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Serum γ-Glutamyltransferase Was Differently Associated with Microalbuminuria by Status of Hypertension or Diabetes: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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Background: **We hypothesized that serum γ-glutamyltransferase (GGT) would positively predict the risk of microalbuminuria, a frequent consequence of both diabetes and hypertension, because serum GGT predicted diabetes and hypertension in dose–response relationships.**

Methods: **In this prospective study, 2478 black and white men and women without microalbuminuria at year 10 provided urine samples 5 years later. Year 10 GGT cutpoints were 12, 18, and 29 U/L.**

Results: **The incidence of microalbuminuria across year 10 GGT categories was U-shaped. Adjusted odds ratios across quartiles of serum GGT were 1.0, 0.39, 0.54, and 0.94 (P <0.01 for quadratic term), but the shape of association depended on the status of hypertension or diabetes (P <0.01 for interaction). Among individuals who ever had hypertension or diabetes, year 10 serum GGT showed a clear positive dose–response association with incident microalbuminuria (P <0.01 for trend), whereas among individuals with neither hypertension nor diabetes during the study, year 10 GGT showed a U-shaped association with it (P = 0.01 for quadratic term). When the long-term risk was evaluated in 3895 participants based on serum GGT at year 0 and prevalence of microalbuminuria at year 10 or year 15, the trends were similar but weaker than those of short-term incidence risk.**

Conclusions: **Serum GGT within the physiologic range predicted microalbuminuria among patients with hypertension or diabetes and may act as a predictor of microvascular and/or renal complications in these vulnerable groups. GGT showed a U-shaped association with microalbuminuria among persons who did not develop either hypertension or diabetes.**

Serum γ-glutamyltransferase (GGT)[5] concentrations within the physiologic range have been strongly associated with most cardiovascular disease risk factors and predicted the development of heart disease, hypertension, stroke, and type 2 diabetes *(1–6)*. In particular, serum GGT concentrations have shown a strong graded relationship with incident diabetes, suggesting a role in the pathogenesis of diabetes *(2, 3)*. Although serum GGT activity has commonly been used as a marker for excessive alcohol consumption or liver diseases *(7)*, neither alcohol consumption nor liver dysfunction likely explain the association between serum GGT and diabetes *(2, 3)*. A series of Coronary Artery Risk Development in Young Adults (CARDIA) studies *(3, 8, 9)* suggested that oxidative stress might explain these associations because serum GGT within the physiologic range had dose–response relationships with serum and/or dietary antioxidant vitamins and markers of oxidative stress such as F_2-isoprostanes. Although the relationship between cellular GGT and serum GGT is not known, cellular GGT has been

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[5] Nonstandard abbreviations: GGT, γ-glutamyltransferase; CARDIA, Coronary Artery Risk Development in Young Adults; OR, odds ratio; and GSH, glutathione.

known to play an important role in antioxidant defense systems *(10–12)*; paradoxically, cellular GGT may also be involved in the generation of reactive oxygen species in the presence of transition metals *(13–16)*. Recently, a role of serum GGT as an early and sensitive marker of oxidative stress was reviewed *(17)*.

Microalbuminuria, slightly increased albumin excretion in the urine, is now considered to be a predictor of atherosclerotic diseases *(18, 19)*. Recent evidence strongly suggested that microalbuminuria is an independent predictor of cardiovascular disease in diabetic or hypertensive patients, in elderly patients, and in the general population *(18, 19)*. The mechanisms linking microalbuminuria and risk for cardiovascular disease are not fully understood; a recent concept is that microalbuminuria is a marker of endothelial dysfunction *(18, 19)*. Generalized endothelial dysfunction has been hypothesized to be the underlying factor for microalbuminuria on the one hand and the underlying factor for increased cardiovascular risk on the other. Accumulating evidence suggests that oxidative stress alters many functions of the endothelium, including modulation of vasomotor tone *(20)*.

