et al. (1990) found that high CRP levels were associated with a more developed stage of colorectal cancer. However, Talwar et al. (1997) reported no difference in CRP level among different stages of NSCLC. Goransson et al. (1996) reported that the sensitivity and specificity of CRP to predict inoperable tumors was 79 percent and 71 percent, respectively, while the sensitivity and specificity of albumin was 94 percent and 54 percent, respectively.
Changes in the CRP concentration of individuals with cancer may be accompanied by changes in the concentration of antioxidants, as well as trace elements and their carrier proteins. In a study of 22 NSCLC cases and 13 healthy controls, cases were grouped by CRP level (less than 35 milligrams per liter, and greater than 35 milligrams per liter (Sattar et al., 1997). The higher CRP group was found to have lower median albumin, zinc, iron, transferrin, and selenium, while copper and ceruloplasmin were higher than both the low CRP group and controls (Sattar et al., 1997). To control for potential APR-related changes in the concentration of proteins, molar ratios of trace elements to their binding proteins were compared among the two cancer groups and controls. The ratio of zinc to albumin was lower in individuals with cancer than in controls, and the ratio of copper to ceruloplasmin was lower in the higher CRP group than in both the lower CRP group and controls (Sattar et al., 1997). Among these same NSCLC cases, Talwar et al. (1997) found that CRP was significantly correlated with retinol ($r = -0.682$), alpha-tocopherol ($r = -0.464$) and lutein ($r = -0.599$). Antioxidants may be consumed as a result of the generation of free radicals (Wiseman et al., 1996; Talwar et al., 1997). The association between CRP levels and trace elements may explain part of the diagnostic and prognostic capacity of CRP, since trace elements alter cancer risk and cancer survival (Strain, 1994; Sattar et al., 1997).

Increases in the concentration of CRP have also been associated with decreased survival from many cancers. The association between CRP and survival was strong in a small study (13 cancer patients, 7 healthy controls) of metastatic colorectal cancer ($r = -0.689$, $p = 0.006$) (Gough et al., 1996). In the same study, albumin also predicted
survival ($r = 0.655, p = 0.011$) as did the ratio of CRP to albumin ($r = -0.758, p = 0.002$) (Gough et al., 1996). High levels of CRP have been associated with shorter survival from myeloma (Bataille et al., 1992), metastatic spread to the liver (Ballou and Kushner, 1992), pancreatic cancer (Falconer et al., 1994), metastatic breast cancer (Albuquerque et al., 1995), and prostate cancer (Ekman and Lewenhaupt, 1991). Additionally, high levels of CRP predict tumor recurrence (Weinstein et al., 1984).
Research Questions

The goal of the present effort is to address four primary research questions concerning the APR. First, are initial mean concentrations of serum albumin and transferrin, the carrier protein for serum iron, different in groups of individuals later diagnosed with cancer or myocardial infarction (MI), and what is the behavior over time of these concentrations prior to cancer or MI diagnoses, compared to concentrations in individuals surviving without cancer or MI diagnoses? In addition, is the (potential) change in the concentration of serum transferrin concurrent with changes in serum iron. The purpose of this investigation is to determine how long prior to cancer and MI diagnoses these changes occur, by examining the intercepts and slopes of serum albumin, transferrin, and iron concentrations over a course of years prior to cancer and MI diagnoses after adjustment for potentially confounding factors, such as age and correlation across observations on the same individual. Negative slopes in the cancer or MI groups, but not in the group without cancer or MI, that differ from zero would suggest that APR-associated concentration changes occur preclinically. Results pertaining to this investigation are important because there are no known studies of the behavior of acute phase proteins during the years prior to cancer or MI diagnoses. Decreasing serum albumin and transferrin levels long before diagnosis would provide some evidence that a prolonged APR occurs early in the development of tumorogenesis and atherosclerosis.

Second, since smoking is associated with the APR, are routinely-measured acute phase reactants (serum albumin and transferrin [assessed as iron binding capacity], white blood cell count [WBCC], and erythrocyte sedimentation rate [ESR]) altered prior to
diagnosis of two smoking-related cancers: bladder and lung cancer? Are 
routinely-measured acute phase reactants measured once at baseline associated with 
increased risk of lung or bladder cancer? Such alterations in risk may, again, suggest that 
a prolonged APR results in serologic changes similar to those witnessed in the short-lived 
APR, and that, like potential results from the first research question, the APR occurs early 
in tumor development. Alterations in WBCC years prior to cancer diagnosis may also add 
support to the expanding literature suggesting that infections play a causal role in the 
development of some cancers.

Third, are concentrations of serum micronutrients altered during the APR after 
control for confounding by dietary factors? APR effects on serum micronutrients have 
been largely ignored, and may be important in identifying the APR as a potential 
confounding factor in case-control studies of serum micronutrients and disease.

Fourth, is the decrease in serum albumin level associated with the APR 
accompanied by an increase in urinary albumin excretion? If so, is urinary albumin 
excretion an indicator of cancer risk? This investigation may shed light on the mechanism 
of albumin loss from serum during the APR. If urinary albumin is related to the APR, but 
control for confounding by low serum albumin removes the association, the two 
ocurrences may reflect one mechanism of albumin loss, transvascular escape. 
Transvascular leakiness is associated with both the APR and cancer (as well as 
atherosclerosis). Urinary albumin excretion, therefore, may be an acute phase reactant, or 
may be a result of the tumor itself.
The four chapters that follow document the investigations of these four research questions.
CHAPTER 2

Serum Transferrin, Iron, and Albumin Concentrations Decrease Prior to Cancer and Myocardial Infarction Diagnoses

Abstract

Serum transferrin and albumin may decrease before diagnosis of cancer and myocardial infarction (MI). These alterations are probably the result of the acute phase response (APR), a systemic cytokine-governed adaptive reaction to cellular injury during which the pattern of protein synthesis in the liver is altered. To determine how long alterations in concentrations of serum transferrin and albumin occur prior to cancer and MI diagnoses, data were analyzed from The Lipid Research Clinics Coronary Primary Prevention Trial and follow-up study (n = 3,806 hyperlipidemic men). During an average in-trial follow-up of 7.4 years and an average combined in-trial and post-trial follow-up of 13.4 years, three groups of study participants were identified: 1) those first diagnosed with cancer (n=245); 2) those first diagnosed with MI (n=437); 3) those diagnosed with neither cancer nor heart disease (n=1,651). Changes in serum albumin, transferrin (assessed by iron binding capacity [IBC]), and iron were plotted against number of years prior to diagnosis (or the end of the trial, for the group developing neither cancer not heart disease) that each serum assessment occurred. Eight years before cancer and MI diagnoses mean serum albumin is significantly lower (cancer: 4.18 g/dl, 95 percent
confidence interval [CI]: 4.13 - 4.23; MI: 4.23, 95 percent CI: 4.19 - 4.27), and mean IBC significantly higher (cancer: 349.8, 95 percent CI: 341.6 - 358.1; MI: 350.9, 95 percent CI: 327.6 - 330.0), than eight years prior to the end of the trial in the group of study participants not diagnosed with cancer or heart disease (albumin: 4.31, 95 percent CI: 4.29 - 4.33; IBC: 330.3, 95 percent CI: 327.6 - 330.0). In addition, mean serum iron is significantly lower eight years prior to cancer diagnosis. To adjust serum transferrin, iron, and albumin over time for age at randomization, a mixed (both random and fixed factors) model regression was used. Serum albumin levels decrease slightly in each of the three groups. A statistically significant decrease in both IBC and serum iron is found in the years prior to cancer and MI diagnoses, while a statistically significance increase in IBC and serum iron is found in the group of study participants not diagnosed with cancer or heart disease. This long preclinical APR may explain discrepancies in results of studies of iron status and both cancer and MI risk. Potential mechanisms for APR-associated decreases include an increase in vascular permeability caused by the activity of vascular endothelial growth factor.
Introduction

The acute phase response (APR) is a cytokine-governed systemic adaptive reaction to cellular injury during which the pattern of protein synthesis in the liver is altered (Kushner, 1982; Cruickshank et al., 1989; Koj, 1996; Mackiewicz, 1997). In addition, the APR is characterized by leukocytosis (an elevated white blood cell count) and increased vascular permeability. The APR can be short-lived (lasting a few days to a few weeks) or can become chronic in individuals with cancer and/or chronic inflammation (Koj, 1996; Mackiewicz, 1997). Acute phase proteins (APP's - proteins whose concentrations are altered by at least 25 percent in response to inflammation) may alter risk for cancer (Stevens et al., 1988; Stevens, 1990; Knect et al., 1997; Selby and Friedman, 1998) and myocardial infarction (MI) (Jensen, 1995). These studies, however, were based on single measurements of APP's. Concentration changes of APP's have not been investigated over a period of years prior to cancer and MI diagnoses. Although changes in the concentrations of several APP's, such as C-reactive protein and alpha 2-macroglobulin, more specifically distinguish the APR, the present investigation concerns two routinely-measured APP's - serum transferrin and albumin.

Serum transferrin is the APP that binds and carries serum iron from absorption and storage sites to tissues requiring iron, although serum transferrin has also been found to be required for cell differentiation (Konijn, 1994; Means, 1995). Transferrin is often assessed by measuring iron binding capacity (IBC) - the number of binding sites available on transferrin (Gambino et al., 1997). The concentration of serum transferrin characteristically decreases during the APR (Mackiewicz, 1997). However, it is possible
that decreases in serum transferrin result from the anemia of chronic disease (ACD), a
type of iron storage (on another APP - ferritin), resulting in both
decreased serum iron and serum transferrin (Bior et al., 1998; Feelders et al., 1998). The
ACD is related to the APR via a similar mechanism of regulation - an activated cytokine
cascade (Bior et al., 1998; Feelders et al., 1998).

The principle mechanism by which iron contributes to carcinogenesis and
atherosclerosis may be its capacity to catalyze free-radical reactions (Okada, 1996;
Toyokuni, 1996; Weinberg, 1996). Iron-induced oxidative stress leads to redox regulation
failure and results in lipid peroxidation and oxidative DNA damage (Okada, 1996;
Weinberg, 1996). Changes in the concentrations of iron-associated proteins are important
because they impact the availability of serum iron to tissues, and because several
investigations have suggested a positive association between increased iron stores and
cancer risk (Stevens et al., 1986; Knekt et al., 1994). However, many of the results
concerning iron are conflicting. For example, the results of Wurzelmann et al. (1996)
suggested a positive association between serum iron and colorectal cancer risk (especially
among females), while Kato et al. (1999) found no association between serum iron and
colorectal cancer risk among females. Similarly, the results of Tuomainen et al (1998)
suggested an increased MI risk from high iron stores, while Sempos et al. (1994) found a
decreased MI risk from high iron stores, and Baer et al. (1994) found no association.
Results pertaining to serum iron and iron-associated proteins may be confounded by the
APR, in that decreases in APR-associated serum iron concentrations may occur prior to
cancer and MI diagnoses. Therefore, risk for cancer from serum iron may depend on how near time of diagnosis serum assessments were made.

Interest in concentration changes of serum transferrin and albumin, in the present study, stems from the fact that simultaneous changes in both of these proteins increase the likelihood that both effects reflect one APR, whereas changes in only one may result from a cause unrelated to APR.

Serum albumin maintains most of the colloid osmotic pressure of plasma (Hastings and Wolf, 1992; Wagner and DAmelio, 1993), carries various dissimilar ligands including vitamins, free fatty acids, amino acids, and pharmaceuticals, and serves as a precursor to many tissue proteins (Rothschild, 1988; Dowelko and Nompleggi, 1991). Decreases in the concentration of serum albumin can result from tobacco smoking (Das, 1985), dehydration (Hastings and Wolf, 1992; Wagner and DAmelio, 1993), and increasing age (Keating et al., 1969; Leto et al., 1970; Greenblatt, 1979; Pickart, 1983; Campion et al., 1988; Salive et al., 1992). The finding that higher levels of serum albumin afford longer survival from cancers at many sites has been reported in numerous investigations (Elder et al., 1986; Coates et al., 1990; Yamasaki et al., 1992; Sirott et al., 1993; Falconer et al., 1994; Parker et al., 1994; Espinosa et al., 1995; Hespanhol et al., 1995; Warwick et al., 1995; Fountzilas et al., 1996; Su et al., 1996; Bastion et al., 1997; Citterio et al., 1997; Kouroumalis et al., 1997; Maestu et al., 1997; Maltoni et al., 1997; Schwartzbaum et al., In Press, 1999). Similarly, risk for MI is lower and survival from MI is longer among individuals with higher levels of serum albumin (Kuller et al., 1991; Corti et al., 1996; Kuller, et al., 1996).
It is unclear whether concentrations of serum transferrin and albumin decrease prior to onset of disease. In epidemiologic studies, the sequencing of events has been difficult to establish. That is, the changes associated with the APR (altered concentrations of APP's) may be results of preclinical neoplasia or atherosclerotic lesions, or may occur with their development. Pre-diagnostic serial serum measurements provide the opportunity to analyze the behavior of these APP's with respect to the time before cancer and MI diagnoses. If serum transferrin decreases as the result of the ACD, and only indirectly as a result of the APR, it would be expected that adjustment for confounding by decreasing serum iron would explain (reduce or eliminate) the decrease in serum transferrin. Analogously, if decreases in serum transferrin and albumin both occur as a result of the APR and concurrently, adjustment for confounding by one may explain the decrease in the other.

This investigation was conducted to determine how long prior to diagnoses of cancer and MI APP concentrations decrease, and to determine whether a (potential) decrease in one APP can be explained by a decrease in the other. Because changes in the concentrations of both serum transferrin and ferritin affect the concentration of serum iron, and because decreasing serum iron concentrations may explain discrepancies among investigations of serum iron and cancer risk, an additional aim of this investigation was to determine whether the concentration of serum iron decreases preclinically with serum transferrin.
Methods

*Study Population.* To examine serial serum albumin, transferrin, and iron prior to diagnoses of cancer and MI, data were analyzed from The Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) and follow-up study, a 12-center double-blind North American clinical trial of the effect of lowering serum cholesterol on coronary heart disease (CHD) incidence. The design, implementation, and results of the LRCCPPT and follow-up study are described more thoroughly elsewhere by others (Lipid Research Clinics Program, 1982; Lipid Research Clinics Program 1983; Lipid Research Clinics Program, 1984; Probstfield and Rifkind, 1991; Schaefer et al., 1994). In brief, during a 33-month period between 1973 and 1976, 3,806 males 35 to 59 years of age without clinically apparent CHD (no history of hypertension, MI, or angina), and without history of clinically apparent cancer (except for nonmelanoma skin cancer) and with (only) type II-a hyperlipoproteinemia (total cholesterol > 265 mg/dl, low-density lipoprotein [LDL] > 190 mg/dl, and triglycerides < 300 mg/dl) were randomly assigned to two groups. The following conditions resulted in exclusion: men diagnosed with conditions related to type II-a hyperlipoproteinemia including diabetes mellitus, liver disease, thyroid disease, nephrotic syndrome, obesity, and hyperuricemia; those deemed likely to have poor adherence to protocols; and those requiring medications potentially interfering with the study medication. Additionally, study participants were excluded based on repeatedly unusual or out-of-range serologic measurements. Of interest to the current investigation, study participants with pre-randomization levels of serum albumin
less than 3.0 g/dl, serum globulin greater than 4.0 g/dl, or serum iron less than 55 ug/dl were excluded.

