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Associations between γ -glutamyltransferase (GGT) and Biomarkers of Atherosclerosis: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

OBJECTIVE—To evaluate associations between total serum γ -glutamyltransferase activity (GGT) and biomarkers of arteriosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA), including 6,783 participants from four ethnic subgroups, i.e., White, Chinese, Black and Hispanic.

METHODS—Associations between fasting total serum GGT activity and oxidized low-density lipoproteins (oxLDL), interleukin-6 (IL-6), C-reactive protein (CRP), and soluble intercellular adhesion molecule-1 (sICAM-1) were assessed. Following evaluation of linear trends between GGT and biomarkers of interest, multivariable linear regression models were serially adjusted for age, gender, site, ethnicity (M1); M1+lifestyle variables (M2); M2+traditional cardiovascular risk factors plus medications (M3); and M3+metabolic status (M4). Interactions were evaluated between GGT and age and ethnicity in all models.

RESULTS—Linear trends were positive and significant between GGT and oxLDL, IL-6, CRP and sICAM-1 in crude models, and trends remained significant in all ethnic subgroups for CRP

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($p<0.0001$) and sICAM-1 ($p<0.001$), and for IL-6 except in the Chinese. Trends between GGT and oxLDL were significant in the entire cohort and the White subgroup ($p<0.0001$), but not in other ethnic subgroups. Multivariable models demonstrated continuous strong, positive associations between GGT and CRP, IL-6 and sICAM-1. Associations between GGT and oxLDL were attenuated upon adjustment for LDL-C and other traditional risk factors. All models were attenuated with adjustment for metabolic status. No age interactions were evident.

CONCLUSIONS—Our findings support the hypothesis that total serum GGT activity represents the impact of metabolic disease on vascular injury and atherosclerosis.

Keywords

GGT; oxidative stress; oxidized LDL; sICAM; CRP; endothelial dysfunction

Introduction

Vascular inflammation and oxidative stress have been implicated in the origins of endothelial dysfunction, which contributes to the micro-vascular complications of metabolic disease and atherosclerotic disease of the macro-vasculature.^{1–4} Endothelial dysfunction is a cumulative process secondary to increased concentrations of, and variability in, blood glucose and lipids, with subsequent redox dysregulation.^{5,6} Relevant biomarkers of oxidation, immune activation, and subclinical inflammation include malondialdehyde modified low-density lipoproteins (commonly referred to as “oxidized” LDL or oxLDL), cytokines such as interleukin-6 (IL-6), elevations in acute phase inflammatory biomarkers including C-reactive protein (CRP), and increased soluble vascular adhesion molecule (sICAM-1) expression- biomarkers which have all demonstrated increased risk prediction beyond traditionally established risk factors.⁷ However, biomarkers of oxidative-inflammatory stress have limitations in clinical research due to the need for careful sample handling, instrumentation requirements, and the high costs of measurement- factors which limit investigation of these processes in population-based studies of human disease, and create barriers to translating basic science evidence into clinical research.⁸

The tri-peptide reduced glutathione (GSH) is a critical antioxidant defense in human tissues; in the absence of adequate GSH, elevations in superoxide, peroxide and peroxynitrite free radicals persist causing lipid peroxidation, protein modification and DNA adduct formation with varying consequences on membrane receptor and gene functioning, including impaired endothelial-mediated vasodilation.⁹ The evidence available on GSH status and cardiovascular risk is limited to patients with type 2 diabetes, and demonstrates lower concentrations of GSH (measured as erythrocyte GSH) in those with diabetes compared to those without diabetes, and diabetics with micro-vascular complications appear to have still lower concentrations, suggesting the importance of GSH status in complication development.^{4,10,11} Although these relatively small case-control studies have demonstrated differences in GSH status, the validity of erythrocyte GSH as a biomarker remains mostly unknown in large population-based studies of cardiovascular disease. Unfortunately, the need to preserve reduced GSH in stored specimens at the time of collection impedes *post-hoc* measurement of erythrocyte GSH in stored samples, and thus limits evaluations of GSH in cardiovascular disease cohorts.¹²

