RESEARCH COMMUNICATION

Iron, Cholesterol, and the Risk of Cancer in an 18-year Cohort

Brian J Wells[1], Arch G Mainous III[2], Charles J Everett[2], James M Gill[3]

Abstract

The iron catalyzed oxidation of serum lipids is hypothesized to generate oxidative stress, which appears to play an important role in the pathogenesis of many cancers. Previous research has obtained conflicting results regarding the independent contribution of cholesterol and iron on cancer risk. The purpose of this study was to test for an interaction between iron and cholesterol on cancer risk. The present cohort study was an analysis of the National Health and Nutrition Examination Survey I (NHANES I) database linked with the NHANES I Epidemiologic Follow-up Study. Baseline serum iron and total cholesterol values were obtained on 7,448 adults, who were followed for the development of cancer over 18-21 years. Population weights were applied to create Cox proportional hazard models of time to the development of cancer for the entire U.S. adult population (n=72,602,523). Control variables included: age, race, gender, smoking, body mass index, chronic cough, chronic hepatitis, chronic/recurrent colitis or enteritis, and gastrointestinal bleeding. Independent elevations of either iron or total cholesterol were not significantly related to the development of cancer in the adjusted model. However, the combination of iron and total cholesterol above the 75th percentile was associated with a significant increase in the risk of all cancers (HR 1.39, 95% CI 1.00-1.94). Iron and cholesterol above the 80th and 85th percentiles increased the hazard ratio for cancer further to 1.51 (CI 1.10-2.08) and 1.61 (CI 1.07-2.43), respectively. These results support the theory that the iron induced oxidation of serum lipids is important in the pathogenesis of cancer.

Key Words: Iron - cholesterol - cancer - oxidative stress - cohort

Asian Pacific J Cancer Prev, **6**, 505-509

Introduction

Free radicals and oxidants are continuously generated within mammalian cells, but are normally neutralized by the body's anti-oxidant metabolism. Oxidative stress can damage lipids, proteins, and DNA. Researchers have discovered that oxidative stress plays a role in the pathogenesis of cancer (Galli et al., 2005; Halliwell, 2002; Hristozov et al., 2001; Mates and Sanchez-Jimenez, 2000; Poulsen et al., 1998).

Trace metals play an important catalytic role in the formation of reactive oxygen species and oxidative stress. Iron in particular can create oxidative stress by inducing the oxidation of serum lipids (Fischer et al., 2002; Glei et al., 2002; Halliwell and Gutteridge, 1990; Johnson, 2001). Our theory is that the oxidative stress created by the iron catalyzed oxidation of serum lipids results in the production of reactive oxygen species that ultimately cause mutagenic damage to DNA. Therefore, we predict that individuals with high levels of iron and cholesterol will be at an increased risk of cancer. The purpose of this study was to examine the risk of cancer based on levels of serum iron and cholesterol in a nationally representative cohort study.

Materials and Methods

The present cohort was created by merging the National Health and Nutrition Examination Survey I database (NHANES I) (1971-1974) with the NHANES I Epidemiologic Follow-up Studies (NHEFS) (1982-1984, 1986, 1987 and 1992). The NHANES I survey was a multistage, stratified, probability, cluster sampling of non-institutionalized persons between 1-74 years of age. The survey design involved over-sampling of population subgroups, including persons living in poverty areas, women of childbearing age (25-44 years of age), and elderly persons (65 years of age and over). The sampling design of the NHANES I allowed the use of weighting to create population estimates of the entire United States.

The NHEFS is a national longitudinal dataset which allows investigation of the relationships between clinical, nutritional, and behavioral factors assessed at the baseline

[1]The Cleveland Clinic Foundation, Department of Family Medicine; [2]Medical University of South Carolina, Department of Family Medicine; [3]Thomas Jefferson University, Department of Family and Community Medicine, and Department of Health Policy.
Corresponding Author: Brian J. Wells, MD, MS, Department of Family Medicine, The Cleveland Clinic Foundation, ST (10), 9500 Euclid Ave, Cleveland, OH 44195 wellsb@ccf.org Phone: 440-878-3164 Fax: 440-878-3185

of the NHANES I, and subsequent morbidity and mortality. The NHEFS initial population includes the 14,407 participants who were 25-74 years of age when first examined in NHANES I. More than 98% of the individuals in the initial NHANES I cohort were traced and supplied data in the 1992 NHEFS.