We therefore performed a prospective study to examine whether GGT, possibly as a marker of oxidative stress or a generator of oxidative stress itself, is a predictor of microalbuminuria among young adult black and white men and women.

Materials and Methods

STUDY POPULATION

CARDIA is a longitudinal, multicenter epidemiologic study of the impact of lifestyle and other factors on evolution of coronary heart disease risk factors during young adulthood. The study design, recruitment of participants, and methods have been described elsewhere *(21)*. In 1985–1986, at total of 5115 black and white men and women 18–35 years of age were recruited and examined at 4 clinical sites in the United States: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants were reexamined at 2, 5, 7, 10, and 15 years after baseline, with reexamination rates among surviving cohort members of 91%, 86%, 81%, 79%, and 74%, respectively.

In this study, we examined (*a*) the association between year 0 GGT and microalbuminuria at year 10 or year 15 and (*b*) the association between year 10 GGT and microalbuminuria at year 15. For this study, a total of 139 persons were excluded because they were pregnant at year 10 or year 15, had any kidney disease at year 10 or year 15, or had macroalbuminuria ($A/kC \geq 250 mg/g$; see below for definition of A/kC) at year 10 or year 15. For the analysis of year 0 GGT in relation to year 10 or 15 microalbuminuria, we excluded 61 study participants in whom GGT was not measured at year 0 and 1038 who dropped out before year 10 or who did not provide a urine sample at both year 10 and 15, leaving 3895 participants. For the analysis of year 10 GGT in relation to year 15 microalbuminuria, among 3817 participants who attended a year 10 examination, we excluded 75 in whom GGT was not measured at year 10, 1116 who did not return for a year 15 follow-up examination or who did not provide a urine sample at either year 10 or 15 measurements, and 263 who had prevalent microalbuminuria at year 10, leaving 2478 participants for analysis. In both sets of analyses, some individuals satisfied more than 1 exclusion criterion.

QUESTIONNAIRES

Standard questionnaires were used to maintain consistency in the assessment of demographic and behavioral information across CARDIA examination visits. Sex, race, date of birth, weekly alcohol consumption, and cigarette smoking were determined by structured interview or by self-administered questionnaire. A physical activity score was derived from the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire *(22)*. Alcohol intake (mL/day) was computed from the self-reported frequency of beer, wine, and liquor consumed per week.

CLINICAL MEASUREMENTS

All participants were asked to fast at least 12 h and to avoid smoking and heavy physical activity at least 2 h before the examination. After a 5-min rest, blood pressure was measured on the right arm in the sitting position. First- and fifth-phase Korotkoff sounds were recorded 3 times at 1-min intervals by use of a random zero sphygmomanometer (WA Baum Company). The mean of the second and third measurements was used in the analyses. Blood was then collected, with minimal stasis, for GGT and glucose. After plasma or serum separation, aliquots were stored at −70 °C until shipped on dry ice to a central laboratory.

The methods for measuring serum GGT were not comparable between year 0 and year 10. At year 0, liver-related enzymes, including GGT, were measured with a SMAC 12 continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically by a nitroanilide methodology at Linco Research Inc. Therefore, to identify an appropriate recalibration formula, GGT was remeasured at Linco Research Inc. with the year 10 methodology in 103 baseline samples with original GGT values ranging from 3 to 228 U/L that had been stored at −70 °C for 17 years (since 1985–1986). The correlation between measurements made at year 0 and those measured with year 10 methodology was 0.995; accordingly, the year 0 values reported here are 2.7618 plus 1.9004 times the original year 0 values. Year 0 and year 10 glucose was measured by the hexokinase-ultraviolet method at Linco, Inc. Year 0 and year 10 lipids were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Total triglycerides and total HDL-cholesterol were measured by enzymatic

methods. HDL-cholesterol was measured after dextran sulfate–magnesium precipitation. LDL-cholesterol was calculated by use of the Friedewald equation. Body weight with light clothing was measured to the nearest 0.09 kg (0.2 pounds), and body height without shoes was measured to the nearest 0.5 cm. Body mass index was computed as weight divided by height squared (kg/m^2).

