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Original Contribution

IS SERUM γ-GLUTAMYLTRANSFERASE INVERSELY ASSOCIATED WITH SERUM ANTIOXIDANTS AS A MARKER OF OXIDATIVE STRESS?

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Abstract—A series of studies in black and white women and men have suggested that serum γ-glutamyltransferase (GGT) within its normal range might be an early marker of oxidative stress. If serum GGT is a marker of oxidative stress, it might have important implications both clinically and epidemiologically because measurement of serum GGT is easy, reliable, and not expensive. We examined the cross-sectional association between deciles of serum GGT and concentrations of serum antioxidants among 9083 adult participants in the third U.S. National Health and Nutrition Examination Survey. After adjustment for race, sex, age, and total cholesterol, serum concentration of GGT across all deciles was inversely associated with serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin/lutein, lycopene, and vitamin C (p for trend <.01, respectively). Vitamin E was not associated with serum GGT. All these associations were not materially different after additional adjustment for total energy intake, body mass index, smoking status, smoking amount, alcohol intake, and exercise. These associations were similarly observed among most subgroups. In conclusion, the current and previous studies strongly suggest that serum GGT level within its normal range may be an early marker of oxidative stress.

Keywords—γ-Glutamyltransferase, Serum antioxidants, Oxidative stress, Biomarker, Free radicals

INTRODUCTION

A series of studies [1–3] in Coronary Artery Risk Development in Young Adults (CARDIA) subjects have shown that serum γ-glutamyltransferase (GGT) within its normal range might be related to oxidative stress. In these studies, both dietary and serum antioxidants inversely predicted future serum GGT, whereas dietary heme iron positively predicted it [2,3]. In addition, serum GGT predicted C-reactive protein, a marker of inflammation, and F_2-isoprostanes, a marker of oxidative damage to arachidonic acid, measured 15 years later in dose–response assays [1].

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Experimental studies [4–7] have reported that cellular GGT has a central role in glutathione homeostasis by initiating the breakdown of extracellular glutathione (GSH), a critical antioxidant defense for the cell. Paradoxically, there is evidence that, under physiological conditions, GGT is directly involved in the generation of reactive oxygen species, especially in the presence of iron or other transition metals [8–11].

With an increasing interest in oxidative stress, emphasis is now being placed on developing functional biomarkers of oxidative stress status, that is, biomarkers that integrate the effects of exposure to oxidants coupled with the full range of antioxidant protective mechanisms in vivo [12]. Many such biomarkers are being studied, including various measures of lipid, DNA, and protein oxidation [12]. Measurement of serum GGT is reliable, easy, and not expensive [13]. Thus, if serum GGT is a marker of oxidative stress, it might have important implications both clinically and epidemiologically.

However, to our knowledge, only the results from the CARDIA study suggest that serum GGT is a marker of oxidative stress in a human population. Therefore, we examined the association between serum antioxidants and serum GGT level among another group of subjects, namely a representative sample of the U.S. population, using the third National Health and Nutrition Examination Survey (NHANES III).

MATERIALS AND METHODS

The NHANES III was a national examination survey conducted in the United States from 1988 to 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention. It used complex, multistage, stratified, clustered samples of civilian, noninstitutionalized populations age 2 months and older. A detailed description of survey methods and data collection procedures has been published elsewhere [14].

Study sample

Of 18,825 sampled persons age 20 years and older, 16,573 (88%) attended an examination at a mobile examination center (MEC). Participants with missing data on serum GGT concentrations (n = 4514) were excluded. We additionally excluded those with viral hepatitis (positive for serum hepatitis B surface antigen or positive for serum hepatitis C antibody) (n = 453), diabetes (n = 1367), unusually high or low dietary intake values (men, <800 or >4200 calories/d; women, <600 or >3500 calories/d) (n = 2953), rarely encountered races (that is, other than non-Hispanic white, non-Hispanic black, and Mexican American, n = 662 excluded), and pregnant women (n = 288). Finally, 9083 study participants remained for analysis.

