Biomarkers, February 2008; 13(1): 41–51

informa
healthcare

Correlation between two markers of inflammation, serum C-reactive protein and interleukin 6, and indices of oxidative stress in patients with high risk of cardiovascular disease

DORA IL'YASOVA[1], ANASTASIA IVANOVA[2], JASON D. MORROW[3], MATTEO CESARI[4], & MARCO PAHOR[4]

[1]*Duke Comprehensive Cancer Center, Durham, NC, USA,* [2]*Department of Biostatistics, School of Public Health, McGavran-Greenberg Hall, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA,* [3]*Vanderbilt University Medical Center, Nashville, TN, USA and* [4]*Department of Aging and Geriatric Research, University of Florida, Gainesville, FL, USA*

Abstract

As evidence of the involvement of inflammation and oxidative damage in pathogenesis of age-related chronic diseases is growing, epidemiologists need to develop measures of both conditions to study their relationships in human populations. One way of searching for appropriate biomarkers is to examine correlations between different inflammatory markers and oxidative indices. We examined cross-sectional correlations between two inflammatory markers, serum C-reactive protein (CRP) and interleukin (IL)-6, and three oxidative indices, plasma levels of α-tocopherol and β-carotene, and urinary levels of 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (F_2-IsoP), in 60 individuals at high risk of cardiovascular disease. Correlations between the biomarkers were examined graphically and using the Pearson correlation coefficient. No correlation was found between plasma levels of α-tocopherol and either of the inflammatory markers. Plasma β-carotene inversely correlated with IL-6 ($r = -0.46$, $p = 0.0002$) and CRP ($r = -0.41$, $p = 0.001$). Although urinary F_2-IsoP did not correlate with IL-6, this biomarker positively correlated with CRP ($r = 0.31$, $p = 0.002$). As only urinary F_2-IsoP levels have been validated against known oxidative assaults, their positive association with CRP levels is interpreted as evidence of an interconnection between low-level inflammation and oxidative status. Urinary levels of F_2-IsoP and serum levels of CRP represent appropriate biomarkers for future studies of inflammation and oxidative status in humans.

Keywords: *Biological markers, oxidative stress, inflammation, epidemiology*

(Received 6 June 2007; accepted 6 August 2007)

Introduction

Recently, a focal point of epidemiology has been variation in levels of inflammatory markers within the reference range. Interest in this topic was raised by the findings that inflammatory markers predict the risk of multiple age-related chronic conditions:

Correspondence: Dora Il'yasova, Duke Comprehensive Cancer Center, 2424 Erwin Rd, Hock Bldg, Ste 602, Box 2949, Durham, NC 27710, USA. Tel: 919-668-6531. Fax: 919-681-4785. E-mail: dora.ilyasova@duke.edu

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500701617708

cardiovascular disease (Pearson et al. 2003), cancer (Coussens & Werb 2002), macular degeneration (Seddon et al. 2005), type 2 diabetes (Pradhan et al. 2001, Hu et al. 2004a), and physical disability (Ferrucci et al. 1999, Cesari et al. 2004). Inflammation-induced pathogenesis, as indicated by a large body of evidence, involves oxidative stress (Coussens & Werb 2002, Hussain et al. 2003). The pathogenic triangle of inflammation/oxidative stress/chronic disease is an intriguing hypothesis because both adverse factors can be alleviated by specific interventions. Such interventions could be based on measurements of systemic levels of inflammation and oxidative status.

Our question is whether 'normal' variations in levels of inflammatory markers correlate with indices of oxidative status. A correlation between these parameters could provide evidence for a causal cluster, similar to what has been observed between continuous traits in metabolic syndrome (Liese et al. 1998). Also, such correlations can help to identify biomarkers for future epidemiological studies focusing on inflammation and oxidative status.

This study examines correlations between two inflammatory markers – serum levels of interleukin (IL)-6 and C-reactive protein (CRP) – and three indices of oxidative status – plasma levels of α-tocopherol and β-carotene, and urinary levels of 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane (F_2-IsoP). We chose these three oxidative indices for the following reasons: fat-soluble antioxidants α-tocopherol and β-carotene have been used in epidemiological studies as indicators of antioxidant protection (Flagg et al. 1995) and F_2-isoprostanes are currently considered to be the best biomarker of oxidative status in humans (Morrow & Roberts 2002, Basu 2004, National Heart Lung & Blood Institute 2004, Milne et al. 2005).

