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Lipids, Lipoproteins, and Cardiovascular Risk Factors

γ-Glutamyltransferase Is a Predictor of Incident Diabetes and Hypertension: The Coronary Artery Risk Development in Young Adults (CARDIA) Study

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Background: **γ-Glutamyltransferase (GGT), which maintains cellular concentrations of glutathione, may be a marker of oxidative stress, and GGT itself may produce oxidative stress. We performed a prospective study to examine whether serum GGT predicts diabetes and hypertension.**

Methods: **Study participants were 4844 black and white men and women 18–30 years of age in 1985–1986; they were reexamined 2, 5, 7, 10, and 15 years later. Year 0 GGT cutpoints were 12, 17, 25, and 36 U/L (overall 25th, 50th, 75th, and 90th percentiles; the laboratory cutpoints for abnormal are 40 U/L in women and 50 U/L in men). We deleted 32 participants with prevalent diabetes and 140 participants with prevalent hypertension from the respective incidence analyses.**

Results: **After adjustment for study center, race, sex, and age in proportional hazards regression, the hazard ratios across year 0 GGT categories were 1.0, 1.6, 1.7, 4.0 (95% confidence interval, 2.0–8.1), and 5.5 (2.7–11.1) for 15-year incident diabetes and 1.0, 1.2, 1.7 (1.2–2.2), 2.3 (1.7–3.2), and 2.3 (1.7–3.2) for hypertension. Additional adjustment for year 0 alcohol consumption, body mass index, cigarette smoking, and physical activity attenuated this relationship, but GGT remained a significant predictor.**

Conclusions: **Serum GGT within a range regarded as physiologically normal is associated with incident diabetes and hypertension. Considering known functionality of GGT, these associations are consistent with a role for oxidative stress in risk for diabetes and hypertension.**

Serum γ-glutamyltransferase (GGT),[8] even within reference intervals, is associated with several cardiovascular disease risk factors and components of the insulin resistance syndrome *(1–3)*. In addition, in several prospective studies *(4–10)*, the baseline serum GGT concentration has been an independent risk factor for the development of cardiovascular or cerebrovascular diseases.

In our previous prospective studies in healthy Korean men *(10)*, serum GGT concentrations within the reference interval showed a strong dose–response relationship with incident diabetes. This strong relationship was observed even in nondrinkers and individuals without increased concentrations of any other liver enzymes. Therefore, although GGT has been widely used as a marker of alcohol consumption or liver disease *(11)*, neither alcohol nor hepatic dysfunction explained the observed relationships between GGT and diabetes. GGT is also a modest risk factor for hypertension *(9)*. Therefore, the mechanism underlying these observations is not fully understood. An

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[8] Nonstandard abbreviations: GGT, γ-glutamyltransferase; CARDIA, Coronary Artery Risk Development in Young Adults; AST, aspartate aminotransferase; CRP, C-reactive protein; BMI, body mass index; RR, relative risk; and 95% CI, 95% confidence interval.

interesting ancillary observation is that the well-known strong association of either obesity or age with diabetes was observed only among individuals with high-normal GGT at baseline *(10)*.

At present, experimental and epidemiologic studies of GGT are in an early stage; therefore, confirmation of recent findings in other population-based cohort studies is important. We performed a prospective study to examine whether GGT is a predictor of incident diabetes and hypertension among young adult black and white men and women, and to analyze whether the relationships of diabetes and hypertension with obesity or age were modified by baseline GGT concentration.

Materials and Methods

STUDY POPULATION

Coronary Artery Risk Development in Young Adults (CARDIA) is a longitudinal, multicenter epidemiologic study of the impact of lifestyle and other factors on the evolution of coronary heart disease risk factors during young adulthood. The study design, recruitment of participants, and methods have been described elsewhere *(12)*. In 1985–1986, 5115 black and white men and women 18–30 years of age were recruited and examined at four clinical sites in the US: Birmingham, AL Chicago, IL; Minneapolis, MN; and Oakland, CA. Participants were reexamined at 2, 5, 7, 10, and 15 years post-baseline, with reexamination rates among surviving cohort members of 90%, 86%, 81%, 79%, and 74%, respectively.

For this study, we excluded 65 study participants in whom GGT was not measured at year 0 and 212 who never returned for a follow-up examination, leaving 4844 participants for analysis. Participants who never returned had higher baseline GGT and were more likely to be black than those who returned. In addition, for analyses of diabetes, 32 participants who had type 1 or type 2 diabetes (defined below) at year 0 were excluded. For analyses of hypertension, 140 participants with hypertension (defined below) at year 0 were excluded. The final sample sizes entering proportional hazards life table regression analyses with the outcome of incident diabetes or incident hypertension were 4812 and 4704, respectively.