Serum γ -glutamyltransferase activity (GGT) is considered an *in vivo* biomarker of GSH demand because it recycles GSH precursors in nearly all tissues, including the hepatic recycling of reduced GSH to support glutathione-S-transferase-mediated conjugation of GSH to lipid peroxides for detoxification.^{9,13,14} In relation to cardiometabolic disease, an expanding volume of observational research in multiple cohorts demonstrates graded elevations of GGT *within the clinically normal range* are associated with increase risk of incident type 2 diabetes, hypertension and cardiovascular events.^{15–19} Additionally, Sedda et al. clearly demonstrated *in vivo* serum GGT activity was independently associated with lower GSH status and total cardiovascular risk factor burden.²⁰ Our prior research in the MESA cohort demonstrates the strong associations between GGT activity and both individual and composite metabolic disease risk, independent of traditional risk factors for cardiovascular disease.²¹ As a biomarker of GSH status, GGT has several advantages as a biomarker compared to erythrocyte GSH, including not requiring special sample preparation at the time of serum collection, high reliability in previously thawed samples, readily available clinical laboratory measurement methods and instrumentation, and the availability of micro-plate assays methods.^{22–24}

Despite evidence for strong associations between GGT and cardiovascular disease risk, and the practical advantages of GGT measurement compared to many other biomarkers, limited population-based research has attempted to understand the potential mechanisms of these associations by creating and evaluating a conceptual model relating GGT to the available biomarkers of subclinical and progressive arteriosclerotic disease, or to determine whether any associations are dependent upon metabolic status. Therefore, in this cross-sectional study in MESA, we aimed to evaluate the hypothesis that graded increases in serum GGT activity mirror graded increases in oxidative stress, cytokine production, acute phase inflammatory response and endothelial cell adhesion molecule expression by testing for associations between GGT activity and oxLDL, IL-6, CRP and sICAM-1, while adjusting for traditional cardiovascular risk factors. We then evaluate the hypothesis that serum GGT activity may be particularly representative of vascular injury in metabolic disease, by adjusting the observed associations by participants' metabolic status. The MESA cohort is well suited for these analyses because it includes excellent representation from four distinct ethnic groups (i.e., Chinese, Hispanic, Black and Caucasian) across a wide age range in participants with varying degrees of cardiovascular and metabolic risk.

Material and Methods

Study Population

The composition of the MESA cohort has been described elsewhere.²⁵ Briefly, MESA consists of 6,814 participants (53% female, 47% male) aged 44–84 years (Mean=62 years) representing four major ethnic groups including Chinese-American (n=803), African-American (n=1,893), Hispanic (n=1,496) and Caucasian (n=2,622). Participants were without symptomatic cardiovascular disease at baseline. In this study we used results and frozen samples from the baseline examination between July 2000-August 2002.

Questionnaires

Participant demographics, medical history and physical activity were all collected using standardized forms during the baseline examination. Participants brought their medications to the examination and medication names were recorded individually by study staff. Smoking status was recorded as current, former or never, and pack years were calculated for former and current smokers. Alcohol use was also categorized as former, current or never based on self-report and total intake was recorded as the number of standardized drinks (1 beer, 1 glass of wine or 1 shot of spirits) per week. Total intentional exercise (MET-min/week) was calculated based on self-report from the past month.

Measurement of Laboratory Variables

GGT—Serum GGT activity was measured in frozen samples from the baseline MESA examination as described elsewhere.²¹

Interleukin-6 (IL-6)—IL-6 was measured in 6622 participants by an ultra-sensitive ELISA assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) in the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The lower detection limit was <0.094 pg/ml with a detection range of 0.156–10.0 pg/ml and a 6.3% CV for this assay.

C-reactive protein (CRP)—CRP was measured in 6762 participants using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL) in the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT). The assay range was 0.175 – 1100 mg/L. Intra-assay CVs range from 2.3 – 4.4% and inter-assay CVs ranged from 2.1 – 5.7%.

Oxidized LDL (oxLDL)—oxLDL was measured in 999 MESA participants by competitive ELISA assay (Mercodia Oxidized LDL ELISA, Mercodia AB, Uppsala, Sweden) utilizing a specific murine monoclonal antibody to malondialdehyde (MDA)-modified LDL and copper-oxidized LDL as a competitive ligand. This assay was developed by Paul Holvoet et al.^{26,27} and was performed in Dr. Holvoet's laboratory (University of Leuven, Leuven, Belgium). The intra-assay and inter-assay CVs ranged from 5.5–7.3% and 4.0–6.2%, respectively.

Soluble Intercellular Adhesion Molecule-1 (sICAM-1)—sICAM-1 was measured in 2622 participants by an ELISA assay (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). In this assay sICAM-1 is sandwiched by an immobilized monoclonal antibody and the enzyme-linked monoclonal antibody. The amount of ICAM-1 present is determined by colorimetric reaction. The minimum detectable level is < 0.35 ng/ml with an assay range of 2.73 – 49.55 ng/ml, and a 5.0% CV. This measurement was performed in the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT).