The study subjects were at high risk of cardiovascular disease. This population was selected because both low-level inflammation and oxidative stress have been implicated in cardiovascular pathogenesis (Pearson et al. 2003, Stocker & Keaney 2004). Involvement of both factors implies that the relationship between them, if it exists, would be more pronounced in a high-risk population.

Materials and methods

Study population

This study included 60 participants in the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors (TRAIN). The TRAIN study is a double-blind, crossover, randomized, placebo-controlled trial aimed at assessing the biological mechanisms by which angiotensin-converting enzyme (ACE) inhibition may improve clinical outcomes in persons aged >55 years who have a high cardiovascular disease risk profile. The design of the study has been reviewed elsewhere (Cesari et al. 2005). Briefly, the participants were recruited from the communities of Winston-Salem and Greensboro, North Carolina, USA. Eligibility was determined via a phone interview followed by a clinical prescreening visit. Subjects who successfully completed the prescreening visit ($n=401$) were entered in a screening and single-blind run-in phase in which compliance and the tolerability of the ACE inhibitor fosinopril were evaluated. Participants who successfully completed all of the preliminary phase interviews and visits ($n=295$) were randomly assigned to either the placebo or the intervention branch (treatment with fosinopril) of the study during the baseline clinical visit. This study presents baseline cross-sectional data from the first 60 participants

randomized to the placebo or the intervention branch, excluding those with missing values for measurements of inflammatory markers or oxidative indices. All of the participants signed an informed consent form for the study at the screening visit. The Institutional Review Board of Wake Forest University approved the study protocol.

Laboratory measurements

To minimize circadian rhythm and other fluctuations in marker concentrations, all of the participants had their blood drawn by venipuncture on the morning of the screening and of each clinical visit, after fasting for 6 h. Blood specimens were processed in the cold within 1 h of collection. Plasma aliquots were placed into cryovials and frozen at -70°C until the analysis. Special precautions were taken to protect fat-soluble antioxidants from oxidation: processing was performed under subdued light; aliquots for antioxidant analysis were placed into amber vials containing 8 μl of 2.5% 2,6 ditertiarybutyl-4-methyl phenol (BHT) in ethanol; and vials were caped under argon.

Serum levels of IL-6 and CRP were available from the main TRAIN study. Methods for their measurement have been described elsewhere (Ridker et al. 1997, Cesari et al. 2005). Plasma concentrations of α-tocopherol and β-carotene were measured in the Pharmacology Core Laboratory of the Wake Forest University Comprehensive Cancer Center by reverse-phase high-performance liquid chromatography (HPLC) with UV/VIS detection using a modified version of a published method (Hess et al. 1991). The detection (quantitation) limits were 0.1 μg ml^{-1} for α-tocopherol and 0.01 μg ml^{-1} for β-carotene. None of the sample values were below the limit of detection for any of the measured antioxidants. Samples with concentrations above the upper limit of the standard curve were extracted and diluted 1:1 with BHT ethanol prior to injection onto the system.

Morning spot urine samples were collected from the participants at the baseline examination and stored at -70°C. Measurement of F_2-IsoP in urine was performed at Vanderbilt University by gas chromatography with negative ion chemical ionization mass spectrometry (Morrow et al. 1999). The F_2-IsoP values were adjusted by urine creatinine. Urine creatinine was determined by a modified Jaffe method (Kroll et al. 1986).

Other variables

Demographic information, smoking status, body mass index (BMI), and medical history were available from the main TRAIN study. These data were collected during the prescreening clinic visit.

Statistical analysis

The analysis of correlations between inflammatory markers and oxidative indices was conducted using log-transformed variables because the distribution of all examined biomarkers was right-skewed. The relationships between markers were examined graphically and using the Pearson correlation coefficient. Similarly, correlations between the biomarkers and two continuous variables, age and BMI, were examined using the Pearson correlation coefficient. We also calculated and compared geometric

means of oxidative indices by three levels of CRP: low (<1.0 mg l^{-1}), average (1.0–3.0 mg l^{-1}), and high-risk (>3.0 mg l^{-1}) levels. This categorization was developed for cardiovascular diseases (Pearson et al. 2003), for which the relationship with inflammatory markers has been studied more extensively than for other chronic conditions. The distribution of biomarkers by gender, diabetes status, and history of cancer were examined using the Wilcoxon rank test.