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 *(13)*. 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 three times at 1-min intervals, using 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, glucose, insulin, lipids, blood cell counts, uric acid, fibrinogen, C-reactive protein (CRP), and F2-isoprostanes. In this analysis, we used year 0 and year 10 GGT, year 0 glucose, year 0 insulin, year 0 lipids, year 0 blood cell counts, year 5 fibrinogen, year 15 uric acid, year 15 CRP, and year 15 F2-isoprostanes. After plasma or serum separation, aliquots were stored at −70 °C until shipped on dry ice to a central laboratory.

Serum GGT was measured at year 0 and year 10. At year 0, liver-related enzymes, including GGT and aspartate aminotransferase (AST) were measured with a SMA-CII continuous-flow analyzer (Technicon Instruments Corp.) at American Bio-science Laboratories (now Smith-Kline Beecham). At year 10, GGT was measured colorimetrically by the nitroanilide method on a Roche Cobas Mira Plus chemistry instrument at Linco Research Inc. (St. Louis, MO). The methodology for measuring GGT was not comparable between year 0 and year 10. To identify an appropriate recalibration formula, GGT was remeasured at Linco Research with the year 10 methodology in 103 baseline samples with original GGT values of 3–228 U/L that had been stored at −70 °C for 17 years (since 1985–1986). The correlation between measurements made in year 0 and those measured with the year 10 methodology was 0.995; accordingly, the year 0 values reported here are 2.7618 + 1.9004 times the original year 0 values.

Year 0 glucose was measured by the hexokinase-ultraviolet method. Year 0 fasting insulin was measured by RIA on sera frozen for 8 years from year 0. Year 0 lipids were measured by the University of Washington Northwest Lipid Research Clinic Laboratory. Total triglycerides and total HDL-cholesterol were measured by enzymatic procedures. HDL-cholesterol was measured after dextran sulfate–magnesium precipitation. LDL-cholesterol was calculated with use of the Friedewald equation. Year 0 complete blood cell count was performed at each local clinical center with a Coulter counter. Year 5 fibrinogen was measured by the Clauss method at Medlantic Research Foundation (Washington, DC). Year 15 uric acid was measured at Linco Research by the uricase method. CRP was measured at the Pathology Laboratory at the University of Vermont with high-sensitivity ELISAs. Year 15 F2-isoprostanes, a free-radical-dependent oxidative damage product of arachidonic acid metabolism, was measured at the Molecular Epidemiology and Biomarker Research Laboratory in the University of Minnesota by a gas chromatography–mass spectrometry-based method

(14). Body weight with light clothing was measured to the nearest 0.2 pounds, and body height without shoes was measured to the nearest 0.5 cm. Body mass index (BMI) was computed as weight divided by height squared (kg/m^2).

STATISTICAL ANALYSIS

We first examined the distribution and change in GGT, using the natural logarithmic transformation to account for skewness. Year 0 serum GGT concentrations were classified into five groups with use of cutpoints of 12, 17, 25, and 36 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) for study of GGT in relation to baseline and follow-up correlates, as well as GGT as a predictor of incidence of diabetes or hypertension during 15 years. The definition of diabetes incidence was serum fasting glucose ≥1260 mg/L or taking diabetes medication. Of 109 participants who ever reported taking antidiabetic medication during the study, insulin was the only drug in only 29 cases. Because individuals with type 1 diabetes always require insulin medication exclusively, most diabetic participants had type 2 diabetes. The definition of hypertension was systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive medication.

For calculation of incidence density, length of follow-up was calculated as time from the baseline exam to the exam at which disease outcome first occurred or the time of the last follow-up exam. Diabetes or hypertension was assumed to be present starting at the first examination at which it was diagnosed. Thus, "events" could occur after exactly 2, 5, 7, 10, or 15 years of follow-up. Before this diagnosis, the outcome was assumed to be absent, even if the earlier examination was missed. Participants were censored after their last examination. Cox proportional hazard models were used to calculate multivariate-adjusted hazard ratios in separate models for diabetes or hypertension. Covariates were the baseline values of study center, sex, race, age, BMI, alcohol consumption, cigarette smoking, and physical exercise. In some models, baseline values of systolic blood pressure, fasting plasma glucose, or insulin were included as additional covariates. In addition, we examined associations after stratification by race-sex or alcohol consumption.