Information in the NHEFS was gathered in one of three ways. Subjects were interviewed who could be contacted and could participate. If the subject was alive but incapacitated, a slightly modified version of the subject questionnaire was administered to a proxy respondent. A separate proxy questionnaire was used only when the subject was deceased. Finally, for individuals who had died in the time period between the NHANES I index interview and the follow-up interview, information from death certificates was recorded. Individuals 25 and older who had serum iron and cholesterol measured at baseline were included in the cohort. Participants who had previous, physician diagnosed, malignant tumors prior to the NHANES I exam were excluded. These exclusions resulted in an un-weighted cohort of 7,448 individuals.

Independent Variables

Details about specific laboratory procedures followed by the NHANES I are available through the Center for Disease Control's website at http://www.cdc.gov/nchs/data/nhanes/nhanesi/16-71_75.pdf.

Cholesterol

Serum cholesterol was quantified in duplicate from non-fasting blood samples using spectrophotometry. Total serum cholesterol was the only lipid measurement available in the baseline assessment. It is unclear what level of cholesterol might be associated with cancer risk. Thus, serum cholesterol was initially stratified according to the 75th percentile of the population (>248.08 mg/dL).

Serum Iron

Serum iron was measured in the NHANES I baseline by spectrometric analysis of the reaction between Ferrozine and Fe(II). As with total serum cholesterol, it is unclear what level of serum iron is associated with cancer risk. Thus, serum iron was initially stratified according to the 75th percentile of the population (122.44 μg/dL).

Cancer Events

Incidence of cancer was determined by answers to interview questions in the 1982-1984, 1986, 1987 and 1992 NHEFS interviews, plus information from death certificates. The ICD-9 codes associated with cancer mortality were 140 through 239. Non-melanoma skin cancer (ICD-9 codes 173.XX) was not classified as a cancer event.

Control Variables

Demographics

Demographic control variables that were available in the NHANES I baseline included age, gender and race. Four age categories were defined (25-34, 35-52, 53-69 and >69). Race follows the NHANES I designations of "white", "black", or "other."

Cancer Risk Factors

Body mass index (BMI) and smoking were included as additional controls, due to their association with cancer risk. Additionally, obesity has been positively correlated with levels of oxidative stress (Suzuki et al., 2003). Smoking status was classified as "never" or "ever" based on self-report from interviews at baseline or during the 1982-1984 follow-up interview. BMI was calculated with the standard formula (BMI = weight (kg) / height (m^2)) from measured height and weight information. BMI > 30 was defined as "obese."

Comorbidity

Hepatitis and chronic/recurrent colitis were also included as controls due their association with liver cancer and colorectal cancer respectively (Ahmad, 2002; Sharan and Schoen, 2002). Histories of hepatitis and/or colitis were determined by self-report.

In order to reduce the bias potentially caused by undetected cancer at baseline, "chronic cough" and "gastrointestinal bleeding" (which could represent undetected cancer), were also included in the controls. The presence of a chronic cough and/or gastrointestinal bleeding was determined by self-report.

Data Analysis

The population was classified into four groups based upon low or high serum iron, and low or high total serum cholesterol. Because of the exploratory nature of this study, iron and cholesterol were initially dichotomized as "high" or "low" according to the 75th percentile. Subsequently, a sensitivity analyses was performed to determine the optimal relationship between iron, cholesterol and cancer. Specifically, the cutoffs for elevated serum iron and cholesterol were analyzed at the 50th, 80th, and 85th percentiles. Analyses could not be conducted above the 85th percentile due to small cell sizes, which would not allow reliable population estimates for the survival analysis.