Additional laboratory measures—Total cholesterol (TC) and HDL were measured all participants in EDTA plasma using a cholesterol oxidase method (Roche Diagnostics,

Indianapolis, IN) on a Roche COBAS FARA centrifugal analyzer. The laboratory CV for TC and HDL were 1.6% and 2.9% respectively. LDL-cholesterol was calculated in plasma specimens having a triglyceride value <400 mg/dl using the formula of Friedewald et al.²⁸ Triglycerides were measured in EDTA plasma using Triglyceride GB reagent (Roche Diagnostics, Indianapolis, IN) on the Roche COBAS FARA centrifugal analyzer; the laboratory CV was 4.0%.

Statistical Analysis

As we aimed to determine continuous associations between GGT and ED biomarkers, we included all GGT values within the sex-specific 95th percentile in our dataset; the upper 5% were considered likely to have extreme hepatic inflammation secondary to frank hepatic disease. GGT values within the 95th percentile were then used to generate quintiles. Mean values for IL-6, CRP, oxLDL and sICAM-1 were summarized for each GGT quintile and tested for linear trends. Tests for trends were adjusted for age, gender, ethnicity and site. All analyses were then stratified by ethnicity and repeated. Because Black-Americans have genetically determined differences in the binding site of the antibody used to measure sICAM-1²⁹, sICAM-1 observations from Black participants were excluded from the analyses of GGT and sICAM-1 associations. Multivariable linear regression analysis was then used to evaluate the presence or absence of continuous associations between GGT and IL-6, CRP, IL-6, oxLDL and sICAM-1 in four sequential models. Analyses were performed with and without log transformation of skewed variables, and compared. Adjustment variables for Model 1 (M1) included race, sex, study site, and age. Model 2 (M2) added adjustments for alcohol consumption (drinking status and drinks/week), smoking (smoking status and pack years), and physical activity (total metabolic equivalents/week). Model 3 (M3) added adjustments for family history of stroke and myocardial infarction; continuous systolic and diastolic blood pressure; use of diabetes, lipid and blood pressure medications; and continuous lipid values including LDL, HDL and triglycerides. Model 4 (M4) added adjustments for waist circumference (cm), fasting blood glucose (mg/dl) and fasting serum insulin (μ U/l). These analyses were performed across the entire cohort, then stratified by ethnicity and repeated. Multiplicative interactions between GGT and age and ethnicity were evaluated in crude and adjusted models. Multiple F tests were performed to determine the significance of interaction variables. All models were also repeated in subgroups defined by age range, i.e., 45–54, 55–64, 65–74, and 75–84.

Results

Associations between GGT and Baseline Demographic Characteristics and Traditional Risk Factors

The distributions of GGT, CRP, IL-6, sICAM-1, and oxLDL were skewed and therefore log-transformed for analysis. The distribution of GGT was: median=31.8 U/l; (IQR: 25.8–41.8 U/l); 10.4% (male) and 20.8% (female) of values were outside of the FAHC laboratory reference range. Ranges for each quintile of GGT were: Q1: <24.5 U/l, Q2: 24.5–29.3 U/l, Q3: 29.3–35.1 U/l, Q4: 35.1–45.2 U/l; and Q5: >45.2 U/l. Baseline characteristics and crude associations with adjustment variables are detailed in Table 1. Adjustment variables were crudely associated with GGT except current drinking status ($p=0.34$) and physical activity

($p=0.75$, as metabolic equivalent-minutes/week). Female gender, “never” smoking and Chinese and White ethnicities were inversely associated with GGT compared to male gender, “current” or “past” smoking status and either Black or Hispanic ethnicity which showed positive trends. Age and HDL lipoproteins were inversely associated with increasing GGT. All other crude associations with adjustment variables were positive, including LDL lipoproteins, triglycerides, blood pressure, pack years of smoking, number of alcoholic drinks/week, and medication use ($p=0.036$ to <0.0001).

