A Comparison of Associations of Alanine Aminotransferase and Gamma-Glutamyltransferase with Fasting Glucose, Fasting Insulin, and Glycated Hemoglobin in Women With and Without Diabetes

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Associations between biomarkers of nonalcoholic fatty liver disease (NAFLD) alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT), with 3 separate measures of glucose homeostasis: fasting glucose, fasting insulin and glycated hemoglobin (HbA1c) were studied and compared between women with and without diabetes in order to gain insight into the documented associations between NAFLD, insulin resistance and diabetes. Data from the British Women's Health and Heart Study, a random sample of British women aged 60-79 years (N = 3394; 3086 without diabetes and 308 with diabetes) was used. Associations of ALT and GGT with fasting glucose and HbA1c and of ALT with fasting insulin (and homeostasis model assessment of insulin resistance [HOMA]) are stronger in women with diabetes compared to women without diabetes (*P* for interaction < 0.001). GGT is associated with fasting insulin (and HOMA) to the same extent in all women, irrespective of diabetes status. Results excluding hyperinsulinemic women, i.e., in the highest fourth of the fasting insulin distribution, were similar to those obtained for all non-diabetic women as were results excluding women in the highest quartile of the alcohol consumption distribution and for women with ALT and GGT levels within the normal range. Associations did not differ substantially between obese and non-obese non-diabetic women. *Conclusion:* elevation of liver enzymes and hepatic insulin resistance as reflected by fasting insulin occur in the early stages of insulin resistance and highlight the central role of the liver in insulin resistance in the general population. (HEPATOLOGY 2007;46:158-165.)

Recognition of the role of the liver in the pathogenesis of type 2 diabetes has been increasing. Nonalcoholic fatty liver disease (NAFLD), characterized by elevated alanine-aminotransferase (ALT), and gamma-glutamyltransferase (GGT) is now regarded as the hepatic manifestation of the insulin resistance syndrome.[1] Prospective studies have demonstrated that increasing levels of ALT and GGT within the normal range predict incident type 2 diabetes and the metabolic syndrome even when controlling for known risk factors (for example, Sattar et al.[2] and Lee et al.[3]). ALT is the enzyme most closely correlated with liver fat accumulation,[4] and therefore the enzyme most commonly used as a biomarker of NAFLD in large epidemiological studies in which more accurate diagnostic methods (such as liver scans and/or biopsy) are not feasible. Limited data exist on the occurrence and degree of GGT elevation in NAFLD.[5]

Non-fasting levels of plasma insulin are determined by pancreatic beta cell insulin secretion as well as by peripheral and hepatic insulin sensitivity. In a fasting state, the rate of hepatic glucose production is the main determinant of plasma glucose concentration as a majority of glucose is utilized via insulin independent pathways by tissue with obligatory glucose requirements, mainly the brain. The main function of insulin in the fasting state is the suppression of hepatic glucose production. Therefore fasting insulin is a surrogate of hepatic insulin sensitivity.[6,7]

Abbreviations: ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HOMA, homeostasis model assessment of insulin resistance; NAFLD, nonalcoholic fatty liver disease.

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Glycated hemoglobin (HbA1c) measures long-term glucose homeostasis status, reflecting a time-weighted mean over the previous 2 to 3 months. As such HbA1c reflects postprandial and postabsorptive plasma glucose levels, not only fasting levels. Studies in populations with largely normal glucose control that assessed the relationship between fasting glucose and HbA1c have yielded very different estimates ranging from no correlation to a high degree of correlation between these two measurements of glycaemic control (see for example, see Kilpatrick et al[8] and Rohlfing[9]). Among patients with diabetes, the correlation is high ($r = 0.77$)[10] and it seems that the contribution of fasting glucose to HbA1c increases across the distribution of HbA1c.[11] In non-diabetic, the correlations between HbA1c and fasting insulin are low (0.4 and 0.11).[12,13]

In this study, our aim was to provide insight into the pathophysiological processes linking liver enzymes to insulin resistance and diabetes. We hypothesized that ALT and GGT would be more strongly associated with glucose homeostasis measures in women with diabetes compared with women without diabetes. We examined associations between levels of ALT and GGT, and three separate measures of glucose homeostasis: fasting plasma glucose, fasting plasma insulin and HbA1c assessed at the same time, as these measures reflect different aspects of glucose homeostasis.