Results

As expected, the study subjects showed a high prevalence of characteristics associated with the risk of cardiovascular disease (Table I): 73% had hypertension, 59% had a high total cholesterol level, 41% were obese, 24% had diabetes, 13% had a history of stroke and 6% had stable angina. The age of study subjects ranged from 55 to 81 years. The median level of CRP, a known predictor of cardiovascular risk, was in the range of average risk for cardiovascular disease (1.0–3.0 mg l^{-1}) (Pearson et al. 2003) and was higher than the established median reference level in healthy individuals (2.26 vs. 0.64–0.80 mg l^{-1}) (Macy et al. 1997). Thirty-seven percent of the subjects had CRP levels associated with a high risk of cardiovascular disease (>3.0 mg l^{-1})

Table I. Subject characteristics.

	Mean (SD) or %
Age (years)	68 (7)
Range	55–81
Race: White	94%
BMI (kg m^{-2})	29.0 (4.3)
Range	22.5–43.3
Female	33%
Current smokers	3%
Diabetes	24%
Total cholesterol >200 mg dl^{-1}	59%
Hypertension	73%
History of stroke	13%
History of stable angina	6%
History of cancer	25%
CRP (mg l^{-1})	3.55 (4.00)
Range	0.21–19.10
25%–median–75%	1.02–2.26–3.84
IL-6 (pg ml^{-1})	3.91 (2.90)
Range	1.18–13.01
25%–median–75%	2.18–2.99–3.72
α-tocopherol (μg ml^{-1})	10.71 (5.88)
Range	3.45–35.22
25%–median–75%	7.10–8.60–12.84
β-carotene (μg ml^{-1})	0.101 (0.098)
Range	0.018–0.606
25%–median–75%	0.039–0.060–0.131
F_2–IsoP (ng mg^{-1} creatinine)	0.90 (0.94)
Range	0.19–4.47
25%–median–75%	0.37–0.56–0.98

BMI, body mass index; CRP, C-reactive protein; IL, interleukin; F_2–IsoP, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

(Pearson et al. 2003). The levels of IL-6 and CRP positively correlated with each other ($r = 0.61$, $p < 0.0001$) as did the levels of antioxidants, α-tocopherol and β-carotene ($r = 0.37$, $p = 0.004$). The levels of F_2-IsoP did not correlate with either α-tocopherol ($r = -0.21$, $p = 0.11$) or β-carotene ($r = -0.03$, $p = 0.83$).

Plasma antioxidants showed different results with respect to correlation with inflammatory markers. Whereas plasma levels of α-tocopherol did not correlate with either inflammatory marker, β-carotene levels inversely correlated with both inflammatory markers (Figure 1). Adjustment of antioxidant levels for total cholesterol did not change the results (data not shown). Urinary levels of F_2-IsoP did not correlate