Sampling weights were used to calculate prevalence estimates for the civilian, non-institutionalized U.S. population. Because of the complex sampling design of the survey, all analyses were performed using the statistical package SUDAAN (Research Triangle Park, North Carolina). Kaplan-Meier curves were computed from the population estimates generated by SUDAAN. Cox proportional hazards models were created with time to development of cancer for each group controlling for age, gender, race, smoking status, BMI, and co-morbidities. In these models, cancer-free survival time was a continuous variable measured in one-year increments up to 18 years from the baseline. The Schoenfeld test of residuals was computed to confirm the proportionality of hazards

Table 1. Population Characteristics of Adults Age 25 and Older from NHANES[1] I and NHEFS[2] Studies with Normal (≤122 μg/dl) or Elevated (>122 μg/dl) Serum Iron Levels, and Normal (≤248 mg/dl) or Elevated (>248 mg/dl) Cholesterol Levels

	Normal Iron and Normal Cholesterol n=40,741,468	Normal Iron and Elevated Cholesterol n=13,485,541	Elevated Iron and Normal Cholesterol n=13,677,875	Elevated Iron and Elevated Cholesterol n=4,697,639
Cancer Incidence	11.17	15.40	11.56	20.11
Age Group				
25-34	29.67	9.86	34.01	11.03
35-52	41.69	37.03	41.09	40.07
53-69	25.42	46.08	22.01	43.04
>69	3.22	7.04	2.89	5.86
Gender				
Male	45.77	41.44	51.63	50.83
Female	54.23	58.56	48.37	49.17
Race				
White	89.32	89.43	91.95	92.92
Black	9.85	10.46	6.81	4.96
Other	0.82	0.11	1.24	2.12
Ever Smoker				
Yes	61.88	60.51	71.05	71.34
No	38.12	39.49	28.95	28.66
Body Mass Index				
<30 kg/m2	83.51	78.45	89.96	86.72
<30 kg/m2	16.49	21.55	10.04	13.28

[1]NHANES = National Health and Nutrition Examination Survey
[2]NHEFS = NHANES I Epidemiologic Follow-up Study

assumption within the models (Schoenfeld, 1982).

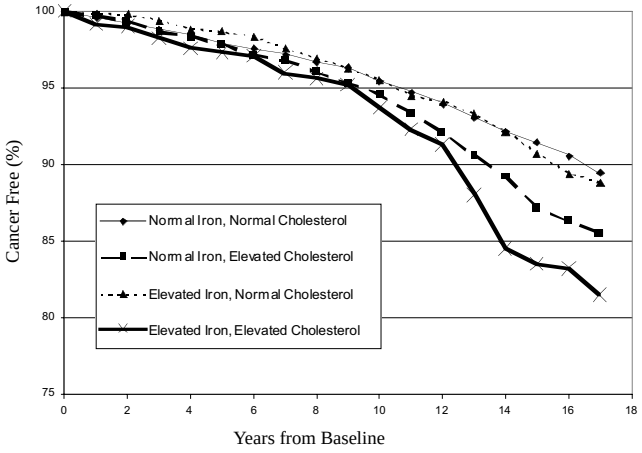
Results

The weighted sample represents 72,602,253 individuals. Table 1 shows the demographic characteristics of the weighted sample. Individuals with elevations of both total cholesterol and serum iron above the 75th percentile represent 6.5% of the U.S. population > 25 years old. Table 2 displays the independent and combined relationships of iron and cholesterol on the risk of cancer. The 75th percentiles for iron and total cholesterol in the adult U.S. population are 122 μg/dl and 248 mg/dl, respectively. Individually, serum iron was not related to the risk of cancer development in either the unadjusted or the adjusted model (Table 2). In the unadjusted model, serum total cholesterol above the 75th percentile was associated with an increased risk of cancer. However, the independent relationship between total