Materials and Methods

Data from the British Women's Heart and Health Study were used. Full details on the selection of participants and measurements used in the study have been previously reported.[14,15] Between 1999 and 2001, 4,286 women (60% of those eligible to participate) aged 60-79 years who had been randomly selected from 23 British towns were interviewed and examined, completed medical questionnaires, and had detailed reviews of their medical records carried out. In the present paper, all analyses were cross-sectional and used data from the baseline assessment of the women.

A diagnosis of diabetes was made according to the World Health Organization criteria (i.e., a doctor's diagnosis and/or fasting plasma glucose equal to or greater than 7 mmol/l). Women were asked to bring all their medications to the baseline examination and the use of insulin was assessed. Weight was measured in light clothing without shoes to the nearest 0.1 kg using Soenhle portable scales (Critikon Service Centre, Berkshire, UK). Body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2). Waist circumference was taken as the midpoint between the lower rib and the iliac crest. Hip circumference was taken as the largest circumference below the waist. In all analyses two measurements of both waist and hip circumference were taken to the nearest millimetre using a flexible metal tape and the mean of these two was used. Central obesity was defined as a waist to hip ratio >0.85.[16] In analyses stratified by obesity status this measure of central obesity was used as the main indicator of obesity as there is evidence that this measure reflects visceral fat mass better than BMI in older populations.[17]

A Dinamap 1846SX (GE Clinical Services, Northampton, UK) vital signs monitor was used to measure blood pressure. Arm circumference was measured and the appropriate cuff size was used. Seated blood pressure was taken twice in succession, using the right arm, supported on a cushion and the mean of the two measurements used in all analyses.

Smoking data, obtained by self-report from either the research nurse interview or the self-completed questionnaire, were analyzed as: never, past and current. Physical activity data was categorized as: less than 2 hours of moderate or vigorous physical activity per week, between 2 and 3 and more than 3 hours per week.[18] Alcohol consumption, based on self-report, was categorized into one of the five mutually exclusive categories: non-drinkers and fourths of the distribution of drinks per week for those who were drinkers. At the research nurse interview all participants brought their current medications and a detailed drugs history was undertaken.

Childhood social class, derived from reported father's occupation, was classified according to the Registrar General's classification: I, II, III–nonmanual, III–manual, IV, or V, with I being the highest (professionals) and V being the lowest (unskilled manual workers). Adult social class was based on own or husband's longest held occupation (which ever was higher) and classified in the same way as childhood social class. Women with missing adult occupation data and women with missing data on father's occupation were included in the lowest social class category for the respective adult and childhood social class as they are likely to be unemployed, married to a husband who was unemployed or have had fathers who were unemployed.[19] Ethnic origin was determined by the research nurse who had to tick a box from a pre-specified list of descriptions (White, Afro-Caribbean, South Asian, Chinese, Other).

Blood samples were taken after a minimum 6 hour fast. Serum was separated on-site within 30 minutes of venipuncture, stored at $-4°C$, and analyzed within 24 hours of venipuncture. Levels of liver enzymes in the serum were determined using an automated analyzer (Technicon Sequential Multiple Analyzer; Technicon Instruments Corporation, Tarrytown, NY). Glucose and insulin were

measured on fasting venous plasma samples. Insulin was measured with a specific ELISA assay that does not cross-react with proinsulin.[20] HbA1c was measured on whole blood using an automated analyzer (Drew Hb Gold instrument, Drew Scientific Ltd, United Kingdom). Assessment of lipids and C-reactive protein was done using standard procedures.[21,22]

Full ethics committee approval for the study was obtained from Local Ethics Committees in each of the 23 participating towns and informed consent to review their general practice medical records was obtained from each participant included in the study.