Crude and Adjusted Associations between GGT and Biomarkers of Oxidative Stress, Immune Activation, Acute Phase Response and Endothelial Dysfunction

Figures 1a–d present trends in oxLDL, IL-6, CRP, IL-6, and sICAM-1 for the entire cohort, as well as in each ethnic subgroup. Crude linear trends between GGT activity and each biomarker of interest were positive and strongly significant in the entire cohort ($p<0.0001$). Linear trends remained strongly positive in the entire cohort in models adjusted for age, gender, race and study site ($p<0.0001$ for all). Tests for linear trends with increasing GGT quintiles within each ethnic subgroup remained positive and significant for CRP and sICAM-1; $p<0.0001$ and <0.001 respectively for each ethnic subgroup, with no evidence of significant interaction between GGT and ethnicity. Trends in IL-6 by GGT quintile were strongly positive for the entire cohort ($p<0.0001$) and remained strongly significant for White ($p<0.0001$, $n=2469$), Black ($p=0.001$, $n=1685$), and Hispanic ($p<0.0001$, $n=1372$) participants, but not for Chinese ($p=0.14$, $n=763$) participants. Although strongly positive in the entire cohort ($p<0.0001$, $n=938$) and in White participants ($p<0.0001$, $n=442$), trends in oxLDL by GGT quintile did not remain statistically significant in Chinese ($p=0.10$, $n=92$), Black ($p=0.14$, $n=188$) or Hispanic ($p=0.11$, $n=216$) ethnic subgroups.

Table 2 details regression coefficients, 95% confidence intervals and associated p-values for all multivariable linear regression analysis performed between GGT and biomarkers of interest. Table 3 details regression coefficients, 95% confidence intervals and associated p-values for M1 and M4 repeated by age subgroups. For every dependent variable evaluated, adjustment for metabolic status attenuated associations with GGT, although significant independent relationships remains between GGT and all variables except oxLDL.

Linear regression results were similar with or without log transformation of skewed variables, i.e., GGT, CRP, IL-6, oxLDL and sICAM-1. Briefly, all four models for CRP demonstrated continuous strong, positive associations with GGT: M1: $\beta_{Adj}=0.04$, 95% CI: 0.03–0.05, $p<0.0001$; M2: $\beta_{Adj}=0.03$, 95% CI: 0.02–0.04, $p<0.0001$; M3: $\beta_{Adj}=0.03$, 95% CI: 0.01–0.04, $p<0.0001$ and M4: $\beta_{Adj}=0.0017$, 95% CI: 0.005–0.03, $p=0.006$. There was no evidence of significant interaction race and GGT in associations with CRP. However a significant interaction was suggested between age and GGT in the minimally adjusted model ($\beta_{Intrxn}=0.04$, $p<0.0001$), although this interaction was no longer present with adjustment for metabolic status. As demonstrated in Table 3, the overall association between CRP and GGT appears to strengthen with increasing age, and is less attenuated with adjustment for metabolic status in older subgroups.

Similar relationships were evident in evaluating continuous associations between GGT and IL-6. Regression coefficients between IL-6 and GGT remained positive and statistically

significant in all four models; M1: $\beta_{Adj.}=0.007$, 95% CI: 0.005–0.009, $p<0.0001$; M2: $\beta_{Adj.}=0.007$, 95% CI: 0.004–0.009, $p<0.0001$; M3: $\beta_{Adj.}=0.006$, 95% CI: 0.003–0.009, $p<0.0001$; M4: $\beta_{Adj.}=0.004$, 95% CI: 0.001–0.006, $p=0.007$. There was no evidence of significant interaction between age and GGT or race and GGT in associations with IL-6. As shown in Table 3, the association between GGT and IL-6 does weaken slightly with age and adjustment for metabolic status does not attenuate associations all age subgroups.

Regression analysis evaluating associations between GGT and oxidized LDL (oxLDL) were not as strong, but remained significant in M1: $\beta_{Adj.}=0.005$, 95% CI: 0.002–0.008, $p=0.004$; and M2: $\beta_{Adj.}=0.006$, 95% CI: 0.003–0.008, $p=0.001$; though were attenuated by M3: $\beta_{Adj.}=0.001$, 95% CI: –0.001–0.004, $p=0.41$. There was no evidence of significant association between oxLDL and age, or interaction between GGT and age or race. Analysis by age subgroups for analyses including oxLDL were significantly limited in sample size (Table 3) and thus definitive conclusions cannot be drawn from these results.

