Prospective Study of Serum γ-Glutamyltransferase and Risk of NIDDM

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OBJECTIVE — Serum γ-glutamyltransferase (GGT) levels are raised in obese individuals, and a particularly strong association with central obesity has been described. We hypothesized that elevated GGT levels are a marker for visceral fat, and specifically for hepatic steatosis (fatty liver), and that hepatic steatosis leads to hepatic insulin resistance. To test this hypothesis, we examined the association between GGT levels and risk of NIDDM.

RESEARCH DESIGN AND METHODS — We carried out a prospective cohort study of incident cases of doctor-diagnosed NIDDM in a group of 7,458 nondiabetic men (aged 40–59 years) followed for a mean of 12.8 years (range 11.5–13.0). The men were randomly selected from general practice lists in 24 British towns. Cases of NIDDM were ascertained by repeated postal questionnaires to the men and by regular systematic review of primary care records.

RESULTS — A total of 194 men developed NIDDM during follow-up. Mean serum GGT at baseline (geometric mean [95% CI]) was significantly higher in the NIDDM patients than in the rest of the cohort (20.9 [19.3–22.6] vs. 15.3 U/l [15.0–15.6], $P < 0.0001$). There was a smooth, graded increase in the age-adjusted risk of NIDDM with increasing GGT levels, with a relative risk in the top fifth of the distribution of 6.8 (3.5–12.9) relative to the bottom fifth (trend $P < 0.0001$). This association was independent of serum glucose and BMI and of other predictors of NIDDM with which GGT is associated, including alcohol intake and physical activity level (adjusted upper to lower fifth relative risk: 4.8 [2.0–11.8], trend $P < 0.0001$]).

CONCLUSIONS — These findings suggest that a raised serum GGT level is an independent risk factor for NIDDM. Serum GGT level may be a simple and reliable marker of visceral and hepatic fat and, by inference, of hepatic insulin resistance.

Serum γ-glutamyltransferase (GGT) is widely used as a marker of alcohol-induced liver disease (1,2). It is known, however, that GGT levels rise, independently of alcohol consumption, with age (3), obesity (4–7), and established diabetes (8). A strong linear association between GGT and waist-to-hip ratio has been described (9), and there is evidence linking raised GGT levels with other cardiovascular disease risk factors, including physical inactivity, hypertension, and dyslipidemia (7). The association of high levels of GGT (and other liver enzymes) with moderate obesity in nondrinking men has been attributed to obesity-related hepatic steatosis (10). In a cross-sectional study of middle-aged male Japanese workers, there were strong associations between serum GGT and hepatic steatosis as determined by ultrasonography (11). In this Japanese study, hepatic steatosis was associated with evidence of insulin resistance on glucose tolerance testing, and a significant association between GGT and plasma insulin was observed.

These interrelations between GGT, waist-to-hip ratio, other cardiovascular disease risk factors, and plasma insulin, raise the possibility that elevated GGT levels are a marker for visceral fat, and specifically for hepatic steatosis, and that hepatic steatosis leads to hepatic insulin resistance. NIDDM is the major clinical manifestation of long-term insulin resistance (12). Hence, a key test of this hypothesis is whether elevated GGT levels predict NIDDM during long-term follow-up. We have examined the association between serum GGT levels and risk of physician-diagnosed NIDDM in a prospective population-based study of British middle-aged men followed for over a decade. Possible associations between other liver enzymes (serum aspartate transaminase [AST] and alkaline phosphatase), serum albumin, and risk of NIDDM have also been examined.

RESEARCH DESIGN AND METHODS

In the British Regional Heart Study, 7,735 men aged 40–59 years were selected at random from the age-sex register of one general practice in each of 24 towns in England, Wales, and Scotland between January 1978 and June 1980 for a prospective study of cardiovascular disease. The criteria for selecting the towns, general practices, and subjects and the methods of data collection have been described (13,14). Men with cardiovascular or other disease or those receiving regular medication were not excluded. The average response rate was 78%, ranging from 70–85% across the 24 towns. Known diabetic patients (n = 121), men diagnosed within the calendar year in which they were screened (n = 14), and those with nonfasting glucose in the diabetic range (11.1 mmol/l, n = 23), were excluded. GGT was not measured in 119 men. The analysis was based on the remaining 7,458 men.