Table 2. Unadjusted and Adjusted Cox Regression Using Serum Iron and Total Cholesterol to Predict Time to the Occurrence of Cancer

	HR (95% CI)
Unadjusted Individual Markers Based on Elevated defined as >75th percentile	
Normal Serum Iron (≤122 μg/dl)	1.00 (1.00-1.00)
Elevated Serum Iron (>122 μg/dl)	1.13 (0.95-1.35)
Normal Cholesterol (≤248 mg/dl)	1.00 (1.00-1.00)
Elevated Cholesterol (>248 mg/dl)	1.52 (1.22-1.88)**
Elevated Serum Iron and Elevated Cholesterol	1.88 (1.36-2.60)**
Adjusted Individual Markers Based on Elevated defined as >75th percentile*	
Normal Serum Iron (≤122 μg/dl)	1.00 (1.00-1.00)
Elevated Serum Iron (>122 μg/dl)	1.17 (0.97-1.41)
Normal Cholesterol (≤248 mg/dl)	1.00 (1.00-1.00)
Elevated Cholesterol (>248 mg/dl)	1.11 (0.89-1.38)
Elevated Serum Iron and Elevated Cholesterol	1.39 (1.00-1.94)**
Adjusted Sensitivity Analysis*	
Serum Iron and Cholesterol >50th Percentile	0.98 (0.77-1.25)
Serum Iron and Cholesterol >75th Percentile	1.39 (1.00-1.94)**
Serum Iron and Cholesterol >80th Percentile	1.51 (1.10-2.08)**
Serum Iron and Cholesterol >85th Percentile	1.61 (1.07-2.43)**

*Adjusted for age, gender, race, smoking status, BMI, chronic cough, chronic hepatitis, chronic/recurrent colitis or enteritis, and gastrointestinal bleeding. **Statistically significant with p<=0.05 HR = Hazard Ratio for the time to the development of cancer CI = Confidence Interval



*Elevated Serum Iron (>122 mg/dl), Elevated Cholesterol (>248 mg/dl)

Figure 1. Kaplan-Meier Curves of time to the Development of Cancer with Elevated Iron and Cholesterol, defined as > the 75th Percentile*

cholesterol and cancer risk disappeared in the adjusted model. The combination of elevated iron and elevated cholesterol was significantly associated with cancer risk in both the unadjusted (HR 1.88, CI 1.36-2.60) and the adjusted models (HR 1.39, CI 1.00-1.94). The hazard ratio increased and remained significant as the combination of high iron and high total cholesterol was stratified at the 80th, and 85th percentiles (Table 2). Figure 1 shows a graphical representation of the unadjusted risks associated with iron and cholesterol above or below the 75th percentile.

Discussion

This study demonstrated, in a nationally representative cohort, that persons with elevations of both serum iron and cholesterol are at an increased risk for the development of cancer. As the levels of both factors increased, the adjusted relative risk of developing cancer increased. The increased risk of cancer was not observed with isolated elevations of either cholesterol or iron. This finding demonstrates an interaction between iron and cholesterol in the development of cancer and supports our theory that the iron induced oxidation of serum lipids is important in carcinogenesis.

Previous research revealed an inverse relationship between cholesterol and cancer in the NHANES I (Schatzkin et al., 1987). Our present study did not find an independent relationship between cholesterol and cancer. The discrepancy between this present study and the study by Schatzkin et al. is likely due to the additional control variables that we have employed to help reduce bias and our weighted analysis performed with SUDAAN.

This study confirms our previous analysis of the Framingham Offspring Cohort that demonstrated a higher risk of cancer in individuals with concomitant elevations of iron and very low density lipoprotein (Mainous et al., 2005). The results of the current study are more representative of the overall U.S. population, due to the weighted analysis.

The proportion of individuals with both elevated iron and cholesterol is not trivial. The present findings suggest that 6.5% of the adult United States population may be at an increased risk for cancer due to these factors. The incidence of cancer in the high-risk group (cholesterol and iron >75th percentile) was 20.1% compared to a incidence of only 12.1% in the rest of the population with the attributable risk of cancer due to high iron and high cholesterol being 8.0% in this study.

