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Associations between total serum GGT activity and metabolic risk: MESA

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Abstract

Aim—To evaluate associations between total serum GGT activity, metabolic risk factors and prevalent metabolic disease in MESA.

Patients & methods—Continuous associations between GGT and fasting blood glucose (FBG), fasting insulin, HbA_{1c} and Homeostasis Model Assessment Index of Insulin Resistance (HOMA–IR) were evaluated in the entire MESA cohort and in metabolic disease subgroups using linear regression models incrementally adjusted for age, gender, site, race, lifestyle, traditional risk factors and medications. Cross-sectional odds of prevalent impaired FBG, metabolic syndrome and Type 2 diabetes were calculated for GGT quintiles in the entire cohort and in subgroups defined by age (< or ≥ 65 years) and ethnicity.

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Author contributions

R Bradley and DR Jacobs Jr researched the data, performed the analysis and wrote the manuscript. D-H Lee and AL Fitzpatrick assisted with the analysis plan, and reviewed/edited the manuscript. NS Jenny developed the GGT measurement assay and reviewed the manuscript.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Results—In multivariable models, significant associations were present between GGT activity and FBG, fasting insulin, HbA_{1c} and HOMA-IR, with the interaction between GGT and BMI affecting the association between GGT and HOMA-IR as well as the association between BMI and HOMA-IR ($p < 0.0001$). Adjusted odds ratios (95% CIs) of prevalent impaired FBG, metabolic syndrome and Type 2 diabetes for quintile 5 versus 1 in the entire cohort were 2.4 (1.7–3.5), 3.3 (2.5–4.5) and 2.8 (1.8–4.4), respectively ($p < 0.0001$). GGT associations weakened with age. The significance of linear trends for increased prevalent metabolic disease by increasing GGT quintile varied by ethnicity.

Conclusion—GGT is strongly associated with both cardiovascular and metabolic risk factors, including prevalent metabolic disease, in the MESA cohort.

Keywords

γ -glutamyltransferase; GGT; glutathione; metabolic syndrome; oxidative stress; Type 2 diabetes

There has been a resurgence of interest in the enzyme GGT owing to the results of several observational studies identifying associations between graded elevations in its activity in serum and increased risk of adverse cardiovascular and metabolic outcomes, including metabolic syndrome (MetS) [1], Type 2 diabetes [2–7], hypertension [6,8], congestive heart failure [9] and vascular events [10,11], plus increased mortality from cardiovascular disease and diabetes [12,13].

A known physiologic function of GGT is to contribute to *in vivo* antioxidant homeostasis through recycling extracellular glutathione (GSH) and its precursor amino acids for intracellular reconversion to reduced GSH [14]. GGT and its relationship to redox balance *in vivo* has been thoroughly summarized by Whitfield [15], and several specific research findings provide a convincing rationale that increased GGT activity represents increased oxidative stress, including:

- GSH depletion appears to be a prerequisite condition to induce GGT [16];
- NADPH oxidase-produced reactive oxygen species and reactive nitrogen species both induce GGT expression [17,18];
- Mitochondria of GGT-knockout mice have depleted GSH, increased reactive oxygen species formation, depleted energy stores and impaired oxidative phosphorylation (thus impaired ATP production), which can be attenuated by *N*-acetylcysteine, a GSH precursor [19];
- GGT-knockout mice die prematurely with complications associated with increased oxidative stress that can be attenuated with *N*-acetylcysteine [20].

Translating this hypothesis to research in humans, and further supporting a relationship between GGT and GSH, Sedda *et al.* demonstrated inverse associations between GGT activity and plasma total GSH concentration in people with established cardiovascular risk and, after multivariate adjustment for individual risk factors, male gender, the total number of risk factors present and plasma total GSH remained the only independent variables associated with GGT activity [21].

Commonly classified as a ‘liver enzyme’ associated with gall bladder disease, alcoholism and frank hepatitis, liver tissue GGT expression does not always correlate with serum GGT activity [22,23]. GGT is expressed in multiple human tissues other than the liver, with total serum GGT activity corresponding to the sum of activity from at least four fractions (i.e., small [s-GGT], medium [m-GGT], big [b-GGT] and free [f-GGT] GGT), patterns of which may identify the tissue sites of origin, as well as the nature of pathological changes [24–28]. However, despite the insights gained from fractionating GGT, the relationship between tissue GGT activity and serum GGT activity remains under active investigation.

As GSH depletion, and subsequent oxidative modification of cellular proteins, lipids and nucleotides, is implicated in the development of insulin resistance, impaired insulin production and the microvascular complications of diabetes [29–31], it is logical to question the relationship between increases in GGT activity and metabolic disease. Supporting a relationship in humans, Bonnet *et al.* demonstrated strong associations between human insulin resistance measured by euglycemic–hyperinsulinemic clamp and GGT activity [32]. However, additional data are needed from larger human cohorts regarding the relationships between GGT activity and specific risk factors with established prognostic value in metabolic disease, including the magnitude, direction and interdependency of any associations, as well as the generalizability of associations found in different ethnic groups.

Therefore, the objective of the current study was to evaluate cross-sectional associations between fasting serum GGT activity, individual metabolic risk factors (e.g., fasting blood glucose [FBG], fasting insulin [FI], HbA_{1c} and insulin resistance) and prevalent disease (e.g., impaired FBG [IFG], MetS and Type 2 diabetes) in the MESA cohort. The MESA cohort was especially useful for this evaluation because it includes participants within a broad range of ages and metabolic risk statuses, plus unlike many other US cohorts, it allows for evaluations within age and ethnic subgroups because of robust representation from four ethnicities, that is, black, Chinese, Hispanic and white. If GGT activity is a valid, generalizable biomarker of metabolic disease severity, we would anticipate total serum GGT activity to be positively associated with risk factors and disease, proportional to the severity of disease (and thus to the hypothetical burden of oxidative stress), and generalizable across all vulnerable ethnic groups.