After four screening pre-randomization visits, one group (n=1,906) was asked to orally self-administer a total of 24 grams of cholestyramine, a serum cholesterol-lowering bile acid sequestrant, daily and the other (n=1,899), a placebo. All study participants were prescribed a cholesterol-lowering diet of approximately 400 milligrams of daily cholesterol intake and a polyunsaturated-to-saturated fat ratio of 0.8. Thereafter, general medical histories were ascertained and physical examinations were conducted at bimonthly visits. More detailed medical histories were taken and examinations performed at annual visits. Information about other cancer and CHD risk factors and potential risk factors, such as the use of tobacco and alcohol was also collected at annual visits. At semiannual visits, a 24-hour dietary recall questionnaire was used to obtain estimated nutrient intakes, and blood was drawn for serologic assessment, including assessment of serum albumin and iron. Although serum transferrin was not measured, the investigators used iron binding capacity (IBC), which was measured, as an index of serum transferrin because it is highly correlated with serum transferrin (Gambino et al., 1997). The trial concluded in April, 1983. After the trial, study participants were invited for yearly physical examinations and medical histories for an additional six years. The present investigation reflects both the in-trial and post-trial portions of the study. However, serum was collected at in-trial visits only. Data from the post-trial portion affect only case (cancer and MI) accrual.
Comparison Groups (Cancer, MI, and Neither). International Classification of Diseases (ICD) codes were collected about all diagnoses associated with hospitalizations, both in-trial and post-trial. To create exclusive groups, only study participants' first diagnoses were considered. For example, if a study participant was diagnosed with cancer, then experienced an MI, he would be included only in the cancer group. The LRCCPPT and follow-up study coded CHD endpoints as either definite or suspect. That is, for example, a study participant may have been coded as having either a “definite MI” or a “suspect MI”. An MI case, for this analysis, was defined as any study participant experiencing a definite MI in-trial or post-trial. A cancer case was defined as a study participant with an ICD code, either in-trial or post-trial, ranging from 140 to 209. (These ICD codes exclude benign neoplasms [ICD codes 210 to 229], carcinomas in situ [ICD codes 230 to 234], and neoplasms of uncertain behavior [ICD codes 235 to 240]). In addition, a cancer case must have experienced any of the following definite CHD-related events or symptoms after the cancer diagnosis, if at all: CHD death, MI, brain infarction, transient cerebral ischemic attack, congestive heart failure, hospitalized intermittent claudication, coronary bypass surgery, resuscitated coronary collapse, peripheral vascular disease, intraoperative MI, coronary angioplasty, or other coronary-related hospitalization. A study participant was included in the “neither” group if he survived to the end of the trial, and was not diagnosed with cancer or any definite or suspect CHD-related disease or symptom (including angina) during the trial or follow-up. These three groups do not exhaust the 3,806 LRCCPPT study participants because the healthy (“neither”) group was restricted to exclude study participants with even suspect
CHD-related diagnoses and symptoms, while the cancer group, to maintain a reasonable sample size, excluded only study participants with definite CHD-related diagnoses and symptoms prior to a cancer diagnosis. Study participants first experiencing a non-MI CHD event or symptom were not included in any of the three groups.

*Statistical Methods - Descriptive.* To describe differences between the three groups described above, average pre-diagnostic means and standard errors of demographic, serologic, and dietary factors were calculated. Means of pre-diagnostic serial serum albumin, IBC, and serum iron were compared over time between the three groups (cancer, MI, and “neither”). Because many study participants were followed (in-trial and post-trial) for over 13 years, it was possible for serum assessments to have occurred, at most, 14 years prior to the diagnosis of cancer or MI. The distances between the time of cancer or MI diagnosis (or, for the healthy group, the end of the trial) and the time of serum assessment were grouped into 14 one-year categories. The means (y-axis) were plotted against the greater of the two years between which the serum assessment occurred (x-axis). That is, for example, a five on the x-axis would correspond to the mean of serum assessments which occurred between the fourth and fifth year prior to diagnosis. Using this method, decreasing serum albumin, IBC, or serum iron can be observed graphically by a negatively-sloped line. Because study participants surviving to the end of follow-up without a cancer or MI diagnosis had no definitive endpoints during the post-trial portion of the trial, only the years for which a serum assessment was made (in-trial) could have corresponding means. For this reason, the method described for the cancer and MI groups produced fewer graphical points for the “neither” group. Therefore,
for the cancer and MI groups, eight one-year categories were examined, so that comparisons with the control group could be made.

**Statistical Methods - Inferential.** While these graphical analyses display changes in APP concentrations, there are at least three disadvantages to this type of analysis - the inability to test hypotheses that slopes differ from zero, no consideration that measurements are repeated on study participants, and no capacity for adjustment of confounding factors. A standard repeated measures analysis could not be conducted due to considerable missing data. Therefore, to test the hypotheses that slopes reflecting APP concentration changes prior to cancer and MI diagnoses differ from zero, a method described by Wolfinger (1997) was used. This method permits adjustment for confounding within a repeated measures design using a mixed (both random and fixed factors) model, given both missing data and unequal distances between measurements. Serum albumin, IBC, and serum iron were modeled as dependent variables, while the distance between serologic assessment and cancer or MI diagnosis (or, for the healthy group, the number of days since randomization) was modeled as an independent variable, along with confounding factors. Briefly, using Statistical Analysis System (SAS) implementation though the MIXED procedure, Wolfinger (1997) proposed comparing nine models - the general mean model, and eight mixed models differing by the structure of their covariance matrix. If the covariance structure is not reasonable or parsimonious, the data will not converge. Among those models converging, the best model is proposed to be the one with the largest Bayesian Information Criterion (BIC) statistic (Wolfinger, 1997).
Sufficient incidence of skin, lung, prostate, and colorectal cancers permitted subgroup analyses of study participants diagnosed with these cancers. Because the purpose of this investigation was to determine whether slopes of changes in serum albumin, IBC, and serum iron differ from zero in seven groups (all cancers combined. MI. “neither”, skin cancer, colorectal cancer, prostate cancer, and lung cancer), seven models were analyzed, for serum albumin, IBC, and serum iron. (It was possible to approach this analysis with one model containing dichotomous variables reflecting potential assignment into one or two of the seven groups. However, sample size did not permit such an analysis.) All statistical methods were conducted using Statistical Analysis System (SAS).

Selection of Potential Confounders. Because they have been found to be associated with alterations in acute phase reactants, as well as cancer and MI risk, age and tobacco smoking (Das, 1985; Carter et al., 1997) were considered potential confounders. In addition, treatment arm (cholestyramine versus placebo) was considered a potentially confounding variable. Both dietary energy intake and body weight decrease as a result of (or in conjunction with) the APR. For example, McCarthy et al. (1985) found that rats injected with interleukin-1 (IL-1) (a mediator of the APR) demonstrated an APR, became anorexic, and lost significant body weight. Both energy intake and body weight were considered intermediate variables because adjustment for these factors may remove some of the APR-related changes in acute phase reactants. Although protein intake may decrease along with energy intake, because it has been suggested that serum albumin decreases as a direct result of insufficient dietary protein intake (Phillips et al., 1989), and because, in rats, severe dietary protein restriction has been found to induce interleukin-6
(IL-6) (another mediator of the APR), protein intake was considered a potentially confounding variable. The potential confounders were: age at baseline, treatment arm, number of cigarettes smoked per day, and daily protein intake. The selection of confounders was accomplished by determining the confounding factor with the greatest effect on the parameter estimate (slope). This process was repeated for the remaining confounding factors until the addition of remaining factors to the model had no (or a negligible) effect on the parameter estimate.
Results

*Descriptive Statistics.* During an average in-trial follow-up of 7.4 years and an average combined in-trial and post-trial follow-up of 13.4 years, the following three exclusive groups of study participants were identified: 245 study participants diagnosed with cancer, 437 diagnosed with MI, and 1,651 diagnosed with neither cancer nor any definitive or suspect cardiovascular event or disease. Table 2.1 shows the breakdown of the 245 cancer diagnoses by site.

Pre-diagnostic means of selected demographic and dietary characteristics of the cancer and MI groups are compared to means of these same factors among study participants diagnosed with neither cancer nor MI in Table 2.2. The three groups are similar with respect to treatment arm, as well as race, each consisting of approximately 97 percent white study participants (not shown in Table 2.2). Study participants in the "neither" group are significantly younger and smoke fewer cigarettes per day than study participants in both the cancer and MI group, and study participants in the MI group are younger than those in the cancer group. Among dietary factors, study participants in the MI group consume significantly less protein than those in the "neither" group.

Figure 2.1 shows means of serum albumin by time prior to either cancer diagnosis, MI diagnosis, or the end of the trial ("neither" group) with fitted regression lines. Serum albumin declines for all three groups (for cancer group, $R^2 = 0.33$ [p = 0.14]; for MI group, $R^2 = 0.16$ [p = 0.33]; for "neither" group, $R^2 = 0.37$ [p = 0.11]), although mean serum albumin is lowest eight years prior to cancer diagnosis compared to both eight years prior to MI and, for the "neither" group, eight years prior to the end of the
trial. Figure 2.2 shows means of IBC by time prior to either cancer diagnosis, MI diagnosis, or the end of the trial. Mean IBC decreases prior to both cancer and MI diagnosis (for cancer group, $R^2 = 0.43$ [$p = 0.08$]; for MI group, $R^2 = 0.34$ [$p = 0.13$]), but increases in the healthy group ($R^2 = 0.93$ [$p = 0.00$]). Serum iron (Figure 2.3), like IBC, is found to decrease in both cancer and MI groups, and to increase in the "neither" group. Means of both IBC and serum iron are lowest eight years prior to the end of the trial ("neither" group) compared to eight years prior to both cancer and MI diagnoses. Because these data are derived from a clinical trial of an agent purported to lower serum cholesterol, separate graphs were constructed for study participants in the two treatment arms, as well as for study participants with serum cholesterol above and below the median at randomization. These analyses reveal that the behavior of serum albumin and IBC are unrelated to treatment arm (placebo versus cholestyramine), or serum cholesterol (stratified at the median value) (figures not presented).

*Inferential Statistics.* Only two of the eight models proposed by Wolfinger (1997) permit convergence of the data: the general linear model and the model with compound symmetry. Here, compound symmetry refers to a common variance and covariance for each study participant. While the two models produce similar parameter estimates, the compound symmetry model is selected because its BIS is larger and because, conceptually, the compound symmetry model permits independence of intercepts across study participants, allowing baseline serum albumin, IBC, and serum iron concentrations to vary across study participants. After adjustment for age at baseline, no additional potential confounders require adjustment. Adjustment for confounding by number of
cigarettes smoked per day, protein intake, or treatment arm has negligible effects on estimates of intercepts or slopes.

Tables 2.3 through 2.5 show estimates of both intercepts and slopes and their 95 percent confidence intervals reflecting changes in serum albumin, IBC, and serum iron, respectively, prior to cancer or MI diagnosis, or prior to the end of the trial. Eight years before cancer and MI diagnoses mean serum albumin is significantly lower than eight years prior to the end of the trial in the group of study participants not diagnosed with cancer or heart disease, evidenced by non-overlapping 95 percent confidence intervals. After adjustment for age at randomization, serum albumin decreases prior to all events - cancer, MI, and, for the “neither” group, prior to end of the trial. However, the decrease is at least marginally statistically significant only for the following groups: all cancers combined, prostate cancer, MI, and “neither”. The steepest slope is seen in the group of study participants developing prostate cancer.

Eight years before cancer and MI diagnoses mean IBC is significantly higher than eight years prior to the end of the trial in the group of study participants not diagnosed with cancer or heart disease (Table 2.4). Age-adjusted statistically significant decreases in IBC are witnessed for all cancers combined, for each of the four specific cancer sites, and for the MI group. However, in the “neither” group, IBC increases with statistical significance. Similar results are found for serum iron concentrations (Table 2.5). Age-adjusted statistically significant decreases in serum iron are seen in the following groups: all cancers combined, skin and prostate cancers, and MI. Like IBC, serum iron increases in the “neither” group. The decreases in IBC and serum iron prior to both cancer
and MI diagnoses are stronger than decreases in serum albumin, and differences between the “neither” group and the other groups are more apparent for changes in IBC and serum iron.

If the concentrations of serum albumin and transferrin both decrease during the APR, it would be expected that control for confounding by one would diminish or remove the decrease in the other. To determine whether changes in IBC are weakened by control for confounding by serum albumin, the IBC models were rerun adding serum albumin to the model. Estimates of slopes resulting from these analyses are only negligibly different from those presented. IBC may decrease prior to cancer and MI diagnoses as the result of mechanism(s) not associated with either the APR.
<table>
<thead>
<tr>
<th>Site of Malignant Neoplasm</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(%) of 245</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>56</td>
<td>(22.86)</td>
</tr>
<tr>
<td>(melanoma and other neoplasms of skin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon and Rectum</td>
<td>42</td>
<td>(17.14)</td>
</tr>
<tr>
<td>Prostate</td>
<td>37</td>
<td>(15.10)</td>
</tr>
<tr>
<td>Lung</td>
<td>34</td>
<td>(13.88)</td>
</tr>
<tr>
<td>Lymphatic/Hematopoietic Tissue</td>
<td>15</td>
<td>(6.12)</td>
</tr>
<tr>
<td>Bladder</td>
<td>15</td>
<td>(6.12)</td>
</tr>
<tr>
<td>Brain</td>
<td>10</td>
<td>(4.08)</td>
</tr>
<tr>
<td>Lip, Oral Cavity, Pharynx</td>
<td>7</td>
<td>(2.86)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>5</td>
<td>(2.04)</td>
</tr>
<tr>
<td>Larynx</td>
<td>4</td>
<td>(1.63)</td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
<td>(1.63)</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>(1.22)</td>
</tr>
<tr>
<td>No Site Specified</td>
<td>3</td>
<td>(1.22)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2</td>
<td>(0.82)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Eight cancer sites are excluded from the table. These eight following cancer sites were diagnosed in one (0.41%) study participant each: breast, connective/soft tissue of thorax, eye, gall bladder/extrahepatic bile duct, non-brain nervous system, parathyroid, small intestine, and thyroid.

Table 2.1 Distribution of cancer by site among 245 Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) and follow-up study participants
<table>
<thead>
<tr>
<th>Variable and Category</th>
<th>Cancer (n = 245)</th>
<th>MI* (n = 437)</th>
<th>Neither* (n = 1,651)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Arm (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>50.20</td>
<td>49.89</td>
<td>51.67</td>
</tr>
<tr>
<td>Cholestyramine</td>
<td>49.80</td>
<td>50.11</td>
<td>48.33</td>
</tr>
<tr>
<td>Age at Baseline (year)</td>
<td>50.66 (49.92-51.40)</td>
<td>47.06 (46.45-47.67)</td>
<td>46.00 (45.69-46.31)</td>
</tr>
<tr>
<td>Body Mass Index*&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.63 (2.59-2.67)</td>
<td>2.67 (2.65-2.69)</td>
<td>2.65 (2.63-2.67)</td>
</tr>
<tr>
<td>Cigarettes Smoked/Day&lt;sup&gt;d&lt;/sup&gt;</td>
<td>20.80 (18.86-22.74)</td>
<td>19.94 (18.67-21.21)</td>
<td>16.53 (15.82-17.24)</td>
</tr>
<tr>
<td>Energy Intake&lt;sup&gt;d&lt;/sup&gt; (kcal/day)</td>
<td>2.134.1 (2.078.4-2.245.5)</td>
<td>2.112.5 (2.068.5-2.156.5)</td>
<td>2.166.2</td>
</tr>
<tr>
<td>Energy Intake&lt;sup&gt;d&lt;/sup&gt; (kcal/day)</td>
<td>2.134.1 (2.078.4-2.245.5)</td>
<td>2.112.5 (2.068.5-2.156.5)</td>
<td>2.166.2</td>
</tr>
<tr>
<td>Protein Intake&lt;sup&gt;d&lt;/sup&gt; (g/day)</td>
<td>88.65 (86.63-90.67)</td>
<td>87.90 (85.87-89.13)</td>
<td>90.34 (89.48-91.20)</td>
</tr>
<tr>
<td>Monounsaturated Fat Intake&lt;sup&gt;d&lt;/sup&gt; (g/day)</td>
<td>34.32 (33.14-35.50)</td>
<td>34.77 (33.87-35.55)</td>
<td>35.20 (34.75-35.65)</td>
</tr>
<tr>
<td>Saturated Fat Intake&lt;sup&gt;d&lt;/sup&gt; (g/day)</td>
<td>29.34 (28.38-30.30)</td>
<td>29.38 (28.60-30.16)</td>
<td>30.24 (29.83-30.65)</td>
</tr>
<tr>
<td>Polyunsaturated Fat Intake&lt;sup&gt;d&lt;/sup&gt; (g/day)</td>
<td>19.88 (19.14-20.62)</td>
<td>19.99 (19.38-20.60)</td>
<td>20.90 (20.60-21.20)</td>
</tr>
<tr>
<td>Cholesterol Intake&lt;sup&gt;d&lt;/sup&gt; (g/day)</td>
<td>342.81 (329.70-355.92)</td>
<td>335.92 (325.96-345.88)</td>
<td>343.40 (338.07-348.73)</td>
</tr>
</tbody>
</table>

<sup>a</sup> MI = myocardial infarction.
<sup>b</sup> Study participants in the "Neither" group were diagnosed with neither cancer nor any cardiovascular event during the trial or follow-up.
<sup>c</sup> Mean (95% confidence interval).
<sup>d</sup> For "cancer" and "MI" groups, means reflect only pre-diagnostic assessments.
<sup>e</sup> Body mass index = (height/weight)<sup>2</sup> x 1.000.