Measurements

The NHANES III data collection included a standardized home interview followed by a detailed physical examination in a MEC or the participant = s home. Information on a wide variety of sociodemographic, medical history, nutritional history, and family history questions, such as self-reported age, race/ethnicity, gender, history of smoking, alcohol consumption, use of vitamin supplements, and 24-h dietary recall, were obtained during the home interview.

A venous blood sample was collected and shipped weekly at −20°C. Serum GGT concentration was assayed using a Hitachi 737 analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA) at White Sands Research Center. Serum antioxidant concentrations were assayed using a Waters HPLC system (Waters Chromatography, Division of Millipore Corp., Marlboro, MA, USA) at the NHANES Laboratory. Serum α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, and vitamin E were measured by isocratic HPLC with detection at 300, 325, and 450 nm. Serum extract was prepared in the vial for vitamin C analysis by diluting 500 μl serum with 2.0 ml freshly prepared 6 mg/dl metaphosphoric acid diluent and thoroughly mixing the resulting solution of clear liquid and white precipitated proteins. Serum vitamin C was measured by isocratic HPLC with electrochemical detection at 650 mV. Serum total cholesterol was measured using a Hitachi 704 analyzer (Boehringer Mannheim Diagnostics) at the Johns Hopkins University Hospital Lipoprotein Analytical Laboratory and White Sands Research Center.

Statistical analysis

Serum GGT levels were classified as deciles; cutoff points of deciles of serum GGT were 12, 14, 16, 19, 21, 25, 29, 38, and 57 U/l (normal range 11–51 U/l for men, 7–33 U/l for women). Some serum antioxidant levels were right-skewed, so results are presented as geometric means of serum antioxidant levels across deciles of serum GGT levels. One previous study [15] with NHANES III has reported the inverse association between abnormal levels of serum alanine aminotransferase (ALT) and serum antioxidants. In this study, for a comparison with serum GGT, we reanalyzed the association between serum ALT within its normal range and serum antioxidants. Therefore, serum ALT levels were also classified as deciles; cutoff points of deciles of ALT were 8, 9, 10, 12, 14, 15, 18, 21, and 29 U/l (normal range <40 U/l for men, <31 U/l for women).

Adjustment for potential confounding was done by linear regression. In this study, the internal validity was a more important issue than generalization to the total U.S. population, therefore we did not use a specific analytic method to take into account the sampling frame of NHANES III. Adjusting variables were race–ethnicity, gender, and age (years). The values of lipophilic antioxidants were additionally adjusted for cholesterol concentrations because the distribution of the lipophilic antioxidant vitamins across various fat depots in the body is influenced by circulating lipoprotein concentrations [16]. In fully adjusted models, we additionally adjusted for body mass index (BMI) (kg/m^2), smoking status (never smoker, ex-smoker, and current smoker), smoking amount (packs), alcohol intake (g/d), total energy intake (kcal/d), and exercise (continuous).

We repeated the same analyses after stratifying by race (non-Hispanic white, non-Hispanic black, vs. Mexican American), sex (men vs. women), alcohol consumption status (nondrinkers vs. drinkers), smoking

status (nonsmokers vs. current smokers), BMI (<25 vs. ≥25), and supplement use (nonuser vs. user of vitamin supplements).

RESULTS

The average age of the sample was 47.6 years. The proportions of ethnic groups were 47.3% for non-Hispanic white, 28.1% for non-Hispanic black, and 24.6% for Mexican American. There were more females (53.6%) than males (46.4%) in the sample.

After adjusting for race–ethnicity, gender, age, and serum cholesterol level, serum GGT levels across most deciles were inversely associated with serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, and vitamin C (Table 1, p for trend <.01, respectively). Fig. 1 shows a clear inverse association of the sum of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene) with serum GGT levels. All these associations were not materially different after additional adjustment for total energy intake, BMI, smoking status, smoking amount, alcohol intake, and exercise (data not shown). Additional adjustment for coffee consumption did not change the results. Serum concentration of vitamin E was not associated with serum GGT levels (Table 1). The inverse associations of serum GGT levels and serum antioxidants were similarly observed among most subgroups, including all races and sexes, nondrinkers, drinkers, nonsmokers, smokers, lean subjects, or obese subjects (data not shown).