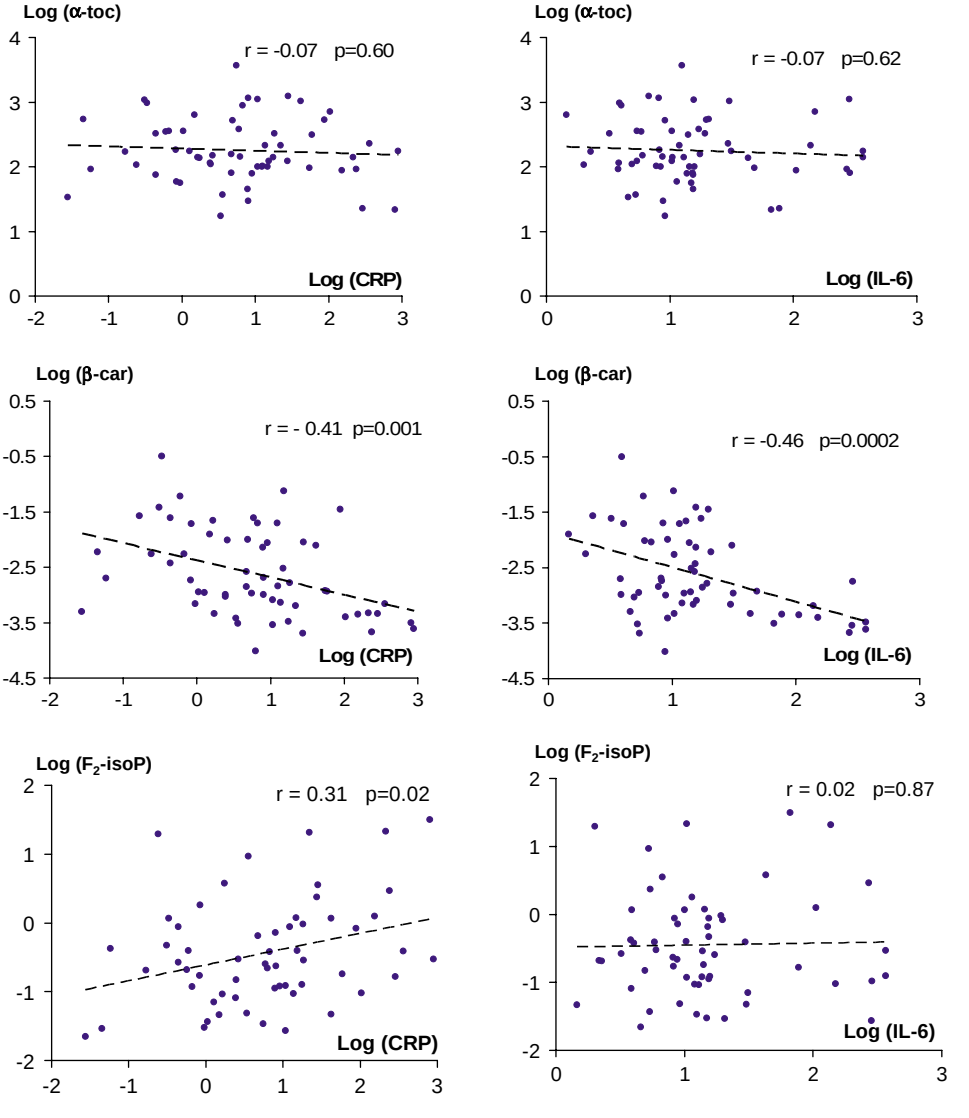

Figure 1. Correlation between inflammatory markers and oxidative indices: all variables are log-transformed, presented are Pearson correlation coefficients. CRP, C-reactive protein; IL, interleukin; F_2–IsoP, 2,3-dinor-5,6-dihydro-15-F_{2t}-isoprostane.

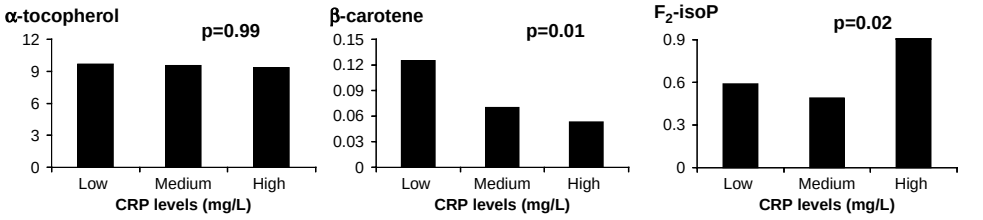

Figure 2. Geometric means of oxidative indices by C-reactive protein (CRP) levels: low (<1.0 mg l^{-1}), average (1.0–3.0 mg l^{-1}), and high-risk (>3.0 mg l^{-1}) levels, presented are *p*-values for Kruskal–Wallis test.

with IL-6 but positively correlated with CRP levels (Figure 1). Consistent with the correlation results, the α-tocopherol geometric mean did not change with CRP levels; the geometric mean of β-carotene decreased and that of F_2-IsoP increased at higher levels of CRP (Figure 2).

Although not the focus of this analysis, correlations between the measured biomarkers and several characteristics of the subjects are noteworthy. F_2-IsoP levels correlated with BMI ($r=0.33$, $p=0.01$) and were higher among women (geometric means were 1.01 vs. 0.49 in men, $p=0.0003$). Levels of β-carotene were higher among diabetic participants (geometric means were 0.084 vs. 0.048, $p=0.01$). Participants with a history of cancer had higher levels of plasma α-tocopherol (geometric means were 11.76 vs. 8.73, $p=0.01$). Other characteristics did not correlate with the examined biomarkers.

Discussion

There is strong interest in correlations between inflammatory and oxidative markers because of the popular idea that both factors contribute to the pathogenesis of many chronic conditions. The epidemiological questions are, first, whether the relationship between these two phenomena exists at a systemic level, and second, how this relationship can be studied. Logically, if inflammation and oxidative status are two pathological factors of the same causal pathway, we should be able to find a correlation between their measurements. Finding such correlated measurements can facilitate epidemiological studies of these clustering factors in human populations.