Results of regression analysis of continuous associations between GGT and soluble intracellular adhesion molecule-1 (sICAM-1) demonstrated strong positive associations in each model; M1: $\beta_{Adj.}=0.87$, 95% CI: 0.68–1.06, $p<0.0001$; M2: $\beta_{Adj.}=0.68$, 95% CI: 0.45–0.91, $p<0.0001$; M3: $\beta_{Adj.}=0.68$, 95% CI: 0.45–0.91, $p<0.0001$; M4: $\beta_{Adj.}=0.51$, 95% CI: 0.27–0.75, $p<0.0001$. Adjustment for lifestyle factors and traditional risk factors did little to attenuate the regression coefficient in either model. Although there was suggestion of interaction between GGT and age related to sICAM-1 ($\beta_{Intrxn} = 0.47$, $p=0.03$) in the minimally adjusted M1, all interaction with age was attenuated in more fully adjusted models.

Discussion

In this cross-sectional analysis performed in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, we saw evidence of several significant associations between γ -glutamyltransferase (GGT), traditional cardiovascular risk factors, and biomarkers of oxidative stress, immune activation, acute phase response and endothelial dysfunction. Specifically, we saw evidence of strong associations for increasing trends in oxLDL, IL-6, CRP, and sICAM-1 with graded increases in GGT in the entire cohort. Continuous associations between GGT and all biomarkers of interest were significant after adjustment for age, race, gender and study site, and remained significant for IL-6, CRP, and sICAM-1 after adjustment for risky lifestyle factors and traditional risk factors. Although the strength of all associations was attenuated by adjustment for metabolic status, most associations remained significant and independent. Associations between GGT and oxLDL were significant in the entire cohort after adjustment for demographics and risky lifestyle factors, but not following adjustment for traditional risk factors, likely secondary to moderate collinearity between LDL and oxLDL [i.e., correlation coefficient = 0.58 between LDL-C and $\ln(\text{oxLDL})$ in MESA]. Our findings are strengthened by the use of the MESA cohort for our analyses, which is unique amongst population cohorts in its excellent ethnic representation and its collection of emerging biomarkers like oxLDL. No known prior studies have evaluated associations between GGT and oxLDL, IL-6, and sICAM-1, nor have any prior studies evaluated associations with CRP in ethnic subgroups.

Our findings are supported by those of prior investigations in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort study which demonstrated similar associations between GGT and CRP and fibrinogen, as well as additional support for GGT associations with lipid peroxidation products, e.g., F2-isoprostanes.¹⁶ Combined with our findings confirming associations with CRP, and demonstrating novel associations with oxLDL, IL-6, and sICAM-1, these data support the hypothesis that increased GGT activity represents immune stimulation (i.e., IL-6) of hepatic acute phase response (i.e., CRP and fibrinogen), possibly due to increased lipid peroxidation products (i.e., oxLDL and F2-isoprostanes)^{30,31}. For the first time, our findings extend this conceptual model to include vascular endothelial involvement, i.e., sICAM-1.

These findings are limited in several important ways, most notably that we evaluated cross-sectional associations between GGT and our biomarkers of interest measured only from the baseline examination. Evidence of correlations between longitudinal changes in GGT and CRP, sICAM-1, etc. would strengthen our hypothesis of GGT as a biomarker of oxidative stress, vascular inflammation and endothelial dysfunction. The suggestion that GGT activity may correlate with endothelial dysfunction requires confirmation by evaluating associations between GGT and a physiologic measure of endothelial function like flow-mediated dilation or reactive hyperemia index.

Our findings are also limited in that we do not adjust for serum AST or ALT in our analyses. Unfortunately, neither AST nor ALT data are available in the MESA cohort for inclusion. While adjustment for AST and ALT would have assisted in reducing the contribution of hepatic disease to our findings, as suggested in our prior work²¹, serum GGT activity is not correlated with hepatic expression of GGT.³² Also we partially accounted for liver disease in two ways: by restricting our analyses to the lower 95th percentile of the GGT activity range (thus likely eliminating severe hepatic disease) and by adjusting for continuous alcohol intake. We were not interested in adjusting our results for fatty liver disease, as fatty liver is highly correlated with hepatic insulin resistance, and thus adjusting for it would have eliminated the influence of metabolic risk fundamental to our hypothesis.

Some tests for associations within ethnic subgroups may have been limited in statistical power, especially in evaluations with oxLDL in which racial and age subgroups were very small, i.e., Chinese (n=92) and Black (n=188). Our findings are further limited by our inability to evaluate associations between GGT and sICAM-1 in Blacks due to limitations in sICAM-1 measurement methodology, thus limiting the generalizability of the measured associations to non-Black adults without symptomatic cardiovascular disease. However, given the strength of the associations between GGT and sICAM-1 in all other ethnic groups, and no evidence of select interactions with ethnicity, the absence of a similar relationship in Blacks would be surprising.

