

Prevalence of and Risk Factors for Hepatic Steatosis and Nonalcoholic Fatty Liver Disease in People With Type 2 Diabetes: the Edinburgh Type 2 Diabetes Study

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OBJECTIVE—Type 2 diabetes is an established risk factor for development of hepatic steatosis and nonalcoholic fatty liver disease (NAFLD). We aimed to determine the prevalence and clinical correlates of these conditions in a large cohort of people with type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 939 participants, aged 61–76 years, from the Edinburgh Type 2 Diabetes Study (ET2DS)—a large, randomly selected population of people with type 2 diabetes—underwent liver ultrasonography. Ultrasound gradings of steatosis were compared with magnetic resonance spectroscopy in a subgroup. NAFLD was defined as hepatic steatosis in the absence of a secondary cause (screened by questionnaire assessing alcohol and hepatotoxic medication use, plasma hepatitis serology, autoantibodies and ferritin, and record linkage to determine prior diagnoses of liver disease). Binary logistic regression was used to analyze independent associations of characteristics with NAFLD.

RESULTS—Hepatic steatosis was present in 56.9% of participants. After excluding those with a secondary cause for steatosis, the prevalence of NAFLD in the study population was 42.6%. Independent predictors of NAFLD were BMI, lesser duration of diabetes, HbA_{1c}, triglycerides, and metformin use. These remained unchanged after exclusion of participants with evidence of hepatic fibrosis from the group with no hepatic steatosis.

CONCLUSIONS—Prevalences of hepatic steatosis and NAFLD were high in this unselected population of older people with type 2 diabetes, but lower than in studies in which ultrasound gradings were not compared with a gold standard. Associations with features of the metabolic syndrome could be used to target screening for this condition.

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Nonalcoholic fatty liver disease (NAFLD), defined as hepatic steatosis in the absence of a secondary cause, is the commonest cause of liver disease in westernized countries, affecting up to 33% of the general population (1,2) and up to 75% in some subgroups such as obese patients (3). An association between NAFLD and type 2 diabetes is well established. Diabetes has been

shown to be a risk factor for the development of NAFLD and its progression to more advanced liver disease including fibrosis, cirrhosis, and hepatocellular carcinoma (4,5). Furthermore, data suggest that some classes of antidiabetic agents (6,7) may be used as treatments in NAFLD.

Despite the recognized association between type 2 diabetes and NAFLD, few large-scale studies of the prevalence of NAFLD in unselected populations of people with type 2 diabetes are available. Studies estimating the prevalence using liver ultrasound have been performed in secondary care rather than community setting and have generally examined small numbers of patients (8). A large study in Italian outpatients with type 2 diabetes reported that 85% had hepatic steatosis and that 70% met the criteria for NAFLD (defined in that study as hepatic steatosis without evidence of excess alcohol consumption, viral hepatitis, or causative medications) (9). This study, however, was confined to patients attending a hospital clinic and also failed to systematically identify and exclude participants with less common causes of liver disease such as autoimmune hepatitis. Furthermore, ultrasound measurements were not compared with a gold standard in the population studied—an important factor given the variable sensitivity and specificity of ultrasound assessment for hepatic steatosis (10).

Given the rising incidence and prevalence of type 2 diabetes, an accurate estimate of the prevalence of NAFLD, as well as its clinical correlates, is important to predict the number of patients who will require to be monitored for more advanced liver disease or who may benefit from future disease-modifying agents. The aim of the current study was to determine the prevalence of NAFLD in a large, randomly selected population of older people with type 2 diabetes, using magnetic resonance spectroscopy (MRS)

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to confirm ultrasound grading classifications and thorough screening for secondary causes of liver disease, and to examine the correlation of NAFLD with clinical and biochemical characteristics.