Patients & methods

This ancillary study to the parent MESA cohort was approved by the Institutional Review Board at the University of Washington (WA, USA) as study #34269 prior to performing all study procedures and analyses.

Study population

The design and objectives of the MESA cohort study have been reported elsewhere [33]. Briefly, MESA consists of 6814 participants recruited from the general US population (53% female; 47% male) aged 44–84 years (mean: 62 years) representing four major ethnic groups, Chinese (n = 803), black (n = 1893), Hispanic (n = 1496) and white (n = 2622). All samples and data were collected during the baseline MESA examination (i.e., exam 1

performed July 2000–August 2002), except for HbA_{1c}, which was measured in samples collected during MESA exam 2 (i.e., September 2002–February 2004).

Participant-reported questionnaires

Participant demographics, medical history including current medications and physical activity were collected using standardized forms during exam 1. Smoking status was recorded as current, former or never and pack-years calculated for former and current smokers. Alcohol use was categorized as former, current or never based on self-report and total intake was recorded as the number of standardized drinks (i.e., one beer, one glass of wine or one shot of spirits) per week. Total intentional exercise (MET-min/week) was calculated based on self-report over the previous month.

Clinical measurements

GGT—Using methods by Silber *et al.*, a microplate assay was developed for measuring GGT activity based on changes in the absorption of p-nitroaniline at 405 nm read by an automated plate reader run at 37°C under specified conditions [34]. A total of 6783 frozen samples were available for GGT measurement (99.5% of the cohort). The assay was conducted at 23°C, had a detectable range of 0.274–199.8 U/l and a coefficient of variation (CV) <5%. In order to calibrate the microplate assay to routine clinical methods, 20 test samples were measured using an automated clinical instrument at the Fletcher Allen Health Care (FAHC) laboratory at the University of Vermont (VT, USA) and then repeated using the plate assay. A calibration equation was developed ($GGT_{\text{clinical}} = [2.034 \times GGT_{\text{plate}}] + 15.93$; $R^2 = 0.98$), and applied to all values measured using the microplate method. To confirm the calibration equation, GGT was remeasured in the original 20 test samples at the Collaborative Studies Clinical Laboratory at Fairview University Medical Center (MN, USA) and found similar to the FAHC data set (Pearson's correlation = 0.99). Therefore, the original FAHC-calibrated data set was used for subsequent analyses.

Additional laboratory measures—Total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol were measured in ethylenediaminetetraacetic acid plasma using a cholesterol oxidase method on a COBAS FARA centrifugal analyzer (Roche Diagnostics, IN, USA); CV for TC and HDL cholesterol was 1.6 and 2.9%, respectively. Plasma low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald formula in specimens with triglycerides <400 mg/dl [35]. Plasma triglycerides were measured using Triglyceride GB reagent (Roche Diagnostics) on the Roche COBAS FARA centrifugal analyzer; CV = 4.0%. FBG was measured by rate reflectance spectrophotometry using a thin film adaptation of the glucose oxidase method on the VITROS[®] analyzer (Johnson & Johnson Clinical Diagnostics, Inc., NY, USA); CV = 1.1%. FI was determined by radioimmunoassay using the Linco Human Insulin Specific RIA Kit (Linco Research, Inc., MO, USA); lower limit of sensitivity was 2 U/l and CV = 4.9%. HbA_{1c} was measured on the Tosoh A_{1c} 2.2 Plus Glycohemoglobin Analyzer (Tosoh Medics, Inc., CA, USA) using automated HPLC; CV = 1.4–1.9%. All measurements were performed at the Collaborative Studies Clinical Laboratory at Fairview University Medical Center. Homeostasis Model Assessment Index of Insulin Resistance (HOMA-IR) was calculated using FBG and FI according to Matthews *et al.*, excluding insulin users [36].

Definitions of metabolic disease subgroups

IFG—Nondiabetic participants were defined as having IFG if their FBG was ≥ 100 mg/dl but <126 mg/dl at their baseline visit [37].

MetS—Both National Heart Lung and Blood Institute (NHLBI) and the 2009 joint International Diabetes Federation (IDF)/NHLBI/American Heart Association (AHA)/International Atherosclerosis Society (IAS)/International Association for the Study of Obesity (IASO) (i.e., ‘2009 Joint’) definitions were used for MetS [38]. Participants were defined as having MetS if they met three or more of the standard criteria including: FBG ≥ 100 mg/dl or antidiabetic treatment, triglyceride ≥ 150 mg/dl or treatment, HDL <40 mg/dl for males or <50 mg/dl for females, blood pressure $\geq 130/85$ mmHg or blood pressure treatment, and/or increased waist circumference. For the NHLBI definition, waist circumference was defined as increased if ≥ 102 cm for males or ≥ 88 cm for females; gender- and ethnicity-specific thresholds were applied for the 2009 Joint definition [38]. As MESA is an ethnically diverse sample, we used the 2009 Joint definition in subsequent analyses because it includes ethnicity-specific thresholds for waist circumference. Participants were not classified as having MetS if they met criteria for Type 2 diabetes.

Type 2 diabetes—Because HbA_{1c} was not measured until exam 2, we defined Type 2 diabetes based on FBG ≥ 126 mg/dl [37], or if participants were taking insulin or oral diabetes medications at exam 1.