We next looked at the short-term risk of GGT measured at year 10 when participants were 28–40 years of age. Year 10 serum GGT cutpoints of 12, 18, 29, and 50 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) were used for study of association of year 10 GGT with year 15 uric acid, CRP, and F2-isoprostanes and for prediction of incident diabetes and hypertension, the latter using proportional hazards regression. Among 3950 individuals who attended a year 10 examination, we excluded 80 study participants in whom GGT was not measured at year 10 and 540 who did not return for a year 15 follow-up examination, leaving 3352 participants for analysis. We excluded 98 and 388 participants with any diagnosis of diabetes or hypertension, respectively, at or before year 10, the baseline for incident analysis. This analysis provided both a shorter term follow-up and a baseline for GGT at a mean age of 35 years.

Finally, we assessed whether the associations between age, BMI, and disease outcomes were modified by baseline serum GGT concentration. We evaluated both long-term risk during 15 years (baseline to year 15) and short-term risk during 5 years (year 10 to year 15). The median serum GGT value at either year 0 or year 10 was used as the cutoff point in these stratified analyses.

Results

BASELINE CHARACTERISTICS

Year 0 serum GGT was strongly associated with year 10 GGT ($r = 0.67$). GGT increased during 10 years [mean (SD) change, 1.1 (1.7) U/L]. At baseline, most cardiovascular risk factors showed clear positive or negative relationships with serum GGT concentration (Table 1). In addition to alcohol consumption, black race, male gender, older age, lower educational attainment, cigarette smoking, and higher BMI were positively associated with baseline serum GGT concentration. Among clinical variables, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, insulin, triglycerides, LDL-cholesterol, red blood cell count, hematocrit, hemoglobin, and white blood cell count showed a positive association with baseline GGT level, whereas HDL-cholesterol showed a negative association.

Year 0 and year 10 GGT also showed positive associations with known markers of oxidative stress or inflammation, including year 5 fibrinogen, year 15 uric acid, year 15 CRP, and year 15 F2-isoprostanes (Table 2). These associations were shown among nondrinkers, drinkers, and individuals with AST concentrations within the reference interval (<30 U/L; data not shown).

INCIDENCE OF DIABETES AND HYPERTENSION BY BASELINE GGT CONCENTRATION

Incidence densities of diabetes and hypertension were 2.4 (157 cases) and 11.6 (708 cases) per 1000 person-years, respectively. There were positive associations between baseline GGT and both incident diabetes and hypertension, but the association with diabetes was the stronger of the two (Table 3). After minimal adjustment for study center, race, sex, and age, the relative risks (RRs) of incident diabetes were 1.0, 1.6, 1.7, 4.0, and 5.5, respectively. Additional adjustment for alcohol consumption did not change the relationship. Further adjustment for known risk factors for diabetes attenuated this relationship, but GGT remained a significant risk factor in the model that included BMI, smoking, and physical activity. Additional adjustment for baseline fasting serum glucose or baseline fasting insulin concentration did not materi-

Table 1. Adjusted mean[a] values for demographic, health behavior, and clinical characteristics by serum GGT at baseline in the CARDIA Study, 1985–1986.

	GGT at 0 year					
	<25% (n = 847)	25% to <50% (n = 1465)	50% to <75% (n = 1503)	75% to <90% (n = 557)	≥90% (n = 472)	*P*[b]
Demographic variables						
Black, %	28.3	39.7	56.8	72.9	75.4	<0.001
Male, %	21.7	37.4	52.8	63.5	68.1	<0.001
Age, years	24.3	24.4	25.0	25.5	26.4	<0.001
Education, years	14.0	13.9	13.9	13.7	13.3	<0.001
Health behavior variables (year 0)						
Alcohol, g/day	7.6	9.5	12.6	15.1	22.5	<0.001
Smoker, %	22.4	26.4	30.9	32.8	47.5	<0.001
Physical activity, exercise units	414.9	421.4	422.8	423.9	401.5	0.643
BMI, kg/m^2	23.3	23.9	24.6	26.0	26.2	<0.001
Clinical variables (year 0)						
SBP,[c] mmHg	108.9	109.1	110.7	112.4	113.8	<0.001
DBP, mmHg	67.3	67.9	68.7	69.9	71.0	<0.001
Fasting plasma glucose, mg/L	824	819	822	843	835	0.019
Insulin, μIU/L	10.1	10.8	11.6	13.6	13.9	<0.001
Triglycerides,[d] mg/L	568	589	644	738	828	<0.001
LDL-C, mg/L	1034	1069	1099	1158	1152	<0.001
HDL-C, mg/L	538	532	530	513	542	0.002
RBC, $\times 10^{10}$/L	477	478	480	485	485	<0.001
Hematocrit, %	41.7	42.0	42.3	42.6	42.8	<0.001
Hemoglobin, g/L	141	142	143	143	144	<0.001
WBC, $\times 10^9$/L	5.7	6.0	6.2	6.4	6.5	<0.001
Platelets, $\times 10^9$/L	265	271	274	280	280	<0.001