In this study, we selected participants at high risk of cardiovascular disease (Table I) because both oxidative stress and inflammation are involved in cardiovascular pathogenesis. The prevalence of many chronic conditions in this study population is higher than in the general population. For example, 24% of our study subjects have diabetes, a condition that has been clearly associated with oxidative stress and inflammation. Selection of such a study population maximizes the chances of finding correlations between biomarkers of oxidative stress and inflammation because of the increased chance that participants will have both conditions simultaneously. Healthy individuals are unlikely to have both of these conditions. Therefore, investigating the relationship between oxidative stress and inflammation would be compromised if healthy individuals were selected as the study population.

Our results clearly indicate that circulating levels of α-tocopherol do not correlate with inflammatory markers (Figures 1 and 2). This finding is consistent with previously published results from large studies (Ford et al. 2003a, Walston et al.

2006). Also consistent with previous studies (Boosalis et al. 1996, Erlinger et al. 2001, Ford et al. 2003a, Hu et al. 2004b, Walston et al. 2006), circulating β-carotene inversely correlated with both CRP and IL-6 levels (Figures 1 and 2). From the standpoint of biological significance, these results are surprising, given that vitamin E and its main component α-tocopherol are classified as 'essential antioxidants in humans' (Halliwell 1996, McCall & Frei 1999), while the biological role of β-carotene as an antioxidant in vivo is questionable (Rice-Evans et al. 1997, Halliwell 1999). On the other hand, circulating α-tocopherol levels are not a valid indicator of oxidative status because they do not decrease, but rather increase, in response to oxidative stressors in animals (carbon tetrachloride treatment) (Kadiiska et al. 2000) and in humans (exercise training) (Aguilo et al. 2005, Watson et al. 2005). Such increase in α-tocopherol levels in response to oxidative stress has been conceptualized as mobilization of antioxidant defence (Watson et al. 2005). Perhaps, systemic α-tocopherol levels present complicated dynamics between its nutritional intake, storage, and the presence of oxidative assault. With respect to our study, the fact that plasma α-tocopherol levels are not a valid oxidative index can explain the lack of correlation with the inflammatory markers.

The results on plasma β-carotene – a nutrient with questionable antioxidant functions (Rice-Evans et al. 1997) – are puzzling. The enigma of β-carotene is not limited to this study, but rather permeates the entire history of antioxidant research. Besides consistent correlations with levels of inflammatory markers (Boosalis et al. 1996, Erlinger et al. 2001, Ford et al. 2003a, Hu et al. 2004b, Walston et al. 2006), circulating β-carotene levels inversely correlate with an impressively large number of risk factors for different chronic conditions: smoking (Wallstrom et al. 2001, Ford et al. 2002), metabolic syndrome (Ford et al. 2003b) and its components – obesity (Strauss 1999) and insulin resistance (Ylonen et al. 2003), hypertension (Chen et al. 2002), pulmonary function (Schunemann et al. 2001), and measures of sarcopenia (Semba et al. 2003). This list is not exhaustive because a comprehensive review of β-carotene is beyond the scope of this manuscript. Contrary to expectations based on finding such correlations, supplementation with β-carotene failed to demonstrate a protective effect in randomized trials (Mayne 1996). One of the popular explanations for such a contradiction is the hypothesis that β-carotene levels are an indicator of consumption of fruits and vegetables known to be associated with lower risk of many chronic conditions (Mayne 1996). For example, carotenoids alone did not increase resistance of low-density lipoproteins to copper-induced oxidation (measured as formation of conjugated dienes and loss of tryptophan fluorescence), whereas this effect was demonstrated in experiments with plant-derived phenolic compounds (Milde et al. 2007). A combination of carotenoids with phenolic compounds produced a supra-additive effect, suggesting that consumption of fruits and vegetables, but not of carotenoids alone, can produce a beneficial effect. Indeed, healthier dietary patterns have been associated with both higher levels of β-carotene (Mayne 1996) and lower levels of CRP (Lopez-Garcia et al. 2004) thus providing a possible explanation to our findings. An alternative explanation could be inflammation-induced changes in the dynamics of intestinal absorption and transport of β-carotene by the lipoprotein system.