Statistical Analysis

ALT, GGT, fasting insulin and triglycerides were naturally logged in order to normalize distributions. Partial age-adjusted correlations for liver enzymes and glucose homeostasis measures were estimated. Linear regression models were constructed for each outcome (fasting glucose, fasting insulin, and HbA1c) on each liver enzyme (ALT and GGT). In all the regression models liver enzymes and glucose homeostasis measures were entered as units of each measure's standard deviation to allow comparison of the effect for the different exposures (liver enzymes) on the same outcome and for the same exposure on different outcomes (glucose homeostasis measures). We adjusted for potential confounders (age, childhood and adult social class, smoking, physical activity, alcohol consumption, and medication), for BMI and waist to hip ratio, and for other components of the metabolic syndrome (systolic blood pressure, high density lipoprotein cholesterol and triglycerides) that are possible mediating factors of the association between ALT, GGT, and glucose homeostasis measures. Data of women with and without diabetes were analyzed separately and a formal test for interaction between age adjusted ALT and GGT (each) and diabetes in their association with fasting glucose, fasting insulin and HbA1c was conducted. As evidence shows that the fat accumulation in the liver is associated with obesity (for example, Pietilainen et al[23]), we also conducted analyses stratified by central obesity for data of women without diabetes.

Results

Of the 4,286 women in the British Women's Health and Heart Study, 3829 women (89%) had an adequate fasting blood glucose test. These women had a slightly lower mean BMI (31.4 versus 32.4, $P = 0.01$), a smaller chance of belonging to a manual labour social class in adulthood (56.2% versus 66.4%, $P < 0.0001$) and of being a current smoker (10.8% versus 16.5%, $P < 0.0002$) compared to women without an adequate glucose test (N = 457). They did not differ in any other respect, including age. Complete data (on ALT, GGT, fasting plasma glucose and insulin, HbA1c, and covariables) was available for 3,421 women (89% of those with an adequate blood glucose test) of which 335 (9.8%) had diabetes that was diagnosed after the age of 30. Of these 335 women 174 (52%) had not been previously diagnosed with diabetes (based on self report and review of medical records); they were diagnosed here on the basis of 1999 World Health Organisation criteria (a fasting blood glucose of ≥7 mmol/l). Twenty-seven diabetic women were treated with insulin and excluded from the current analysis. Therefore the present analysis includes 3086 non-diabetic women and 308 diabetic women. Characteristics of non-diabetic and diabetic women with complete data are presented in Table 1.

Table 1. Means and Standard Deviations of Characteristics of Diabetic and Non-Diabetic Women

	Women Without Diabetes (N = 3,086)	Women with Diabetes (N = 308)
Age	68.7 (5.5)	69.3 (5.5)
ALT* (U/l)	12.5 (1.5)	16.0 (1.6)
GGT* (U/l)	21.9 (1.9)	30.8 (1.9)
Fasting plasma glucose (mmol/l)	5.7 (0.50)	9.0 (3.4)
Fasting plasma insulin* (μU/l)	6.3 (1.8)	14.1 (2.2)
HbA1c (g/dl)	4.9 (0.6)	6.1 (1.5)
BMI (kg/m^2)	27.2 (4.8)	29.7 (5.9)
Waist: hip ratio	0.81 (0.07)	0.85 (0.07)
Alcohol consumption per week		
None (%)	35	42
Highest quartile of drinkers** (%)	16	14
Current smokers (%)	11	10
Physical activity		
< 2 hours per week (%)	18	25
Manual-labour social class		
Childhood (%)	78	85
Adulthood (%)	54	59
Triglycerides* (mmol/l)	1.6 (1. 6)	2.1 (1.6)
HDL (mmol/l)	1.7 (0.5)	1.5 (0.4)
Systolic blood pressure (mm Hg)	146.1 (24.7)	155 (26.0)
C-reactive protein* (mg/l)	1.7 (3.2)	2.4 (3.3)

Abbreviation: HDL, high density lipoprotein cholesterol.