measurement of urine albumin and creatinine

A single, untimed (spot) urine sample was collected at the year 10 examination when convenient during the clinic visit, usually shortly after arrival at the clinic. Albumin (A) and creatinine (C) were measured, and the term A/*k*C adjusted for race and gender bias, which was reported previously *(23)*. Urinary creatinine concentration (mg/dL) in men was multiplied by $k = 0.68$ *(23, 24)* and in blacks by $k = 0.88$; the constant in black men is therefore 0.68×0.88 *(23, 25)*. Applying these adjustments allowed the use of 25 mg/g A/*k*C as the cutpoint for microalbuminuria in each of the 4 race–gender groups of CARDIA *(23)*.

statistical analysis

Year 0 serum GGT concentrations were first classified into 4 groups based on cutpoints of 12, 18, and 26 U/L (the 25th, 50th, and 75th percentiles computed over the entire sample) for study of GGT as a predictor of microalbuminuria 10 or 15 years later. Because there was no earlier measure of microalbuminuria, we could not be sure that all cases were incident; we will therefore use the term "prevalent microalbuminuria" for this analysis. Logistic regression models were used to calculate multivariate-adjusted odd ratios (ORs). Covariates in the minimally adjusted model were the values of study center, sex, race, and baseline age. The second model added baseline body mass index, alcohol consumption, cigarette smoking, physical exercise, and education. The third model added LDL-cholesterol, HDL-cholesterol, and triglycerides. Given the strong possibility that diabetes and hypertension are intermediate in the causal pathway between GGT and microalbuminuria *(26)*, we examined associations after stratification by status of diabetes or hypertension (never experiencing diabetes or hypertension during follow-up compared with having diabetes or hypertension during follow-up). The definition of diabetes incidence was serum fasting glucose ≥1260 mg/L or taking diabetes medication, and the definition of hypertension was systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive medication. In tests for trend, the quartile number of the serum GGT value was treated as a continuous variable.

We next examined the shorter term risk of GGT measured at year 10 when participants were 28–40 years of age. Year 10 serum GGT cutpoints of 12, 18, and 29 U/L (the 25th, 50th, and 75th percentiles computed over the entire sample) and year 10 values of all covariates were used for study of association of year 10 GGT with year 15 incident microalbuminuria (omitting participants who had microalbuminuria at year 10).

Results

year 0 ggt and year 10 or 15 prevalent microalbuminuria

The risk of prevalent microalbuminuria was increased in the highest quartile of year 0 GGT (Table 1). After minimal adjustment for study center, race, sex, and age, the ORs of year 10 or year 15 prevalent microalbuminuria across quartiles of year 0 GGT were 1.0, 0.97, 1.02, and 1.82 (model 1; $P < 0.01$ for trend; $P < 0.01$ for quadratic term). After further adjustment for alcohol consumption, cigarette smoking, physical activity, education, body mass index, education, LDL-cholesterol, HDL-cholesterol, and triglycerides, the ORs were 1.0, 0.93, 0.92, and 1.44 ($P = 0.03$ for trend; $P = 0.01$ for quadratic term).

The shape of association, however, depended on the status of hypertension or diabetes ($P < 0.01$ for interaction). Among the 777 participants who ever had either hypertension or diabetes, the distribution of first diagnosis was 14.0% at year 0, 6.1% at year 2, 10.3% at year 5, 10.6% at year 7, 18.9% at year 10, and 40.2% at year 15. Thus, 59% of the diagnoses of hypertension or diabetes were seen at year 10 or later. Of the participants with

Table 1. Adjusted ORs for prevalent microalbuminuria at year 10 or year 15 by quartile of serum GGT at baseline (year 0) in the CARDIA Study: 1985–1986.[a]