Unlike serum GGT levels, serum ALT levels within its normal range were not inversely associated with serum antioxidants, as shown for the sum of the five carotenoids in Fig. 1. The associations between serum ALT and carotenoids or vitamin C seemed to be inverse U shapes (p for quadratic terms <.01, respectively) (Table 2). Serum ALT levels were clearly and positively associated with serum levels of vitamin E (p for trend <.01) (Table 2).

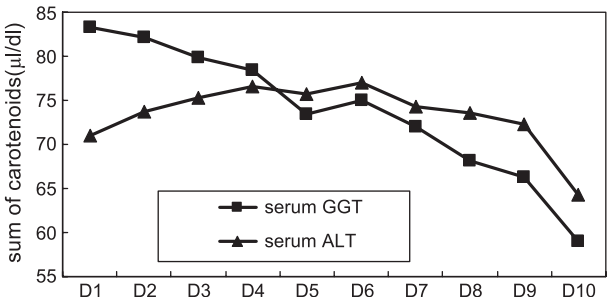

Fig. 1. Geometric means of sum of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin/lutein, and lycopene) by deciles of serum γ-glutamyltransferase (GGT) (p for trend <.01) and alanine aminotransferase (ALT) (p for quadratic term <.01) levels, after adjustment for race, sex, age, and serum total cholesterol.

DISCUSSION

In this sample of the U.S. population, we documented that serum carotenoids and vitamin C concentrations are strongly and inversely associated with serum GGT levels within its normal range, but serum vitamin E concentration was not associated with serum GGT levels. These inverse associations were consistently demonstrated in most subgroups. Our parallel study in the CARDIA subjects also reported inverse associations of serum concentrations of some carotenoids and α-tocopherol with serum GGT level cross-sectionally [2]. It is not possible to discern direction of

Table 1. Adjusted[a] Geometric Means (Standard Error) of Serum Antioxidants by Deciles of Serum γ-Glutamyltransferase (GGT) Levels

Serum carotenoids and vitamins	Deciles of serum GGT levels										Regression coefficient	p_{trend}
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10		
α-Carotene, μg/dl	4.31 (0.12)	3.95 (0.11)	3.72 (0.10)	3.55 (0.09)	3.39 (0.08)	3.36 (0.09)	3.30 (0.08)	2.85 (0.08)	2.80 (0.07)	2.40 (0.06)	−0.056	<.01
β-Carotene, μg/dl	18.8 (0.47)	18.5 (0.47)	17.2 (0.41)	16.8 (0.40)	15.2 (0.35)	15.6 (0.38)	14.6 (0.34)	12.8 (0.32)	12.5 (0.30)	10.0 (0.24)	−0.063	<.01
β-Cryptoxanthin, μg/dl	10.2 (0.20)	9.6 (0.20)	9.4 (0.18)	9.0 (0.17)	8.8 (0.16)	8.6 (0.17)	8.2 (0.15)	7.8 (0.16)	7.4 (0.15)	6.4 (0.13)	−0.044	<.01
Zeaxanthin/lutein, μg/dl	22.4 (0.34)	22.6 (0.34)	21.9 (0.32)	21.7 (0.31)	20.8 (0.29)	21.3 (0.32)	20.5 (0.29)	19.9 (0.30)	19.3 (0.28)	17.9 (0.26)	−0.023	<.01
Lycopene, μg/dl	20.7 (0.37)	20.2 (0.37)	20.6 (0.35)	20.1 (0.34)	19.3 (0.32)	19.6 (0.34)	18.7 (0.31)	18.7 (0.33)	18.0 (0.31)	16.7 (0.29)	−0.021	<.01
Vitamin C, mg/dl	0.63 (0.02)	0.64 (0.02)	0.59 (0.02)	0.61 (0.02)	0.59 (0.02)	0.60 (0.02)	0.53 (0.02)	0.55 (0.02)	0.50 (0.01)	0.40 (0.01)	−0.040	<.01
Vitamin E, mg/dl	1059 (9.68)	1077 (9.99)	1056 (9.33)	1070 (9.29)	1063 (8.99)	1078 (9.72)	1068 (9.10)	1060 (9.58)	1060 (9.44)	1044 (9.30)	−0.001	.22