* Geometric means.

** Quartiles of alcohol consumption (among non-abstainers): lowest quartile: average of 0.5-1 drinks per week (N = 635); 2nd quartile: 2 drinks per week (N = 441); 3rd quartile: 3-6 drink per week (N = 594); highest quartile: >6 drinks per week (N = 536).

Using a clinical threshold of >19 U/l,[24] the prevalence of elevated ALT in the study population was 11% (N = 338) among non-diabetic women and 31% (N = 94) among diabetic women. Using a cutoff of 60 U/l to define elevated GGT,[25] the prevalence of elevated GGT was 7% (N = 215) and 16% (N = 48) among non-diabetic and diabetic women, respectively. Three percent (N = 95) of non-diabetic women and 10% (N = 32) of diabetic women had both elevated ALT and GGT.

Table 2. Partial Age-Adjusted Correlation Coefficients (*P* Values) for Liver ALT, GGT, Fasting Glucose, Fasting Insulin and Glycated Haemoglobin

	ALT	GGT	Fasting plasma glucose	Fasting plasma insulin
Women without diabetes (N = 3086)				
GGT	0.44 (<0.001)			
Fasting glucose	0.08 (<0.001)	0.10 (<0.001)		
Fasting insulin	0.20 (<0.001)	0.25 (<0.001)	0.26 (<0.001)	
HbA1c	0.07 (<0.001)	0.10 (<0.001)	0.21 (<0.001)	0.17 (<0.001)
Women with diabetes (N = 308)				
GGT	0.50 (<0.001)			
Fasting glucose	0.24 (<0.001)	0.20 (0.001)		
Fasting insulin	0.33 (<0.001)	0.20 (<0.001)	0.29 (<0.001)	
HbA1c	0.27 (<0.001)	0.25 (<0.001)	0.78 (<0.001)	0.29 (<0.001)

The age-adjusted partial correlation coefficients between ALT, GGT, fasting glucose, fasting insulin and HbA1c in women with and without diabetes are presented in Table 2. In women without diabetes the correlation between fasting glucose and HbA1c was low (partial r = 0.21) while in women with diabetes the correlation was higher (partial r = 0.78).