RESEARCH DESIGN AND METHODS

The recruitment and baseline examination of subjects for the Edinburgh Type 2 Diabetes Study (ET2DS) have been described previously (11). Briefly, subjects recorded as having type 2 diabetes and aged 60–74 years were selected at random by sex and 5-year age bands in years 2006–2007 from the Lothian Diabetes Register. This is a comprehensive database of people with type 2 diabetes living in Lothian, a region in the south-east of Scotland that includes the city of Edinburgh and its surrounding towns and countryside, including both patients attending a hospital clinic and those managed solely in primary care. Study participants ($n = 1,066$) have been shown previously to be representative, in terms of age, HbA_{1c}, duration of diabetes, insulin treatment, and total cholesterol, of all those randomly selected to participate ($n = 5,454$) and therefore of the target population of older men and women with type 2 diabetes living in the general population (12). At the time of recruitment into the study, participants attended a baseline examination that included measurement of demographic and anthropometric variables (age, sex, BMI, and waist circumference), plasma HbA_{1c}, total cholesterol, platelets, and hyaluronic acid. One year after baseline examination, 1,054 participants were invited to attend a year 1 clinic for assessment of liver structure and function. A total of 12 participants were not invited due to withdrawal ($n = 2$), refusal of consent for contact ($n = 5$), unsuitable for contact ($n = 3$), or death ($n = 2$). A total of 939 subjects (88% of the original cohort) participated in the year 1 clinic. Of those subjects invited but who did not attend the year 1 clinics ($n = 114$), 19 were uncontactable, 61 unable or unwilling to attend, 21 repeatedly cancelled or failed to attend appointments, and 13 had died.

The ET2DS was approved by the local research ethics committee, and all participants gave written informed consent for baseline and year 1 examinations.

Ultrasound examination and comparison with MRS

Subjects attended the year 1 research clinic after a 4-h fast for an ultrasound

examination of abdomen. All ultrasound examinations were performed by a single ultrasonographer, who was unaware of the clinical and laboratory results of the participants, using a Sonoline Elegra Ultrasound Imaging System (Siemens Medical Systems, Issaquah, WA), software version 6, with a 3.5-MHz transducer. The liver was graded for markers of hepatic steatosis using established criteria (13–15) including a bright hepatic echo pattern (compared with the echo response of the right kidney), increased attenuation of the echo beam, and the presence of focal fatty sparing. Participants were given an overall liver grading based on a subjective measurement of the severity of steatosis: grade 0, normal appearance of liver on ultrasound and initially graded as a “normal ultrasound”; grade 1, possible slight increase in echogenicity or slightly impaired visualization of the diaphragm or intrahepatic vessels, or difficulty in grading as a result of a diseased or absent right kidney—initially termed an “indeterminate ultrasound”; grade 2, definite increase in echogenicity and/or definite impaired visualization of the intrahepatic vessels and diaphragm, no or little evidence of focal fatty sparing, initially graded as “evidence of mild steatosis on ultrasound”; grade 3, marked increase in echogenicity and/or poor or no visualization of the diaphragm and intrahepatic vessels, with or without focal fatty sparing, initially graded as “evidence of severe steatosis on ultrasound.” Evidence of hepatic cirrhosis was also sought systematically.

Comparison of the ultrasound gradings for hepatic steatosis with ¹H MRS, the noninvasive gold standard for quantification of hepatic fat, in a subgroup of 58 participants has previously been described in detail (16). In brief, 17 participants with a grade 0 ultrasound grading, 19 with grade 1 or 2, and 22 with grade 3 underwent MRS. A grade 0 grading on ultrasound was associated with a “normal” hepatic fat fraction on MRS of <6.1% (17,18) in 14 of 17 cases (and a fat fraction of <9% [19,20] in all cases) and was accepted as “no steatosis.” A grading of grade 3 on ultrasound was associated with and MRS hepatic fat fraction $\geq 6.1\%$ in all cases, and this was therefore taken as “definite steatosis.” The group with grade 1 or 2 on ultrasound had an MRS fat fraction of <6.1% in 13 of 19 cases, and a fat fraction of < 9% in 16 of 19 cases, thus displaying considerable overlap with those regarded as

normal. In view of this, the group with grade 1 or 2 on ultrasound scan was considered to have a “probable normal” scan. If a liver FF of $\geq 6.1\%$ on MRS was used to denote hepatic steatosis, sensitivity, specificity, and positive and negative predictive values (adjusted for the portion of the whole study cohort receiving each grading) of ultrasound in detecting “definite steatosis” were 86.8%, 100%, 100%, and 80.0%, respectively.

Clinical examination

Average alcohol intake per week over the previous year, a history of alcohol excess, and use of hepatotoxic medications within the previous 6 months were determined by questionnaire. Average alcohol intake was determined using two questions adapted from the AUDIT-C screening tool (21): “How often did you have a drink containing alcohol in the past year? Consider a “drink” to be a can or a bottle of beer, a glass of wine, or one cocktail or a measure of spirits (like scotch, gin, or vodka)” (a drink was considered to be the equivalent of one unit of alcohol); and “How many drinks did you have on a typical day when you were drinking in the last year?”

Those participants with evidence of hepatic steatosis or abnormal blood tests of liver function had further investigations performed including serology for hepatitis B and C, antinuclear antibody (ANA), antismooth muscle antibody, antimitochondrial antibody, and ferritin. All participants had brachial blood pressure, serum triglycerides, and HDL cholesterol measured.