In this study, urinary F_2-IsoP levels did not correlate with IL-6 but positively correlated with CRP (Figures 1 and 2). Two facts are important for interpreting these results. First, CRP is the best studied inflammatory marker in prediction of

cardiovascular diseases with the defined risk categories (low, average, high correspond to <1.0, 1.0–3.0 and >3.0 mg l^{-1}, respectively). In our previously published study, CRP levels more consistently associated with different cancer outcomes, showing a pronounced dose–response relationship with total cancer incidence, when compared with IL-6 levels (Il'yasova et al. 2005). Second, among the three examined indices of oxidative status, only urinary F_2-IsoP levels have been validated against known oxidative assaults in animals (carbon tetrachloride treatment) (Kadiiska et al. 2005) and humans (exercise training) (Mastaloudis et al. 2001, Nieman et al. 2004). These findings emphasize the credibility of a positive correlation between urinary F_2-IsoP and CRP as an indication of the interconnection between low-level inflammation and oxidative status. Important for future studies, urinary F_2-IsoP levels have a number of favourable qualities as an epidemiological marker such as chemical stability, independence of dietary lipid content, and low within-person variability (Richelle et al. 1999, Gopaul et al. 2000, Kanabrocki et al. 2002, Morrow & Roberts 2002).

Finally, we would like to out point several limitations of this study. Remembering that 'association is not causation', we interpret the correlation between CRP and urinary F_2-IsoP as evidence of an interconnection, but not of a causal relationship, between low-level inflammation and oxidative status. Such a correlation may be the result of an unknown causal factor that induces both inflammation and high oxidative status. Establishing a causal relationship between inflammation and oxidative status is beyond the scope of any single investigation. Other limitations of this study are the specifics of the study population and the small sample size.

Obviously, the study subjects are not entirely representative of the general population because they were selected for a specific clinical trial. As noted above, we selected this study population assuming that if a relationship between low-level inflammation and oxidative status exists, it should be detectable in the population at high risk of cardiovascular disease, because both factors are known to be involved in cardiovascular pathogenesis. According to this logic, if no correlations were found between the biomarkers in this study, it is unlikely that they would be found in the general population; however, any correlation found in this study may be attenuated in the general population.

Although the sample size ($n=60$) is large enough to detect correlations, it is insufficient for a full investigation of relationships between the biomarkers including interactions with other characteristics and non-linear dose responses. Lastly, we examined only frequently used biomarkers, whereas inflammation and oxidative status both involve a complicated network messengers and metabolites. This study describes a simple method for initial evaluation of potential epidemiological markers, which can be used to evaluate other existing and new emerging biomarkers.

In conclusion, first we found that plasma levels of α-tocopherol did not correlate with levels of inflammatory markers; therefore, α-tocopherol does not appear to be useful for epidemiological studies of oxidative stress and inflammation. Second, plasma β-carotene levels inversely correlated with levels of both markers of inflammation that were studied; however, plasma β-carotene is a highly questionable marker of oxidative status and therefore the observed inverse correlations might not reflect a true relationship between oxidative stress and inflammation. Finally, urinary F_2-IsoP levels are a valid index of oxidative status in humans and are positively correlated with CRP levels. Thus, based on our results, we recommend using systemic F_2-IsoP and CRP levels to study the relationships between oxidative status,

inflammation, and chronic diseases. Both of these biomarkers can be assessed on a large scale. This is an important practical consideration for epidemiological studies and supports our recommendation.

Acknowledgements

This work was supported through the National Institute on Aging and the Wake Forest University Claude D. Pepper Older Americans Independence Center (NIA grant P30-AG-021332-02) and the following grants: DK48831, CA77839, ES13125 and GM15431.

References

Aguilo A, Tauler P, Fuentespina E, Tur JA, Cordova A, Pons A. 2005. Antioxidant response to oxidative stress induced by exhaustive exercise. Physiology & Behavior 84:1–7.

Basu S. 2004. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radical Research 38:105–122.

Boosalis MG, Snowdon DA, Tully CL, Gross MD. 1996. Acute phase response and plasma carotenoid concentrations in older women: findings from the nun study. Nutrition 12:475–478.

Cesari M, Penninx BW, Pahor M, Lauretani F, Corsi AM, Rhys WG, Guralnik JM, Ferrucci L. 2004. Inflammatory markers and physical performance in older persons: the InCHIANTI study. Journals of Gerontology Series A Biological Sciences & Medical Sciences 59:242–248.

Cesari M, Kritchevsky SB, Baumgartner RN, Atkinson HH, Penninx BW, Lenchik L, Palla SL, Ambrosius WT, Tracy RP, Pahor M. 2005. Sarcopenia, obesity, and inflammation – results from the Trial of Angiotensin Converting Enzyme Inhibition and Novel Cardiovascular Risk Factors study. American Journal of Clinical Nutrition 82:428–434.