[a] Adjusted for study center, race, sex, and age.
[b] *P* values are based on *F*-test for any difference among GGT categories.
[c] SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; RBC, red blood cells; WBC, white blood cells.
[d] Geometric mean.

ally alter the association with GGT; because it is possible that increased GGT was in the causal pathway for hyperinsulinemia at baseline, this model may be an overadjustment. Incident hypertension was also associated with baseline GGT, with RRs of 1.0, 1.2, 1.5, 2.0, and 1.9 after adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, respectively (Table 3). The positive association between baseline GGT and incident diabetes and/or hypertension was observed in both nondrinkers and drinkers (data not shown).

After adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, RRs for AST with cutpoints of 18, 23, 28, and 37 U/L (the 25th, 50th, 75th, and 90th percentiles computed over the entire sample) were 1.0, 1.0 [95% confidence interval (95% CI), 0.6–1.6], 1.2 (95% CI, 0.8–2.0), 1.4 (95% CI, 0.8–2.4), and 2.0 (95% CI, 1.1–3.5) for prediction of diabetes and 1.0, 1.2 (95% CI, 0.9–1.5), 1.0 (95% CI, 0.8–1.3), 1.2 (95% CI, 0.9–1.5), and 1.4 (95% CI, 1.1–1.8) for prediction of hypertension. The dose–response relationship between GGT concentration and incidence of diabetes and/or hypertension was observed among individuals with AST concentrations within the reference interval (data not shown).

The positive association between baseline GGT and incident diabetes and/or hypertension was observed among all race and sex subgroups, although power was reduced in the subgroup analyses and the subgroup relationship did not always reach statistical significance. For example, compared with individuals with GGT below the median in each race-sex group, after adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, RRs for diabetes among individuals with year 0 GGT at or above the 75th percentile were 2.5 (95% CI, 1.1–5.7) in black men, 1.8 (95% CI, 1.0–3.3) in black women, 1.6 (95% CI, 0.6–3.7) in white men, and 8.8 (95% CI, 1.1–72.7) in white women. Similarly, RRs for hypertension among individuals with GGT at or above the 75th percentile were 1.6 (95% CI, 1.1–2.2) in black men, 1.5 (95% CI, 1.1–2.1) in black women, 1.6 (95% CI, 1.0–2.5) in white men, and 1.4 (95% CI, 0.7–2.5) in white women.

The RRs for year 10 serum GGT with incident diabetes or hypertension at year 15 (Table 4) were higher than the RRs for the 15 year risk that started with year 0 GGT as

Table 2. Adjusted mean[a] values for inflammatory and/or oxidative stress markers by year 0 and/or year 10 GGT in the CARDIA Study, 1985–2001.

	GGT at year 0						
	<25%	25% to <50%	50% to <75%	75% to <90%	≥90%	Difference,[b] %	*P* for trend
Fibrinogen (year 5), mg/L	2607 (n = 758)	2607 (n = 1268)	2634 (n = 1297)	2674 (n = 471)	2728 (n = 414)	5	<0.001
Uric acid (year 15), mg/L	46 (n = 639)	48 (n = 1099)	50 (n = 1083)	52 (n = 411)	52 (n = 339)	13	<0.001
CRP (year 15),[c] mg/L	1.3 (n = 642)	1.4 (n = 1101)	1.4 (n = 1085)	1.6 (n = 411)	1.7 (n = 340)	31	<0.001
F2-Isoprostanes (year 15), ng/L	56.0 (n = 535)	59.1 (n = 921)	59.3 (n = 909)	60.9 (n = 332)	63.3 (n = 282)	13	0.003
	GGT at year 10					**Difference,[b] %**	***P* for trend**
	<25%	**25% to <50%**	**50% to <75%**	**75% to <90%**	**≥90%**		
Uric acid (year 15), mg/L	45 (n = 677)	47 (n = 901)	50 (n = 868)	52 (n = 511)	54 (n = 311)	20	<0.001
CRP (year 15),[c] mg/L	1.2 (n = 676)	1.4 (n = 905)	1.5 (n = 869)	1.7 (n = 512)	1.6 (n = 311)	33	<0.001
F2-Isoprostanes (year 15), ng/L	54.7 (n = 572)	57.6 (n = 757)	59.3 (n = 738)	62.4 (n = 427)	68.2 (n = 253)	25	<0.001