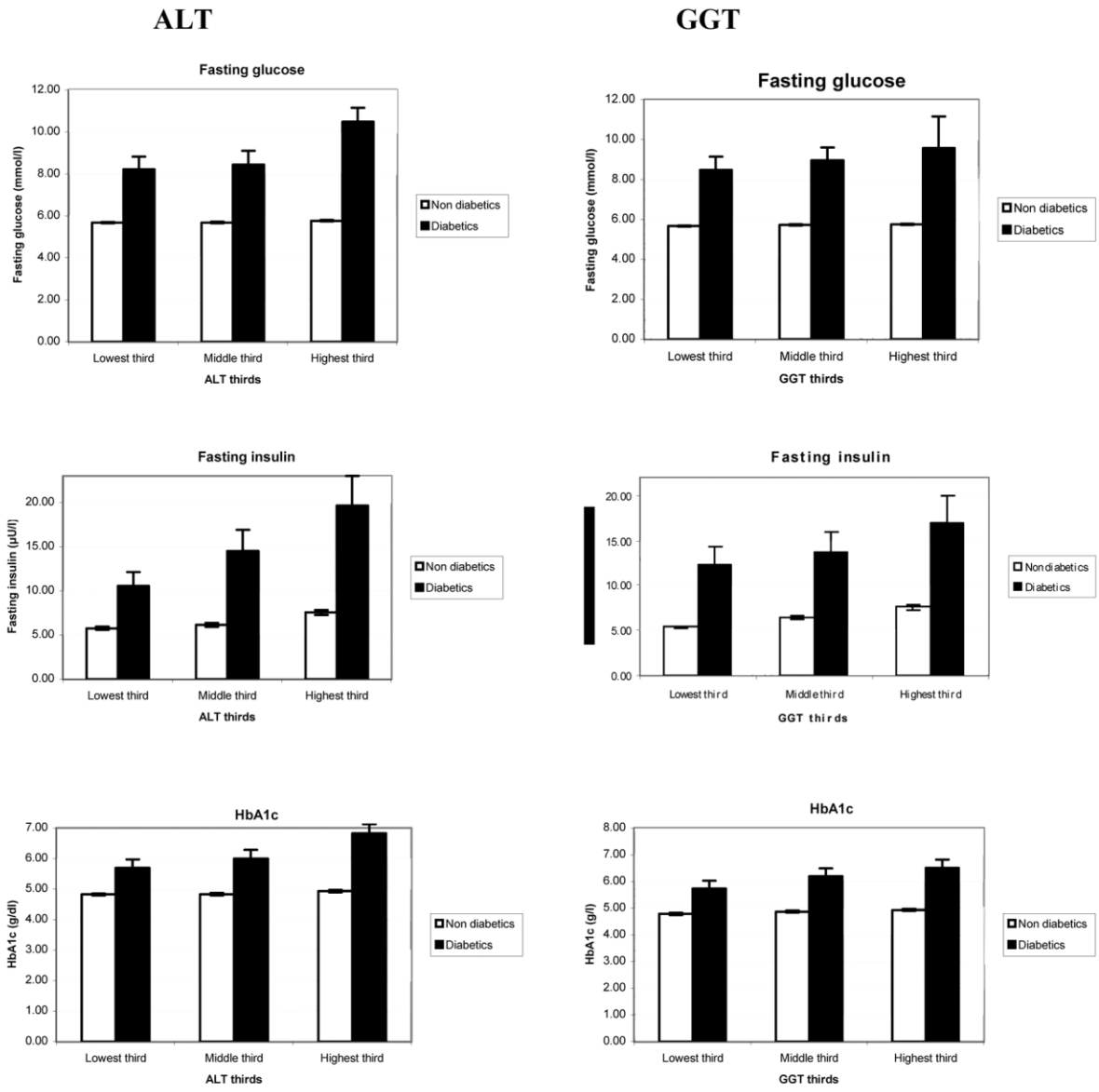
Age-adjusted mean fasting glucose, fasting insulin and HbA1c per thirds of the ALT and GGT distribution in non-diabetic and diabetic women are presented in Fig. 1. Levels of fasting insulin, fasting glucose and HbA1c increased across increasing thirds of the ALT and GGT distribution, among both non-diabetic and diabetic women (all *P* for trend <0.001).

Mean differences in standardized fasting glucose, fasting insulin, and HbA1c per increase of one standard deviation (SD) of ALT and GGT, stratified by diabetic status are presented in Table 3. In women without diabetes, ALT and GGT were positively associated with all three outcomes: fasting glucose, fasting insulin and HbA1c. However, the magnitude of the association of ALT and GGT with fasting insulin was consistently greater than with fasting glucose or HbA1c. In women with diabetes, ALT and GGT were also associated with all three outcomes. All associations were of greater magnitude in women with diabetes than in women without diabetes, except for the association between GGT and fasting insulin. For example, an increase in one SD of ALT was associated with a 0.02 SD increase in fasting glucose in women without diabetes and a 0.38 SD increase in fasting glucose in women with diabetes (Table 3, model 3). Formal evidence for interactions ($P \leq 0.001$) between ALT and diabetic status in their relation with all three outcomes was found in all models (models 1 through 3). Strong evidence ($P < 0.001$) was also found for interactions between GGT and diabetic status in their relation to fasting glucose and HbA1c (in all models). No evidence was noted for an interaction between GGT and diabetic status in their relation with fasting insulin (model 1, *P* for interaction = 0.69).