Statistical analyses

All skewed variables (i.e., GGT, glucose, insulin, triglycerides, HOMA-IR and HbA_{1c}) were log-transformed for linear regression analyses. GGT data were divided into equal quintiles. Crude cross-sectional associations between GGT and individual demographic, lifestyle and risk variables were determined by calculating the mean value for each within GGT quintiles, and then applying an unadjusted linear regression model to determine the significance of linear trends across quintiles. Sequential multivariable linear regression models were developed to evaluate continuous associations between GGT and clinical metabolic risk variables, that is, FBG, FI, HbA_{1c} and HOMA-IR, adjusted for hypothesized confounders. The first model (M1) adjusted for continuous age plus gender, ethnicity and study site as categorical variables. The second model (M2) added lifestyle covariates including categorical alcohol use (current/former/never), continuous drinks per week, continuous intentional exercise (MET-min/week) and categorical smoking (current/former/never), plus continuous exposure as pack-years. The third model (M3) added traditional cardiovascular risk covariates including continuous measures of LDL, HDL, triglycerides, systolic and diastolic blood pressure, and binary variables for diabetes medication, lipid-lowering medication and blood pressure medication, as well as family history of stroke and myocardial infarction. Waist circumference was added to M3 for HOMA-IR (M4_{HOMA}). We also added BMI to the HOMA-IR model *post hoc* and tested for interaction with GGT based on previous findings of an interaction [3]. Users of exogenous insulin were omitted from all models. We tested for multiplicative interactions between continuous GGT and age and ethnicity; tests for interactions with age were specified *a priori* based on past findings [13], while tests for interactions with ethnicity were added *post hoc* as exploratory tests.

Wald tests were applied to test for nonzero regression coefficients for each ethnic subgroup, and to test the equivalency of coefficients. All analyses were repeated within metabolically normal, MetS and Type 2 diabetes subgroups, and in subgroups defined by age < or ≥ 65 years.

The cross-sectional prevalence of metabolic disease was calculated and reported as the number of cases and proportion of the total cohort meeting each definition. The Mantel–Haenszel odds ratio of IFG, MetS and Type 2 diabetes were calculated within each quintile, adjusted for age, gender, ethnicity, waist circumference and study site, and additional adjustment for alcohol, smoking and exercise. Statistical significance was determined for linear trends across all quintiles and for point estimates for odds of disease within each quintile. Analyses were repeated in subgroups defined by ethnicity and age < or ≥ 65 years. We also evaluated multiplicative interaction between GGT and age (specified *a priori*) and ethnicity (*post hoc*, exploratory).

Experimental results

Distribution of GGT

GGT activity was significantly different between males and females ($p < 0.0001$ for comparisons of log-transformed means by unpaired, two-sided t-test). Because neither liver ultrasound nor biopsy data were available in MESA, we included only GGT values <95th percentile for each gender in order to exclude extreme liver disease in our final data set ($n = 6446$), corresponding to an upper limit of 80.9 and 99.7 U/l for females and males, respectively. Gender-specific medians and interquartile ranges (IQRs) for the overall distribution of GGT were: 29.1 U/l (23.8–37.3 U/l) for females and 35.5 U/l (28.6–46.6 U/l) for males, with the median and IQR of the entire cohort equal to 31.8 U/l (25.8–41.8 U/l).

Prevalence of metabolic disease

We identified a considerable prevalence of metabolic disease within members of the MESA cohort. IFG and glucose-defined diabetes were present in 15.9 ($n = 945$) and 12.5% ($n = 852$) of participants, respectively. The prevalence of MetS was highly concordant between definitions ($\kappa = 96\%$): NHLBI was 29.9% ($n = 2038$) and 2009 Joint was 28.4% ($n = 1935$). Individual components of MetS were highly prevalent at baseline, including increased waist circumference (62.9% per NHLBI and 62.2% per 2009 Joint definition), elevated blood pressure ≥ 135/85 or treatment (51.0%), low HDL cholesterol (34.3%), elevated triglycerides or treatment (28.6%) and glucose (15.9%).

Crude & adjusted cross-sectional associations between GGT, demographic, lifestyle & metabolic risk factors

In unadjusted analyses, positive trends were evident between increasing GGT activity and numerous demographic and lifestyle variables, including riskier profiles in each traditional risk category, except physical activity (Table 1). In adjusted analyses (Table 2), the positive associations remained strong between GGT activity and increased FBG, FI, HbA_{1c} and HOMA–IR for the entire cohort, despite adjustment for demographic, lifestyle and

traditional cardiovascular risk covariates. In *post hoc* tests, there was a strong interaction with BMI in estimating HOMA-IR from GGT ($p < 0.0001$). In each subgroup defined by metabolic status (normal, MetS and Type 2 diabetes), all associations remain strongly positive between individual risk factors and GGT activity in adjusted models through M3, except in the Type 2 diabetes subgroup in which associations with FBG and HbA_{1c} were attenuated.

There was no evidence of an interaction between ethnicity and continuous GGT in M3 for FBG, FI or HOMA-IR models. However, there was an interaction with race/ethnicity in estimating HbA_{1c} from GGT, in that Hispanics had a different association than the other race/ethnic groups ($p < 0.0001$; Wald: $p = 0.006$ and 0.19). A significant interaction was evident between continuous age and continuous GGT in FI and HOMA-IR models ($p = 0.004$ for both); however, the interaction was attenuated in M4_{HOMA} with adjustment for waist circumference. Continuous interaction with age in ethnic subgroups was apparent only in the white subgroup for FI and HOMA-IR models ($p < 0.05$ for both), although was similarly attenuated in M4_{HOMA} ($p = 0.12$). Although there were no significant interactions between continuous age and GGT for adults <65 years, there were significant interactions with age in estimating FI, HbA_{1c} and HOMA-IR models from GGT ($p = 0.001$ – 0.006), in that adults <65 years had different association than adults ≥ 65 years, which remained significant in M4_{HOMA} ($p = 0.008$). The negative β -coefficients on this interaction variable indicate the adjusted associations between GGT and metabolic risk factors generally weaken as age increases beyond 65 years.