[a] Adjusted for study center, race, sex, and age.
[b] Lowest vs highest.
[c] Geometric mean.

baseline (Table 3). Because year 10 serum GGT was increased, the minimally adjusted RRs for diabetes were 1.0, 6.0, 10.4, 24.4, and 28.3, and those for hypertension were 1.0, 2.5, 2.5, 3.8, and 3.4 (Table 4). After adjusting for study center, race, sex, age, alcohol consumption, BMI, smoking, and physical activity, the RRs were 1.0, 4.5, 6.3, 11.0, and 15.5 for diabetes and 1.0, 2.4, 2.2, 3.1, and 2.8 for hypertension.

Table 3. Incidence density and adjusted RR for incident diabetes and hypertension from year 0 to year 15 by serum GGT at baseline in the CARDIA Study, 1985–2001.

	GGT at year 0					
Outcome	**<25%**	**25% to <50%**	**50% to <75%**	**75% to <90%**	**≥90%**	***P* for trend**
Incident cases of diabetes[a]						
Cases/person-years	11/11 552	32/19 729	37/19 879	35/7262	42/6127	
Incidence density, per 1000 person-years	1.0	1.6	1.9	4.8	6.9	
Adjusted RR						
Model 1[b]	1.0	1.6 (0.8–3.3)	1.7 (0.9–3.4)	4.0 (2.0–8.1)	5.5 (2.7–11.1)	<0.01
Model 2[c]	1.0	1.7 (0.8–3.3)	1.8 (0.9–3.6)	4.2 (2.1–8.5)	6.1 (3.0–12.3)	<0.01
Model 3[d]	1.0	1.5 (0.8–3.0)	1.4 (0.7–2.7)	2.6 (1.3–5.3)	3.3 (1.6–6.8)	<0.01
Model 4[e]	1.0	1.5 (0.7–3.4)	1.5 (0.7–3.4)	2.6 (1.2–6.0)	2.9 (1.2–6.9)	0.002
Incident cases of hypertension[f]						
Cases/person-years	65/11 271	150/19 043	240/18 762	133/6394	120/5310	
Incidence density, per 1000 person-years	5.8	7.9	12.8	20.8	22.6	
Adjusted RR						
Model 1[b]	1.0	1.2 (0.9–1.6)	1.7 (1.2–2.2)	2.3 (1.7–3.2)	2.3 (1.7–3.2)	<0.01
Model 2[c]	1.0	1.2 (0.9–1.6)	1.6 (1.2–2.2)	2.3 (1.7–3.2)	2.3 (1.7–3.2)	<0.01
Model 3[d]	1.0	1.2 (0.9–1.6)	1.5 (1.1–2.0)	2.0 (1.5–2.7)	1.9 (1.4–2.7)	<0.01
Model 4[e]	1.0	1.3 (0.9–1.8)	1.5 (1.1–2.1)	1.9 (1.4–2.8)	1.5 (1.0–2.2)	0.003

[a] Fasting plasma glucose ≥1260 mg/L or medication from year 0 to year 15.
[b] Model 1: minimal adjustment for study center, race, sex, and age.
[c] Model 2: model 1 plus adjustment for alcohol consumption.
[d] Model 3: model 2 plus adjustment for BMI, cigarette smoking, and physical activity.
[e] Model 4: model 3 plus adjustment for fasting serum glucose and insulin for diabetes and systolic blood pressure and insulin for hypertension.
[f] Blood pressure ≥140/90 mmHg or medication from year 0 to year 15.

Table 4. Incidence density and adjusted RR for incident diabetes and hypertension from year 10 to year 15 by serum GGT at year 10 in the CARDIA Study, 1995–2001.