Information on previous diagnoses of chronic liver disease was collected from participants by questionnaire at year 1 and supplemented using data on liver diagnoses, which had been obtained at baseline via record linkage to hospital discharges at the Information and Services Division (ISD) of NHS Scotland.

Definition of NAFLD

NAFLD was defined as the presence of definite hepatic steatosis on ultrasound scan (i.e., grade 3) in the absence of a secondary cause for hepatic steatosis. Secondary causes were defined as alcohol consumption ≥ 14 units/week (22) or participant report of current/previous alcohol excess; use of hepatotoxic medication (2) (glucocorticoids, isoniazid, methotrexate, amiodarone, and tamoxifen) within the 6 months prior to the year 1 clinic; positive hepatitis B or C

serology; ferritin concentration $\geq 1,000$ $\mu\text{g/L}$ (milder hyperferritinemia can be associated with obesity, insulin resistance, and NAFLD [2,23]); clinically significant positive immunology titers (antismooth muscle antibody titer $\geq 1:160$ [24] or antimitochondrial antibody titer $\geq 1:40$ [25]); or a previous diagnosis of a persistent secondary cause for chronic liver disease. Subjects were considered to have a previous diagnosis of a secondary cause for chronic liver disease if ISD linkage revealed such a diagnosis, or if a participant report of a diagnosis was confirmed by their medical records. Subjects were excluded from calculations on the prevalence of NAFLD if data on the above measures were missing such that a secondary cause could not be excluded.

Definition of metabolic syndrome

As per Adult Treatment Panel III criteria, subjects were considered to have the metabolic syndrome if, in addition to type 2 diabetes, they had at least two of the following: blood pressure $\geq 130/85$ or on antihypertensive treatment; triglycerides ≥ 1.7 mmol/L or taking a fibrate; HDL cholesterol < 1.04 mmol/L (men) or 1.29 mmol/L (women); waist circumference > 102 cm (men) or 88 cm (women).

Statistical analysis

Analysis was performed using SPSS software version 14.0. Data that did not conform to the normal distribution (duration of diabetes and triglycerides) were log-transformed prior to parametric analysis. Statistical analysis included the independent *t* test and χ^2 tests to compare characteristics between groups. Binary logistic regression analysis was used to assess the independence of variables in their association with hepatic steatosis and NAFLD. Variables included in this model were age, sex, BMI, duration of diabetes, HbA_{1c}, systolic blood pressure, HDL and LDL cholesterol, triglycerides, and the use of metformin, sulfonylureas, thiazolidinediones, incretin mimetics, and insulin. Alcohol use ≥ 14 units/week or previous alcohol misuse was used as a categorical variable in the model examining hepatic steatosis, but not that examining NAFLD. Results were reported as estimated odds ratios (ORs) and 95% CI.

RESULTS

Subject characteristics

The characteristics of 939 participants (aged 61–76 years) attending the year 1

clinic are shown in Table 1. Baseline characteristics of subjects attending the year 1 clinical examination were similar to those of the total ET2DS population suggesting that the study population attending for liver assessment remained representative of the target general population with type 2 diabetes.

Prevalence of hepatic steatosis and NAFLD

Of the 939 subjects undergoing abdominal ultrasound, data on steatosis grading was incomplete for 2 subjects, and these were excluded from the analysis. Hepatic steatosis was graded as definite (grade 3) on ultrasound examination in 56.9% ($n = 533$) of the remaining participants. The appearance of the liver was normal (grade 0) in 23.5% ($n = 220$) of participants. Twenty-two subjects (2.3%) were graded as grade 1 (in three of these cases, it was commented that the liver was difficult to scan as it was located high up under the costal margin) and a further 16.9% ($n = 158$) were graded as grade 2, giving a total of 180 subjects (19.2%) who were classified as “probable normal.” In addition, 0.4% subjects ($n = 4$) had a liver in which ultrasound identified cirrhosis—steatosis gradings in these subjects were grade 0 in two participants, grade 1 in one participant, and grade 2 in one participant.