Chen J, He J, Hamm L, Batuman V, Whelton PK. 2002. Serum antioxidant vitamins and blood pressure in the United States population. Hypertension 40:810–816.

Coussens LM, Werb Z. 2002. Inflammation and cancer. Nature 420:860–867.

Erlinger TP, Guallar E, Miller ER III, Stolzenberg-Solomon R, Appel LJ. 2001. Relationship between systemic markers of inflammation and serum {beta}-carotene levels. Archives of Internal Medicine 161:1903–1908.

Ferrucci L, Harris TB, Guralnik JM, Tracy RP, Corti MC, Cohen HJ, Penninx B, Pahor M, Wallace R, Havlik RJ. 1999. Serum IL-6 level and the development of disability in older persons. Journal of the American Geriatric Society 47:639–646.

Flagg EW, Coates RJ, Greenberg RS. 1995. Epidemiologic studies of antioxidants and cancer in humans. Journal of the American College of Nutrition 14:419–427.

Ford ES, Liu S, Mannino DM, Giles WH, Smith SJ. 2003a. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. European Journal of Clinical Nutrition 57:1157–1163.

Ford ES, Gillespie C, Ballew C, Sowell A, Mannino DM. 2002. Serum carotenoid concentrations in US children and adolescents. American Journal of Clinical Nutrition 76:818–827.

Ford ES, Mokdad AH, Giles WH, Brown DW. 2003b. The metabolic syndrome and antioxidant concentrations: findings from the third National Health and Nutrition Examination Survey. Diabetes 52:2346–2352.

Gopaul NK, Halliwell B, Anggard EE. 2000. Measurement of plasma F2-isoprostanes as an index of lipid peroxidation does not appear to be confounded by diet. Free Radical Research 33:115–127.

Halliwell B. 1996. Antioxidants in human health and disease. Annual Review of Nutrition 16:33–50.

Halliwell B. 1999. Antioxidant defence mechanisms: From the beginning to the end (of the beginning). Free Radical Research 31:261–272.

Hess D, Keller HE, Oberlin B, Bonfanti R, Schuep W. 1991. Simultaneous determination of retinol, tocopherols, carotenes and lycopene in plasma by means of high-performance liquid chromatography on reversed phase. International Journal of Vitamin & Nutrition Research 61:232–238.

Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. 2004a. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 53:693–700.

Hu P, Reuben DB, Crimmins EM, Harris TB, Huang MH, Seeman TE. 2004b. The effects of serum beta-carotene concentration and burden of inflammation on all-cause mortality risk in high-functioning older persons: MacArthur studies of successful aging. Journals of Gerontology Series A: Biological Sciences and Medical Sciences 59:M849–M854.

Hussain SP, Hofseth LJ, Harris CC. 2003. Radical causes of cancer. Nature Reviews. Cancer 3:276–285.

Il'yasova D, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, Kritchevsky SB. 2005. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. Cancer Epidemiology, Biomarkers &Prevention 14:2413–2418.

Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, Nyska A, Wachsman JT, Ames BN, Basu S. 2005. Biomarkers of oxidative stress study II: Are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radical Biology and Medicine 38:698–710.

Kadiiska MB, Gladen BC, Baird DD, Dikalova AE, Sohal RS, Hatch GE, Jones DP, Mason RP, Barrett JC. 2000. Biomarkers of oxidative stress study: are plasma antioxidants markers of CCl4 poisoning? Free Radical Biology and Medicine 28:838–845.

Kanabrocki EL, Murray D, Hermida RC, Scott GS, Bremner WF, Ryan MD, Ayala DE, Third JL, Shirazi P, Nemchausky BA, Hooper DC. 2002. Circadian variation in oxidative stress markers in healthy and type II diabetic men. Chronobiology International 19:423–439.

Kroll MH, Chesler R, Hagengruber C, Blank DW, Kestner J, Rawe M. 1986. Automated determination of urinary creatinine without sample dilution: theory and practice. Clinical Chemistry 32:446–452.

Liese AD, Mayer-Davis EJ, Haffner SM. 1998. Development of the multiple metabolic syndrome: an epidemiologic perspective. Epidemiologic Reviews 20:157–172.

Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, Hu FB. 2004. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. American Journal of Clinical Nutrition 80:1029–1035.

Macy EM, Hayes TE, Tracy RP. 1997. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clinical Chemistry 43:52–58.