	GGT at year 10					
Outcome	**<25%**	**25% to <50%**	**50% to <75%**	**75% to <90%**	**≥90%**	***P* for trend**
Incident cases of diabetes						
Cases/person-years	1/3789	8/5034	15/4739	19/2697	14/1610	
Incidence density, per 1000 person-years	0.3	1.6	3.2	7.0	8.7	
Adjusted RR						
Model 1[a]	1.0	6.0 (0.8–48.5)	10.4 (1.4–80.4)	24.4 (3.2–187.2)	28.3 (3.6–223.4)	<0.001
Model 2[b]	1.0	6.0 (0.7–48.5)	10.7 (1.4–82.0)	26.1 (3.4–199.3)	34.3 (4.3–270.02)	<0.001
Model 3[c]	1.0	4.5 (0.6–36.2)	6.3 (0.8–48.8)	11.0 (1.4–85.4)	15.5 (1.9–122.9)	<0.001
Model 4[d]	1.0	3.5 (0.4–28.9)	5.1 (0.7–39.6)	7.0 (0.9–55.4)	8.7 (1.1–71.3)	0.010
Incident cases of hypertension						
Cases/person-years	21/3675	72/4672	77/4202	68/2348	38/1268	
Incidence density, per 1000 person-years	5.7	15.4	18.3	29.0	30.0	
Adjusted RR						
Model 1[a]	1.0	2.5 (1.5–4.1)	2.5 (1.5–4.0)	3.8 (2.3–6.4)	3.4 (1.9–6.0)	<0.001
Model 2[b]	1.0	2.5 (1.5–4.1)	2.5 (1.5–4.0)	3.9 (2.3–6.4)	3.5 (2.0–6.1)	<0.001
Model 3[c]	1.0	2.4 (1.5–4.0)	2.2 (1.3–3.6)	3.1 (1.8–5.3)	2.8 (1.6–5.1)	<0.001
Model 4[d]	1.0	2.3 (1.4–3.9)	2.0 (1.2–3.3)	2.8 (1.6–4.8)	2.1 (1.1–3.8)	0.034

[a] Model 1: minimal adjustment for study center, race, sex, and age.
[b] Model 2: model 1 plus adjustment for alcohol consumption.
[c] Model 3: model 2 plus adjustment for BMI, cigarette smoking, and physical activity.
[d] Model 4: model 3 plus adjustment for fasting serum glucose and insulin for diabetes and systolic blood pressure and insulin for hypertension.

INTERACTION BETWEEN GGT AND AGE OR BMI ON INCIDENCE OF DIABETES AND HYPERTENSION

When a 15-year risk of developing diabetes was examined, starting at a mean age of 25 years, there were no interactions between BMI and GGT on the development of diabetes and hypertension. However, when a 5-year risk was examined, starting with year 10 as the baseline when the mean age was 35 years, the association of year 10 BMI with incident diabetes and/or hypertension was different depending on year 10 GGT (Figs. 1 and 2). Compared with individuals with year 10 GGT below the median, year 10 BMI among individuals with year 10 GGT above the median was more strongly associated with incident diabetes and/or hypertension than for those

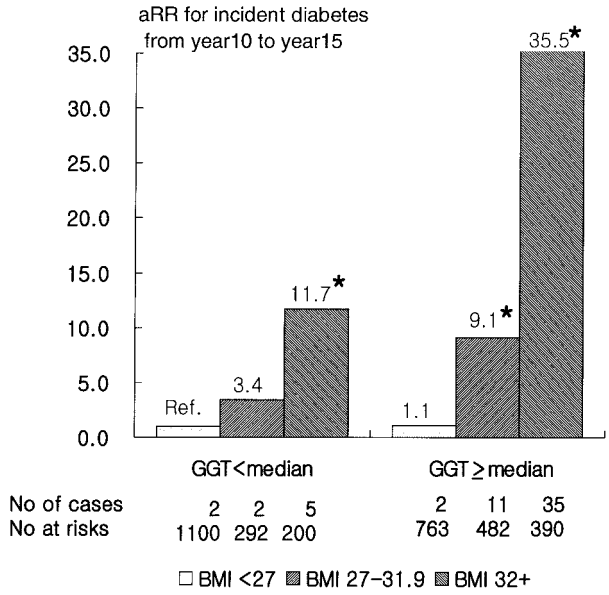

Fig. 1. Adjusted RR for incident diabetes by BMI at year 10 and serum GGT at year 10 in the CARDIA Study.

Adjusted for study center, race, sex, age, alcohol consumption, BMI, cigarette smoking, and physical activity. *, 95% CI for the adjusted RR does not include 1.