Among those with evidence of definite hepatic steatosis (grade 3), 123 subjects had evidence of one or more secondary causes for steatosis: 78 had alcohol intake ≥ 14 units/week or a history of excess; 40 had used a hepatotoxic medication, as outlined above, in the previous 6 months; 1 each had serological evidence of hepatitis B and C; 3 had ferritin levels $\geq 1,000$ $\mu\text{g/L}$; 1 had antismooth muscle antibody titer $\geq 1:160$; 1 had antimitochondrial antibody titer $\geq 1:40$; and 7 had previously diagnosed liver disease (2 had alcoholic liver disease, 1 hemochromatosis, 1 autoimmune hepatitis, 1 recurrent cholangitis, 1 biliary cirrhosis, and 1 hepatic carcinoid metastases). In a further 19 subjects data were missing such that a secondary cause could not be excluded. In addition, a further 160 participants had at least one positive immunology titer $\geq 1:40$, which was not considered significant. Of these, most had borderline ANA or antismooth muscle titers ($\leq 1:80$; $n = 120$); 40 participants had ANA titer $\geq 1:160$.

Using the predefined criteria, 391 out of 918 participants with a full dataset had definite hepatic steatosis on ultrasound grading with no secondary cause, giving a prevalence of NAFLD of 42.6% in this study population.

Table 1—Characteristics of subjects at year 1 clinic

Characteristic	Year 1
<i>n</i>	939
Age (years)	68.9 \pm 4.2
Sex, % (<i>n</i>) male	52.0 (488)
Race, % (<i>n</i>) Caucasian	98.3 (923)
BMI measured at baseline clinic (kg/m ²)	31.3 \pm 5.7
Waist circumference measured at baseline clinic (cm)	106.7 \pm 12.8
Duration of diabetes (years)	9.0 \pm 6.4
HbA _{1c} (%)	7.19 \pm 1.06
Diet controlled, % (<i>n</i>)	19.4 (182)
Oral antidiabetic agent users, % (<i>n</i>)	74.4 (699)
Insulin users, % (<i>n</i>)	15.8 (148)
Systolic blood pressure (mmHg)	138.1 \pm 18.5
Diastolic blood pressure (mmHg)	74.1 \pm 9.6
Total cholesterol (mmol/L)	4.15 \pm 0.81
HDL cholesterol (mmol/L)	1.23 \pm 0.34
LDL cholesterol (mmol/L)	2.17 \pm 0.68
Triglyceride (mmol/L)	1.66 \pm 0.90
Statin users, % (<i>n</i>)	81.6 (767)
Aspirin users, % (<i>n</i>)	67.7 (636)
ACE inhibitor users, % (<i>n</i>)	52.0 (488)
Current or ex-smokers, % (<i>n</i>)	60.0 (563)

Data are mean \pm SD or proportions in whole cohort of participants unless otherwise indicated.

Clinical and biochemical associations with hepatic steatosis and NAFLD

Physical and biochemical characteristics of study participants according to steatosis groups are shown in Table 2. In univariate analysis, participants in the group with definite steatosis (grade 3) were significantly younger and had lesser duration of diabetes than the combined normal/probably normal groups (grades 0, 1, and 2) ($P < 0.05$). BMI, waist circumference, HbA_{1c}, diastolic blood pressure, triglycerides, and prevalence of alcohol intake over 14 units/week were significantly higher, and HDL cholesterol significantly lower, in the definite steatosis group. Metformin use was more common in the definite steatosis group. No significant differences in the use of other antidiabetic agents (sulfonylureas, glitazones, incretin mimetics, or insulin) were found.

In multivariate analysis, independent predictors of definite hepatic steatosis (compared with those classed as normal/probable normal) on ultrasound scan were BMI (OR 1.07 [95% CI 1.04–1.10]), lesser duration of diabetes (OR for log duration diabetes 0.53 [0.30–0.92]), HbA_{1c} (OR 1.35 [1.15–1.59]), triglycerides (OR for log triglycerides 20.76 [0.85–50.85]), alcohol intake ≥ 14 units/week (OR 3.13 [1.80–5.43]), and use of metformin (OR 2.19 [1.59–3.00]).

Similar results were obtained when those with secondary causes for liver

disease were excluded from analysis. Independent predictors of NAFLD were BMI (OR 1.07 [95% CI 1.03–1.10]), lesser duration of diabetes (OR for log duration diabetes 0.46 [0.25–0.83]), HbA_{1c} (OR 1.29 [1.08–1.54]), triglycerides (OR for log triglycerides 17.57 [6.68–46.18]), and use of metformin (OR 2.25 [1.60–3.17]).

Of the 400 participants who had gradings of normal/probable normal liver parenchyma on ultrasound scan, 74 had at least one of the following features suggestive of possible hepatic fibrosis or cirrhosis: spleen size ≥ 13 cm (16 participants); hyaluronic acid >75 ng/mL (the upper end of normal on the laboratory reference range) in the absence of joint disease (54 participants); or platelet count $<150 \times 10^9/L$ (20 participants). If these participants were excluded from regression analysis, independent predictors of definite steatosis and NAFLD were unchanged (data not shown).