Baseline assessment

Research nurses administered a standard questionnaire and completed an examination of each man, which included a resting electrocardiogram (15). The questionnaire included questions on alcohol intake, smoking habits, usual pattern of physical activity, medical history, and regular medication. Details of the classification of alcohol intake, smoking habits, physical activity, measurement of blood pressure, and other physical measurements have been reported (13,14,16–18). The men were classified into

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Abbreviations: AST, aspartate transaminase; CHD, coronary heart disease; FEV_{1sec}, forced expiratory volume in 1 s; GGT, γ-glutamyltransferase.

five groups according to their current alcohol intake: none, occasional (<1 U/week), light (1–15 U/week), moderate (16–42 U/week), and heavy (>42 U/week) (19,20). The men were characterized on the basis of current smoking status as never smokers, former smokers, and current smokers, with the latter group further subdivided by the number of cigarettes smoked daily (16). A physical activity score was derived from an exercise questionnaire administered at the screening examination, based on the frequency and intensity of the activities reported (17,18,21). Based on the score, the men were grouped into six broad physical activity categories: inactive, occasional, light, moderate, moderately vigorous, and vigorous. Blood pressure was recorded with a London School of Hygiene sphygmomanometer. Two successive recordings were taken, and the mean was used in the analysis with adjustment for interobserver variation (22). BMI calculated as weight divided by height squared was used as an index of relative weight. Heart rate was determined from the electrocardiogram (23). Forced expiratory volume in 1 s (FEV_{1sec}) was measured in the seated position, using a Vitalograph spirometer, and values were height standardized.

Prevalent coronary heart disease (CHD) at screening was defined on the basis of any or all of the following criteria: recall of doctor diagnosis of angina or heart attack, a World Health Organization (Rose) questionnaire response indicating angina or possible myocardial infarction, and electrocardiographic evidence of definite or possible myocardial ischemia or infarction (15,24).

GGT

Nonfasting blood samples were obtained between 8:30 A.M. and 6:30 P.M. The time of arrival at the examination center was noted, and the estimated time of venipuncture (at the end of the examination) was 35 min later (25). Serum was separated on-site within 30 min of venipuncture, stored at −4°C, and analyzed by noon of the following day. GGT was analyzed in serum (together with AST and alkaline phosphatase) using an automated analyzer (Technicon SMA 12/60). There was minimal diurnal variation in GGT levels, with 0.2% of the variance attributable to time of sampling (25). The methods of analysis for serum glucose, lipids, uric acid, albumin, and hematocrit have been described (25–28). Because triglyceride concentrations were not determined for men in the first six towns, data on this variable were available for only 5,327 men.

Table 1—*Incidence of doctor-diagnosed NIDDM per 1,000 person-years of follow-up by fifths of serum GGT distribution*

GGT (U/l)	Men (*n*)	Events	Rate per 1,000 person-years
<10	1,336	10	0.6
10	1,660	25	1.3
13	1,602	33	1.7
17	1,369	52	3.2
24	1,491	74	4.3

Follow-up

All men have been followed up for all-cause mortality and for cardiovascular morbidity up to December 1991, a mean period of 12.8 years (range 11.5–13.0) (29). Information on death was collected through the established flagging procedures provided by the National Health Service registers in Southport (England and Wales) and Edinburgh (Scotland). New cases of NIDDM were ascertained by means of *1*) a postal questionnaire sent to the men at year 5 of follow-up for each individual, 2) systematic reviews of primary care records in 1990 and 1992, 3) a further questionnaire sent to 6,582 surviving members of the cohort resident in Britain in 1992, and 4) review of all death certificates for any mention of diabetes (21). The questionnaire at year 5 achieved a response rate of 98% and the 1992 questionnaire a response rate of 90%. A diagnosis of diabetes was not accepted on the basis of questionnaire data unless confirmed in the primary care records.