	Quartile of GGT at year 0, U/L				*P* for trend[a]	*P* for quadratic term
	<12	12 to <18	18 to <26	≥26		
Cases/No. of participants	61/682	106/1196	115/1191	138/826		
Relative risk, %	8.9	8.9	9.7	16.7		
OR[c]						
Model 1	1.0	0.97 (0.69–1.35)	1.02 (0.73–1.43)	1.82 (1.28–2.57)	<0.01	<0.01
Model 2	1.0	0.93 (0.67–1.30)	0.95 (0.67–1.34)	1.59 (1.11–2.27)	<0.01	<0.01
Model 3	1.0	0.93 (0.66–1.30)	0.92 (0.65–1.30)	1.44 (1.00–2.08)	0.03	0.01

[a] Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

[b] *P* for trend was calculated in a model without a quadratic term.

[c] 95% confidence limits in parentheses.

hypertension, 43.9% took antihypertensive medication, and 21.4% of participants took antidiabetes medication. Among participants who ever had hypertension or diabetes, the association between year 0 GGT and year 10 or 15 prevalent microalbuminuria was positive in a dose–response pattern, even after full adjustment (Table 2); adjusted ORs across quartiles of serum GGT were 1.0, 1.08, 1.50, and 1.94 ($P = 0.02$ for trend). Separate analyses for participants with diabetes but no hypertension and those with hypertension but no diabetes had results very similar to those for participants with either diabetes or hypertension (data not shown). However, among participants with neither hypertension nor diabetes during the study, year 0 GGT showed a shallow, nonsignificant, U-shaped association; adjusted ORs across quartiles of serum GGT were 1.0, 0.94, 0.80, 1.05 ($P = 0.26$ for quadratic term).

year 10 GGT and year 15 incident microalbuminuria

Year 10 serum GGT showed a clear U-shaped association with year 15 incident microalbuminuria (Table 3; $P < 0.01$ for quadratic term in all models). The risk of incident microalbuminuria among participants in the second or third quartile of GGT was less than one-half of that in the lowest year 10 GGT quartile. Similar with year 0 serum GGT, the shape of association was different depending on the status of hypertension or diabetes ($P < 0.01$ for interaction). Among participants with neither hypertension nor diabetes during the study, year 10 GGT also showed a U-shaped association with year 15 incident microalbuminuria (Table 4). However, among participants who ever had hypertension or diabetes, the association between year 10 GGT and year 15 incident microalbuminuria was positive in a dose–response pattern. The short-term relative risk (between year 10 GGT and year 15 incident microalbuminuria) appeared to be stronger than the long-term relative risk (year 0 GGT and year 10 or 15 prevalent microalbuminuria) irrespective of the shape of association, linear or U-shaped.

Discussion

We performed this study with the hypothesis that serum GGT concentrations within the physiologic range positively predict future development of microalbuminuria because serum GGT has been strongly associated with most cardiovascular disease risk factors and predicted the development of heart disease, hypertension, stroke, and type 2 diabetes *(1–6)*. However, in general, the shape of association between serum GGT and microalbuminuria was closer to a U-shaped association than a linear positive association. This finding was much clearer in the association between year 10 GGT and year 15 incident microalbuminuria than in the association between year 0 GGT and year 10 or 15 prevalent microalbuminuria.

Interestingly, the shape of association was clearly different depending on the status of hypertension or diabetes. Consistent with our previous hypothesis, serum GGT was positively associated with microalbuminuria among participants who were ever diagnosed with hypertension or diabetes during the 15 years of study. Also similar to our finding predicting diabetes in a previous CARDIA study *(3)*, the 5-year short-term risk of serum GGT was stronger than the 10- to 15-year long-term risk of serum GGT. However, serum GGT showed a U-shaped association with microalbuminuria among participants with nei-

Table 2. Adjusted ORs for prevalent microalbuminuria at year 10 or year 15 by quartile of serum GGT at baseline (year 0) stratified by the status of hypertension or diabetes in CARDIA.[a]