Our findings in the Multi-Ethnic Study of Atherosclerosis (MESA) support the hypothesis that increases in serum GGT activity occur in parallel with increases in oxidative stress, immune activation, acute phase response, and vascular inflammation- and serum GGT activity may represent the influence of metabolic status on vascular injury. These results are consistent with *in vivo* findings that increased GGT activity represents a reduction in

available GSH,²⁰ potentially leaving substrates, e.g., LDL, vulnerable to oxidative modification, which then activate immune targets. Based on the strength and consistency of the associations demonstrated here, combined with current understanding of the oxidative/inflammatory mechanisms of endothelial dysfunction, we conclude serum GGT activity should be considered a continuous biomarker of increased glutathione demand relevant in the development of endothelial dysfunction and subsequent arteriosclerosis. The low cost of measuring GGT and its stability in stored samples make it ideal for continued confirmatory research in large population-based cohort studies. Yet, given the accumulation of population-based research findings suggesting cross-sectional associations with established cardiometabolic risk factors, as well as increased cardiometabolic event prediction from graded elevations in GGT activity, we suggest translational clinical research is now needed to determine the mechanistic determinants of serum GGT activity.

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Abbreviations

GGT	total serum γ -glutamyltransferase activity
oxLDL	oxidized LDL
sICAM-1	soluble intercellular adhesion molecule
IL-6	interleukin 6
CRP	C-reactive protein
GSH	glutathione

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Highlights

- We evaluated associations with GGT and biomarkers of lipoprotein oxidation, immune activation, acute phase response and atherosclerosis in the Multi-Ethnic Study of Atherosclerosis cohort.
- We specifically investigated associations between GGT and oxLDL, IL-6, CRP and sICAM-1, in crude and multivariable models adjusted for demographics, lifestyle, traditional cardiovascular risk parameters and metabolic status.
- Associations were measured in four ethnic subgroups, i.e., Chinese, Hispanic, Black and Caucasian, including tests for interactions
- Strong associations were measured between GGT and all biomarkers under evaluation, which were attenuated with adjustment for metabolic status.
- Associations and interactions with age were also attenuated with adjustment for metabolic status.

Figure 1.
Trends in Biomarkers of Arteriosclerosis by Increasing GGT Quintiles

Table 1

Crude Associations between GGT and Baseline Characteristics

Baseline Characteristic (n=6,446)	GGT-Q1 <24.5 U/l n (%) or Mean (SD)	GGT-Q2 24.5–29.3 U/l n (%) or Mean (SD)	GGT-Q3 29.3–35.1 U/l n (%) or Mean (SD)	GGT-Q4 35.1–45.2 U/l n (%) or Mean (SD)	GGT-Q5 >45.2–99.7 U/l n (%) or Mean (SD)	P-value for trend
Age (yr)	62.4 (10.9)	63.5 (10.3)	62.8 (10.0)	62.1 (10.0)	61.0 (9.7)	<0.0001
Gender: Male	325 (25.2%)	523 (40.7%)	635 (49.1%)	724 (52.5%)	840 (65.1%)	<0.0001
Race						
White	665 (30.0%)	543 (24.5%)	465 (20.9%)	435 (19.6%)	408 (18.4%)	Referent
Chinese	194 (21.9%)	173 (19.6%)	164 (18.6%)	123 (13.9%)	119 (13.5%)	0.91
Black	226 (12.9%)	308 (17.6%)	389 (22.3%)	430 (24.6%)	395 (22.6%)	<0.0001
Hispanic	204 (14.5%)	262 (18.6%)	274 (19.5%)	301 (21.4%)	368 (26.2%)	<0.0001
Smoking Status						
Never	746 (22.9%)	663 (20.4%)	695 (21.4%)	615 (18.9%)	533 (16.4%)	Referent
Current	105 (14.9%)	135 (19.2%)	159 (22.6%)	172 (24.4%)	228 (32.4%)	<0.0001
Past	432 (18.2%)	483 (20.4%)	435 (18.3%)	498 (21.0%)	525 (22.1%)	<0.0001
Pack Years (yr)	21.1 (23.2)	21.6 (21.6)	24.8 (27.1)	23.4 (25.5)	24.5 (33.7)	0.021
Current Alcohol Use (%)	699 (10.3%)	669 (9.8%)	672 (10%)	719 (10.6%)	782 (11.5%)	0.34
Current Drinks/Week	2.4 (3.7)	3.5 (5.1)	3.5 (5.1)	4.4 (6.8)	5.9 (8.0)	<0.0001
Physical Activity (Total MET-min/Wk)	1499 (2131)	1529 (2286)	1548 (2196)	1558 (2580)	1609 (2841)	0.75
Diabetes Medications						
None	1,218 (94%)	1,184 (92%)	1,159 (89%)	1,150 (89%)	1,124 (87%)	Referent
Insulin	11 (1%)	19 (2%)	20 (2%)	21 (2%)	33 (3%)	<0.0001
Oral	60 (5%)	83 (6%)	113 (9%)	118 (9%)	133 (10%)	<0.0001
Total Cholesterol (mg/dl)	192.0 (32.6)	192.8 (35.7)	193.5 (35.3)	195.3 (34.8)	196.8 (37.6)	<0.0001
LDL-C (mg/dl)	114.1 (29.4)	116.6 (31.1)	118.3 (31.0)	119.6 (31.9)	118.7 (32.5)	<0.0001
HDL-C (mg/dl)	56.7 (15.3)	52.4 (15.5)	49.4 (14.1)	48.4 (13.3)	48.0 (13.8)	<0.0001