Table 2.2 Prediagnostic means of characteristics of three groups of study participants first diagnosed with cancer, myocardial infarction, and neither cancer nor any cardiovascular event throughout the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) and follow-up.
Figure 2.1 Mean serum albumin by number of years in the trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)
Figure 2.2 Mean iron binding capacity by number of years in the trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)
Figure 2.3 Mean serum iron by number of years in the trial for three exclusive groups of Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT) study participants: diagnosed first with cancer (n = 245), diagnosed first with myocardial infarction (n = 437), and diagnosed with neither cancer nor any cardiovascular event (n = 1,651)
<table>
<thead>
<tr>
<th>Site of Cancer</th>
<th>Serum Albumin Intercept (in g/dl) (95% CI)</th>
<th>Serum Albumin Slope Estimate X 10^7 (in g/dl/day) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Cancers Combined</td>
<td>4.18 (4.13 to 4.23)</td>
<td>-137.9 (-254.7 to -21.1)</td>
</tr>
<tr>
<td>Skin</td>
<td>4.20 (4.16 to 4.24)</td>
<td>-141.3 (-348.2 to 65.6)</td>
</tr>
<tr>
<td>Colon/Rectum</td>
<td>4.14 (4.02 to 4.26)</td>
<td>-8.7 (-150.3 to 132.9)</td>
</tr>
<tr>
<td>Prostate</td>
<td>4.24 (4.15 to 4.33)</td>
<td>-337.0 (-620.0 to -53.0)</td>
</tr>
<tr>
<td>Lung</td>
<td>4.12 (3.99 to 4.25)</td>
<td>-56.5 (-380.6 to 267.6)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>4.23 (4.19 to 4.27)</td>
<td>-74.8 (Unobtainable CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p-value = 0.1112)</td>
</tr>
<tr>
<td>Neither</td>
<td>4.31 (4.29 to 4.33)</td>
<td>-172.0 (Unobtainable CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p-value = 0.0001)</td>
</tr>
</tbody>
</table>

Table 2.3 Age-adjusted estimates of intercepts and slopes reflecting the change in serum albumin (g/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT)
<table>
<thead>
<tr>
<th>Iron Binding Capacity</th>
<th>Slope Estimate $X 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(in ug/dl) (95% CI)</td>
</tr>
<tr>
<td>All Cancers Combined</td>
<td>349.8 (341.6 to 358.1)</td>
</tr>
</tbody>
</table>

**Site of Cancer:**

<table>
<thead>
<tr>
<th>Site of Cancer</th>
<th>Intercept (in ug/dl) (95% CI)</th>
<th>Slope Estimate $X 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>356.5 (344.3 to 377.7)</td>
<td>-9.3 (-12.3 to -6.3)</td>
</tr>
<tr>
<td>Colon/Rectum</td>
<td>336.5 (318.2 to 354.8)</td>
<td>-6.8 (-10.8 to -2.8)</td>
</tr>
<tr>
<td>Prostate</td>
<td>349.1 (325.6 to 372.6)</td>
<td>-9.3 (-13.4 to -5.2)</td>
</tr>
<tr>
<td>Lung</td>
<td>354.0 (337.44 to 370.6)</td>
<td>-8.6 (-12.8 to -4.4)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>350.9 (343.7 to 358.2)</td>
<td>-9.8 (-11.1 to -8.5)</td>
</tr>
<tr>
<td>Neither</td>
<td>330.3 (327.6 to 333.0)</td>
<td>17.0 (16.4 to 17.6)</td>
</tr>
</tbody>
</table>

Table 2.4 Age-adjusted estimates of intercepts and slopes reflecting the change in iron binding capacity (ug/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT)
<table>
<thead>
<tr>
<th>Site of Cancer</th>
<th>Serum Iron Intercept (in ug/dl) (95% CI)</th>
<th>Slope Estimate X 10⁻³ (in ug/dl/day) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Cancers Combined</td>
<td>113.6 (107.6 to 119.6)</td>
<td>-2.7 (-4.0 to -1.4)</td>
</tr>
<tr>
<td>Skin</td>
<td>121.2 (111.8 to 130.6)</td>
<td>-2.2 (-4.6 to 0.2)</td>
</tr>
<tr>
<td>Colon/Rectum</td>
<td>105.4 (94.4 to 116.1)</td>
<td>-4.1 (-7.2 to -1.1)</td>
</tr>
<tr>
<td>Prostate</td>
<td>113.7 (101.2 to 126.2)</td>
<td>-6.6 (-9.7 to -3.5)</td>
</tr>
<tr>
<td>Lung</td>
<td>109.1 (92.2 to 126.0)</td>
<td>-1.8 (-5.4 to 1.9)</td>
</tr>
<tr>
<td>Myocardial Infarction</td>
<td>108.1 (103.5 to 112.7)</td>
<td>-3.2 (-4.3 to -2.2)</td>
</tr>
<tr>
<td>Neither</td>
<td>104.4 (102.8 to 106.0)</td>
<td>5.4 (4.9 to 6.0)</td>
</tr>
</tbody>
</table>

Table 2.5 Age-adjusted estimates of intercepts and slopes reflecting the change in serum iron (ug/dl) prior to a first diagnosis of either cancer or myocardial infarction or, for study participants diagnosed with neither, prior to the end of the trial, derived from repeated measures analyses with modeling of a random intercept across study participants from the Lipid Research Clinics Coronary Primary Prevention Trial (LRCCPPT)
Discussion

Results from this investigation have shown that eight years before cancer and MI diagnoses, mean serum albumin is significantly lower, and mean IBC significantly higher, than eight years prior to the end of the trial in the group of study participants not diagnosed with cancer or heart disease. In addition, mean serum iron is significantly lower eight years prior to cancer diagnosis. Results have also shown that serum albumin, IBC (a proxy for serum transferrin), and serum iron decrease prior to both cancer and MI diagnoses. A decrease in serum albumin is also observed for the group of study participants surviving to the end of follow-up without a cancer or MI diagnosis, while the concentrations of both IBC and serum iron increase in this group. While the findings that IBC and serum iron increase among the "neither" group invite speculation, no known limitation or source of bias could be identified to explain the finding.

The general findings of APR-related concentration changes are in agreement with other investigations of skin cancer (Gruner et al., 1992; Feelders et al., 1998), lung cancer (Sattar et al., 1997; Wojciechowska-Lacka et al., 1997), colorectal cancer (Fearon et al., 1991; Heys et al., 1998), prostate cancer (Nakashima et al., 1998), as well as MI (Pannitteri et al., 1997; Wanner et al., 1997). However, there are no known investigations of serial assessments of acute phase reactants over the course of years prior to cancer or MI diagnosis for comparison.

Several theories have been proposed to explain decreases in the concentration of serum albumin. Although it has been speculated that decreased albumin synthesis explains the decreased concentrations of serum albumin witnessed during the APR, recent
evidence suggests that there is no decrease in the rate of albumin synthesis during the APR and, in fact, albumin synthesis rates may be increased (Fearon et al., 1998). There is a body of evidence indicating that increased degradation of albumin, specifically by tumors, may both cause decreases in serum albumin and serve as nitrogen and energy sources to tumors (Rothschild et al., 1972; Andersson et al., 1990; Stehle et al., 1997). The decrease in serum albumin may result from insufficient replacement following increased degradation by tumors.

To explain decreased serum albumin in individuals with cancer and cardiovascular disease, Fleck et al. (1985) proposed that greater vascular permeability permits the leakage of serum albumin out of vessels. Fleck et al. (1985) found that during the APR to cardiovascular surgery, septic shock, and cancer, the loss of serum albumin from the vasculature to tissue spaces was accounted for by increases in vascular permeability. Vascular permeability is increased during the APR, probably as a result of vascular endothelial growth factor (VEGF) (Abramov et al., 1997; Rizk et al., 1997; Webb et al., 1998). VEGF, a tumor-derived angiogenic cytokine, has been shown to induce fenestrations in endothelial cells (Roberts and Palade, 1997; Roberts et al., 1998), and increase the vascular permeability of endothelial cells both in vivo (Senger et al., 1993; Roberts and Palade, 1995) and in vitro (Hippenstiel et al., 1998). Hippenstiel et al. (1998) found that the hydraulic conductivity (measured as the amount of water able to pass, with consistent pressure applied, through an endothelial monolayer) was greatly increased, and that the albumin reflection coefficient (a measure of passage selectivity of the monolayer and the difficulty of albumin passage) was decreased as a result of VEGF.
The vascular permeability of serum albumin has been shown, in vitro, to be increased by VEGF (Kevil et al., 1998). The results of Hippenstiel et al. (1998) suggest that VEGF directly alters vascular permeability, rather than working through mechanisms reflecting indirect effects of VEGF (VEGF-induced increased filtration pressure, and VEGF-induced activation of monocytes). Indeed, VEGF has been found in the sera of individuals with prostate cancer (Ferrer et al., 1997), colorectal cancer (Yamamoto et al., 1996; Fox et al., 1998; Fujisaki et al., 1998; Ishigami et al., 1998), and lung cancer (Yamamoto et al., 1996; Takigawa et al., 1998). VEGF is up-regulated by androgens in the stroma surrounding the prostate (Levine et al., 1998), and this may explain why the strongest decrease in serum albumin is witnessed among study participants developing prostate cancer. This does not, however, explain the decrease in serum albumin among the healthy study participants or those in the MI group. In fact, since VEGF is a potent angiogenic factor (providing the potential benefit of collateral circulation to thwart developing atherosclerosis), it could be expected that individuals with atherosclerosis (and impending MI) may lack the ability to induce or up-regulate VEGF. If it is true that serum albumin leaks from the vasculature as a result of the actions of VEGF, such an inability would be expected to correspond with no change in serum albumin prior to MI. However, on the other hand, perhaps up-regulation of VEGF in the MI group is occurring, but to insufficient ends. Because all study participants are hyperlipidemic and, therefore, at greater risk for coronary heart disease, study participants in the healthy group may also experience an up-regulation of VEGF, permitting serum albumin vasculature escape.
It is possible that a chronic APR plays a causal or promoting role in the
developments of cancer and heart disease. For example, with regard to MI, an increased
vascular permeability may permit, not only the escape of albumin through the vessel wall,
but the insudation of lipids into vessel walls, making decreased serum albumin a potential
marker of vascular lipid influx (Jensen, 1995).

Theories concerning the causes of changes in the concentration of serum
transferrin (assessed, here, by changes in IBC) have been less speculated upon. It may be
that the synthesis of transferrin is sacrificially decreased during an APR to provide
production capacity for positive APP’s, such as C-reactive protein (Mackiewicz, 1997;
Koj, 1998). As above, it is also possible that the actions of VEGF permit vasculature
leakage of serum transferrin. However, the decreases in IBC are not explained by either
decreases in serum albumin or serum iron, suggesting that the mechanisms governing the
decreases of serum albumin and IBC are either different or not occurring simultaneously,
and that ACD does not fully explain the decreasing IBC. In the short-lived APR (over the
course of one week), serum albumin and serum transferrin have been found to decrease in
concentration concurrently (Feelders et al., 1998). It may be that, in the long-lasting APR,
serum albumin levels return to normal, while serum transferrin remains sacrificed as the
result of anemia. Another explanation of decreasing serum transferrin prior to cancer and
MI diagnoses is that transferrin is taken up and used by tumor cells. Transferrin is an
angiogenic factor (Carlevaro et al., 1997) and is required for cellular proliferation
(Cherington et al., 1979). In fact, small cell lung cancer cells have been found to
synthesize transferrin for proliferation (Vostrejs et al., 1988). Lastly, it may be that the
decreases in serum transferrin concentrations are associated with the cause(s) of cancer and MI. If serum transferrin decreases in concentration, serum iron may remain unbound, permitting a greater opportunity for iron-associated free radical damage - a factor associated with the development of both cancer and MI.

The difference in behavior of serum transferrin and iron concentrations among the cancer and MI group, and the “neither” group may explain conflicting results previously found in studies of iron status and both cancer and MI risk. The risks associated with serum transferrin and iron depend on how long prior to diagnosis iron assessments were determined, as well as how long the comparison group was followed before making the determination of iron status (Figure 2.3). A portion of the difference in iron-related factors between the cancer and MI groups, and the “neither” group may be accounted for by regression to the mean. That is, measurements of individuals below the mean on a first assessment tend to increase (or approach the mean) on subsequent assessments, and individuals above the mean tend to decrease on subsequent assessments.

Results from this investigation are limited by inconsistently-spaced assessments of serum albumin, IBC, and serum iron, as well as small samples. Because the cancer site-specific analyses are based on few observations, it is difficult to interpret, with any certainty, the behavior of acute phase reactants at specific points in time. However, this is the first known investigation of long-term serial changes in the concentrations of acute phase proteins. The APR appears to have a long preclinical phase.

Further research is required to determine, more specifically, when concentrations of serum albumin, IBC, and serum iron, as well as more specific APR proteins (such as
C-reactive protein), change prior to cancer and MI diagnoses. To answer this question precisely requires sufficient data (a large sample, frequent APR assessments, and consistent spacing between assessments) to conduct a change-point analysis, so that inflection point(s) in regression lines of APR-related concentration changes can be identified. Because of changes in the concentrations of serum transferrin and iron, it may be necessary to obtain serial measurements of these factors and to treat them as time-dependent covariates in investigations of iron status and both cancer and MI risk. Moreover, it would be beneficial to investigate previous iron-related findings within a meta-analysis that adjusted for the length of time between iron status assessment and either cancer or MI diagnosis. Lastly, it may be worthwhile to examine the screening capacity of easily- and routinely-measured serial acute phase reactants, both individually and in combinations. The identification of APR-related changes occurring long before cancer and MI diagnoses may elucidate very early systemic changes associated with neoplasia and atherosclerosis.
CHAPTER 3

Concentrations of Acute Phase Reactants Are Altered At Least Three Years Prior to Lung and Bladder Cancer Diagnoses

Abstract

Serum constituents are altered at the time of cancer diagnosis as a result of the acute phase response (APR), a cytokine-governed response to tissue injury, infection, or tobacco smoking. The purpose of this investigation was to determine whether four routinely-measured serum constituents (white blood cell count [WBCC], serum albumin, iron binding capacity [IBC], and erythrocyte sedimentation rate [ESR]) are associated with risk for two smoking-related cancers: lung and bladder cancers. Data from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study (NHEFS) (n = 13,370) were used to obtain Cox proportional hazards regression analysis-derived risk ratios (RR). After excluding study participants diagnosed with lung or bladder cancer in the three-year period following baseline acute phase serum assessment, 155 lung cancer cases (median follow-up of 9.4 years) and 41 bladder cancer cases (median follow-up of 8.4 years) were identified. Alterations in risk for lung and bladder cancer vary considerably by sex. For example, men in the upper quartile of WBCC have eight times the bladder cancer risk of those in the remaining three quartiles.
(RR = 8.24, 95 percent confidence interval [CI]: 3.39 - 20.04), but women in the upper quartile of WBCC are not at greater bladder cancer risk (RR = 0.76, 95 percent CI: 0.10 - 5.76). Associations between the APR and both bladder and lung cancer should be further evaluated.
Introduction

An acute phase response (APR), mediated primarily by the cytokines tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and interleukin-1 (IL-1), may occur as a result of tumor growth and leads to alterations in serum constituents, including at least 30 serum proteins (Elmquist et al., 1997; Mackiewicz, 1997; Heinrich et al., 1998; Koj, 1998). Initially, the APR is essential in responding to tissue trauma, infection, or inflammation, but a prolonged APR results in overproduction of proinflammatory cytokines (Grimble and Tappia, 1998; Koj, 1998). Tobacco smoking is associated with an ongoing APR, demonstrated by increased C-reactive protein (a positive acute phase protein, a protein whose concentration is increased by at least 25 percent as part of the APR) levels in the sera of smokers (Das, 1985). If smoking-related cancers, such as lung and bladder cancers, are associated with a prolonged APR, serum constituents may be altered early in their development. It is known that many routinely-measured acute phase reactants are altered at the time of cancer diagnosis.