	Quartile of GGT at year 0, U/L				*P* for trend[b]	*P* for quadratic term
	<12	12 to <18	18 to <26	≥26		
Neither hypertension nor diabetes						
Cases/No. of participants	52/610	81/1041	62/941	46/526		
Relative risk, %	8.5	7.8	6.6	8.8		
OR[c]						
Model 1	1.0	0.93 (0.64–1.34)	0.80 (0.53–1.19)	1.07 (0.68–1.68)	0.93	0.20
Model 2	1.0	0.93 (0.64–1.35)	0.80 (0.53–1.20)	1.09 (0.69–1.74)	0.98	0.19
Model 3	1.0	0.94 (0.65–1.37)	0.80 (0.53–1.20)	1.05 (0.66–1.68)	0.82	0.26
Either hypertension or diabetes						
Cases/No. of participants	9/72	25/155	53/250	92/300		
Relative risk, %	12.5	16.1	21.2	30.7		
OR						
Model 1	1.0	1.32 (0.58–3.00)	1.79 (0.83–3.88)	2.92 (1.36–6.28)	<0.01	0.55
Model 2	1.0	1.12 (0.49–2.59)	1.60 (0.83–3.88)	2.25 (1.02–4.94)	<0.01	0.70
Model 3	1.0	1.08 (0.47–2.50)	1.50 (0.68–3.29)	1.94 (0.87–4.31)	0.02	0.80

[a] Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline serum triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

[b] *P* for trend was calculated in a model without a quadratic term.

[c] 95% confidence limits in parentheses.

Table 3. Adjusted ORs for incident microalbuminuria at year 15 by quartile of GGT at year 10 in CARDIA.[a]

	Quartile of GGT at year 10, U/L				*P* for trend[b]	*P* for quadratic term
	<12	12 to <18	18 to <29	≥29		
Cases/No. of participants	29/489	18/694	28/684	46/611		
Relative risk, %	5.9	2.6	4.1	7.5		
OR[c]						
Model 1	1.0	0.44 (0.24–0.81)	0.68 (0.38–1.20)	1.36 (0.79–2.36)	0.06	<0.01
Model 2	1.0	0.39 (0.21–0.72)	0.54 (0.30–0.99)	0.95 (0.53–1.71)	0.51	<0.01
Model 3	1.0	0.39 (0.21–0.73)	0.54 (0.29–0.99)	0.94 (0.51–1.75)	0.60	<0.01

[a] Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL- cholesterol concentrations.

[b] *P* for trend was calculated in a model without a quadratic term.

[c] 95% confidence limits in parentheses.

ther hypertension nor diabetes during the study, especially in the 5-year short-term risk. These different findings by status of hypertension or diabetes may have arisen by chance, particularly given the small numbers of participants who were both in the lowest quartile of serum GGT and had either hypertension or diabetes, but this seems unlikely in light of the high degree of statistical significance for the positive trends seen among hypertensive or diabetic participants and for the U-shape for 5-year risk of incident microalbuminuria starting with year 10 GGT.

In this study, we used a single, untimed spot urine sample for measurement of albumin in urine. This may have led to some participants being misclassified; generally, nondifferential misclassification leads to a null association rather than a spurious association. If we had used a more rigorous design with urine samples collected over 24 h or collected in the first morning urine, we might have expected stronger associations.