Baseline Characteristic (n=6,446)	GGT-Q1 <24.5 U/l n (%) or Mean (SD)	GGT-Q2 24.5–29.3 U/l n (%) or Mean (SD)	GGT-Q3 29.3–35.1 U/l n (%) or Mean (SD)	GGT-Q4 35.1–45.2 U/l n (%) or Mean (SD)	GGT-Q5 >45.2–99.7 U/l n (%) or Mean (SD)	P-value for trend
Triglycerides (mg/dl) *	94.0 (68–127)	104.0 (74–146)	111.0 (78–158)	119.0 (83–174)	132.0 (89–191)	<0.0001
Lipid-lowering Medications	181 (14%)	203 (16%)	213 (16.5%)	230 (18%)	222 (17%)	0.036
SBP (mm Hg)	121.9 (21.6)	125.7 (22.0)	127.7 (21.7)	128.1 (21.0)	129.2 (20.5)	<0.0001
DBP (mm Hg)	68.3 (10.0)	70.4 (9.9)	72.4 (10.1)	74.4 (10.1)	73.9 (10.2)	<0.0001
Current HTN	466 (36%)	531 (41%)	591 (46%)	642 (50%)	650 (50.4%)	<0.0001
HTN Medications	395 (31%)	454 (35%)	485 (38%)	537 (42%)	517 (40%)	<0.0001
Waist circumference (cm)	91.9 (14.3)	96.2 (14.3)	99.0 (14.0)	101.1 (13.8)	101.4 (13.4)	<0.0001
Glucose (mg/dl) *	85.0 (80–91)	88.0 (82–95)	91.0 (84–100.5)	92.0 (85–103)	93.0 (86–106)	<0.0001
Insulin (μU/l) *	3.8 (2.7–5.5)	4.8 (3.3–7.2)	5.6 (3.8–8.6)	6.2 (4.1–9.4)	7.1 (4.4–11.1)	<0.0001

* Skewed variable; descriptive statistics reported as median and inter-quartile range (IQR).

Table 2

Adjusted Associations between CRP, IL-6, oxLDL and sICAM-1 and GGT

CRP = $\beta_{Adj.}^* \text{ GGT} + \beta_n X_n \dots + \beta_0$, n=6,415	$\beta_{Adj.}^*$	95% CI	p-value for GGT
M1 (n=6,415)	0.042	0.03–0.05	<0.0001
M2 (n=3,819)	0.032	0.02–0.04	<0.0001
M3 (n=3,774)	0.025	0.01–0.04	<0.0001
M4 (n=3,772)	0.017	0.005–0.03	0.006
IL-6 = $\beta_{Adj.}^* \text{ GGT} + \beta_n X_n \dots + \beta_0$, n=6,289	$\beta_{Adj.}^*$	95% CI	p-value for GGT
M1 (n=6,289)	0.0069	0.005–0.009	<0.0001
M2 (n=3,745)	0.0065	0.004–0.009	<0.0001
M3 (n=3,701)	0.0060	0.003–0.009	<0.0001
M4 (n=3,699)	0.0036	0.001–0.006	0.007
oxLDL = $\beta_{Adj.}^* \text{ GGT} + \beta_n X_n \dots + \beta_0$, n=935	$\beta_{Adj.}^*$	95% CI	p-value for GGT
M1 (n=935)	0.005	0.002–0.008	0.004
M2 (n=583)	0.006	0.003–0.010	0.001
M3 (n=573)	0.001	–0.001–0.004	0.41
M4 (n=572)	0.0007	–0.002–0.004	0.64
sICAM-1 = $\beta_{Adj.}^* \text{ GGT} + \beta_n X_n \dots + \beta_0$, n=938	$\beta_{Adj.}^*$	95% CI	p-value for GGT
M1 (n=938)	0.87	0.68–1.06	<0.0001
M2 (n=585)	0.68	0.45–0.91	<0.0001
M3 (n=575)	0.68	0.45–0.93	<0.0001
M4 (n=574)	0.51	0.27–0.75	<0.0001