Odds of prevalent metabolic disease by GGT quintile

Prevalent metabolic disease increased significantly across GGT quintiles in the entire cohort (Table 3). Strong associations were observed for the entire cohort and in ethnic subgroups for minimally adjusted models including age, gender and study site ($p < 0.0001$ – 0.01 for linear trends; data not shown). However, several associations were attenuated in models adding adjustment for lifestyle variables (i.e., alcohol use, smoking and exercise). Figure 1A & 1B provides graphical representations of the odds of MetS and Type 2 diabetes by GGT quintile in ethnic subgroups. In general, linear trends for increased disease by quintile remained significant, except in the Chinese subgroup in which trends were attenuated for all outcomes. The trend also became borderline for IFG in the black subgroup ($p = 0.05$) and for Type 2 diabetes in the white subgroup ($p = 0.10$).

The significance of the point estimates for increased odds of IFG, MetS and Type 2 diabetes was also greatly attenuated with adjustment for lifestyle variables, especially in the Chinese subgroup, which demonstrated no significant increases in prevalent disease by quintile. For prevalent IFG, the odds of disease were also attenuated in the white, black and Hispanic subgroups in all but the fifth quintile for the white and Hispanic subgroups. In all ethnic subgroups except the Chinese, the odds of MetS increased with GGT quintile, generally reached statistical significance in the third quintile. In general, point estimates for increased odds of Type 2 diabetes increased with GGT quintile, becoming statistically significant only in the fifth quintile, except for Hispanic cases, who reached significance in the third quintile. No interaction between GGT and ethnicity was evident for metabolic disease outcomes in

models adjusted for age, gender, study site and lifestyle variables. Odds of diabetes, but not MetS, were consistently greater in for those aged <65 years compared with those aged ≥ 65 for each quintile, although linear trends remained strong in both subgroups for both outcomes (Figure 1C & 1D).

Discussion

GGT activity is strongly associated with individual and composite cardiovascular and metabolic risk factors in the MESA cohort. The observed increases in prevalent metabolic diseases across GGT quintiles are highly consistent with our unadjusted analyses demonstrating positive associations between GGT and 'riskier' profiles for nearly every individual risk variable. The results of our multivariable analysis suggest the associations between GGT and individual metabolic risk factors, especially FI and HOMA-IR, are very stable and remain independent of standard clinical measures and lifestyle variables. Similar to previous reports, we measured a significant interaction between GGT, BMI and HOMA-IR, and weaker associations in adults >65 years of age [3,13]. As these associations are so consistent, strongly significant and relatively independent of ethnic group, we suggest total serum GGT activity as a continuous generalizable biomarker of composite metabolic risk in those adults without clinically evident vascular disease, with particular utility in people under the age of 65 years, even after considering differences in established demographic, behavioral and clinical risk factors.

Although several previous investigators have demonstrated strong associations between GGT activity and both cardiovascular and metabolic risk factors, our report is novel in several important ways. This is the first evaluation of GGT activity in MESA, a very well-executed cohort study with excellent data quality. Additionally, we have carefully evaluated both individual and composite risk factors, in one study, systematically controlling first for demographics in our models, followed by lifestyle variables, and then including traditional risk factors to determine the independence of any observed relationships. We also evaluated associations in four ethnic subgroups simultaneously in a cohort with sufficient power to do so. Finally, replication research is valuable, and in this case, we clearly confirm previously reported relationships between GGT and individual cardiovascular and metabolic risk factors, while also reporting strong, independent, residual association with composite risk.

This investigation is also strengthened by the fact that the MESA cohort includes an ethnically diverse sample of adults in the USA, and contains rich data on potential confounders. This combination allowed us to differentiate the prevalence of metabolic disease between ethnic subgroups, and detect potential meaningful ethnic differences. To our knowledge, our research provides evidence of GGT activity-associated metabolic risk in the largest known sample of Hispanics that has been reported. MESA also allowed for well-powered tests for interaction between GGT and ethnicity, which we did not detect upon adjustment for lifestyle covariates. The apparent differences in associations between GGT and odds of metabolic disease within ethnic subgroups deserve future investigation, although residual confounding due to lifestyle differences remains likely.

Notably, the values of GGT activity reported here are higher than have been reported in other cohorts. Although this difference may be due to methodological variability, that is, using a research assay compared with a clinical assay, and differences in assay temperature, our plate assay was validated by two separate clinical laboratories. Rather than assay methodology, we suggest that the primary differences in the GGT range measured for this cohort are attributable to the greater than average non-white representation in the MESA cohort, combined with the high prevalence of metabolic risk. Table 1 demonstrates the association between black and Hispanic ethnicity and GGT activity, which account for 28 and 22% of the cohort respectively, that is, 50% of the total cohort. Regarding the prevalence of metabolic risk factors, increased waist circumference was highly prevalent in participants (i.e., 62.9% per NHLBI and 62.2% per 2009 Joint definition), as was elevated blood pressure 135/85 or treatment (51.0%), low HDL cholesterol (34.3%), elevated triglycerides or treatment (28.6%), and elevated glucose (15.9%). Combined, we suggest these differences resulted in a higher than expected mean GGT activity than would be expected in a 'healthy' cohort.