Age-adjusted associations (Table 3, model 1) were not attenuated when childhood and adult social class, smoking, duration of physical activity and alcohol consumption were entered as covariables (model 2). Adjustment for components of the metabolic syndrome and C-reactive protein (model 3) attenuated associations. The addition of terms for the use of statins, beta-blockers, ACE inhibitors, non-steroidal anti-inflammatory drugs and anti-hypertensive medication (data not shown), slightly attenuated associations further, however positive associations remained.

We repeated analyses for women without diabetes excluding women with high insulin resistance, i.e. in the highest fourth of the fasting insulin distribution (N = 632), as these women may be pre-diabetic and therefore similar to women with diabetes with regard to fasting insulin. Results were similar to those obtained for all non-diabetic women. For example, in the fully adjusted model (equivalent to model 3, Table 3), an increase of one SD of GGT was associated with a 0.08 SD increase in fasting insulin (95% CI: 0.06-0.11) and a 0.02 SD (95% CI: 0.01-0.03) increase in fasting glucose in this group of women without hyperinsulinemia.

We also analysed data on women without diabetes, stratifying by central obesity (defined by waist-to-hip ratio >0.85). In both non-obese (N=2359) and obese women (N=727), associations of ALT and GGT with fasting insulin were greater than for fasting glucose and HbA1c. For example, in the equivalent to Table 3, model 3, an increase of one SD of ALT was associated with a mean increase of 0.10 SD (95%CI: 0.07-0.13) of fasting insulin and with an increase of 0.02 SD (95%CI: 0.00-0.03) in fasting glucose in non-obese women. The magnitude of association of ALT and GGT with fasting insulin was similar in non-obese and obese women. In non-obese women a one SD increase in ALT was associated with a 0.10 SD (95% CI: 0.07-0.13) increase in fasting insulin and in obese women with an increase of 0.13 SD (95% CI: 0.07-0.19, P for interaction 0.08). An increase in one SD of GGT was associated with an increase of 0.11 SD (95% CI 0.8-0.14) and a 0.15 SD (95% CI 0.09-0.22) increase in non-obese and obese women,



* Geometric means for fasting insulin
All p for trend <0.001
Thirds of ALT in non-diabetic women: 3-11 U/L; 12-14 U/L; 15-209 U/L.
Thirds of ALT in diabetic women: 3-13 U/L; 14-19 U/L; 20-146 U/L
Thirds of GGT in non-diabetic women: 5-16 U/L; 17-25 U/L; 26-474 U/L.
Thirds of GGT in diabetic women: 7-22 U/L; 23-38 U/L; 39-288 U/L

Fig. 1. Age-adjusted mean* fasting glucose, fasting insulin and HbA1c by thirds of ALT and GGT.

respectively (*P* for interaction 0.03). Results were essentially unaltered when obesity was defined as BMI >30.

Excluding women with cardiovascular disease (N = 497 of the women without diabetes and N = 78 of the women with diabetes) from all analyses did not substantially alter results compared with those presented in Table 3. Excluding women in the top quartile of alcohol consumption (N = 492 women without diabetes and N = 42 with diabetes) and of unknown or non-white ethnic origin (N = 9 women without diabetes and N = 2 women with diabetes) also did not affect results. All analyses were also conducted using naturally logged homeostasis model assessment of insulin resistance (HOMA) calculated as the product of fasting glucose (mmol/l) and insulin (μU/ml) divided by the constant 22.5.[26] Results were essentially the same as those obtained for fasting insulin and therefore not presented.

Finally, we repeated the main analysis for women with ALT and GGT levels within the laboratory (Royal Free, London) "normal range" (ALT≥5 and ≤40 U/l; GGT

Table 3. Regression Coefficients (95%CI) of Fasting Glucose, Logged Fasting Insulin, and Glycated Haemoglobin Per Change of One Standard Deviation of Logged ALT, GGT in Non-Diabetic and Diabetic Women, British Women's Heart and Health Study (1999-2001)