Statistical analysis

The men were divided into five groups, representing approximate fifths of the distribution of serum GGT. Cox's proportional hazards models were used to assess the independent contribution of serum GGT concentration at baseline to the risk of NIDDM during follow-up and to estimate the relative risk of NIDDM in each fifth of the GGT distribution relative to the first, adjusted for other risk factors (30). Alcohol intake (four levels), smoking (five levels), physical activity (six levels), and preexisting ischemic heart disease (yes/no) were fitted as categorical variables. Age, BMI, systolic blood pressure, heart rate, uric acid, FEV_{1sec}, HDL cholesterol, and triglyceride concentration were fitted as continuous variables in the proportional hazards model. Hematocrit was fitted as four dummy variables for five hematocrit groups, based on absolute levels of hematocrit: <42.0, 42.0–43.9, 44.0–45.9, 46.0–47.9, and ≥48%. Because GGT, glucose, and triglyceride concentrations were not normally distributed, the data were log-transformed, and GGT data are presented as geometric means. Because of the marked diurnal variation in serum triglyceride levels (25), the log-transformed data on this variable were adjusted for time of sampling (31).

To illustrate the separate effects of allowing for key lifestyle-related and biological variables, the GGT-NIDDM relationship was adjusted for potential confounding factors in four cumulative stages. In tests for trend, GGT was fitted as a continuous variable. Possible interactions between GGT and both BMI and alcohol consumption in the development of NIDDM were explored in stratified analyses and by fitting interaction terms in Cox's proportional hazards models.

RESULTS — At 12.8 years of follow-up, there were 194 cases of NIDDM in the group of 7,458 nondiabetic men. Mean serum GGT level at baseline (geometric mean [95% CI]) was significantly higher in men who subsequently developed NIDDM than in the rest of the cohort: 20.9 (19.3–22.6) vs. 15.3 U/l (15.0–15.6), $P < 0.0001$. Table 1 shows the incidence of doctor-diagnosed NIDDM per 1000 person-years of follow-up (unadjusted) by fifth of serum GGT, and Fig. 1 shows the relative risk of NIDDM with 95% CI in each fifth of the serum GGT distribution relative to the first, adjusted for age and separately for age and BMI. There was a strong, graded association between serum GGT concentration and the subsequent risk of NIDDM, with a greater than fourfold increased risk among men with serum GGT in the upper fifth of the distribution relative to the lower fifth after adjustment for age and BMI (Table 2).

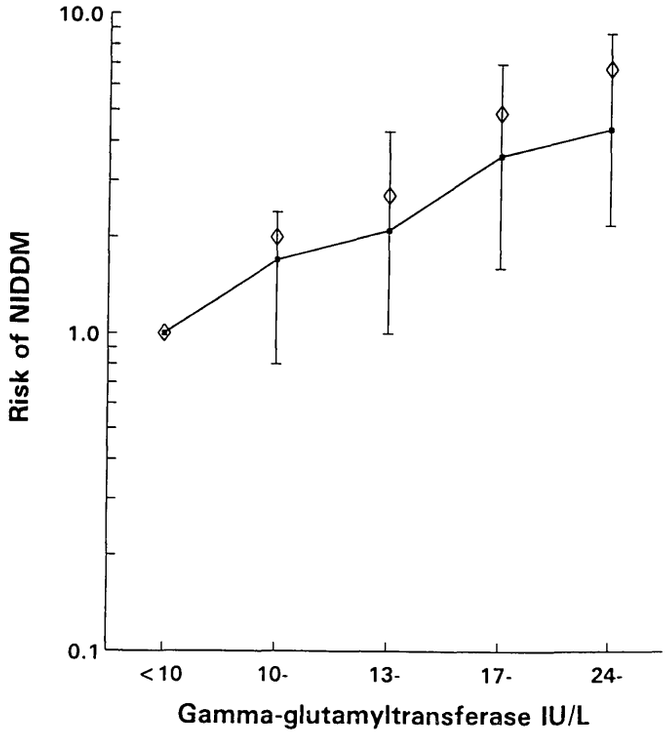

Figure 1—*Relative risk of NIDDM (log scale) adjusted for age and BMI with 95% CI, by fifth of baseline serum GGT. ◇ indicates each relation adjusted for age alone.*