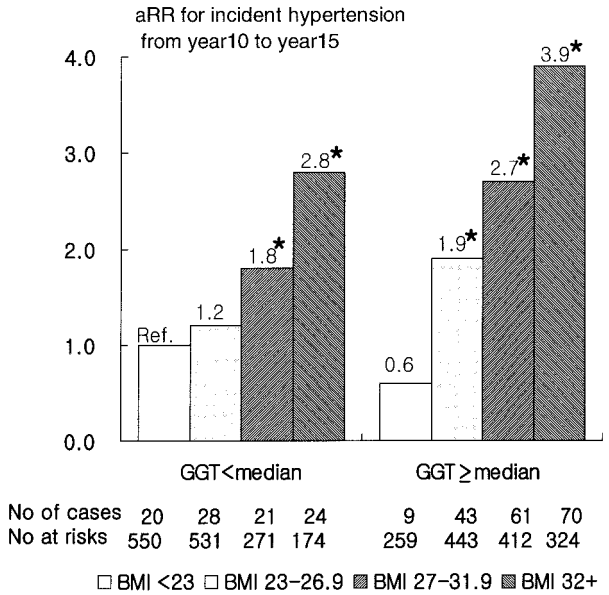

Fig. 2. Adjusted RR for incident hypertension by BMI at year 10 and serum GGT at year 10 in the CARDIA Study.

Adjusted for study center, race, sex, age, alcohol consumption, BMI, cigarette smoking, and physical activity. *, 95% CI for the adjusted RR does not include 1.

with GGT below the median. No such interaction was seen in the 5-year risk (incident diabetes or hypertension at year 2 or 5), using year 0 GGT as baseline (data not shown). Although the stronger gradient of risk starting at age 35 than starting at age 25 (comparing Tables 3 and 4) is suggestive of an interaction between age and GGT and gradients of risk associated with age for both diabetes and hypertension were slightly higher among individuals with high-normal GGT, we observed no clear interaction of age and GGT on the development of diabetes or hypertension (data not shown).

Discussion

In this prospective study of young black and white men and women, serum GGT concentrations measured at ages 18–30 years and mostly within the reference interval predicted the development of diabetes and/or hypertension during 15 years of follow-up in a dose–response relationship. These associations were not confounded by other lifestyle factors examined and did not differ materially by race or sex. Moreover, the coefficient for serum GGT and the 5-year risk of diabetes or hypertension, starting at a mean age of 35 years, was higher than that for long-term risk, starting at age 25 years. Our data are in agreement with results of previous prospective studies *(6, 7, 9, 10)*, which showed that baseline serum GGT was an independent risk factor for the development of type 2 diabetes and hypertension.

Increased GGT is conventionally interpreted as a marker of alcohol abuse and/or liver damage *(11)*. However, neither of these interpretations explains the association of GGT within its reference interval with incident diabetes and/or hypertension. In this study, the associations of GGT with incident diabetes and hypertension were independent of alcohol intake and were present in nondrinkers. Recently, fatty liver with a broad spectrum of pathologic conditions has also been linked to insulin resistance syndrome and/or type 2 diabetes *(15, 16)*. GGT might therefore be interpreted as a marker for hepatic steatosis and hepatic insulin resistance in the pathogenesis of type 2 diabetes. However, in our participants, the dose–response relationship between GGT and incidence of diabetes and/or hypertension was observed among individuals with AST concentrations within the reference interval (<30 U/L); AST usually increases in cases of hepatic steatosis. In addition, the associations between AST and disease outcomes were weaker than those of GGT and were restricted to the highest AST concentrations; note that the highest category of AST is above the reference interval used by laboratories. Thus, neither alcohol consumption nor liver damage appear to explain the association of GGT with diabetes and/or hypertension.

Emerging evidence has shown that serum GGT might be an important enzyme in the pathogenesis of cardiovascular diseases. Consistent with such a role, population-based studies *(1–3)* have found a strong association between serum GGT concentrations and many cardiovascular disease risk factors. Even in this young adult population, after adjusting for alcohol consumption, serum GGT concentration was associated with many cardiovascular disease risk factors, including black race, male gender, older age, cigarette smoking, BMI, higher blood pressure, higher fasting blood sugar, higher fasting blood triglycerides, higher blood LDL-cholesterol, and lower blood HDL-cholesterol. In addition, other variables, such as white blood cell count, red blood cell count, hematocrit, and hemoglobin, were positively associated with GGT.