The results of this study may also help to explain the conflicting results of previous studies regarding iron levels and cancer risk iron has well described carcinogenic properties (Weinberg, 1996). However, previous studies have suggested only a weak positive association between iron levels and the risk of cancer with significant independent risk appearing only at very high levels (Knekt et al., 1994; Stevens et al., 1994; Stevens et al., 1988; Wu et al., 2004). These previous studies may have found small and inconsistent results mainly because they were focusing on an un-differentiated population with unknown lipid levels. Lipid peroxidation is a common product of iron induced oxidation and may be expected to occur at the highest rate when both cofactors are readily available.

Discoveries in genetic research support the findings of this study. Genetic mutations of chromosome 8p22, which codes for the macrophage scavenger receptor 1 (MSR1) gene, has been linked to the development of prostate cancer (Xu et al., 2002). The MSR1, also known as the class A macrophage scavenger receptor (SR-A), enables macrophages to recognize and eliminate oxidatively damaged low density lipoprotein (LDL)(Platt and Gordon, 2001). Similarly, CD36 plays a role as a scavenger of oxidized LDL in the colon. Mutations of CD36 have been linked to the risk of colorectal cancer (Kuriki et al., 2005). Inability to properly dispose of oxidized LDL could increase cancer risk by increasing exposure to reactive oxygen species (Xu et al., 2002). Future research could examine the relationship between iron, cholesterol, and cancer in individuals with MSR1 or CD36 mutations.

The findings in this study may also partially explain the recent discovery that the use of Hydroxymethylglutaryl-CoA Reductase Inhibitors (ie. Statins) is associated with lower odds of colorectal cancer (Poynter et al., 2005) and prostate cancer (Shannon et al., 2005).

Limitations of the study include the use of a one-time laboratory value to assess iron and cholesterol. We have no information on iron or cholesterol levels after the baseline measurement. However, elevated iron levels are likely to remain elevated unless there are major dietary changes, since the propensity to over-absorb iron has a substantial genetic component (Witte et al., 1996). In addition, serum iron and total cholesterol levels are relatively crude markers. Other indicators such as serum ferritin and more specific lipid markers were unavailable in the NHANES I. However, using nonspecific predictors of oxidative stress may only serve to minimize the effect size observed in this study. A second limitation of the study is the use of family-reports, self-

reports, and death certificate information to obtain cancer incidence/mortality. These methods are subject to ascertainment bias.

Strengths of this study include the large sample size, population weighting, use of extensive control variables, rigorous methodology of the NHANES, and long length of follow-up.

In summary, elevations in serum iron and total cholesterol interacted to increase cancer risk in this cohort. These findings support our hypothesis that the iron induced oxidation of serum lipids is important in carcinogenesis. If this finding can be replicated in other studies, this would suggest the need for interventional studies to reduce iron and/or lipid levels in persons with dual elevations of these substances.

Acknowledgements

This work was supported in part by grants from the Health Resources and Services Administration (1D12HP00023-02 and 8 D54 HP 00023-04), the National Cancer Institute (K 12 CA 76917-08), and from the Delaware Division of Public Health.

References

Ahmad K (2002). Hepatitis B risk factors for liver cancer. *Lancet Infect Dis,* **2,** 511.

Fischer JG, Glauert HP, Yin T, et al (2002). Moderate iron overload enhances lipid peroxidation in livers of rats, but does not affect NF-kappaB activation induced by the peroxisome proliferator, Wy-14,643. *J Nutr,* **132,** 2525-31.

Galli F, Piroddi M, Annetti C, et al (2005). Oxidative stress and reactive oxygen species. *Contrib Nephrol,* **149,** 240-60.

Glei M, Latunde-Dada GO, Klinder A, et al (2002). Iron-overload induces oxidative DNA damage in the human colon carcinoma cell line HT29 clone 19A. *Mutat Res,* **519,** 151-61.