	Non-diabetic women (N = 3086)			Diabetic women (N = 308)		
	Fasting glucose (SD = 1.48)	Fasting insulin (SD = 0.64)	HbA1c (SD = 0.83)	Fasting glucose (SD = 1.48)	Fasting insulin (SD = 0.64)	HbA1c (SD = 0.83)
			ALT(SD=0.41)			
Model 1	0.03 (0.02, 0.04)	0.18 (0.15,0.22)	0.06 (0.03,0.08)	0.49 (0.27,0.71)	0.36 (0.25,0.38)	0.43 (0.25,0.60)
Model 2	0.03 (0.02, 0.04)	0.19 (0.16,0.22)	0.07 (0.04,0.09)	0.52 (0.30,0.74)	0.37 (0.26,0.48)	0.44 (0.27,0.61)
Model 3	0.02 (0.01, 0.03)	0.11 (0.08,0.14)	0.04 (0.02,0.07)	0.38 (0.16,0.61)	0.30 (0.18,0.41)	0.31 (0.14,0.49)
			GGT (SD = 0.63)			
Model 1	0.03 (0.02, 0.05)	0.23 (0.20,0.26)	0.07 (0.05,0.10)	0.46 (0.20,0.72)	0.25 (0.11,0.39)	0.47 (0.27,0.67)
Model 2	0.03 (0.02, 0.04)	0.23 (0.20,0.26)	0.07 (0.04,0.09)	0.48 (0.22,0.74)	0.24 (0.10,0.38)	0.50 (0.30,0.70)
Model 3	0.02 (0.01, 0.04)	0.12 (0.10,0.15)	0.04 (0.01,0.07)	0.30 (0.02,0.58)	0.12 (-0.03,0.26)	0.32 (0.10,0.54)

Model 1 – adjusted for age.

Model 2 – model 1 plus potential confounders: childhood social class adulthood social class, smoking, physical activity, alcohol consumption.

Model 3 – model 2 plus components of the metabolic syndrome: BMI, waist: hip ratio, systolic blood pressure, high density lipoprotein cholesterol, triglycerides and C-reactive protein.

$\geq$8 and $\leq$35), as NAFLD is associated with a milder liver enzyme elevation than other potential causes of liver enzyme elevation such as alcohol induced liver disease, viral hepatitis and autoimmune liver disease.[27,28] Results for both ALT (N = 3051 women without diabetes and N = 322 women with diabetes) and GGT (N = 2516 women without diabetes and N = 213 women with diabetes) were essentially the same as those presented in Table 3, with a single exception. We did not find evidence of an association between GGT and fasting glucose in women with diabetes in this sub-sample.

Discussion

Although the association between levels of liver enzymes and measures of glucose homeostasis has been documented in several cross sectional, and prospective studies (for example, Sattar et al.[2] and Kim et al.[29]), to our knowledge this is the first study to examine these associations in detail and to compare them between diabetic and non-diabetic populations.

The magnitude of associations between ALT and GGT differed between outcomes and between women with and without diabetes. In women without diabetes, both ALT and GGT were associated with all three outcomes but to a greater magnitude with fasting insulin than with fasting glucose and HbA1c, when adjusting for potential confounders and mediating factors. In women with diabetes, both ALT and GGT were also associated with all three outcomes, and associations were stronger than in women without diabetes.

Our results show that levels of ALT and GGT are positively associated with levels of fasting insulin required to maintain normal hepatic glucose production, even in normogylcaemic women. This association was also observed in a sub-group of women without hyperinsulinemia. In diabetes, beta cell dysfunction is coupled with increasing insulin resistance for which insulin production cannot compensate. Therefore, among diabetic women fasting glucose and HbA1c are also elevated and we found that liver enzymes were more strongly associated with these measures in diabetic women in addition to being associated with fasting insulin.