At present, studies on GGT are at an early stage. Although the mechanism underlying the above associations remains largely unknown, some possible mechanisms exist. Previous experimental studies *(17–19)* have reported that GGT plays an important role in antioxidant systems with the primary function of maintaining intracellular concentrations of glutathione, a critical antioxidant defense for the cell. Increases in GGT activity can be a response to oxidative stress, marking increased transport of glutathione into cells. In addition, GGT is leaked into the serum, possibly as a result of normal cell turnover and cellular stresses. Thus, increased serum GGT may identify those individuals with low but persistent increases in oxidative and other cellular stresses. On the other hand, recent experimental studies *(20–23)* indicated that under physiologic conditions, GGT is involved directly in the generation of reactive oxygen species in the presence of iron or other transition metals. GGT might alternatively be a specific marker of oxidative stress, e.g., as a result of iron overload, because several experimental and epidemiologic studies have suggested a close relationship between iron overload and cellular or serum GGT activity *(24, 25)*. Interestingly, a recent review *(26)* pointed out important influences of iron metabolism in type 2 diabetes. Diabetes and hypertension are interrelated diseases that strongly predispose affected individuals to atherosclerotic cardiovascular disease. Regardless of the type of diabetes, hypertension is two to three times more common among diabetic individuals than in nondiabetic individuals *(27)*. Furthermore, both diabetes and hypertension are among the numerous pathologic conditions that are associated with increased vascular production of reactive oxygen species *(28–30)*. Vascular oxidant stress, particularly interactions between NO and oxygen-derived radicals, represents a common pathologic mechanism in many risk factors for atherosclerosis.

In this study, serum GGT concentrations measured at ages 18–30 years predicted 15-year incidence of diabetes and/or hypertension and the future concentrations of oxidative stress and inflammation markers such as fibrinogen, uric acid, CRP, and F2-isoprostanes, which were measured at various times during the 15 years of follow-up. These observations suggest that GGT might be an early marker of oxidative or other cellular stress and that it is possibly directly related to the pathogenesis of

diabetes and hypertension, perhaps as an oxidative stressor itself.

Another interesting finding of this study was an interaction between BMI and GGT, both measured at a mean age of 35 years, in the prediction of 5-year incident diabetes. This analysis was motivated by our previous finding in Korean men, in whom there were interactions between age and/or BMI and GGT in the development of diabetes during 4 years *(10)*. In partial agreement with our previous findings, we observed that BMI was a stronger risk factor for incident diabetes and/or hypertension among individuals with GGT concentrations greater than the median. No such interaction was observed between year 0 GGT and year 0 BMI, measured at a mean age of 25 years, and 15-year risk. A possible interpretation of this interaction is that obese individuals with high-normal GGT have already suffered subclinical pathologic changes as a result of obesity, whereas obese individuals with low-normal GGT are at an earlier stage of pathogenesis. According to this interpretation, the serum GGT concentration might be an intervening factor in the association between obesity and diabetes. However, relatively high GGT was not predictive of either incident diabetes or and/or hypertension among the leaner participants. Therefore, some other condition may be necessary for pathogenesis, in addition to relatively high GGT alone; among the possible conditions is high body iron *(20–23)*. For example, GGT may play an antioxidant or a prooxidant role, depending on the presence of iron *(20–23)*. In this regard, it is interesting that population studies have reported a positive association between BMI and/or age and serum ferritin, a marker of body iron storage *(31, 32)*. In our previous study of Korean men *(10)*, age was a strong risk factor of diabetes only among individuals with high-normal GGT; CARDIA data, however, failed to show an interaction of GGT and diabetes or hypertension with age. This divergence of findings may arise in part because of the difference in age distribution between the two cohorts. In our previous study, the interaction with age was largely restricted to participants who were 45 years of age or older. The oldest CARDIA participant was 45 years of age at year 15 of follow-up. Furthermore, age did play a role in the interaction of GGT with BMI and incident diabetes: the interaction was found only for GGT and BMI measured at a mean age of 35 years.

Although this report shows that GGT provides significant prognostic value above and beyond that provided by traditional risk factors, GGT alone has inadequate sensitivity to be used as a screening tool in clinical management. Furthermore, although the exact shape of the relationship between GGT and either diabetes or increased blood pressure is not known, the evidence presented is consistent with an increase in risk that is gradual as GGT increases. At present, it is uncertain whether serum GGT has a role in the causal pathway of diabetes or hypertension; GGT may be only a marker of risk. However, the information presented is consistent with such a role and enhances the importance of further study of GGT, either as a marker of oxidative or other stress, such as iron overload, or as an etiologic agent in itself.

Single measurements of fasting blood sugar or blood pressure for diagnosis of diabetes or hypertension in our study is a limitation typical of epidemiologic studies. Although diagnosis based on a single measurement is not acceptable clinically in individual patients because of random fluctuations, it is generally accepted as a diabetes criterion in epidemiologic studies. Random error attributable to a single determination usually leads to an attenuated estimate of the strength of association.

In conclusion, this study suggests that serum GGT is a strong predictor of diabetes and hypertension. Neither alcohol consumption nor liver damage likely explains this association. We speculate that it might be involved in the pathogenesis of diabetes and hypertension through a mechanism related to oxidative stress. In addition, the well-known associations of BMI with diabetes and hypertension may be modified by serum GGT concentrations.

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