We speculate that the current findings are interpretable from the perspective of the cellular role of GGT, which may connect serum GGT to oxidative stress. Although the relationship between cellular GGT and serum GGT is not known, experimental studies have shown that cellular GGT activity plays a role in maintaining intracellular glutathione (GSH) as an antioxidant defense mechanism *(10–12)*. Cellular GGT is widely distributed in the human body and is frequently localized to the plasma membrane with its active site directed into the extracellular space *(27)*. The highest activity was in the kidneys, where GGT was localized to the luminal surface of the proximal tubule cells; the distal tubules and glomeruli gave negative results. Although serum GGT is known as one of the liver enzymes, cellular GGT activity in homogenates of

Table 4. Adjusted ORs for incident microalbuminuria at year 15 by quartile of serum GGT at year 10 stratified by the status of hypertension or diabetes in CARDIA.[a]

	Quartile of GGT at year 10, U/L				*P* for trend[b]	*P* for quadratic term
	<12	12 to <18	18 to <29	≥29		
Neither hypertension nor diabetes						
Cases/No. of participants	28/462	14/585	12/533	14/420		
Relative risk, %	6.1	2.4	2.3	3.3		
OR[c]						
Model 1	1.0	0.43 (0.22–0.84)	0.42 (0.20–0.89)	0.70 (0.34–1.47)	0.20	<0.01
Model 2	1.0	0.41 (0.21–0.81)	0.40 (0.19–0.84)	0.60 (0.28–1.32)	0.12	0.01
Model 3	1.0	0.40 (0.21–0.80)	0.39 (0.18–0.83)	0.56 (0.25–1.27)	0.09	0.01
Either hypertension or diabetes						
Cases/No. of participants	1/27	4/109	16/151	32/191		
Relative risk, %	3.7	3.7	10.6	16.8		
OR						
Model 1		1.0[d]	2.88 (1.01–8.23)	5.24 (1.92–14.32)	<0.01	0.98
Model 2		1.0[d]	2.65 (0.88–7.95)	3.97 (1.38–11.43)	<0.01	0.46
Model 3		1.0[d]	2.66 (0.88–8.09)	4.38 (1.48–12.93)	<0.01	0.54

[a] Model 1: minimal adjustment for study center, race, sex, and age. Model 2: model 1 plus adjustment for alcohol consumption, cigarette smoking, physical activity, education, and body mass index. Model 3: model 2 plus adjustment for baseline triglyceride, LDL-cholesterol, and HDL-cholesterol concentrations.

[b] *P* for trend was calculated in a model without a quadratic term.

[c] 95% confidence limits in parentheses.

[d] First and second quartiles are combined because there was only 1 case in the first quartile.

liver was approximately one-fifth that in kidney. The primary role of GGT ectoactivity is to metabolize extracellular reduced GSH, allowing precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis; in this way, a continuous "GSH cycling" across the plasma membrane occurs in many cell types *(28)*. Thus, cellular GGT favors the intracellular supply of GSH, the most important nonprotein antioxidant of the cell, suggesting that increased serum GGT activity might eventually minimize oxidative stress and the consequent pathologic changes attributable to oxidative stress. Small increases in serum GGT might therefore reflect a successful defense response and, particularly in the short run (e.g., 5 years, second and third quartiles of year 10 GGT), might lead to less endothelial dysfunction and microalbuminuria than would have been the case if the GGT concentration had remained low (first quartile of year 10 GGT). However, larger increases in serum GGT suggest an environment in which there is more oxidative stress, in which the primary role of GGT in the antioxidant defense of cells might be overwhelmed, leading to a U-shaped association.

However, recent experimental studies *(13–16)* indicate that cellular GGT can also be involved in the generation of reactive oxygen species. This effect of cellular GGT occurs when it is produced in the presence of free iron or other transition metals. In vitro experimental studies have reported that free iron can be released from iron storage proteins such as ferritin by superoxide radicals or nitric oxide *(29–31)*. It is well known that substantial oxidative stress exists in diabetes *(32, 33)* and hypertension *(34, 35)*. Therefore, patients with diabetes or hypertension might have a potential to have free iron released from iron storage protein, and in this case, cellular GGT might act as a prooxidant.

In conclusion, this study showed that serum GGT within the physiologic range was differently associated with the risk of microalbuminuria depending on the status of diabetes or hypertension. We speculate that these complicated associations might be related to the dual roles of cellular GGT as antioxidant or prooxidant, depending on the presence of iron or other transition metals.

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