Methods for measuring subfractions of total GGT activity (i.e., s-GGT, m-GGT, b-GGT and f-GGT) have been reported by Franzini *et al.* [24], and disease-specific patterns of GGT subfractions and ratios of subtypes are emerging, for example, increased b-GGT has recently been associated with cardiovascular risk [27], whereas s-GGT was increased in alcoholics [39]. Our research is limited by not having this GGT subfraction data, which should be applied in future studies to better differentiate subgroups within larger cohorts by disease status, including those with isolated hepatic disease and those with multiorgan disease, that is, concomitant nonalcoholic fatty liver disease, metabolic disease and cardiovascular disease. This innovative method of GGT subfractionation will also be critical in future determinations of the organ-specific mechanisms responsible for observed increases in serum GGT activity. Despite the potential to improve upon our methods by subfractionating GGT, the associations demonstrated here with total serum GGT activity remain valid. Other studies have demonstrated that participants with increased total serum GGT activity have increased GGT subfractions more associated with disease (i.e., non-f-GGT fractions), total serum GGT activity increased with increases in each subfraction (i.e., except f-GGT) and total serum GGT activity remains the most accessible biomarker to clinicians [26,27,39].

Although we accounted for many confounders, including smoking and alcohol intake, previous research has also identified associations between GGT and exposures unaccounted for in this study, including environmental pollutants [40] and dietary factors [41]. Our findings are also limited due to their cross-sectional design. Future research to evaluate associations between GGT activity, dietary factors and incident disease is planned in the MESA cohort. Our study is also limited by not having ALT data available to evaluate whether similar trends exist between ALT and the metabolic risk factors evaluated in our research, and to potentially adjust for other causes of liver disease. However, previous reports do not support the hypothesis that ALT is associated with cardiovascular outcomes in the same manner as GGT activity, as demonstrated in a recent meta-analysis by Fraser *et al.* [11]. Fraser *et al.* have also demonstrated GGT as a better predictor of incident diabetes, compared with ALT [7]. Similarly, analyses by Lim *et al.* do not demonstrate associations

between ALT and dietary antioxidant intake, as have been demonstrated with GGT activity [42]. These findings suggest differential causes of ALT and GGT activity elevation; however, we are unable to confirm this hypothesis in the present study.

Given the importance of redox balance to cellular metabolism in all tissues, it is conceivable that total GGT activity is a biomarker of multiorgan GSH depletion, whereas increases in tissue-specific GGT activity subfractions suggesting chronic tissue-specific GSH depletion. Extending this research may even result in the ability to differentiate isolated liver, kidney and vascular disease, as well as chronic diseases with multiple affected organs (i.e., Type 2 diabetes). If this differentiation is confirmed by additional experimental research, it would fill an important research gap in population-based cohort studies [43].

Conclusion

The data presented in this study, combined with the accumulation of past evidence, support total serum GGT activity as a significant biomarker of cardiovascular and metabolic risk, including strong associations with established individual risk factors of vascular disease, plus composite risk conditions including MetS and Type 2 diabetes. The established role of GGT in GSH homeostasis supports the need for translational investigations of the behavioral and environmental causes of increased GGT activity in humans, including triggers, regulators and more precise understanding of its relationship to GSH status in single- and multi-organ pathology.

Future perspective

Despite decades of basic science research supporting an oxidative stress-based theory of vascular disease and aging, experimental research on ‘antioxidant’ therapies has been disappointing. A contributor to these disappointing findings is a lack of valid biomarkers to determine an individual’s current burden of oxidative stress, and thus to identify individuals who may respond to targeted antioxidant therapies and/or behavioral approaches to reduce exposure to increased oxidative stress; for example, dietary change. In the future, GGT may be validated as a biomarker of oxidative stress relevant in cardiovascular and metabolic disease. GGT is a prime candidate biomarker for this role due to its parallel functions in recycling GSH to meet intracellular antioxidant needs, as well as for hepatic conjugation reactions of xenobiotics, including lipid peroxides and persistent organic pollutants. Relative increases of GGT activity, within a range now considered clinically normal, might be used to identify people who are at risk for metabolic disease and who are candidates for antioxidant therapy, dietary changes and/or priority individuals for reducing medications and environmental pollutants that require GSH for conjugation. Further fractionation of GGT will help identify tissue sites of injury, and will improve clinicians’ ability to differentiate multiorgan pathology from site-organ pathology. Our current research results clearly demonstrate the validity of total serum GGT activity as a biomarker for increased metabolic risk, even within values now considered clinically normal. Future research will identify the specific contributors to total and tissue-specific increases GGT activity, including the relative contributions of exogenous oxidants versus endogenous redox dysregulation. Additional research is needed to determine priority interventions for GGT lowering. In the

future, GGT lowering will be considered a clinical goal, similar to LDL lowering, and subsequent lowering of GGT will reduce the risk for incident cardiovascular and metabolic diseases, and adverse cardiovascular outcomes.

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▪▪ of considerable interest

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Executive summary

GGT activity & its association with riskier cardiovascular risk profiles

- Cross-sectional associations demonstrate strong, positive relationships between GGT activity and male gender, higher risk ethnic subgroups, age, smoking history, waist circumference, low-density lipoprotein, triglyceride level, blood pressure and use of medications for diabetes, hypertension and cholesterol. Negative associations were evident for high-density lipoprotein level.