GGT and other risk factors for NIDDM

Significant associations were observed between GGT and a range of cardiovascular disease risk factors that have been shown to predict NIDDM (7,21). A significant positive association was observed between GGT and alcohol intake, cigarette smoking, and prevalent CHD, and an inverse association was seen with physical activity level (7). Positive associations were observed between GGT and BMI (r = 0.21), systolic blood pressure (r = 0.14), serum triglyceride (r = 0.1), FEV_{1sec} (r = 0.09), heart rate (r = 0.12), uric acid (r = 0.28), and hematocrit (r = 0.23), all $P < 0.0001$. Weak but significant associations were seen with serum glucose (r = 0.05, $P < 0.0001$) and HDL cholesterol (r = 0.04, P = 0.0007) (7).

Multivariate analyses

Adjustment for confounders additional to age and BMI was not associated with appreciable further attenuation of the association between serum GGT levels and the subsequent risk of NIDDM (Table 2). Data on serum triglyceride, an important predictor of NIDDM in this cohort (21) were available from a subgroup of 5,025 men. We examined, in a separate model, the effect of adjustment for triglyceride in addition to the lifestyle and other biological predictors of NIDDM in this subgroup. In this analysis, there remained a more than fourfold increased risk of NIDDM in the upper relative to the lower fifth of the GGT distribution, with a highly significant linear trend of increasing risk of NIDDM with increasing GGT levels.

GGT and NIDDM, stratified by obesity and alcohol consumption

The GGT-NIDDM association was examined in three strata of BMI (Table 3). These data were adjusted for other potential confounders, with the exception of triglyceride. In each stratum, there was evidence of a graded independent association between GGT and risk of NIDDM, associations that attained statistical significance in the second and third strata. In further analyses, the GGT-NIDDM association was examined by alcohol consumption in three categories: *1*) none or occasional, 2) light, and 3) moderate and heavy drinkers (Table 4). Serum GGT was a significant independent predictor of NIDDM in each category. On exclusion of cases diagnosed within the first 5 years of follow-up from the analysis, the association between GGT level and risk of NIDDM was essentially unchanged, with a fully adjusted relative risk in the upper fifth of the distribution of 3.1 (1.3–7.2) relative to the bottom fifth.

Other liver enzymes and serum albumin

GGT was strongly correlated with AST (r = 0.44) and with serum alkaline phosphatase, (r = 0.19). A weakly positive but significant association was seen between AST and risk of NIDDM after adjustment for age, with a relative risk in the top fifth of the distribution of 1.8 (1.2–2.7) relative to the bottom fifth. This increased risk was attenuated after adjustment for BMI (relative risk: 1.2 [0.8–1.9]). A similar weakly positive association was seen between serum alkaline phosphatase and risk of NIDDM that was also attenuated after adjustment for age and BMI (top versus bottom fifth relative risk: 1.4 [0.9–2.4]). A significant positive association was seen between serum albumin and risk of NIDDM, even after adjustment for age and BMI: the relative risk was 1.6 (1.1–2.4) in the top fifth relative to the bot-

Table 2—*Relative risk of incident cases of doctor-diagnosed NIDDM by fifth of serum GGT at baseline*

GGT (U/l)	A	B	C
<10	1.0	1.0	1.0
10	1.7 (0.8–2.4)	1.8 (0.8–3.7)	1.8 (0.8–4.0)
13	2.1 (1.0–4.3)	2.1 (1.0–4.2)	1.6 (0.7–3.5)
17	3.6 (1.6–7.0)	3.6 (1.8–7.3)	3.2 (1.5–6.8)
24	4.4 (2.2–8.8)	4.7 (2.4–9.4)	3.5 (1.8–7.7)
Trend (*P*)	<0.0001	<0.0001	<0.0001

Data are relative risk (95% CI). A, adjusted for age and BMI (n = 7,456; 194 cases); B, adjusted for the factors in A plus physical activity, alcohol consumption, cigarette smoking, and preexisting CHD (n = 7,339; 189 cases); C, adjusted for the factors in A and B plus systolic blood pressure, heart rate, HDL cholesterol, uric acid, FEV_{1sec}, hematocrit, and blood glucose (n = 6,618; 171 cases).