* Corresponding units for regression coefficients are: CRP: mg/L/unit GGT activity; IL-6: pg/ml/unit GGT activity; oxLDL: mg/dl/unit GGT activity; and sICAM-1: ng/ml/unit GGT activity.

M1 adjusts for age (years), gender, ethnicity, and study site.

M2 equals M1 + alcohol use (current/former/never and drinks/week) + exercise (MET-min/week) + smoking (current/former/never and pack years).

M3 equals M2 + lipids (LDL-C, HDL-C, triglycerides in mg/dl), lipid-lowering medications, systolic and diastolic blood pressures (mmHg), anti-hypertensive medications, diabetes medications, and family history of heart attack or stroke.

M4 equals M3 + waist circumference (cm), fasting blood glucose (mg/dl) and fasting insulin (μ U/l).

Table 3

Associations between CRP, IL-6, oxLDL and sICAM-1 and GGT within age subgroups

	CRP β_{GGT} 95% CI (n)	p-value for GGT	IL-6 β_{GGT} 95% CI (n)	p-value for GGT	oxLDL β_{GGT} 95% CI (n)	p-value for GGT	sICAM-1 β_{GGT} 95% CI (n)	p-value for GGT
Age 45–54								
M1	0.04 0.03–0.06 (n=1,782) ---	<0.0001	0.008 0.005–0.01 (n=1,764) ---	<0.0001	0.008 0.002–0.01 (n=345) ---	<0.0001	0.80 0.5–1.1 (n=762) ---	<0.0001
M4	0.01 –0.009–0.03 (n=1,168)	0.27	0.005 0.0002–0.009 (n=1,153)	0.04	0.002 –0.004–0.007 (n=230)	0.56	0.31 –0.08–0.7 (n=483)	0.12
Age 55–64								
M1	0.02 –0.0002–0.04 (n=1,102) ---	0.053	0.006 0.003–0.01 (n=1,728) ---	0.001	0.004 –0.001–0.01 (n=299) ---	0.24	1.2 0.9–1.6 (n=641) ---	<0.0001
M4	–0.006 –0.03–0.02 (n=1,086)	0.60	0.002 –0.003–0.007 (n=1,064)	0.45	0.001 –0.005–0.008 (n=177)	0.65	0.7 0.2–1.1 (n=387)	0.005
Age 65–74								
M1	0.05 0.03–0.07 (n=1,936) ---	<0.0001	0.008 0.003–0.01 (n=1,887) ---	0.001	0.001 –0.006–0.008 (n=220) ---	0.71	0.63 0.22–1.0 (n=454) ---	0.003
M4	0.04 0.02–0.06 (n=1,046)	0.001	0.006 0.0002–0.01 (n=1,020)	0.04	–0.001 –0.008–0.005 (n=122)	0.70	0.5 0.02–1.1 (n=266)	0.04
Age 75–84								
M1	0.07 0.04–0.10 (n=931) ---	<0.0001	0.006 0.00009–0.01 (n=910) ---	0.05	–0.005 –0.02–0.008 (n=74) ---	0.47	0.50 –0.09–1.1 (n=184) ---	0.09
M4	0.05 0.01–0.08 (n=472)	0.01	0.005 –0.003–0.013 (n=462)	0.24	–0.01 –0.04–0.01 (n=45)	0.24	0.4 –0.4–1.3 (n=110)	0.31

M1 adjusts for age (years), gender, ethnicity, and study site.

M4 equals M1 + alcohol use (current/former/never and drinks/week) + smoking (MET-min/week) + smoking (current/former/never and pack years), lipids (LDL-C, HDL-C, triglycerides in mg/dl), lipid-lowering medications, systolic and diastolic blood pressures (mmHg), anti-hypertensive medications, diabetes medications, family history of heart attack or stroke, waist circumference (cm), fasting blood glucose (mg/dl) and fasting insulin (μ U/l).