GGT activity & its associations with metabolic risk factors after adjustment for demographics, lifestyle factors & traditional risk factors

- Multivariable linear regression models demonstrated positive and significant cross-sectional associations between GGT activity and fasting blood glucose (FBG), fasting insulin, HbA_{1c} and the homeostasis model assessment of insulin resistance (HOMA-IR).
- Linear regression models adjusting for age, gender, study site, ethnicity, alcohol intake, smoking, physical activity, medications and traditional cardiovascular risk factors remained significant for FBG, insulin, HbA_{1c} and HOMA-IR.
- Associations with FBG, insulin, HbA_{1c} and HOMA-IR were significant in participants without metabolic disease, as well as in those with prediabetes and metabolic syndrome.
- Associations with insulin and HOMA-IR remained significant in those participants with Type 2 diabetes.

GGT activity & odds of prevalent metabolic syndrome & Type 2 diabetes

- Odds of prevalent metabolic syndrome and Type 2 diabetes increased significantly with increasing quintiles of GGT activity.

GGT activity & its relationship to ethnicity & age

- Significant trends for increased prevalent metabolic disease were evident in all ethnic groups (i.e., white, black and Hispanic), except the Chinese.
- An age greater than 65 years reduced the strength of the associations between GGT activity and disease risk.

Conclusion

- Total serum GGT activity is strongly and positively associated with increased composite metabolic risk in this large cohort of mixed ethnicity adults without symptomatic cardiovascular disease.
- Future observational and clinical translational research is necessary to determine the exact relationship between total serum GGT activity and the

availability of reduced glutathione and other antioxidants to further validate GGT as a biomarker of oxidative stress.

- Future clinical translational research is necessary to determine the exact culprits for increasing GGT activity *in vivo*, as well as environmental, behavioral and medical approaches to reduce oxidative stress.

Figure 1. Odd ratios of metabolic disease by GGT quintile in subgroups defined by ethnicity and age

(A) Demonstrates odds of metabolic syndrome by GGT activity quintile, in ethnic subgroups and in the entire cohort; (B) demonstrates odds of Type 2 diabetes by GGT activity quintile, in ethnic subgroups and in the entire cohort; (C) demonstrates odds of metabolic syndrome by GGT activity quintile, in subgroups defined by age <65 or ≥ 65 year and in the entire cohort; (D) demonstrates odds of Type 2 diabetes by GGT activity quintile, in subgroups defined by age <65 or ≥ 65 years and in the entire cohort.

Table 1

Trends between GGT and demographic, lifestyle and clinical risk variables.

Baseline characteristics (n = 6446)	GGT-Q1 (n = 1289)	GGT-Q2 (n = 1286)	GGT-Q3 (n = 1292)	GGT-Q4 (n = 1289)	GGT-Q5 (n = 1290)	p-value for trend
Age (years), mean (SD)	62.4 (10.9)	63.5 (10.3)	62.8 (10.0)	62.1 (10.0)	61.0 (9.7)	<0.0001
Age <65 years, n (%)	699 (54)	647 (50)	686 (53)	726 (56)	804 (62)	Referent
Age ≥65 years, n (%)	590 (46)	639 (50)	606 (47)	563 (47)	486 (38)	<0.0001
Male/female, n (%)	325 (25)/964 (75)	523 (41)/763 (59)	635 (49)/657 (51)	724 (56)/565 (44)	840 (65)/450 (35)	<0.0001
Ethnicity:						
White, n (%)	665 (52)	543 (42)	465 (36)	435 (36)	408 (32)	Referent
Chinese, n (%)	194 (15)	173 (13)	164 (13)	123 (13)	119 (9)	0.66
Black, n (%)	226 (18)	308 (24)	389 (30)	430 (33)	395 (31)	<0.0001
Hispanic, n (%)	204 (16)	262 (20)	274 (20)	301 (23)	368 (29)	<0.0001
Smoking status:						
Never, n (%)	746 (58)	663 (52)	695 (54)	615 (48)	533 (41)	Referent
Past, n (%)	432 (34)	483 (38)	435 (34)	498 (39)	525 (41)	<0.0001
Current, n (%)	105 (8)	135 (11)	159 (12)	172 (13)	228 (18)	<0.0001
Pack-years (current or past smokers, excludes never), mean (SD)	21.1 (23.2)	21.6 (21.6)	24.8 (27.1)	23.4 (25.5)	24.5 (33.7)	0.02
Current alcohol, mean (SD)	699 (71)	669 (68)	672 (66)	719 (66)	782 (71)	0.34
Drinks/week, mean (SD)	2.4 (3.7)	3.5 (5.1)	3.5 (5.1)	4.4 (6.8)	5.9 (8.0)	<0.0001
Total intentional exercise (MET-min/week), mean (SD)	1499 (2131)	1529 (2286)	1548 (2196)	1558 (2580)	1609 (2841)	0.75
Glucose (mg/dl), median (IQR) [†]	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), median (IQR) [†]	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
HbA _{1c} (%), median (IQR) [†]	5.4 (5.1–5.6)	5.5 (5.2–5.8)	5.5 (5.2–5.9)	5.6 (5.3–6.0)	5.6 (5.3–6.1)	<0.0001
HOMA-IR, median (IQR) [†]	0.8 (0.6–1.2)	1.0 (0.7–1.6)	1.3 (0.8–2.1)	1.4 (0.9–2.3)	1.6 (1.0–2.7)	<0.0001