[a] Concentrations of carotenoids and vitamin E were adjusted for race, sex, age, and serum total cholesterol. Concentrations of vitamin C were adjusted for race, sex, and age.

association from cross-sectional studies. However, in the CARDIA study [2], these serum antioxidants also predicted GGT longitudinally, whereas baseline GGT did not predict future serum antioxidant levels [2]. Moreover, in the CARDIA study, dietary heme iron positively predicted future serum GGT level [3]; free iron is a critical catalyst in generating reactive oxygen species [17] and most dietary antioxidants inversely predicted future serum GGT level [3]. Inverse associations between serum antioxidants and serum GGT in the present study might be interpreted in two ways: low serum antioxidants can cause more oxidative stress or high levels of oxidative stress can also deplete serum antioxidants. Therefore, taking this NHANES study and CARDIA study together, a fundamental mechanism for the associations between serum antioxidants and GGT might be that low serum antioxidants mark or promote oxidative stress.

Recently, F_2-isoprostanes, a set of isomers produced by oxidative damage to arachidonic acid, have been regarded as a reliable marker of lipid peroxidation. In assessing oxidative stress in vivo, usefulness of F_2-isoprostanes has been enthusiastically discussed among researchers due to the mechanism of their formation, chemical stability, and availability of sensitive and noninvasive methods for their estimation [18,19]. Thus, it is interesting to note that serum GGT level within its normal range predicted F_2-isoprostanes, which were measured 5 or 15 years later in a dose–response assay in the CARDIA subjects [1]. In addition, serum GGT also predicted C-reactive proteins, uric acid, and fibrinogens with similar patterns [1]. All these findings suggest that serum GGT might be an early marker of oxidative stress.

Although serum GGT has been well known as a marker of alcohol consumption or liver dysfunction, serum GGT level within its normal range is associated with many cardiovascular disease risk factors and components of metabolic syndrome in the general population [20–22]. Population studies have found that serum GGT predicted cardiac mortality or nonfatal myocardial infarction, especially among ischemic patients with established coronary atherosclerosis and previous myocardial infarction, as well as diabetes or stroke [21–26]. Our current and previous findings suggest that the associations of serum GGT with cardiovascular events and/or metabolic syndrome might be explained by a mechanism related to oxidative stress. In addition, serum GGT might be used as an early marker for predicting development of cardiovascular events and/or metabolic syndrome [27,28].

Although the relationship between serum GGT and cellular GGT is unknown, cellular GGT has been known to play an important role in antioxidant defense systems [4–7]. Cellular GGT catalyzes the initial step in the degradation of extracellular GSH, thereby providing a supply of constituent amino acids for uptake and reutilization in intracellular GSH synthesis. The well-known elevation of serum GGT after alcohol consumption might arise from oxidative stress in the liver, due to reduced glutathione in the hepatocyte [29,30]. However, recent experimental studies [8–11] clearly indicate that cellular GGT may also be involved in the generation of reactive oxygen species. A GGT-dependent lipid peroxidation process was induced in the lipids of biological membranes of living cells retaining their antioxidant defense systems and sustained by the endogenous GGT, highly expressed at the surface of

Table 2. Adjusted[a] Geometric Means (Standard Errors) of Serum Antioxidant Level by Deciles of Serum Alanine Aminotransferase (ALT) Levels