One of these, white blood cell count (WBCC), is increased as a result of the APR (Kushner and Rzewnicki, 1994; Koj, 1996). Tobacco smoking increases WBCC by approximately 25 percent (Parry et al., 1997; Terashima et al., 1997). A high WBCC predicts shorter survival from small cell lung cancer (SCLC) (Kawahara et al., 1997), unresectable non-small cell lung cancer (NSCLC) (Borges et al., 1996) and inoperable lung adenocarcinoma (Sroensen et al., 1989). In addition to WBCC, serum albumin and transferrin are altered during the APR.
Serum albumin is a negative acute phase protein (Elmquist et al., 1997; Mackiewicz, 1997). Serum albumin is lower in individuals with NSCLC compared to healthy controls (Talwar et al., 1997), and in NSCLC patients experiencing an APR, compared to both NSCLC patients not experiencing an APR and healthy controls (Sattar et al., 1997). In addition, low serum albumin is associated with shorter survival from bladder carcinoma (Hannisdal et al., 1993), NSCLC (Hespanhol et al., 1995), and SCLC (Maestu et al., 1997). The concentration of serum transferrin, the protein that carries serum iron, also decreases during the APR (Elmquist et al., 1997; Mackiewicz, 1997). Knekt et al. (1994) and Selby and Friedman (1998) found that lung cancer risk is inversely related to iron binding capacity (IBC) (a proxy for serum transferrin) and Stevens et al. (1988) found that risk for all cancers combined was inversely related to IBC.

In addition to these acute phase reactants, the erythrocyte sedimentation rate (ESR) (the distance red blood cells sediment through plasma in a specified amount of time, usually one hour) is increased as a result of the APR (Saadeh, 1998). The ESR is a good prognostic indicator of survival from primary lung cancer (Engan and Hannisdal, 1990), NSCLC (Hannisdal and Engan, 1991), and bladder carcinoma (Hannisdal et al., 1993). Among blood donors, ESR increases in tobacco smokers (Gudmundsson and Bjelle, 1993).

The purpose of this investigation was to determine whether routinely measured acute phase reactants are altered at least three years prior to diagnosis with two smoking-related cancers: lung and bladder cancers. APR-associated alterations in four
acute phase reactants (WBCC, serum albumin, serum transferrin, and ESR) were examined to determine whether risk ratios (RR) for lung and bladder cancers were higher among individuals with high APR levels. Study participants diagnosed in the three-year period following assessment of acute phase reactants were excluded from this study. The primary objective of this study was to determine whether the concentrations of both serum albumin and serum transferrin are decreased, and ESR and WBCC count increased, (as would be expected during the APR) prior to lung or bladder cancer diagnosis.
Methods

*Study Population.* Data from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study (NHEFS) were used to ascertain risk ratios for both lung and bladder cancers. The design and implementation of NHANES I is described in detail elsewhere (Engel et al., 1978; McDowell et al., 1981; Madans et al., 1986; Cohen et al., 1987; Finucane et al., 1990; Cox et al., 1992). Briefly, NHANES I is a longitudinal study of a national probability sample of 20,729 United States non-institutionalized citizens ages 25 to 74 years. The study was designed to assess the effects of clinical, nutritional, and behavioral factors on subsequent morbidity, mortality, and institutionalization. Between 1971 and 1974, members of this sample were administered a survey including a medical examination and several health-related questionnaires. At baseline, blood and urine samples were collected for all medically examined study population members to obtain biochemical, serologic, and hematologic measurements. Serum albumin, ESR, and WBCC were measured for all examined study participants. Serum transferrin was not directly measured. However, iron binding capacity (IBC), which was measured, is highly correlated with serum transferrin ($R^2 = 0.94$), and is a reasonable index of serum transferrin (Gambino et al., 1997). In addition, blood samples from a sub-sample ($n = 5,854$ study participants) were analyzed for more detailed assessments, including a peripheral blood film analysis. Four follow-up studies have been conducted since the baseline study. From 1982 to 1984, data on morbidity and mortality were gathered and classified by ICD code from a follow-up questionnaire for the 14,407 medically examined traceable study population members. For the second follow-up, data
and ICD codes were gathered during 1986 for 3,980 traceable individuals who were ages 55 to 74 years at baseline and not known to be deceased in the 1982 to 1984 follow-up.

For the third and fourth follow-up studies, data and ICD codes were gathered during 1987 for 11,750 non-deceased traceable individuals, and in 1992, for 11,195 of this same cohort. During each follow-up, personal interviews were conducted with study participants or their proxies.

*Case Identification and Exclusions.* Incident lung and bladder cancer cases were identified from follow-up interviews and hospital records using ICD codes. The original group of study participants consisted of the 14,407 study participants ages 25 to 74 who were medically examined at baseline (1971-1975). Of the 14,407 study participants in the original sample, 231 developed lung cancer and 68 developed bladder cancer. The following groups of study participants were excluded from analyses: individuals reporting a current or previous malignant tumor, individuals who, over the study period, developed either benign neoplasm(s), carcinoma *in situ* (s), or neoplasm(s) of uncertain behavior/ unspecified nature, individuals with unmeasured or unreported acute phase reactants, and individuals diagnosed within the three-year period following serum assessment. These exclusions resulted in a final sample size of 12,534 study participants who did not develop cancer during follow-up, 155 lung cancer cases and 41 bladder cancer cases. Study participants diagnosed with non-lung, non-bladder cancers were excluded from analyses, so as to maintain a cancer-free control series.

*Statistical Methods.* Cox proportional hazards regression analyses were used to obtain risk ratios (RR) to compare lung and bladder cancer risks in quartiles of acute
phase reactants characterizing changes that occur during the APR. Risks among study participants in case-generated lower quartiles of serum albumin and serum transferrin, and case-generated upper quartiles of ESR and WBCC, were each compared with risks among study participants in the remaining three quartiles. Quartile cut-points were case-generated because there were too few cases in categories generated by control cut-points to produce RR's (Greenland, Schwartzbaum, and Finkle, Unpublished Manuscript, 1998). Adjustment for potential confounding by sex, race, age at baseline, body mass index, and pack-years of tobacco smoking was accomplished by including factors in models. Potentially confounding factors were initially included in models and were removed only if the removal failed to affect the regression coefficient of the acute phase reactant of interest. All analyses were conducted separately for males and females. All statistical analyses were conducted using Statistical Analysis System (SAS).
Results

Table 3.1 presents adjusted RR’s for lung cancer case-generated lower quartiles of serum albumin and IBC, and lung cancer case-generated upper quartiles of ESR and WBCC. For each acute phase reactant, the referent category consists of study participants outside the quartile characterizing the APR. Control for confounding is limited to age at baseline and pack-years of tobacco smoking. Among males, lower quartile serum albumin (less than 4.1 g/dl) does not alter lung cancer risk, and lower quartile IBC (less than 313 ug/dl) is associated with a marginally statistically significant decrease in lung cancer risk - a finding opposite to that expected if serum transferrin decreases during both the APR and lung tumorogenesis. Both upper quartile ESR (greater than 25 mm/hour) and WBCC (greater than 9,350/mm³) are associated with increased lung cancer risk among males. Among females, each acute phase reactant marks an increase in lung cancer risk, although RR’s reach only marginal statistical significance.

Table 3.2 presents adjusted RR’s for bladder cancer case-generated lower quartiles of serum albumin and IBC, and bladder cancer case-generated upper quartiles of ESR and WBCC. As above, control for confounding is limited to age at baseline and pack-years of tobacco smoking. Among males, lower quartile serum albumin (less than 4.1 g/dl), upper quartile ESR (greater than 25 mm/hour), and upper quartile WBCC (greater than 9,440/mm³) are associated with increased bladder cancer risk, although only the RR for upper quartile WBCC reaches statistical significance. Among males, upper quartile WBCC is associated with over an eight-fold increase in bladder cancer risk from WBCC greater than 9,350/mm³ among males. Among females, lower quartile IBC (less
than 319 ug/dl) is associated with a marginally statistically significant decrease in bladder cancer risk.

Because, among males, upper quartile WBCC is associated with increased lung and bladder cancer risks, data from a sub-sample of study participants (n = 5,854, including 93 lung cancer cases and 17 bladder cancer cases) who had peripheral blood film analyses, as part of a more detailed hematologic examination, were analyzed. The following five cell types were counted and presented as a percentage of total cells: segmented neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Among males, of these five cell types, an eosinophil count greater than two percent is associated with increased lung cancer risk (age- and pack-years of tobacco smoking-adjusted RR = 1.79, 95 percent confidence interval [CI]: 1.05 - 3.07), and a lymphocyte count greater than 33 percent is associated with decreased lung cancer risk (age- and pack-years of tobacco smoking-adjusted RR = 0.60, 95 percent CI: 0.37 - 1.00). Among females, none of the percentages of cell types is associated with altered lung cancer risk. Too few cases prohibit investigations of bladder cancer risks associated with white blood cell types.
<table>
<thead>
<tr>
<th>Acute Phase Reactant</th>
<th>R.R.(^a)</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males (n = 106):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Albumin(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4.1 g/dl</td>
<td>1.14</td>
<td>0.76 - 1.71</td>
</tr>
<tr>
<td>Iron Binding Capacity(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 313 μg/dl</td>
<td>0.74</td>
<td>0.50 - 1.10</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation Rate(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25 mm/hour</td>
<td>1.59</td>
<td>0.90 - 2.81</td>
</tr>
<tr>
<td>White Blood Cell Count(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 93.5 hundred/mm(^3)</td>
<td>2.95</td>
<td>1.90 - 4.56</td>
</tr>
<tr>
<td><strong>Females (n = 49):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Albumin(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4.1 g/dl</td>
<td>1.71</td>
<td>0.97 - 3.04</td>
</tr>
<tr>
<td>Iron Binding Capacity(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 313 μg/dl</td>
<td>1.48</td>
<td>0.81 - 2.57</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation Rate(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 25 mm/hour</td>
<td>1.51</td>
<td>0.78 - 2.95</td>
</tr>
<tr>
<td>White Blood Cell Count(^c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 93.5 hundred/mm(^3)</td>
<td>1.71</td>
<td>0.85 - 3.45</td>
</tr>
</tbody>
</table>

\(a\). Adjusted for age at baseline and pack-years of tobacco smoking.  
\(b\). Study participants in case-generated lower quartiles of serum albumin and iron binding capacity are compared to study participants in the remaining three quartiles.  
\(c\). Study participants in case-generated upper quartiles of erythrocyte sedimentation rate and white blood cell count are compared to study participants in the remaining three quartiles.

Table 3.1 Adjusted risk ratios (RR) for lung cancer from acute phase reactants among 155 incident lung cancer cases and 12,534 non-case National Health and Nutrition Examination Survey (NHANES) I study participants.
<table>
<thead>
<tr>
<th>Acute Phase Reactant</th>
<th>R.R.(^a)</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males (n = 22):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Albumin(^b) &lt; 4.1 g/dl</td>
<td>1.81</td>
<td>0.76 - 2.00</td>
</tr>
<tr>
<td>Iron Binding Capacity(^b) &lt; 319 µg/dl</td>
<td>1.26</td>
<td>0.54 - 2.90</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation Rate(^c) &gt; 25 mm/hour</td>
<td>1.98</td>
<td>0.66 - 5.96</td>
</tr>
<tr>
<td>White Blood Cell Count(^c) &gt; 94.4 hundred/mm(^3)</td>
<td>8.24</td>
<td>3.39 - 20.04</td>
</tr>
<tr>
<td><strong>Females (n = 19):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Albumin(^b) &lt; 4.1 g/dl</td>
<td>0.46</td>
<td>0.13 - 1.59</td>
</tr>
<tr>
<td>Iron Binding Capacity(^b) &lt; 319 µg/dl</td>
<td>0.33</td>
<td>0.10 - 1.14</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation Rate(^c) &gt; 25 mm/hour</td>
<td>0.95</td>
<td>0.34 - 2.65</td>
</tr>
<tr>
<td>White Blood Cell Count(^c) &gt; 94.4 hundred/mm(^3)</td>
<td>0.76</td>
<td>0.10 - 5.76</td>
</tr>
</tbody>
</table>

\(a\). Adjusted for age at baseline, and pack-years of tobacco smoking.

\(b\). Study participants in case-generated lower quartiles of serum albumin and iron binding capacity are compared to study participants in the remaining three quartiles.

\(c\). Study participants in case-generated upper quartiles of erythrocyte sedimentation rate and white blood cell count are compared to study participants in the remaining three quartiles.

Table 3.2 Adjusted risk ratios (RR) for bladder cancer from acute phase reactants among 41 incident bladder cancer cases and 12,534 non-case National Health and Nutrition Examination Survey (NHANES I) study participants
Discussion

The results from this study suggest associations between acute phase reactants measured at least three years prior to diagnosis and both lung and bladder cancer risks. The results vary considerably by sex. It is difficult to compare findings from this investigation with previously reported results because the exclusion of study participants diagnosed within three years of serum assessment may have profound effects on findings. However, the finding that APR-associated serum constituents are altered in individuals at the time of lung cancer diagnosis is supported by the results of Sattar et al. (1997), and the finding that bladder cancer is associated with APR-induced changes at the time of diagnosis is supported by the results of Pejovic et al. (1997).

There are no known reports of sex differences in APR’s among humans. However, Coe et al. (1981) found that female hamsters have a high serum concentration of an acute phase protein similar to C-reactive protein in humans, and that, in males, the concentration of this protein is low. During an APR, the concentration in male hamsters increases three-fold, while it decreases to half its original concentration in female hamsters (Coe et al., 1981). It is possible that the sex differences witnessed in the present investigation result from a similar scenario, although there is no evidence to support this notion.

There are at least two theories explaining the associations between increased lung and bladder risks and WBCC. First, higher WBCC may result from infections associated with lung and bladder cancers. Recent evidence suggests that both lung and bladder cancers are associated with infectious agents. Lung cancer has been related to chlamydia
pneumoniae infection (Laurila et al., 1997), as well as human immunodeficiency virus (HIV) infection (Vyzula and Remick, 1996). Bladder cancer has been associated with Epstein-Barr virus, cytomegalovirus, and herpes simplex virus type 2 (Gazzaniga et al., 1998), as well as human papillomavirus (Boucher and Anderson, 1997; Cooper et al., 1997) and schistosomiasis (Cooper et al., 1997). Second, WBCC may be increased as a defense against the growth of the tumor. It may be that an ultimately unsuccessful battle is underway, during which WBCC is increased, but, given the negative association between lymphocyte percentage and lung cancer risk, the percentage of lymphocytes is too low to mediate the battle. The finding that, among males, greater than two percent eosinophils is positively associated with lung cancer risk is supported by anecdotal evidence reported by Matsumoto et al. (1992), who found that the serum of a 55-year-old male diagnosed with squamous cell carcinoma of the lung could stimulate eosinophil production. Matsumoto et al. (1992) speculate that the tumor produced a factor causing eosinophil proliferation.