Table 3—*Fully adjusted relative risk of incident cases of doctor-diagnosed NIDDM by fifth of serum GGT in three BMI strata (first and second, third and fourth, and upper fifth of the BMI distribution)*

GGT (U/l)	BMI 1	BMI 2	BMI 3
<10	1.0	1.0	1.0
10	1.5 (0.3–6.4)	2.4 (0.6–8.7)	1.5 (0.4–5.9)
13	1.5 (0.3–6.8)	2.2 (0.6–7.9)	1.0 (0.3–4.0)
17	1.7 (0.3–8.2)	3.6 (1.0–12.8)	3.0 (0.8–10.6)
24	3.0 (0.6–13.5)	5.6 (1.6–19.7)	2.9 (0.8–10.0)
Trend (P)	0.3	0.0004	0.01

Data are relative risks (95% CI) and are adjusted for age, physical activity, alcohol consumption, cigarette smoking, preexisting CHD, systolic blood pressure, heart rate, HDL cholesterol, uric acid, FEV_{1sec}, hematocrit, and blood glucose. BMI 1, ≤24.56 kg/m², *n* = 2,676, 23 cases; BMI 2, 24.57–27.8 kg/m², *n* = 2,627, 70 cases; and BMI 3, ≥27.9 kg/m², *n* = 1,315, 78 cases.

tom fifth of the distribution. However, serum albumin and GGT were not highly correlated (r = 0.06), and adjustment for serum albumin did not alter the association between GGT and risk of NIDDM.

Prediction of NIDDM

BMI and glucose are the dominant predictors of NIDDM in this cohort (21). Using a risk score based on age, BMI, and nonfasting glucose, 112 of the 194 cases (58%) were placed in the upper fifth of the distribution of risk. The performance of this predictive model was not appreciably enhanced by the addition of GGT. One additional case was placed in the upper fifth of the distribution of risk.

CONCLUSIONS — In this prospective study of a general population sample of middle-aged men, we have found a strong, independent, and graded association between serum GGT levels at baseline and the incidence of NIDDM during follow-up that has extended for over a decade. The association was observed across the normal distribution of GGT and in each of three strata of BMI and alcohol consumption. In contrast, significant associations between serum AST and alkaline phosphatase levels and risk of NIDDM were not observed in multivariate analysis. In this cohort, the risk of NIDDM falls significantly with increasing alcohol consumption to moderate levels and is similar in heavy and occasional drinkers (21). Hence, the association between GGT and risk of NIDDM cannot be attributed to residual confounding due to alcohol intake. Although it is known that GGT levels are increased in individuals with established NIDDM, this is the first longitudinal study to show that serum GGT levels predict the subsequent development of diabetes. It should be noted, however, that despite this strong independent association between GGT and risk of NIDDM, measurement of GGT will not substantially improve prediction of diabetes in the clinical setting, given data on BMI and glucose level. This reflects the overwhelming importance of the latter factors as predictors of NIDDM (21). Nonetheless, the observation that serum GGT levels predict NIDDM has significant implications for our understanding both of interrelations between NIDDM and cardiovascular disease and of the pathogenesis of NIDDM.

GGT, NIDDM, and cardiovascular disease

In previous studies GGT levels have been linked with cardiovascular disease risk factors, such as obesity, physical inactivity, hypertension, and dyslipidemia (4–7,32). Higher GGT levels have also been linked with hyperinsulinemia (7,32). In a previous prospective study based on this cohort, an association between GGT and CHD events has been reported (7). Given these earlier studies, the current findings suggest that an elevated GGT level should be added to the cluster of vascular risk factors that form the insulin resistance syndrome and that link NIDDM and cardiovascular disease (33,34).