The exact mechanism or mechanisms linking NAFLD and insulin resistance are not fully understood. There is evidence that hepatic fat content as determined by magnetic resonance imaging is closely correlated with fasting insulin and is a main determinant of the sensitivity of endogenous glucose production to insulin in both non-diabetics and diabetic patients.[6,30,31] A study conducted in rats has provided evidence for a causal relationship between liver fat content and hepatic insulin resistance as well as a dose-response relationship between the two.[32] Therefore NAFLD may cause hepatic insulin resistance even in the absence of peripheral insulin resistance. An alternative explanation is that NAFLD is a consequence of primary generalized insulin resistance through an increased supply of fatty acids to the liver due to peripheral insulin resistance.[33,34]

Progression towards overt type 2 diabetes occurs with deterioration in both insulin resistance and secretion (due to worsening beta cell dysfunction)[35,36] but there is evidence that once diabetes has occurred there is no further marked deterioration in insulin resistance, rather poor insulin secretion becomes the main pathological process.[37,38] This may explain our finding that the magnitude of the associations of ALT and GGT with fasting insulin were similar in non-diabetic and diabetic women, whereas the associations of ALT and GGT with glucose and

HbA1c were weaker in non-diabetic compared to diabetic women.

We also found that associations of ALT and GGT with glucose homeostasis measures were similar among obese and non-obese non-diabetic women. Although obesity is an established risk factor for NAFLD, there is also evidence of associations of the degree of liver fat content (as measured by proton spectroscopy) and fasting insulin in non-obese men.[6] Furthermore, in a small study examining the effects of weight loss in obese women (N = 23), liver fat content before weight loss was not correlated with intra-abdominal or subcutaneous fat but was correlated with the percent of fat and saturated fat of total caloric intake. The decrease in liver fat content after weight loss was also not correlated with changes in the volumes of intra-abdominal or subcutaneous fat depots.[39] Therefore, it is possible that other factors besides obesity, such as genetic factors or dietary fat intake determine liver fat content and its association with insulin resistance.

Although the cross sectional nature of this study does not allow us to determine temporal relationships between elevation of liver enzymes and measures of glucose homeostasis, our decision to look at liver enzymes as the explanatory variables and glucose homeostasis measures as outcome variables was based on data from prospective studies showing that levels of liver enzymes predict diabetes risk.[2,3]

Another limitation of this study is our inability to differentiate between hepatic and peripheral insulin resistance. In a study of non-obese, non-diabetic biopsy proven NAFLD patients and age and body composition matched controls, NAFLD was associated with insulin resistance determined by euglycaemic clamp and involved both the liver and peripheral tissues.[40] However, the association between levels of liver enzymes and fasting insulin observed here among normoglycaemic women without hyperinsulinemia suggests that both elevation of liver enzymes, as well as hepatic insulin resistance (as measured by fasting insulin or HOMA[7]) occur in the early stages of the development of insulin resistance and diabetes. Similarly, a prospective study has shown that elevation of liver enzymes occurs before hyperglycaemia.[41]

Prospective studies with repeated measures of both liver enzymes, or preferably using imaging techniques to diagnose NAFLD, and euglycaemic clamp determined glucose homeostasis measures with separate liver specific and peripheral tissue measurements could shed light on the temporal relations between elevation of liver enzymes and the development of hepatic and peripheral insulin resistance.

This study included women only and therefore its results cannot necessarily be generalized to men, particularly as differences in the associations of liver enzymes and diabetes by gender, have been demonstrated.[42] An additional limitation is that data on potential causes of liver enzymes elevation such as viral hepatitis status and iron overload were not available in this study. However the prevalence of both these conditions are extremely rare in older British women.[43-45] In addition, when we limited the analysis to women with ALT and GGT within the normal range for the laboratory that completed the assay (as NAFLD is associated with more modest elevation than other causes of liver disease), results were essentially the same as those presented.

In conclusion, associations of ALT and GGT with fasting glucose and HbA1c and of ALT with fasting insulin (and HOMA) are stronger in women with diabetes compared to women without diabetes, whereas GGT is associated with fasting insulin (and HOMA) to the same extent in all women, irrespective of diabetes status. Associations of liver enzymes with glucose homeostasis measures are observed in both obese and non-obese women without diabetes and suggest that additional factors may determine liver fat content. These findings highlight the liver's involvement in insulin resistance in the general population.

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