Serum albumin has been found to be marginally associated with increased lung cancer risk among females, and increased bladder cancer risk among males. There is debate concerning the cause of the decrease in serum albumin. One theory purports that vascular endothelial growth factor (VEGF), a tumor-derived angiogenic cytokine, is responsible for the decrease. VEGF increases the vascular permeability of endothelial cells both in vivo and in vitro (Senger et al., 1993; Roberts and Palade, 1995; Roberts and Palade, 1997; Roberts et al., 1998) and serum albumin may leak from the vasculature as a result. Another theory suggests that hepatocyte synthesis of serum albumin is sacrificed
early in tumorigenesis to provide capacity for cytokine-mediated protein synthesis (Mackiewicz, 1997). Both may be correct.

The concentration of serum transferrin is also suggested to decrease during the APR as the result of decreased hepatocyte synthesis (Elmquist et al., 1997; Mackiewicz, 1997; Heinrich et al., 1998; Koj, 1998). The results of this investigation do not show an association between lung and bladder cancers and decreased levels of serum transferrin. Among females, lower quartile IBC is associated with a decrease in bladder cancer risk, and, among males, a decrease in lung cancer risk. These findings are opposite to that which would be expected if IBC is a reasonable proxy for serum transferrin, serum transferrin decreases during the APR, and APR occurs with the growth of lung and bladder tumors. The decreases in risk for bladder cancer (among females) and lung cancer (among males) from the lower quartile of IBC are not supported by the results of Knekt et al. (1994), Sattar et al. (1997), or Selby and Friedman (1988), who find increases in cancer risk from low IBC. It is possible that excluding cancer cases diagnosed within three years of assessment caused this discrepancy. It may be that serum transferrin declines within this period, closer to diagnosis. Further, since IBC is inversely related to body iron stores, it may be that the difference in findings results from tumor-associated alterations of iron stores in the three-year period prior to diagnosis.

The slight and marginally statistically significant association between ESR and lung cancer risk among males may be explained by the actions of the positive acute phase protein, fibrinogen, during the APR. Increased fibrinogen and immunoglobulins attach to erythrocytes and cause them to aggregate. This aggregation causes erythrocytes to
precipitate more quickly (increased ESR) (Chien, 1973; Reinhart and Nagy, 1995). In addition, serum albumin may either increase or decrease the ESR, depending on the presence of fibrinogen and immunoglobulins in serum (Yamamoto, 1986; Lacombe et al., 1988; Reinhart and Nagy, 1995).

There are at least three recognized sources of bias or limitation in this study. First, because ICD codes distinguish between site, but not type, of tumor, it is possible that effects within each site reflect several types of tumors. Second, it is possible that, although every attempt was made to control confounding, confounding by a factor related to both the APR and lung or bladder cancer remained uncontrolled. Third, it is possible that study participants who survived the (at least) three-year period between APR assessment and diagnosis are a healthier subset of lung and bladder cancer cases. The presence of an APR is associated with decreased survival from both lung and bladder cancers. Therefore, an APR may have been mounted by a greater number of study participants not surviving the three-year interval, compared to those who did survive the three-year interval. The exclusion of study participants diagnosed within the three-year interval may pull RR’s toward the null, in which case the RR’s are underestimated.

These results suggest that APR-induced changes, especially a greater WBCC (among males), occur at least three years prior to clinical presentation with lung and bladder cancers. Larger studies of the behavior of these easily-measured APR factors prior to lung and bladder cancer diagnoses are warranted. As demonstrated by the approximately three- and eight-fold increases in lung and bladder cancer risks (among
males), respectively, the relationship between WBCC and the APR should be further investigated.
CHAPTER 4

Serum Micronutrient Concentrations Are Decreased During Acute Phase Response

Abstract

While there is strong evidence that the concentrations of many serum proteins are altered during, or as a result of, the acute phase response (APR), there is only limited and disease-specific information about non-protein affects of the APR. This investigation was conducted to determine whether the concentrations of several serum micronutrients are altered in individuals experiencing an APR, after control for confounding by age, sex, body mass index, current smoking status, and dietary factors. Data from the National Health and Nutrition Examination Survey (NHANES) III were used to obtain logistic regression-derived adjusted prevalence odds ratios (POR) for the presence of an APR (indicated by a C-reactive protein level greater than 3.5 mg/dl). Statistically significant associations between the APR and the following serum micronutrients are not removed by control for confounding: serum iron (POR = 0.946, 95 percent confidence interval [CI]: 0.933 - 0.960), serum selenium (POR = 0.959, 95 percent CI: 0.939 - 0.980, serum vitamin C (POR = 0.375, 95 percent CI: 0.164 - 0.857), serum vitamin A (POR = 0.943, 95 percent CI: 0.921 - 0.966), serum α-carotene (POR = 0.819, 95 percent CI: 0.710 -
0.945), serum β-carotene (POR = 0.967, 95 percent CI: 0.938 - 0.998), and serum lycopene (POR = 0.937, 95 percent CI: 0.902 - 0.972). That is, for example, for every unit increase in serum iron (in ug/dl) the log of the odds of having a marked APR decreases by 0.0555. Potential mechanisms governing the decreased levels of serum micronutrients include: 1) serum micronutrients may escape the vasculature during an APR; 2) they may be consumed by free radicals; 3) their concentrations may be altered by APR-associated changes in transport and/or binding proteins.
Introduction

There is a large, consistent, and expanding literature supporting the idea that characteristic changes in the concentrations of serum proteins during the acute phase response (APR) occur in response to tumorogenesis or atherosclerosis (Matei, 1997). The concentrations of at least 30 proteins are known to be altered during the APR (Matei, 1997; Mackiewicz, 1997; Koj, 1998). Non-protein effects of the APR, especially alterations in serum vitamin concentrations, have been largely ignored, perhaps because these effects may be secondary to APR-associated protein concentration changes, such as changes in concentrations of binding and transport proteins. For example, Sattar et al. (1997) found that circulating concentrations of serum iron were significantly lower in a group of non-small cell lung cancer (NSCLC) patients experiencing an APR than in NSCLC patients not experiencing an APR, but that the molar ratio of serum iron to its binding protein, transferrin, was not significantly different between the two groups. Results from several investigations of post-surgical or post-injury study participants have shown APR-associated decreases in the concentrations of trace elements such as iron, zinc, and copper (Fraser et al., 1989; Taggart et al., 1990; Shenkin, 1995).

In examining potential associations between serum vitamin and mineral levels and the APR, previous investigations have neglected to control for confounding by factors such as age, sex, body mass index, dietary energy intake and dietary factors assessing micronutrient intakes. Control for such confounding is important because decreased serum micronutrient concentrations may occur as the result of preclinical diseases (such as cancer) associated with both the APR and serum micronutrients. For example, control
for confounding by dietary factors is important because the concentrations of some serum micronutrients are strongly associated with their dietary intake. For example, Drewnowski et al. (1997) found that serum vitamin C concentrations were associated with dietary fruit intake, as well as negatively associated with age and body mass index. Tobacco smoking may also confound associations between the APR and serum micronutrient concentrations, in that tobacco smoking has been associated with altered serum antioxidant vitamin status (Ross et al., 1995; Torun et al., 1995; Drewnowski et al., 1997) and tobacco smoking is the strongest risk factor for NSCLC. Because these confounding factors may be associated with both the APR, diseases characterized by the APR, and serum micronutrient levels, then control is essential. The purpose of the present investigation was to assess APR-associated changes in serum micronutrients.
Methods

*Study Population.* Data from the National Health and Nutrition Examination Survey (NHANES) III was used for this analysis. The design and implementation of NHANES III are explained in more detail by others (National Center for Health Statistics, 1994; US Department of Health and Human Services, 1996). Briefly, a nationwide probability sample of 33,994 individuals over 2 months of age was obtained between 1988 and 1994. The survey was designed to obtain nationally representative information on the health and nutritional status of the civilian, noninstitutionalized population of the United States through interviews and direct physical examinations. Study participants (or proxies) were asked if they had ever been diagnosed by a physician with any of the following health conditions: arthritis, congestive heart failure, stroke, asthma, chronic bronchitis, emphysema, hay fever, cataracts, goiter, thyroid disease, lupus, gout, skin cancer, or other cancer, and if they currently have these conditions/diseases. A 24 hour dietary recall questionnaire was used to ascertain nutrient intakes. General biochemistry tests, including assessments of serum micronutrients and acute phase reactants, were conducted on the blood of adult study participants.

*Exclusions.* Because some diseases and disease treatments are associated with alterations in serum micronutrients, study participants suspected of having an infection during the medical examination or who were found by a physician to be in less than "very good" physical condition were excluded from analyses. In addition, study participants previously diagnosed with non-skin cancer, congestive heart failure, myocardial
infarction, and/or stroke were excluded. Lastly, only study participants between the ages of 18 and 90 were included.

Statistical Procedures. The APR was characterized conservatively by a CRP level greater than 3.5 mg/dl (Sattar et al., 1997). Two groups of study participants were characterized - one experiencing an APR (CRP level greater than or equal to 3.5 mg/dl) and one not experiencing an APR. For continuous factors, to compare differences in means of selected factors between the two groups of study participants, either two-sample t-tests or pooled t-tests, depending on tests of equality of variances, were used. To compare differences in proportions in the two groups, chi-square tests were used. To control confounding, logistic regression was used to determine if the APR was associated with changes in the concentrations of selected serum micronutrients. To determine if serum micronutrient concentrations are altered during a mild, compared to a marked, APR, prevalence odds ratios (POR’s) were generated for C-reactive protein levels between one and 3.5 mg/dl, and greater than 3.5 mg/dl. (Levels of CRP in sera less than 1 mg/dl are considered clinically unimportant (Morley and Kushner, 1982; Gabay and Kushner, 1999). Potential confounders included age, sex, body mass index, current smoking status, and dietary factors. To assess current smoking status serum cotinine levels were used. A level of 50 ng/ml has been suggested as a cutpoint, above which study participants were considered to be current tobacco smokers (Apseloff et al., 1994). The selection of confounders was accomplished by assessing the effect of the removal of a potential confounder on the regression coefficient of the serum micronutrient of interest. (Confounding by binding proteins [such as calcium-binding protein,
carotenoid-binding protein, retinol-binding protein, and selenium-binding protein], and transport proteins [such as serum albumin and serum transferrin] was neglected in this analysis because APR-associated changes in the concentrations of these factors are potentially intermediate factors in the causal pathway between the APR and serum micronutrient concentration changes. Special control for confounding by intermediate factors may be obtained using G-estimation within a survival analysis [Robins et al., 1992] - a technique requiring serial assessments of binding and transport proteins, as well as serial assessments of serum micronutrients.) All statistical procedures were conducting using Statistical Analysis System (SAS).
Results

After excluding study participants with a physical examination report of less than very good health, or with (at least suspected) current infection, a history of cancer or cardiovascular disease, or who have missing or unreported CRP levels, 7,096 study participants remain for the analyses. Table 5.1 shows demographic and potentially confounding characteristics of study participants by APR status (e.g. CRP level greater than or equal to 3.5 mg/dl indicates an APR). The group of study participants experiencing an APR are, on average, older and have a greater body mass index. The proportion of white study participants is not statistically different between groups, although the group experiencing an APR contains a greater proportion of females. Although the group of study participants with evidence of an APR is comprised of a greater percentage of tobacco smokers, the difference between the groups is not statistically significant.

To demonstrate that other APR factors are altered in the group with elevated CRP levels, Table 5.2 presents differences in four hematologic factors (white blood cell count, hemoglobin, hematocrit, and platelet count) and three acute phase proteins (serum albumin, serum ferritin, and plasma fibrinogen). Differences between the groups are found for each factor, and the differences occur in the expected direction, given an APR in the group with CRP greater than or equal to 3.5 mg/dl. That is, for example, concentrations of serum albumin, as witnessed, decrease during the APR, while concentrations of serum ferritin and plasma fibrinogen increase. These differences do not vary considerably according to sex or body mass index, but among study participants less
than 51 years of age differences between groups for both platelet count and serum ferritin, although increased in the group with high CRP, do not reach statistical significance (not shown in tables).

Because there is evidence that individuals diagnosed with diseases associated with the APR (such as cancer) have altered nutrient intakes, and because these alterations may confound associations between the APR and serum micronutrients, Table 5.3 shows differences in mean nutrient intakes between the two groups. Study participants experiencing an APR consume, on average, fewer calories than study participants not experiencing an APR. The lower caloric intake among study participants experiencing an APR may account for the observed differences in the remainder of the nutrient intakes. Mean intakes of monounsaturated fat, vitamin A, carotenes, and vitamin C, although lower in the group experiencing an APR, are not statistically different from the comparison group. When stratified by sex and mean body mass index, only statistical significance is affected; lower dietary intakes remain apparent for all subgroups experiencing an APR (not shown in tables).

Table 5.4 shows that mean levels of all serum micronutrients except folate and vitamin E are considerably lower in study participants experiencing an APR. To determine if these differences are attributable to confounding by age, sex, tobacco smoking, body mass index, and/or dietary factors, Table 5.5 shows adjusted POR’s for both a mild and marked APR from each serum micronutrient. Because only 51 study participants are experiencing a marked APR, and because six factors confound the associations, serum micronutrients are modeled as continuous, rather than categorical,

79
factors. The POR's are interpreted as follows: for every unit increase in serum iron (in ug/dl) the log of the odds of having a marked APR decreases by 0.0555. For example, the ratio of the odds of having a marked APR if serum iron is 100 ug/dl to the odds if serum iron is 70 ug/dl is $e$ to the power of the product of beta (-0.0555) and the difference in the serum iron levels of interest (30), or 0.189. Control for confounding removes associations only between the APR and serum calcium. Serum folate and serum vitamin E maintain their lack of association with the APR after control for confounding. Associations between the APR and serum iron, selenium, vitamin C, vitamin A, α-carotene, β-carotene, and lycopene could not be removed by control for confounding.
<table>
<thead>
<tr>
<th>Factor and Category</th>
<th>No APR CRP &lt; 3.5 mg/dl (n=7,045)</th>
<th>APR CRP &gt;= 3.5 mg/dl (n=51)</th>
<th>p^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% Female)</td>
<td>50.06</td>
<td>62.75</td>
<td>0.0711</td>
</tr>
<tr>
<td>Race (% White)</td>
<td>72.33</td>
<td>74.51</td>
<td>0.7266</td>
</tr>
<tr>
<td>Age (Years)^a</td>
<td>44.08 (0.23)</td>
<td>51.73 (2.77)</td>
<td>0.0081</td>
</tr>
<tr>
<td>Body Mass Index (kg/m^2)^a</td>
<td>26.43 (0.06)</td>
<td>28.74 (1.11)</td>
<td>0.0436</td>
</tr>
<tr>
<td>Current Smoking Status</td>
<td>24.76</td>
<td>29.41</td>
<td>0.4429</td>
</tr>
<tr>
<td>(% with cotinine &gt; 50 ng/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive Protein (mg/dl)^a</td>
<td>0.37 (0.01)</td>
<td>5.19 (0.07)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

a. Mean (standard error).
b. p-value for t-test of difference between means, or chi-square test for difference between proportions.