GGT, body fat, and insulin resistance

Although the association between GGT and NIDDM was independent of BMI, it is clear that GGT levels are closely linked with body fat (4–6). In a cross-sectional study involving a group of ~21,000 men attending for routine health screening, a strong linear association between GGT and BMI was observed that was independent of alcohol consumption and other potential confounders in multivariate analysis (6). In the Trömsø Study (Norway) (5), BMI was the dominant predictor of GGT levels in both men and women in multivariate analyses that included alcohol intake and major cardiovascular disease risk factors. In the latter study, the association between GGT and BMI (adjusted for potential confounders) was exponential in men. In contrast, for women, in whom GGT levels were lower, the association between GGT and BMI was nonlinear, with an increase in GGT levels seen only in the upper fifth of the BMI distribution (5). This suggests an association between GGT and male pattern (central) obesity, which has emerged as a strong and independent predictor of NIDDM in a number of studies (35,36). In a cross-sectional study involving 69 randomly selected healthy men aged 38 years, GGT and waist-to-hip ratio were highly correlated (r = 0.5), an association that was independent of BMI (9). In a liver biopsy study involving a group of morbidly obese patients, waist-to-hip ratio accounted for most of the variance in liver fat content, whereas significant associations with other measures of adiposity were

Table 4—*Fully adjusted relative risk of incident cases of doctor-diagnosed NIDDM by fifth of serum GGT in three alcohol consumption categories*

GGT (U/l)	None or occasional	Light	Moderate and heavy
<10	1.0	1.0	1.0
10	1.6 (0.6–4.7)	6.7 (0.9–53.2)	0.6 (0.1–2.8)
13	1.4 (0.5–4.1)	3.5 (0.4–28.9)	0.9 (0.2–3.6)
17	3.0 (1.1–8.6)	7.0 (0.9–54.9)	1.7 (0.5–6.0)
24	3.6 (1.3–10.2)	8.0 (1.0–62.6)	2.1 (0.6–7.1)
Trend (P)	0.009	0.04	0.01

Data are relative risks (95% CI) and are adjusted for age, physical activity, alcohol consumption, cigarette smoking, preexisting CHD, systolic blood pressure, heart rate, HDL cholesterol, uric acid, FEV_{1sec}, hematocrit, and blood glucose. None or occasional, <1 U/week, *n* = 1,972, 60 cases; light, 1–15 U/week, *n* = 2,195, 49 cases; moderate and heavy, ≥16 U/week, *n* = 2,451, 62 cases.

not detected (37). Ikai et al. (11) have reported significant and biologically important associations between hepatic steatosis as determined by ultrasonography and elevated GGT, insulin resistance, and hyperinsulinemia. In this Japanese study, the well-described association between serum GGT levels and blood pressure (7,32) was attenuated on adjustment for plasma insulin levels (11). In the British Regional Heart Study cohort, baseline data on nonfasting serum insulin are available for a subgroup of 5,550 nondiabetic men from 18 of the 24 towns (31). In this subgroup, a significant association between nonfasting serum insulin and GGT was observed, with a linear trend in geometric mean GGT (adjusted for age and BMI) by fifth of serum insulin (13.7, 15.2, 15.5, 15.5, and 16.4 U/l, $P < 0.001$). Against this background, the findings in the current study of a continuous association between GGT levels and risk of NIDDM during a decade of follow-up are consistent with the hypothesis that GGT is a marker for visceral fat, hepatic steatosis, and hepatic insulin resistance. The findings are also consistent with the hypothesis that hepatic insulin resistance is an early and fundamental abnormality in the pathogenesis of NIDDM and in the constellation of vascular risk factors that form the insulin resistance syndrome (33,34,38–40). Further studies should assess the independent contribution of GGT levels to the risk of NIDDM after adjustment for other measures of visceral fat.

Summary

We have found a strong, graded, and independent association between the concentration of GGT in serum and the risk of NIDDM during long-term follow-up. At levels of GGT considered well within the normal range, there was a substantial and significant increase in risk of NIDDM relative to baseline levels. The findings suggest that the GGT concentration should be added to the list of risk factors that are common to NIDDM and cardiovascular disease (21). The findings are consistent with the hypothesis that GGT is a marker for hepatic steatosis and hepatic insulin resistance, and with an early and fundamental role for hepatic insulin resistance in the pathogenesis of NIDDM.

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