Mastaloudis A, Leonard SW, Traber MG. 2001. Oxidative stress in athletes during extreme endurance exercise. Free Radical Biology and Medicine 31:911–922.

Mayne ST. 1996. Beta-carotene, carotenoids, and disease prevention in humans. FASEB Journal 10:690–701.

McCall MR, Frei B. 1999. Can antioxidant vitamins materially reduce oxidative damage in humans? Free Radical Biology and Medicine 26:1034–1053.

Milde J, Elstner EF, Grassmann J. 2007. Synergistic effects of phenolics and carotenoids on human low-density lipoprotein oxidation. Molecular Nutrition & Food Research 51:956–961.

Milne GL, Musiek ES, Morrow JD. 2005. F2-isoprostanes as markers of oxidative stress in vivo: an overview. Biomarkers 10(Suppl. 1):S10–S23.

Morrow JD, Zackert WE, Yang JP, Kurhts EH, Callewaert D, Dworski R, Kanai K, Taber D, Moore K, Oates JA, Roberts LJ. 1999. Quantification of the major urinary metabolite of 15-F2t-isoprostane (8-iso-PGF2alpha) by a stable isotope dilution mass spectrometric assay. Anals of Biochemistry 269:326–331.

Morrow JD, Roberts LJ. 2002. The isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. American Journal of Respiratory and Critical Care Medicine 166:25S–30.

National Heart Lung and Blood Institute. 2004. NHLBI Workshop on Oxidative Stress/Inflammation and Heart, Lung, Blood, and Sleep Disorders. Available at http://www.nhlbi.nih.gov/meetings/workshops/oxidative-stress.htm

Nieman DC, Henson DA, McAnulty SR, McAnulty LS, Morrow JD, Ahmed A, Heward CB. 2004. Vitamin E and immunity after the Kona Triathlon World Championship. Medicine & Science in Sports & Exercise 36:1328–1335.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107:499–511.

Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. 2001. C-Reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA: The Journal of the American Medical Association 286:327–334.

Rice-Evans CA, Sampson J, Bramley PM, Holloway DE. 1997. Why do we expect carotenoids to be antioxidants in vivo? Free Radical Research 26:381–398.

Richelle M, Turini ME, Guidoux R, Tavazzi I, Metairon S, Fay LB. 1999. Urinary isoprostane excretion is not confounded by the lipid content of the diet. FEBS Letters 459:259–262.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. 1997. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. The New England Journal of Medicine 336:973–979.

Schunemann HJ, Grant BJB, Freudenheim JL, Muti P, Browne RW, Drake JA, Klocke R, Trevisan M. 2001. The relation of serum levels of antioxidant vitamins C and E, retinol and carotenoids with pulmonary function in the general population. American Journal of Respiratory and Critical Care Medicine 163:1246–1255.

Seddon JM, George S, Rosner B, Rifai N. 2005. Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers. Archives of Ophthalmology 123:774–782.

Semba RD, Blaum C, Guralnik JM, Moncrief DT, Ricks MO, Fried LP. 2003. Carotenoid and vitamin E status are associated with indicators of sarcopenia among older women living in the community. Aging Clinical Experimental Research 15:482–487.

Stocker R, Keaney JF Jr. 2004. Role of oxidative modifications in atherosclerosis. Physiological Reviews 84:1381–1478.

Strauss RS. 1999. Comparison of serum concentrations of [alpha]-tocopherol and [beta]-carotene in a cross-sectional sample of obese and nonobese children (NHANES III). The Journal of Pediatrics 134:160–165.

Wallstrom P, Wirfalt E, Lahmann PH, Gullberg B, Janzon L, Berglund G. 2001. Serum concentrations of {beta}-carotene and {{alpha}}-tocopherol are associated with diet, smoking, and general and central adiposity. American Journal of Clinical Nutrition 73:777–785.

Walston J, Xue Q, Semba RD, Ferrucci L, Cappola AR, Ricks M, Guralnik J, Fried LP. 2006. Serum antioxidants, inflammation, and total mortality in older women. American Journal of Epidemiology 163:18–26.

Watson TA, MacDonald-Wicks LK, Garg ML. 2005. Oxidative stress and antioxidants in athletes undertaking regular exercise training. International Journal of Sport Nutrition & Exercise Metabolism 15:131–146.

Ylonen K, Alfthan G, Groop L, Saloranta C, Aro A, Virtanen SM, the Botnia Research Group. 2003. Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. American Journal of Clinical Nutrition 77:1434–1441.