Table 4.1 Demographic and potentially confounding characteristics of National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than or equal to 3.5 mg/dl) - evidence of an acute phase response
<table>
<thead>
<tr>
<th>Acute Phase Reactant</th>
<th>No APR(^a)</th>
<th>APR(^a)</th>
<th>(p)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRP &lt; 3.5 mg/dl</td>
<td>CRP &gt;= 3.5 mg/dl</td>
<td></td>
</tr>
<tr>
<td>White Blood Cell Count (thousands/mm(^3))</td>
<td>7.10 (0.03)</td>
<td>8.96 (0.43)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>(n=6,896)</td>
<td>(n=49)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.96 (0.02)</td>
<td>13.26 (0.20)</td>
<td>0.0484</td>
</tr>
<tr>
<td></td>
<td>(n=6,896)</td>
<td>(n=49)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41.57 (0.05)</td>
<td>39.67 (0.58)</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td>(n=6,895)</td>
<td>(n=49)</td>
<td></td>
</tr>
<tr>
<td>Platelet Count (thousands/mm(^3))</td>
<td>281.96 (0.84)</td>
<td>329.56 (13.49)</td>
<td>0.0018</td>
</tr>
<tr>
<td></td>
<td>(n=6,893)</td>
<td>(n=49)</td>
<td></td>
</tr>
<tr>
<td>Serum Albumin (g/dl)</td>
<td>4.26 (0.01)</td>
<td>3.88 (0.06)</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>(n=6,969)</td>
<td>(n=50)</td>
<td></td>
</tr>
<tr>
<td>Serum Ferritin (ng/ml)</td>
<td>126.48 (1.57)</td>
<td>173.10 (16.88)</td>
<td>0.0120</td>
</tr>
<tr>
<td></td>
<td>(n=7,035)</td>
<td>(n=51)</td>
<td></td>
</tr>
<tr>
<td>Plasma Fibrinogen (mg/dl)</td>
<td>306.08 (1.38)</td>
<td>478.91 (19.44)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>(n=3,502)</td>
<td>(n=34)</td>
<td></td>
</tr>
</tbody>
</table>

\(a.\) Mean (standard error).
\(b.\) \(p\)-value for \(t\)-test of difference between means.

Table 4.2 Known acute phase reactants in National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than or equal to 3.5 mg/dl) - evidence of an acute phase response
<table>
<thead>
<tr>
<th>Dietary Factor</th>
<th>No APR(^a) CRP &lt; 3.5 mg/dl</th>
<th>APR(^a) CRP &gt;= 3.5 mg/dl</th>
<th>p(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2.143 (13.12)</td>
<td>1.763.58 (127.25)</td>
<td>0.0179</td>
</tr>
<tr>
<td>Protein (gm)</td>
<td>81.72 (0.55)</td>
<td>71.73 (5.38)</td>
<td>0.1391</td>
</tr>
<tr>
<td>Total Fat (gm)</td>
<td>82.70 (0.63)</td>
<td>69.44 (6.01)</td>
<td>0.0332</td>
</tr>
<tr>
<td>Saturated Fat (gm)</td>
<td>28.04 (0.23)</td>
<td>23.70 (2.05)</td>
<td>0.0408</td>
</tr>
<tr>
<td>Monounsaturated Fat (gm)</td>
<td>30.92 (0.25)</td>
<td>20.36 (2.42)</td>
<td>0.1431</td>
</tr>
<tr>
<td>Polyunsaturated Fat (gm)</td>
<td>17.30 (0.16)</td>
<td>13.70 (1.49)</td>
<td>0.0202</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>311.16 (3.27)</td>
<td>257.80 (27.88)</td>
<td>0.0637</td>
</tr>
<tr>
<td>Carbohydrate (gm)</td>
<td>258.30 (1.58)</td>
<td>212.32 (16.29)</td>
<td>0.0175</td>
</tr>
<tr>
<td>Fiber (gm)</td>
<td>16.87 (0.14)</td>
<td>13.15 (1.26)</td>
<td>0.0202</td>
</tr>
<tr>
<td>Vitamin A(^c)</td>
<td>998.79 (21.18)</td>
<td>878.76 (134.71)</td>
<td>0.3833</td>
</tr>
<tr>
<td>Carotenes(^c)</td>
<td>539.66 (12.04)</td>
<td>508.44 (25.59)</td>
<td>0.8324</td>
</tr>
<tr>
<td>Vitamin E (α-tocopherol equivalents)</td>
<td>9.05 (0.11)</td>
<td>6.54 (0.73)</td>
<td>0.0015</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>109.40 (1.36)</td>
<td>97.87 (15.10)</td>
<td>0.4880</td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>282.69 (2.64)</td>
<td>216.53 (20.09)</td>
<td>0.0021</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>807.42 (6.85)</td>
<td>629.84 (59.92)</td>
<td>0.0051</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>14.75 (0.12)</td>
<td>12.10 (1.07)</td>
<td>0.0177</td>
</tr>
</tbody>
</table>

a. Mean (standard error).
b. p-value for t-test of difference between means.
c. Vitamin A and carotenes are assessed in retinol equivalents.

Table 4.3 Univariate mean dietary intakes in National Health and Nutrition Examination Survey (NHANES) III study participants with (n=45) and without (n=6,718) considerable levels of C-reactive protein (greater than or equal to 3.5 mg/dl) - evidence of an acute phase response
<table>
<thead>
<tr>
<th>Serum Micronutrient</th>
<th>No APR(^a)</th>
<th>APR(^a)</th>
<th>p(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRP &lt; 3.5 mg/dl</td>
<td>CRP &gt;= 3.5 mg/dl</td>
<td></td>
</tr>
<tr>
<td>Serum Iron (ug/dl)</td>
<td>89.52 (0.45)</td>
<td>45.82 (4.22)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(n=7,035)</td>
<td>(n=50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Folate (ng/ml)</td>
<td>5.92 (0.06)</td>
<td>6.15 (0.66)</td>
<td>0.7483</td>
</tr>
<tr>
<td>(n=7,036)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Calcium (mmol/l)</td>
<td>2.30 (0.01)</td>
<td>2.26 (0.02)</td>
<td>0.0351</td>
</tr>
<tr>
<td>(n=6,744)</td>
<td>(n=48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Selenium (ng/ml)</td>
<td>121.93 (0.19)</td>
<td>112.64 (1.97)</td>
<td>0.0000</td>
</tr>
<tr>
<td>(n=6,909)</td>
<td>(n=50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Vitamin C (mg/dl)</td>
<td>0.69 (0.01)</td>
<td>0.53 (0.05)</td>
<td>0.0030</td>
</tr>
<tr>
<td>(n=6,787)</td>
<td>(n=47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Vitamin A (ug/dl)</td>
<td>57.53 (0.19)</td>
<td>46.67 (1.98)</td>
<td>0.0000</td>
</tr>
<tr>
<td>(n=6,997)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Vitamin E (ug/dl)</td>
<td>1,101.27 (5.32)</td>
<td>1,094.90 (59.58)</td>
<td>0.9188</td>
</tr>
<tr>
<td>(n=6,997)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (\alpha)-carotene (ug/dl)</td>
<td>4.38 (0.06)</td>
<td>2.84 (0.37)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(n=6,997)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum (\beta)-carotene (ug/dl)</td>
<td>18.84 (0.24)</td>
<td>14.10 (1.83)</td>
<td>0.0129</td>
</tr>
<tr>
<td>(n=6,997)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Lycopene (ug/dl)</td>
<td>22.18 (0.14)</td>
<td>14.88 (1.08)</td>
<td>0.0001</td>
</tr>
<tr>
<td>(n=6,997)</td>
<td>(n=51)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Mean (standard error).
b. p-value for t-test of difference between means.

Table 4.4 Univariate mean levels of serum micronutrients in National Health and Nutrition Examination Survey (NHANES) III study participants with and without considerable levels of C-reactive protein (greater than or equal to 3.5 mg/dl) - evidence of an acute phase response
<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Mild APR 1 mg/dl &lt; CRP &lt; 3.5 mg/dl POR(^a) (95% CI)</th>
<th>Marked APR CRP &gt;= 3.5 mg/dl POR(^a) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron</td>
<td>0.984 (0.980-0.988)(^b)</td>
<td>0.946 (0.933-0.960)(^b)</td>
</tr>
<tr>
<td>Serum Folate</td>
<td>1.011 (0.993-1.030)</td>
<td>1.011 (0.973-1.051)</td>
</tr>
<tr>
<td>Serum Calcium</td>
<td>0.836 (0.641-1.091)</td>
<td>1.101 (0.558-2.171)</td>
</tr>
<tr>
<td>Serum Selenium</td>
<td>0.990 (0.982-0.998)(^b)</td>
<td>0.959 (0.939-0.980)(^b)</td>
</tr>
<tr>
<td>Serum Vitamin C</td>
<td>0.729 (0.545-0.976)(^c)</td>
<td>0.375 (0.164-0.857)(^c)</td>
</tr>
<tr>
<td>Serum Vitamin A</td>
<td>0.983 (0.975-0.991)(^b)</td>
<td>0.943 (0.921-0.966)(^b)</td>
</tr>
<tr>
<td>Serum Vitamin E</td>
<td>1.000 (1.000-1.000)(^b)</td>
<td>1.000 (0.999-1.000)</td>
</tr>
<tr>
<td>Serum α-carotene</td>
<td>0.919 (0.880-0.960)(^b)</td>
<td>0.819 (0.710-0.945)(^b)</td>
</tr>
<tr>
<td>Serum β-carotene</td>
<td>0.970 (0.959-0.981)(^b)</td>
<td>0.967 (0.938-0.998)(^c)</td>
</tr>
<tr>
<td>Serum Lycopene</td>
<td>0.970 (0.959-0.982)(^b)</td>
<td>0.937 (0.902-0.972)(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age, sex, body mass index, current smoking status, dietary energy intake, and dietary intake of specific micronutrient being assessed (iron, folate, calcium, vitamins C, A, and E, or carotenoids).

\(^b\) p-value less than 0.01.

\(^c\) p-value less than 0.05.

Table 4.5 Adjusted prevalence odds ratios for a mild APR (C-reactive protein level between 1 and 3.5 mg/dl) and a marked APR (C-reactive protein level greater than or equal to 3.5 mg/dl) from serum micronutrients among National Health and Nutrition Examination Survey (NHANES) III study participants.
Discussion

Results from this investigation have shown that the APR is associated with decreased concentrations of serum iron, selenium, vitamin C, vitamin A, $\alpha$-carotene, $\beta$-carotene, and lycopene, and that the decreased concentration of serum calcium can be explained by sex, age, body mass index, current smoking status, and dietary factors. The fact that serum micronutrients are altered during both mild and marked APR's, and that the POR's for a mild APR are nearer the null, but still statistically significant, suggest that the effect may be continuous, rather than discrete. All but one of the known previous investigations of non-protein changes associated with the APR have examined study participants who were either diagnosed with cancer (Georgiannos et al., 1993; Sattar et al., 1997; Talwar et al., 1997; Feelders et al., 1998), malaria (Das et al., 1996), or cystic fibrosis (Duggan et al., 1996), or who were undergoing surgery (Louw et al., 1992) or who were either older than 60 years of age (Chiari et al., 1995; Boosalis et al., 1996), or younger than 18 years of age (Filteau et al., 1993; Friis et al., 1997). Results from these studies are consistent with the findings that the following micronutrients are decreased during an APR: serum iron (Chairi et al., 1995; Sattar et al., 1997; Feelders et al., 1998), vitamin C (Louw et al., 1992; Georgiannos et al., 1993; Khaw and Woodhouse, 1995), vitamin A (Louw et al., 1992; Das et al., 1996; Duggan et al., 1996; Friis et al, 1997; Talwar et al., 1997; Filteau et al., 1998), and carotenoids (Boosalis et al., 1996).

The findings of the present investigation may be effects of altered concentrations of other factors, not yet known to be associated with the APR, or the APR may directly affect serum micronutrients. Decreases in most of the serum micronutrients may be
explained by two theories. First, to provide increased availability to assaulted tissues. Serum micronutrients may escape the vasculature during an APR. Second, serum micronutrients may be consumed by free radicals, especially since diseases associated with the APR (such as cancer and atherosclerosis) progressively increase oxidative stress (Wiseman et al., 1996).

The finding of lower serum vitamin C among individuals experiencing an APR may be the result of regulation of the APR (or mediating cytokines) by vitamin C. Ikedo et al. (1998) found that, among rats with a genetic deficiency resulting in the inability to synthesize vitamin C, concentrations of the positive acute phase proteins haptoglobin and alpha 1-acid glycoprotein were significantly increased in the rats fed a vitamin C-deficient diet (compared to their counterparts fed a diet containing 300 mg/kg of vitamin C), while negative acute phase protein concentrations were decreased only among the vitamin C-deficient rats. In addition, interleukin-6 (IL-6 - a regulator of APR-related hepatic protein synthesis) concentrations were found to be significantly elevated only in the vitamin C-deficient rats (Ikedo et al., 1998). The author speculates that serum vitamin C may, at least in part, mediate the APR.

The finding that serum iron is decreased among individuals experiencing an APR may be the result of an APR-associated event - the anemia of chronic disease (ACD). As a result of the ACD, the concentration of serum transferrin (the carrier protein for serum iron) is decreased, and serum iron is moved toward storage (and blocked from tissue release) into serum ferritin, a positive acute phase protein. (Konijn, 1994).
A potential bias of this investigation results from the fact that the APR witnessed here may be the heavily-weighted result of one disease, such as preclinical cancer. Although study participants with any evidence of current poor health or previous disease conditions are excluded from these analyses, it is likely that most, if not all, of the study participants experiencing an APR are responding to some tissue assault (e.g. tumorogenesis, atherosclerosis, infection, etc.). It may be that most of the study participants with an APR have, for example, preclinical cancer and the lower concentrations of serum micronutrients are result of the nutrient needs of the tumor. An additional source of error may be found in the inability to control confounding by intermediate factors such as proteins that bind and transport serum micronutrients.

Further research is needed to determine if the concentration changes in serum micronutrients like those witnessed here are concurrent with changes in concentrations of acute phase proteins, or if the effects are independent but both caused by disease state(s). There are at least three associated lines of inquiry that should be followed. First, since the APR is associated with changes in antioxidants (Louw et al., 1992; Talwar et al., 1997) as well as trace elements and their carrier proteins (Sattar et al., 1997), estimates of risk from cancer case-control studies examining these factors (or adjusting for their confounding) may not reflect the status of individuals prior to the development of cancer. For example, in a case-control study, an APR-induced decrease in serum vitamin A may appear to increase cancer risk, when there may be no association between pre-APR serum vitamin A and serum vitamin A at the time of assessment. Determining the rate of decrease (if it is uniform) of vitamin A may permit the adjustment of serum vitamin A for
the APR. Second, the benefit of vitamin and mineral supplementation (especially vitamin C) among individuals experiencing an APR should be evaluated. Similarly, the impact of vitamin C supplementation on acute phase reactants should be evaluated in individuals diagnosed with APR-associated diseases, such as cancer. Third, G-estimation (Robins et al., 1992) should be used to control confounding by serum proteins functioning as potential intermediates between the APR and altered serum micronutrient concentrations.
CHAPTER 5

Urinary Albumin is Associated with the Acute Phase Response and Increased Risk for Uterine, Lymphatic, and Prostate Cancer Independent of Serum Albumin

Abstract

Previous investigations have suggested that urinary albumin excretion is associated with the acute phase response (APR) among individuals diagnosed with several diseases. These findings may be proxies for APR-associated decreased serum albumin concentrations because changes in urinary albumin and serum albumin may result from the same mechanism, transvascular escape. The purpose of this investigation was to determine whether (1) urinary albumin is associated with the APR independently of serum albumin, (2) serum albumin modifies the association between urinary albumin and the APR, and (3) urinary albumin excretion is associated with increased cancer risk. Data from the National Health and Nutrition Examination Survey (NHANES) III were employed to obtain logistic regression-derived prevalence odds ratios (POR) for questions (1) and (2), and data from NHANES I were used to obtain Cox proportional hazards regression-derived risk ratios (RR) for question (3). Standardized urinary dipstick analysis was used to obtain the level of albumin in urine for both NHANES I and III. Urinary albumin levels greater than 100 μg/dl are independently associated with the APR (indicated by a C-reactive protein level greater than 1 mg/dl) (adjusted POR = 1.80, 95
percent confidence interval [CI]: 1.19 - 2.70), and this association is not altered after control for confounding by serum albumin. Low serum albumin (less than 3.5 g/dl) modifies the association between urinary albumin and the APR, but not as expected. The largest POR is found among study participants with serum albumin less than 3.5 g/dl but a level of albumin in urine less than 100 mg/dl (POR = 4.50, 95 percent CI: 3.30 - 6.12). A urinary albumin level greater than or equal to 100 mg/dl is associated with increased risks of cancers of the prostate (RR = 1.88, 95 percent CI: 0.98 - 3.56), lymphatic/hematopoietic tissue (RR = 2.84, 95 percent CI: 1.31 - 6.17), and uterus (including cervix) (RR = 2.48, 95 percent CI: 0.78 - 7.95). Potential mechanisms explaining these findings include cytokine-induced increases in glomerular capillary permeability, and cytokine-induced glomerular damage.
Introduction

Serum albumin decreases during the course of the acute phase response (APR) (Kushner, 1982; Koj, 1985; Baumann and Gauldie, 1994; Moshage, 1997). The mechanism(s) resulting in this decrease are unclear, and the subject of debate because low serum albumin has been associated with many adverse health outcomes, including shorter lengths of survival from cancer at many sites (Herrmann et al., 1992; Falconer et al., 1994; Su et al., 1996; Bastion et al., 1997; Kouroumalis et al., 1997; Maltoni et al., 1997; Schwartzbaum et al., In Press, 1999). Albumin losses may be due to increased albumin degradation, decreased albumin synthesis (Kushner, 1982; Cruickshank et al., 1989; Mackiewicz, 1997), movement of albumin from plasma to interstitial compartments (Fleck et al., 1985), a plasma dilution effect as the result of an expanded water space (Preston et al., 1987), or external losses (especially in urine) (Kaysen, 1990; Kaysen, 1993). In a study of cachectic pancreatic cancer patients, Fearon et al. (1998) found no net decrease in the synthesis of albumin to explain the decreased serum albumin.