Baseline characteristics (n = 6446)	GGT-Q1 (n = 1289)	GGT-Q2 (n = 1286)	GGT-Q3 (n = 1292)	GGT-Q4 (n = 1289)	GGT-Q5 (n = 1290)	p-value for trend
Waist circumference (cm), mean (SD)	91.9 (14.3)	96.2 (14.3)	99.0 (14.0)	101.1 (13.8)	101.4 (13.4)	<0.0001
Diabetes medications:						
None, mean (SD)	1218 (94)	1184 (92)	1159 (89)	1150 (89)	1124 (87)	Referent
Insulin, mean (SD)	11 (1)	19 (2)	20 (2)	21 (2)	33 (3)	0.001
Oral, mean (SD)	60 (5)	83 (6)	113 (9)	118 (9)	133 (10)	<0.0001
Total cholesterol (mg/dl), mean (SD)	192.0 (32.6)	192.8 (35.7)	193.5 (35.3)	195.3 (34.8)	196.8 (37.6)	<0.0001
LDL cholesterol (mg/dl), mean (SD)	114.1 (29.4)	116.6 (31.1)	118.3 (31.0)	119.6 (31.9)	118.7 (32.5)	<0.0001
HDL cholesterol (mg/dl), mean (SD)	56.7 (15.3)	52.4 (15.5)	49.4 (14.1)	48.4 (13.3)	48.0 (13.8)	<0.0001
Triglycerides, median (IQR) [†]	94.0 (68–127)	104.0 (74–146)	111.0 (78–158)	119.0 (83–174)	132.0 (89–191)	<0.0001
Lipid medications, mean (SD)	171 (13)	210 (16)	216 (17)	234 (18)	218 (17)	0.03
SBP (mmHg), mean (SD)	121.9 (21.6)	125.7 (22.0)	127.7 (21.7)	128.1 (21.0)	129.2 (20.5)	<0.0001
DBP (mmHg), mean (SD)	68.3 (10.0)	70.4 (9.9)	72.4 (10.1)	74.4 (10.1)	73.9 (10.2)	<0.0001
Hypertension (140/90 or treatment), mean (SD)	466 (36)	531 (41)	591 (46)	642 (50)	650 (50)	<0.0001

Q1: <4.5 U/l; Q2: 24.5–29.3 U/l; Q3: 29.3–35.1 U/l; Q4: 35.1–45.2 U/l; Q5: 45.2–99.7 U/l.

[†] Skewed variable.

DBP: Diastolic blood pressure; HOMA-IR: Homeostasis Model Assessment Index of Insulin Resistance; IQR: Interquartile range; Q: Quintile; SBP: Systolic blood pressure; SD: Standard deviation.

Table 2

Adjusted associations between GGT and metabolic risk factors, overall and in metabolic disease subgroups.

Metabolic subgroup risk variable	Entire (n = 6446)	Normal (n = 3783)	MetS (n = 1935)	Diabetes (n = 852)
	$\beta_{Adj.} \times \ln(GGT)$ (95%CI)	$\beta_{Adj.} \times \ln(GGT)$ (95%CI)	$\beta_{Adj.} \times \ln(GGT)$ (95%CI)	$\beta_{Adj.} \times \ln(GGT)$ (95%CI)
	p-value	p-value	p-value	p-value
FBG (mg/dl)				
M1	0.11 (0.10–0.13)	<0.0001	0.03 (0.026–0.04)	<0.0001
M2	0.11 (0.10–0.13)	<0.0001	0.03 (0.02–0.04)	<0.0001
M3	0.07 (0.05–0.09)	<0.0001	0.03 (0.02–0.04)	<0.0001
FI (μU/l)				
M1	0.53 (0.49–0.57)	<0.0001	0.39 (0.34–0.44)	<0.0001
M2	0.55 (0.50–0.60)	<0.0001	0.40 (0.38–0.47)	<0.0001
M3	0.39 (0.34–0.44)	<0.0001	0.33 (0.27–0.39)	<0.0001
HbA_{1c} (%)				
M1	0.06 (0.05–0.07)	<0.0001	0.02 (0.008–0.02)	<0.0001
M2	0.06 (0.05–0.08)	<0.0001	0.02 (0.008–0.02)	<0.0001
M3	0.04 (0.03–0.05)	<0.0001	0.01 (0.004–0.02)	<0.0001
HOMA-IR				
M1	0.64 (0.60–0.69)	<0.0001	0.43 (0.37–0.48)	<0.0001
M2	0.66 (0.60–0.72)	<0.0001	0.44 (0.37–0.51)	<0.0001
M3	0.47 (0.41–0.52)	<0.0001	0.36 (0.29–0.43)	<0.0001
M4 _{HOMA}	0.37 (0.32–0.42)	<0.0001	0.28 (0.22–0.34)	<0.0001

FBG, FI, HbA_{1c}, HOMA-IR and GGT are log transformed. All models exclude insulin users. Regression coefficient units for FBG, FI, HbA_{1c} and HOMA-IR are 10 mg glucose/unit GGT activity, mU insulin/unit GGT activity, %HbA_{1c}/unit GGT activity and 1/unit GGT activity, respectively. M1 adjusts for age, gender, ethnicity and study site. M2 represents M1 plus alcohol use (current/former/never and drinks/week) plus exercise (MET-min/week) plus smoking (current/former/never and pack-years). M3 represents M2 plus lipids (low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides), lipid-lowering medications, systolic and diastolic blood pressures, antihypertensive medications, diabetes medications, and family history of heart attack or stroke. M4_{HOMA} represents M3 plus waist circumference.

$\beta_{Adj.}$: Regression coefficient for GGT in adjusted models; FBG: Fasting blood glucose; FI: Fasting insulin; HOMA-IR: Homeostasis Model Assessment Index of Insulin Resistance; MetS: Metabolic syndrome.