Serum carotenoids and vitamins	Deciles of serum ALT levels										Regression coefficient	$p_{quadratic}$
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10		
α-Carotene, µg/dl	2.75 (0.08)	3.07 (0.07)	3.20 (0.09)	3.58 (0.08)	3.43 (0.11)	3.67 (0.09)	3.61 (0.11)	3.55 (0.09)	3.44 (0.09)	3.00 (0.08)	0.013	<.01
β-Carotene, µg/dl	14.8 (0.37)	15.0 (0.33)	15.4 (0.42)	16.0 (0.35)	15.8 (0.48)	16.2 (0.37)	14.9 (0.40)	15.0 (0.34)	14.7 (0.37)	12.0 (0.30)	−0.014	<.01
β-Cryptoxanthin, µg/dl	7.50 (0.15)	8.27 (0.15)	8.61 (0.19)	8.90 (0.16)	9.14 (0.22)	8.95 (0.16)	8.82 (0.19)	8.54 (0.16)	8.48 (0.17)	7.69 (0.15)	0.002	<.01
Zeaxanthin/lutein, µg/dl	21.0 (0.32)	21.3 (0.28)	21.4 (0.35)	21.4 (0.28)	21.6 (0.39)	21.4 (0.29)	20.3 (0.33)	20.8 (0.28)	19.9 (0.30)	18.6 (0.28)	−0.011	<.01
Lycopene, µg/dl	18.7 (0.33)	19.4 (0.30)	20.3 (0.39)	19.9 (0.31)	19.4 (0.41)	19.6 (0.32)	20.0 (0.38)	18.9 (0.30)	19.1 (0.34)	17.2 (0.30)	−0.007	<.01
Vitamin C, mg/dl	0.45 (0.01)	0.50 (0.01)	0.55 (0.02)	0.58 (0.02)	0.59 (0.02)	0.60 (0.02)	0.60 (0.02)	0.59 (0.02)	0.60 (0.02)	0.56 (0.02)	0.022	<.01
Vitamin E, mg/dl	978 (8.8)	1012 (8.0)	1036 (10.0)	1054 (8.2)	1076 (11.6)	1086 (8.9)	1086 (10.5)	1105 (9.0)	1117 (10.1)	1101 (9.8)	0.013	<.01*

[a] Concentrations of carotenoids and vitamin E were adjusted for race, sex, age, and serum total cholesterol. Concentrations of vitamin C were adjusted for race, sex, and age.

* p value is for linear trend for vitamin E.

human hepatoma cells [31,32]. Moreover, cellular GGT expressed by a number of malignancies can play an important role in modulation of the redox status of cellular protein thiols, with special reference to proteins on the cell surface [33,34].

One recent study [15] using the NHANES III data showed increased prevalence of elevated serum ALT activity accompanying a decrease in antioxidant levels. In the present study, we examined these data more closely. We reanalyzed the dataset to examine association between serum ALT levels within its normal range and serum antioxidants, which was intended to be compared with the parallel relationship with serum GGT levels. Contrary to serum GGT level, serum ALT levels below their median level were positively associated with some serum antioxidants and unrelated to others. The inverse association of serum ALT in its higher deciles with some serum carotenoids might be interpreted as similar to the findings of the previous study [15].

Oxidative stress is apparent in pathology associated with aging and many age-related chronic diseases, including atherosclerosis, diabetes mellitus, rheumatoid arthritis, and neurodegenerative diseases [35]. For the prevention of diseases and control of aging, evaluation and control of oxidative stress in vivo may become essential. A wide variety of functional assays are used in the field of research related with oxidative stress. However, direct detection of reactive oxygen species and other free radicals is difficult, because these molecules are short-lived and highly reactive in a nonspecific manner [35]. Although ongoing oxidative damage is, thus, generally analyzed by measurement of secondary products, including derivatives of amino acids, nucleic acids, and lipid peroxidation [35], biomarkers to reflect minor changes in the pro-oxidant/antioxidant status under normal, nonpathological conditions in humans might be of special interest.

In conclusion, the current and previous studies suggest that serum GGT level within its normal range may be an early marker of oxidative stress. As measurement of serum GGT is accurate, reliable, easy, and inexpensive, this finding might have important implications both clinically and epidemiologically.

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ABBREVIATIONS

ALT — alanine aminotransferase
BMI — body mass index
CARDIA — Coronary Artery Risk Development in Young Adults
GGT — γ-glutamyltransferase
GSH — glutathione
NHANES — National Health and Nutrition Examination Survey