Serum albumin also decreases during the progression of renal disease - the apparent result of insufficient replacement (synthesis) of albumin following losses in urine (Kaysen, 1998). If losses of albumin in urine reflect a decrease in serum albumin, a high concentration of albumin in urine may be indirectly associated with the APR. In fact, there is limited evidence that a urinary albumin excretion is an indicator of the APR among individuals with non-insulin-dependent diabetes mellitus (NIDDM) ( Pickup, et al., 1997; Islam et al., 1998), inflammatory bowel disease (Mahmud et al., 1995; Mahmud et al., 1996), and renal disease (Kaysen, 1998). For example, in patients diagnosed with
inflammatory bowel disease, microalbuminuria (a significant increased rate of albumin excretion in urine, usually assessed as greater than 20 milligrams per minute) was strongly correlated with levels of C-reactive protein (CRP - a protein whose concentration is increased during the APR) (Mahmud et al., 1995). Microalbuminuria has also been associated with both advanced lung cancer stage and shorter survival from lung cancer (Pedersen and Milman, 1998), a greater severity of colonic inflammation (Mahmud et al., 1996), and the prevalence of weight-loss in gastrointestinal cancer patients (Georgiannos et al., 1995). Further, Jensen et al., (1995) found that, in healthy study participants, microalbuminuria was an independent marker of systemic transvascular albumin leakiness, suggesting that APR-associated decreases in serum albumin may be the result of an increased transcapillary escape rate (movement from the intravascular to the extravascular compartment) (Fleck et al., 1985), perhaps mediated by the cytokine, vascular endothelial growth factor (VEGF) (Roberts et al., 1998; Webb et al., 1998).

The purpose of this investigation was three-fold: 1) first, to determine whether urinary albumin is associated with the APR, and to find out whether the association is altered after control for confounding by serum albumin. If the presence of albumin in urine is related to the APR, but is altered after control for confounding by low serum albumin, this result may suggest that the two occurrences are related (and, perhaps, governed by the same factors), and that they reflect one mechanism of albumin loss; 2) second, to determine whether any association between the APR and urinary albumin is modified by low serum albumin. That is, for example, individuals with low serum albumin, but no albumin in urine, may be less likely to be experiencing an APR than are
individuals with both low serum albumin and meaningful levels of albumin in urine; 3) third, to determine whether urinary albumin is associated with increased cancer risk after control for confounding by serum albumin.
Methods

*Study Populations.* Data from the National Health and Nutrition Examination Survey (NHANES) III Epidemiologic Follow-up Study (NHEFS) (description of study is detailed in Chapter 4, Methods Section, as well as by others [National Center for Health Statistics, 1994; US Department of Health and Human Services, 1996]) were used to determine if the APR (indicated by a CRP level greater than 1 mg/dl) is associated with the presence of a high concentration of albumin in urine (indicated by a urinary albumin level greater than or equal to 100 µg/ml) among apparently healthy study participants. (Levels of CRP in sera less than 1 mg/dl have been suggested to be clinically unimportant [Morley and Kushner, 1982; Gabay and Kushner, 1999].) Study participants suspected of having an infection during the medical examination or who were found by a physician to be in less than “very good” physical condition were excluded from analyses. In addition, study participants previously diagnosed with non-skin cancer, congestive heart failure, myocardial infarction, and/or stroke were excluded. Lastly, only study participants between the ages of 18 and 90 were included. (It should be noted that, although all study participants were found to be in “very good” physical condition, it is likely that most individuals with an APR are experiencing some tissue assault, injury, infection, or disease [Kushner, 1982; Cruickshank et al., 1989; Mackiewicz, 1997].)

To determine whether albumin in urine is associated with increased cancer risk, data from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study (NHEFS) (description of study and exclusions are explained in Chapter 3, Methods Section, as well as by others [Engel et al., 1978;
McDowell et al., 1981; Madans et al., 1986; Cohen et al., 1987; Finucane et al., 1990; Cox et al., 1992) were employed.

*Laboratory Procedure.* Urinary albumin measurement in both NHANES I and NHANES III was accomplished by standardized urinary dipstick analysis. During the NHANES III survey, urinary dipstick analysis was conducted on the same day (or very near the same day) as CRP analysis.

*Statistical Methods.* To determine if urinary albumin is associated with the APR, logistic regression was used to obtain prevalence odds ratios (POR). Potential confounders included age, sex, body mass index, and current smoking status. Current smoking status was determined using serum cotinine levels. A level of 50 ng/ml has been suggested as a cutpoint, above which study participants were considered to be current tobacco smokers (Apseloff et al., 1994) The selection of confounders was accomplished by assessing the effect of the removal of a potential confounder on the regression coefficient of the urinary albumin excretion factor. Factors whose removal affected the regression coefficient remained in the model. To determine whether the potential association between urinary albumin and the APR is modified by low serum albumin, stratification was used. Low serum albumin was characterized as less than 3.5 g/dl - a commonly-used clinical reference of the lower limit of normal range (Lowrie and Lew, 1990).

To determine whether albumin in urine marks an increase in cancer risk, Cox proportional hazards regression analysis was used to obtain risk ratios (RR). Risk ratios were generated for cancer at seven sites (lung, colon/rectum, female breast, prostate,
lymphatic/hematopoietic, uterus [including cervix], and bladder) for which there were sufficient incidences. The referent group for each analysis was study participants with urinary albumin less than 100 mg/dl. Adjustment for potential confounding by sex, race, age at randomization, body mass index, pack-years of tobacco smoking, and serum albumin was accomplished, as above, by including factors in models. Potentially confounding factors were selected as above. All statistical procedures were conducting using Statistical Analysis System (SAS).
Results

*Question 1. Urinary Albumin and the APR.* (Results pertaining to question 1 are not presented in tables.) Among 7,096 apparently healthy study participants, 466 are experiencing an APR (as evidenced by a CRP level greater than 1 mg/dl), and a total of 234 study participants are found to have levels of albumin in urine greater than 100 mg/dl). The 234 are not distributed randomly between the groups with the APR and the group without. Among those without an APR, 203 (86.8 percent) are found to have a urinary albumin level greater than or equal to 100 mg/dl, and among those with an APR, 31 (13.2 percent) are found to have a urinary albumin level greater than or equal to 100 mg/dl. Logistic regression reveals an association between the APR and urinary albumin greater than or equal to 100 mg/dl. The age-, sex-, body mass index-, and cotinine-adjusted POR for the APR is 1.80 (95 percent confidence interval: 1.19 - 2.70) for a high concentration versus trace or no albumin in urine. The POR, when further adjusted for serum albumin diminishes, but the association is not entirely eliminated (POR = 1.42 (95 percent confidence interval: 0.93 - 2.17). In addition, urinary albumin greater than or equal to 100 mg/dl is associated with serum albumin. The age-, sex-, body mass index-, and cotinine-adjusted prevalence POR for the presence of albumin in urine is 0.40 (95 percent confidence interval: 0.29 - 0.60).

*Question 2. Modification of Urinary Albumin/APR by Serum Albumin.* Table 5.1 shows adjusted POR’s for the APR from the interaction between serum albumin and urinary albumin. The strongest association with the APR is witnessed among the study participants with serum albumin less than 3.5 g/dl and less than 100 mg/dl of urinary
albumin. Although urinary albumin greater than or equal to 100 mg/dl is associated with the APR across both levels of serum albumin, the strength of the association is reduced among those with normal levels of serum albumin. Paradoxically, compared to study participants with trace or no albumin in urine, the strength of the association between serum albumin and the APR is reduced among those with greater than or equal to 100 mg/dl of albumin in urine. To evaluate the presence of an interaction, the product of the discordant cells (the cell reflecting urinary albumin less than 100 mg/dl and serum albumin less than 3.5 g/dl, and the cell reflecting urinary albumin greater than or equal to 100 mg/dl and serum albumin greater than or equal to 3.5 g/dl) is compared with the non-referent concordant cell (POR = 3.01). The expected POR of the concordant cell is 8.64 - almost three times that which is observed.

**Question 3. Urinary Albumin and Cancer Risk.** Table 5.2 shows the number of NHANES I study participants diagnosed with cancer by site, after exclusions. Table 5.3 presents RR’s for seven cancers from urinary albumin, after control for confounding. Greater than or equal to 100 mg/dl of albumin in urine is found to be associated with increased risk for cancers of the prostate, lymphatic/hematopoietic tissue, and uterus (including cervix). The increased risks for cancer of the prostate (RR = 1.31, 95 percent confidence interval [CI]: 0.69 to 2.50), lymphatic/hematopoietic tissue (RR = 2.06, 95 percent CI: 0.96 to 4.45), and uterus (including cervix) (RR = 1.79, 95 percent CI: 0.56 to 5.69) diminish, but are not eliminated, by control for confounding by serum albumin. A small number of study participants with urinary albumin greater than or equal to 100
mg/dl (n = 96) prevents obtaining risk ratios for more specific cancer sites (such as cervix and leukemia).
<table>
<thead>
<tr>
<th>Urinary Albumin</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt; (95% C.I.)</th>
<th>OR&lt;sup&gt;a&lt;/sup&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 3.5 g/dl</td>
<td>4.50 (3.30 - 6.12)</td>
<td>3.01 (1.03 - 8.77)</td>
</tr>
<tr>
<td>(n = 285)</td>
<td></td>
<td>(n = 22)</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; = 3.5 g/dl</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.92 (1.24 - 2.97)</td>
</tr>
<tr>
<td>(n = 6,577)</td>
<td></td>
<td>(n = 212)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for age, sex, cotinine level greater than 50 ng/ml, and body mass index.  
<sup>b</sup> Referent cell.

Table 5.1 Adjusted odds ratios (OR) for the acute phase response from the stratification of serum albumin and urinary albumin among NHANES III study participants.
<table>
<thead>
<tr>
<th>Site of Neoplasm</th>
<th>Original n</th>
<th>(1)</th>
<th>(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>248</td>
<td>231</td>
<td>231</td>
</tr>
<tr>
<td>Colon/Rectum</td>
<td>229</td>
<td>208</td>
<td>207</td>
</tr>
<tr>
<td>Female Breast</td>
<td>221</td>
<td>197</td>
<td>196</td>
</tr>
<tr>
<td>Prostate</td>
<td>185</td>
<td>176</td>
<td>174</td>
</tr>
<tr>
<td>Lymphatic/Hematopoietic</td>
<td>126</td>
<td>116</td>
<td>110</td>
</tr>
<tr>
<td>Uterus (Including Cervix)</td>
<td>78</td>
<td>74</td>
<td>71</td>
</tr>
<tr>
<td>Bladder</td>
<td>68</td>
<td>65</td>
<td>64</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td><strong>1,609</strong></td>
<td><strong>1,509</strong></td>
<td><strong>1,367</strong></td>
</tr>
</tbody>
</table>

*a. Cancers that occurred in fewer than 60 study participants are not presented. Therefore, columns do not total to number of cancers at all sites.*

Table 5.2 Number of NHANES I study participants developing cancers after sequential exclusions for baseline malignant tumors (1), and incident benign tumors and absence of measurement or reporting of selected acute phase reactants (2)
<table>
<thead>
<tr>
<th>Site of Neoplasm (n)</th>
<th>R.R.</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung(^a) (231)</td>
<td>1.09</td>
<td>0.48 - 2.46</td>
</tr>
<tr>
<td>Colon/Rectum(^b) (207)</td>
<td>0.67</td>
<td>0.21 - 2.10</td>
</tr>
<tr>
<td>Female Breast(^c) (196)</td>
<td>1.03</td>
<td>0.33 - 3.22</td>
</tr>
<tr>
<td>Prostate(^d) (174)</td>
<td>1.88</td>
<td>0.98 - 3.56</td>
</tr>
<tr>
<td>Lymphatic/Hematopoietic(^a) (110)</td>
<td>2.84</td>
<td>1.31 - 6.17</td>
</tr>
<tr>
<td>Uterus (Including Cervix)(^e) (71)</td>
<td>2.48</td>
<td>0.78 - 7.95</td>
</tr>
<tr>
<td>Bladder(^a) (64)</td>
<td>0.62</td>
<td>0.09 - 4.49</td>
</tr>
</tbody>
</table>

- \(^{a}\) Adjusted for sex, age at baseline and pack-years of tobacco smoking.
- \(^{b}\) Adjusted for sex and age at baseline.
- \(^{c}\) Among females, adjusted for age at baseline.
- \(^{d}\) Among males, adjusted for race and age at baseline.

Table 5.3 Adjusted risk ratios (RR) for cancers by site of neoplasm from a urinary albumin level greater than or equal to 100 mg/dl among incident cancer cases and 12,534 non-case NHANES I study participants.
Discussion

The results from this investigation suggest that urinary albumin levels greater than or equal to 100 mg/dl are associated with the APR, that serum albumin does not completely explain (or remove) the association, and that, while serum albumin modifies the association, the modification does not occur as expected. That is, the POR is greater, not in the group of study participants with both serum albumin less than 3.5 g/dl and urinary albumin greater than or equal to 100 mg/dl, but in those with only lower serum albumin. Fewer than the expected number of individuals with both low serum albumin and urinary excretion of albumin mount an APR. Renal damage, such as that of the nephrotic syndrome, has been observed in individuals already diagnosed with non-renal malignancies (Fer et al., 1981; Hoyt and Hamilton, 1987; Wagrowska-Danilewicz and Danilewicz, 1995), and both the nephrotic syndrome and proteinuria are associated with decreased survival from cancers at many sites (Pedersen and Milman, 1996; Wagrowska-Danilewicz and Danilewicz, 1995). However, this investigation provides the first known demonstration that albumin in urine is associated with an increase in risk of cancers of the prostate, lymphatic/hematopoietic tissue, and uterus (including cervix).

The general finding that urinary albumin is associated with the APR is in agreement with results of other investigations (Mahmud et al., 1996; Pickup et al., 1997; Islam et al., 1998). Islam et al. (1998) speculate that the association between the APR and microalbuminuria (at least among white NIDDM patients) may result from an increase in the permeability of glomerular capillaries caused by actions of the APR-regulating cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha.
(TNF-alpha). Because APR-regulating cytokines have been found circulating in the sera of individuals with cancer (Baumann and Gauldie, 1994; Mackiewicz, 1997; Seymour et al., 1997), this increase in permeability may also explain increases in cancer risks from urinary albumin. There is, however, another potential mechanism explaining the association between cancer and urinary albumin. Individuals with cancer are continuously exposed to antigens, stimulating antibody production and forming circulating immune complexes, which have been found deposited on glomerular basement membranes (Norris, 1993; Wagrowska-Danilewicz, 1995; Pedersen and Milman, 1998). This deposition may result in structural and functional changes in the glomerulus (such as thickening of the basement membrane), and may ultimately result in glomerulopathy. Glomerulopathies are thought to be more common in carcinomas and lymphomas (Wagrowska-Danilewicz, 1995). Glomerulopathies have been specifically associated with Hodgkin’s disease (Powderly et al., 1985) and T-cell lymphoma (Belghiti et al., 1981). This may explain the nearly three-fold increase in risk for lymphatic and hematopoietic cancers from urinary albumin levels greater than or equal to 100 mg/dl.