Table 3

Adjusted odds ratios of metabolic disease by GGT quintile in the entire MESA cohort and stratified by ethnic groups.

Metabolic subgroup	GGT-Q1, prevalence	GGT-Q2, OR [†] (95% CI); p-value [‡] ; prevalence	GGT-Q3, OR [†] (95% CI); p-value [‡] ; prevalence	GGT-Q4, OR [†] (95% CI); p-value [‡] ; prevalence	GGT-Q5, OR [†] (95% CI); p-value [‡] ; prevalence	p-value	
						Trend	Interaction with ethnicity
Impaired fasting blood glucose							
All ethnicities (n = 872)	n = 90	1.05 (0.71–1.58); 0.79; n = 131	1.62 (1.11–2.37); 0.01; n = 192	2.09 (1.44–3.04); <0.0001; n = 229	2.40 (1.65–3.48); <0.0001; n = 230	<0.0001	–
White (n = 272)	n = 34	1.26 (0.71–2.24); 0.43; n = 39	1.83 (1.04–3.21); 0.04; n = 60	2.76 (1.61–4.74); <0.0001; n = 70	2.68 (1.54–4.66); 0.001; n = 69	<0.0001	Referent
Chinese (n = 130)	n = 22	0.65 (0.20–2.16); 0.48; n = 22	1.31 (0.48–3.86); 0.62; n = 32	1.16 (0.38–3.55); 0.79; n = 24	1.18 (0.39–3.61); 0.77; n = 30	0.52	0.84
Black (n = 248)	n = 17	1.58 (0.66–3.78); 0.31; n = 41	1.45 (0.62–3.40); 0.39; n = 56	2.15 (0.96–4.84); 0.06; n = 79	1.76 (0.75–4.16); 0.20; n = 55	0.15	0.72
Hispanic (n = 222)	n = 17	0.55 (0.20–1.52); 0.25; n = 29	1.65 (0.71–3.85); 0.25; n = 44	1.60 (0.68–3.78); 0.28; n = 56	3.27 (1.43–7.43); 0.005; n = 76	<0.0001	0.45
Metabolic syndrome							
All ethnicities (n = 1151)	n = 154	1.47 (1.09–1.99); 0.01; n = 210	1.97 (1.47–2.65); <0.0001; n = 246	2.46 (1.83–3.30); <0.0001; n = 256	3.31 (2.46–4.46); <0.0001; n = 285	<0.0001	–
White (n = 466)	n = 76	1.53 (1.03–2.29); 0.04; n = 97	1.92 (1.27–2.91); 0.002; n = 97	2.45 (1.63–3.70); <0.0001; n = 90	3.39 (2.24–5.14); <0.0001; n = 106	<0.0001	Referent
Chinese (n = 127)	n = 22	0.44 (0.12–1.56); 0.21; n = 25	1.64 (0.52–5.17); 0.40; n = 32	1.27 (0.39–4.19); 0.69; n = 26	1.08 (0.30–3.87); 0.90; n = 22	0.41	0.34
Black (n = 229)	n = 24	1.57 (0.76–3.26); 0.22; n = 29	2.30 (1.16–4.56); 0.017; n = 56	2.92 (1.50–5.69); 0.002; n = 70	2.97 (1.46–6.03); 0.003; n = 50	<0.0001	0.83
Hispanic (n = 329)	n = 32	1.75 (0.88–3.49); 0.11; n = 59	2.38 (1.23–4.63); 0.01; n = 61	2.99 (1.53–5.85); 0.001; n = 70	5.19 (2.68–10.03); <0.0001; n = 107	<0.0001	0.30
Type 2 diabetes							
All ethnicities (n = 781)	n = 73	1.14 (0.70–1.86); 0.61; n = 120	1.42 (0.86–1.86); 0.15; n = 168	1.71 (1.09–2.70); 0.02; n = 182	2.81 (1.80–4.39); <0.0001; n = 238	<0.0001	–
White (n = 158)	n = 22	0.93 (0.46–1.92); 0.84; n = 26	0.81 (0.37–1.77); 0.60; n = 28	1.16 (0.57–2.38); 0.68; n = 35	1.62 (0.80–3.25); 0.18; n = 38	0.10	Referent
Chinese (n = 105)	n = 17	0.69 (0.17–2.78); 0.60; n = 23	0.69 (0.18–2.61); 0.59; n = 17	0.67 (0.18–2.49); 0.55; n = 17	0.97 (0.28–3.34); 0.96; n = 22	0.96	0.46
Black (n = 330)	n = 15	1.83 (0.75–4.44); 0.18; n = 45	2.01 (0.85–4.71); 0.11; n = 74	1.67 (0.72–3.91); 0.23; n = 65	4.44 (1.93–10.21); <0.0001; n = 104	<0.0001	0.21
Hispanic (n = 259)	n = 19	1.38 (0.24–7.90); 0.72; n = 26	4.96 (1.09–22.6); 0.04; n = 49	8.93 (2.02–39.38); 0.004; n = 65	8.28 (1.87–36.54); 0.005; n = 74	<0.0001	0.09

Q1: <24.5 U/l (referent group); Q2: 24.5–29.3 U/l; Q3: 29.3–35.1 U/l; Q4: 35.1–45.2 U/l; Q5: 45.2–99.7 U/l.

[†] Adjusted for age, gender, waist circumference, study site, alcohol, smoking and exercise, plus ethnicity in the entire cohort.

[‡] p-value corresponds to the point estimate for odds of disease within each quintile.
OR: Odds ratio; Q: Quintile.