Halliwell B (2002). Effect of diet on cancer development: is oxidative DNA damage a biomarker? *Free Radic Biol Med,* **32,** 968-74.

Halliwell B, Gutteridge J (1990). Oxygen radicals in biological systems. Part B: Oxygen radicals and antioxidants. *Methods Enzymol,* **186,** 1-766.

Hristozov D, Gadjeva V, Vlaykova T, et al (2001). Evaluation of oxidative stress in patients with cancer. *Arch Physiol Biochem,* **109,** 331-6.

Johnson S (2001). The possible crucial role of iron accumulation combined with low tryptophan, zinc and manganese in carcinogenesis. *Med Hypotheses,* **57,** 539-43.

Knekt P, Reunanen A, Takkunen H, et al (1994). Body iron stores and risk of cancer. *Int J Cancer,* **56,** 379-82.

Kuriki K, Hamajima N, Chiba H, et al (2005). Relation of the CD36 gene A52C polymorphism to the risk of colorectal cancer among Japanese, with reference to with the aldehyde dehydrogenase 2 gene Glu487Lys polymorphism and drinking habit. *Asian Pac J Cancer Prev,* **6,** 62-8.

Mainous AG, 3rd, Wells BJ, Koopman RJ, et al (2005). Iron, lipids, and risk of cancer in the Framingham Offspring cohort. *Am J Epidemiol,* **161,** 1115-22.

Mates JM, Sanchez-Jimenez FM (2000). Role of reactive oxygen species in apoptosis: implications for cancer therapy. *Int J Biochem Cell Biol,* **32,** 157-70.

Platt N, Gordon S (2001). Is the class A macrophage scavenger receptor (SR-A) multifunctional? - The mouse's tale. *J Clin Invest,* **108,** 649-54.

Poulsen HE, Prieme H, Loft S (1998). Role of oxidative DNA damage in cancer initiation and promotion. *Eur J Cancer Prev,* **7,** 9-16.

Poynter JN, Gruber SB, Higgins PD, et al (2005). Statins and the risk of colorectal cancer. *N Engl J Med,* **352,** 2184-92.

Schatzkin A, Hoover RN, Taylor PR, et al (1987). Serum cholesterol and cancer in the NHANES I epidemiologic followup study. National Health and Nutrition Examination Survey. *Lancet,* **2,** 298-301.

Schoenfeld D (1982). Residuals for the proportional hazards model. *Biometrika,* **69,** 239-41.

Shannon J, Tewoderos S, Garzotto M, et al (2005). Statins and Prostate Cancer Risk: A Case-Control Study. *Am J Epidemiol,* **162,** 318-325.

Sharan R, Schoen RE (2002). Cancer in inflammatory bowel disease. An evidence-based analysis and guide for physicians and patients. *Gastroenterol Clin North Am,* **31,** 237-54.

Stevens RG, Graubard BI, Micozzi MS, et al (1994). Moderate elevation of body iron level and increased risk of cancer occurrence and death. *Int J Cancer,* **56,** 364-9.

Stevens RG, Jones DY, Micozzi MS, et al (1988). Body iron stores and the risk of cancer. *N Engl J Med,* **319,** 1047-52.

Suzuki K, Ito Y, Ochiai J, et al (2003). Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pac J Cancer Prev,* **4,** 259-66.

Weinberg ED (1996). The role of iron in cancer. *Eur J Cancer Prev,* **5,** 19-36.

Witte DL, Crosby WH, Edwards CQ, et al (1996). Practice guideline development task force of the College of American Pathologists. Hereditary hemochromatosis. *Clin Chim Acta,* **245,** 139-200.

Wu T, Sempos CT, Freudenheim JL, et al (2004). Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. *Ann Epidemiol,* **14,** 195-201.

Xu J, Zheng SL, Komiya A, et al (2002). Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. *Nat Genet,* **32,** 321-5.