At least three sources of possible bias encourage the cautious interpretation of these results. First, a small number of study participants with urinary albumin greater than or equal to 100 mg/dl (n = 96, for NHANES I; n = 234, for NHANES III) has resulted in wide confidence intervals for associations between urinary albumin and the APR, and urinary albumin and cancer risk. Second, it is possible that the preponderance of one pre-clinical disease (such as renal disease) among NHANES III study participants is the cause of associations between the APR and urinary albumin. That is, the association
between urinary albumin excretion and the APR may be strong among individuals with renal disease, and the POR’s may reflect this disease-specific trend, rather than the more general finding pertaining to individuals without pre-clinical disease. Third, although every attempt was made to control confounding, it is possible that an unidentified or unrecognized factor confounds the results.

The results suggest that urinary albumin and serum albumin are independently associated with the APR, perhaps as the result of different mechanisms of albumin loss. More research is required to determine the mechanisms governing both APR-related decreases in serum albumin concentrations, and increases in urinary albumin excretion, especially among individuals with cancer.
CHAPTER 6

Summary and Suggestions for Future Research

The previous four chapters have addressed four primary research questions concerning the APR.

Research Question Number One - What is the behavior of the concentrations of serum albumin, transferrin, and iron prior to cancer and MI diagnoses?

Serum transferrin and albumin may decrease before diagnosis of cancer and myocardial infarction (MI). These alterations are probably the result of the acute phase response (APR), a systemic cytokine-governed adaptive reaction to cellular injury during which the pattern of protein synthesis in the liver is altered. To determine how long alterations in concentrations of serum transferrin and albumin occur prior to cancer and MI diagnoses, data were analyzed from The Lipid Research Clinics Coronary Primary Prevention Trial and follow-up study (n = 3,806 hyperlipidemic men). During an average in-trial follow-up of 7.4 years and an average combined in-trial and post-trial follow-up of 13.4 years, three groups of study participants were identified: 1) those first diagnosed with cancer (n=245); 2) those first diagnosed with MI (n=437); 3) those diagnosed with neither cancer nor heart disease (n=1,651). Changes in serum albumin, transferrin (assessed by iron binding capacity [IBC]), and iron were plotted against number of years
prior to diagnosis (or the end of the trial, for the group developing neither cancer nor heart disease) that each serum assessment occurred.

Eight years before cancer and MI diagnoses mean serum albumin is significantly lower (cancer: 4.18 g/dl, 95 percent confidence interval [CI]: 4.13 - 4.23; MI: 4.23, 95 percent CI: 4.19 - 4.27), and mean IBC significantly higher (cancer: 349.8, 95 percent CI: 341.6 - 358.1; MI: 350.9, 95 percent CI: 327.6 - 330.0), than eight years prior to the end of the trial in the group of study participants not diagnosed with cancer or heart disease (albumin: 4.31, 95 percent CI: 4.29 - 4.33; IBC: 330.3, 95 percent CI: 327.6 - 330.0). In addition, mean serum iron is significantly lower eight years prior to cancer diagnosis. To adjust serum transferrin, iron, and albumin over time for age at randomization, a mixed (both random and fixed factors) model regression was used. Serum albumin levels decrease slightly in each of the three groups. A statistically significant decrease in both IBC and serum iron is found in the years prior to cancer and MI diagnoses, while a statistically significance increase in IBC and serum iron is found in the group of study participants not diagnosed with cancer or heart disease. This long preclinical APR may explain discrepancies in results of studies of iron status and both cancer and MI risk.

Potential mechanisms for APR-associated decreases include an increase in vascular permeability caused by the activity of vascular endothelial growth factor.

There is very little research concerning the prolonged APR. Further research may better determine if concentration changes in characteristic acute phase proteins during the prolonged APR are similar to those witnessed during the short-lived APR. The identification of APR-related changes occurring long before cancer and MI diagnoses
may elucidate very early systemic changes associated with both neoplasia and atherosclerosis.

First, further research is required to determine, more specifically, *when* concentrations of serum albumin, IBC, and serum iron, as well as more specific APR proteins (such as C-reactive protein), change prior to cancer and MI diagnoses. Because it is possible that decreases in serum albumin, IBC, and serum iron just prior to cancer and MI diagnoses cause the slopes of change of these proteins to be different from zero, a change-point analysis is needed. A change-point analysis would estimate inflection point(s) in regression lines of changes in the concentrations of APP’s (like those shown in Figures 2.1 to 2.7). Determining when serum protein concentrations are altered may be beneficial in understanding the biology governing associations between the APR and both tumorogenesis and atherosclerosis. To answer this question precisely requires sufficient data (a large sample, frequent APR assessments, and consistent spacing between assessments).

Second, because concentrations of serum transferrin and iron decrease prior to cancer and MI diagnoses, it may be necessary to obtain serial measurements of these factors and to treat them as time-dependent covariates in investigations of iron status and both cancer and MI risk. Moreover, it would be beneficial to investigate previous iron-related findings within a meta-analysis adjusted for the length of time between iron status assessment and either cancer or MI diagnosis.

Third, it may be worthwhile to examine the screening capacity of easily- and routinely-measured serial acute phase reactants, both individually and in combinations.
Last, in general, the analysis of longitudinal studies containing serial preclinical measurements should be (at least additionally) evaluated with techniques specific for repeated measurements. Often, such data are analyzed at a specific time/event of interest (such as cancer diagnosis), rather than over some course of time prior to the time/event of interest. Assessing changes in serial measurements permits the richer investigation of the behavior of the factor of interest, which may more likely test hypotheses concerning mechanisms of change. The new MIXED procedure offered by SAS provides the technique and implementation by which these investigations can be conducted (Wolfinger, 1997).
Research Question Number Two - Because acute phase reactants have been found to be altered at the time of lung and bladder cancer diagnoses, do routinely-measured acute phase reactants (serum albumin, IBC, white blood cell count [WBCC], and ESR) alter risks for lung and bladder cancers, after individuals diagnosed within the three-year period following assessment of acute phase reactants are excluded?

Serum constituents are altered at the time of cancer diagnosis as a result of the acute phase response (APR), a cytokine-governed response to tissue injury, infection, or tobacco smoking. The purpose of this investigation was to determine whether four routinely-measured serum constituents (white blood cell count [WBCC], serum albumin, iron binding capacity [IBC], and erythrocyte sedimentation rate [ESR]) are associated with risk for two smoking-related cancers: lung and bladder cancers. Data from the National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up Study (NHEFS) (n = 13,370) were used to obtain Cox proportional hazards regression analysis-derived risk ratios (RR). After excluding study participants diagnosed with lung or bladder cancer in the three-year period following baseline acute phase serum assessment, 155 lung cancer cases (median follow-up of 9.4 years) and 41 bladder cancer cases (median follow-up of 8.4 years) were identified.

Alterations in risk are found to vary considerably by sex. Among males, lower quartile serum albumin (less than 4.1 g/dl) does not alter lung cancer risk, and lower quartile IBC (less than 313 ug/dl) is associated with a marginally statistically significant decrease in lung cancer risk, a finding opposite to that expected if serum transferrin decreases during both the APR and lung tumorogenesis. Both upper quartile ESR (greater
than 25 mm/hour) and WBCC (greater than 9,350/mm³) are associated with increased lung cancer risk among males. Among females, each acute phase reactant marks an increase in lung cancer risk, although RR’s reach only marginal statistical significance. Among males, lower quartile serum albumin (less than 4.1 g/dl), upper quartile ESR (greater than 25 mm/hour), and upper quartile WBCC (greater than 9,440/mm³) are associated with increased bladder cancer risk, although only the RR for upper quartile WBCC reaches statistical significance. Among males, upper quartile WBCC is associated with over an eight-fold increase in bladder cancer risk from WBCC greater than 9,350/mm³ among males. Among females, lower quartile IBC (less than 319 ug/dl) is associated with a marginally statistically significant decrease in bladder cancer risk. Among males, an eosinophil count greater than two percent is associated with increased lung cancer risk (age- and pack-years of tobacco smoking-adjusted RR = 1.79, 95 percent confidence interval [CI]: 1.05 - 3.07), and a lymphocyte count greater than 33 percent is associated with decreased lung cancer risk (age- and pack-years of tobacco smoking-adjusted RR = 0.60, 95 percent CI: 0.37 - 1.00). Among females, none of the percentages of cell types is associated with altered lung cancer risk.

The results suggest that APR-induced changes, especially a greater WBCC, occur at least three years prior to clinical presentation with bladder and lung cancers. At least three lines of inquiry should be further investigated. First, larger studies of the behavior of these easily-measured APR factors prior to lung and bladder cancer diagnoses are warranted to confirm the findings of the present investigation. Second, the relationship between WBCC (and, more specifically lymphocytes and eosinophils) and the APR
should be further investigated. Very little is known about the behavior of leukocytes during the APR. The mechanism(s) governing an increased WBCC among males prior to lung and bladder cancer diagnoses should be elucidated. Last, the these easily-measured acute phase reactants should be reconsidered as potential screening agents, either in combination with one another, or in combination with more sophisticated factors.
Research Question Number Three - Are the concentrations of serum micronutrients altered during, or as a result of, the APR after control for confounding by dietary and other factors?

APR effects on serum micronutrients have been largely ignored. The purpose of this study was to better characterize the APR - to determine whether the concentrations of serum micronutrients are altered in individuals experiencing an APR. Data from NHANES III were used to obtain logistic regression-derived adjusted prevalence odds ratios (POR) for the presence of an APR (indicated by a C-reactive protein level greater than or equal to 3.5 mg/dl), after excluding individuals in less than good health, and those previously diagnosed with cancer and cardiovascular disease. Except for serum folate and vitamin E, mean concentrations of all serum micronutrients are found to be lower among the group of study participants experiencing an APR. Statistically significant associations between the APR and the following serum micronutrients are not removed by control for confounding: serum iron (POR = 0.946, 95 percent confidence interval [CI]: 0.933 - 0.960), serum selenium (POR = 0.959, 95 percent CI: 0.939 - 0.980), serum vitamin C (POR = 0.375, 95 percent CI: 0.164 - 0.857), serum vitamin A (POR = 0.943, 95 percent CI: 0.921 - 0.966), serum α-carotene (POR = 0.819, 95 percent CI: 0.710 - 0.945), serum β-carotene (POR = 0.967, 95 percent CI: 0.938 - 0.998), and serum lycopene (POR = 0.937, 95 percent CI: 0.902 - 0.972). Similar results, but nearer the null value, are reported for a mild APR (C-reactive protein levels between one and 3.5 mg/dl). These results are, generally, in agreement with the results of previous investigations of individuals diagnosed with APR-associated diseases. The mechanisms governing the
decreased levels of serum micronutrients (including the notion that serum micronutrients may escape the vasculature during an APR, and may be consumed by free radicals) need to be elucidated with further research.

First, serial assessments of serum micronutrients should be compared to serial assessments of definitive acute phase proteins (such as C-reactive protein). Changes in the concentrations of serum micronutrients concomitant with concentration changes in acute phase proteins, would more explicitly suggest APR-associated changes in serum micronutrient concentrations. Second, based on these findings and the results of other investigations, the benefit of vitamin C supplementation in individuals experiencing an APR should be evaluated. Third, because the APR is a potential confounding factor in the study of serum micronutrients and diseases (potentially) related to the APR (such as cancer), the analysis of such investigations should include adjustment for a definitive acute phase protein. Fourth, G-estimation should be used to control confounding by serum proteins functioning as potential intermediates between the APR and altered serum micronutrient concentrations. Such an analysis may determine the extent to which concentrations of serum micronutrients are altered by their transport and/or binding proteins.
Research Question Number Four - Is urinary excretion of albumin a unique acute phase reactant, or is urinary albumin a proxy for serum albumin, and is urinary albumin associated with an increase in cancer risk?

Previous investigations have suggested that urinary albumin excretion is associated with the acute phase response (APR) among individuals diagnosed with several diseases. These findings may be proxies for APR-associated decreased serum albumin concentrations because changes in urinary albumin and serum albumin may result from the same mechanism, transvascular escape. The purpose of this investigation was to determine whether (1) urinary albumin is associated with the APR independently of serum albumin, (2) serum albumin modifies the association between urinary albumin and the APR, and (3) urinary albumin excretion is associated with increased cancer risk. Data from the National Health and Nutrition Examination Survey (NHANES) III were employed to obtain logistic regression-derived prevalence odds ratios (POR) for questions (1) and (2), and data from NHANES I were used to obtain Cox proportional hazards regression-derived risk ratios (RR) for question (3). Standardized urinary dipstick analysis was used to obtain the level of albumin in urine for both NHANES I and III.

Logistic regression reveals an association between the APR and urinary albumin greater than or equal to 100 mg/dl. The age-, sex-, body mass index-, and cotinine-adjusted POR for the APR is 1.80 (95 percent confidence interval: 1.19 - 2.70) for a high concentration versus trace or no albumin in urine. The POR, when further adjusted for serum albumin diminishes, but the association is not entirely eliminated (POR = 1.42 (95 percent confidence interval: 0.93 - 2.17). Low serum albumin (less than
3.5 g/dl) modifies the association between urinary albumin and the APR, but not as expected. The largest POR is found among study participants with serum albumin less than 3.5 g/dl but a level of albumin in urine less than 100 mg/dl (POR = 4.50, 95 percent CI: 3.30 - 6.12). A urinary albumin level greater than or equal to 100 mg/dl is associated with increased risks of cancers of the prostate (RR = 1.88, 95 percent CI: 0.98 - 3.56), lymphatic/hematopoietic tissue (RR = 2.84, 95 percent CI: 1.31 - 6.17), and uterus (including cervix) (RR = 2.48, 95 percent CI: 0.78 - 7.95). Potential mechanisms explaining these findings include cytokine-induced increases in glomerular capillary permeability, and cytokine-induced glomerular damage.

At least two lines of research should be evaluated pertaining to potential APR-induced increases in urinary albumin excretion. First, associations between cytokines governing the APR (IL-1, IL-6, TNF-alpha), urinary albumin, and glomerular damage should be explored, especially in individuals diagnosed with cancer. Experiments of the effects of cytokines on glomerular damage may provide more conclusive evidence elucidating the mechanisms responsible for APR-induced increases in urinary albumin excretion. Second, there is a need for more investigations of the mechanism(s) governing the loss of albumin from sera of individuals experiencing an APR. Specifically, studies of the transvascular escape and fate of serum albumin are needed, especially among individuals with cancer.
LIST OF REFERENCES


Fraser WD. Taggart DP. Fell GS. Lyon TD. Wheatley D. Garden OJ. Shenkin A. Changes in iron, zinc, and copper concentrations in serum and in their binding to transport proteins after cholecystectomy and cardiac surgery. Clinical Chemistry. 35(11):2243-7, 1989 Nov.


128


Liao Y. Cooper RS. McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study. Amer J of Epidemiology. 139(7):704-12, 1994 Apr.


Schaefer EJ. Lamon-Fava S. Jenner JL. McNamara JR. Ordovas JM. Davis CE. Abolafia JM. Lippel K. Levy RI. Lipoprotein(a) levels and risk of coronary heart disease in men.


135


137


Woodhouse PR.; Meade TW.; Khaw KT. Plasminogen activator inhibitor-1, the acute phase response and vitamin C. Atherosclerosis. 133(1): 71-6, 1997 Aug.