CONCLUSIONS—This is the first study to examine the prevalence of hepatic steatosis and NAFLD in a population of people with type 2 diabetes using an ultrasound classification that has been refined by comparison with MRS and with detailed exclusion of secondary causes of steatosis. The study population included the full clinical spectrum of type 2 diabetes and included people who were being managed in the community as well as in hospital clinics.

Previous studies have estimated that a hepatic fat fraction on MRS of under 6.1–9% is consistent with a normal liver, whereas hepatic steatosis is associated with higher fat fractions (17–20). Comparison of our ultrasound gradings with MRS suggested that the “definite steatosis” grading was an excellent predictor for the presence of hepatic steatosis (16). In contrast, considerable overlap was observed between those graded initially as having “indeterminate” or “mild” steatosis (grade 1 or 2) and the normal group, and it was considered that these participants probably had a normal liver. Our prevalence of hepatic steatosis, at 56.9%, was therefore considerably lower than in previous studies including that of a large Italian population of patients with type 2 diabetes, in which 85.3% were considered to have hepatic steatosis (9). In view of these findings, caution should be used with regard to the prevalences reported by previous studies that have used ultrasound measurements that have been less rigorously corroborated for a diagnosis of hepatic steatosis.

NAFLD was the most common cause for steatosis, accounting for 76% of all cases. In comparison with previous studies, our analysis had the advantage of a more systematic identification and exclusion of secondary causes of liver disease in those with steatosis, both by the use of ISD linkage to identify previously diagnosed chronic liver disease and by the routine measurement of autoantibody

Table 2—Comparison of participant characteristics across gradings of steatosis

Characteristic	Steatosis grade			
	Grade 0 (n = 220)	Grade 1 (n = 22)	Grade 2 (n = 158)	Grade 3 (n = 533)
Age (years)	69.4 \pm 4.2	68.5 \pm 4.8	69.5 \pm 4.4	68.5 \pm 4.0*
Sex, % (n) male	59.9 (132)	40.9 (9)	49.4 (78)	50.1 (267)
BMI measured at baseline clinic (kg/m ²)	28.8 \pm 5.18	35.1 \pm 8.4	30.7 \pm 4.9	32.4 \pm 5.5*
Waist circumference (cm)	102.4 \pm 13.5	112.6 \pm 18.7	104.7 \pm 11.6	108.9 \pm 12.0*
Duration of diabetes (years)	9.6 \pm 7.6	10.5 \pm 6.0	9.8 \pm 6.5	8.4 \pm 5.8*
HbA _{1c} (%)	7.02 \pm 0.97	7.15 \pm 0.93	6.99 \pm 0.98	7.33 \pm 1.12*
Systolic blood pressure (mmHg)	140.1 \pm 21.5	141.2 \pm 22.4	135.9 \pm 18.6	137.9 \pm 16.7
Diastolic blood pressure (mmHg)	73.4 \pm 9.5	76.3 \pm 11.2	72.1 \pm 9.5	74.9 \pm 9.4*
Total cholesterol (mmol/L)	4.14 \pm 0.81	4.53 \pm 0.81	4.01 \pm 0.75	4.17 \pm 0.82
HDL cholesterol (mmol/L)	1.28 \pm 0.69	1.16 \pm 0.27	1.30 \pm 0.35	1.19 \pm 0.32*
LDL cholesterol (mmol/L)	2.24 \pm 0.70	2.57 \pm 0.72	2.08 \pm 0.65	2.14 \pm 0.67
Triglyceride (mmol/L)	1.37 \pm 0.69	1.76 \pm 0.67	1.37 \pm 0.63	1.86 \pm 1.00*
Metabolic syndrome, % (n) present	70.2 (153)	86.4 (19)	78.3 (123)	91.2 (485)*
Alcohol intake, % (n) over 14 units/week	6.4 (14)	0.0 (0)	7.6 (12)	12.9 (69)*
Metformin use, % (n)	48.2 (106)	50.0 (11)	63.3 (100)	70.9 (378)*

Data are mean \pm SD unless otherwise indicated. *Significant difference grade 3 vs. grade 0/1/2 by t test or χ^2 test, $P < 0.05$.

titers and ferritin in all participants with steatosis. The use of these additional measures within the group with steatosis identified four patients with likely hemochromatosis, two with likely autoimmune hepatitis, two with primary biliary cirrhosis, one with recurrent cholangitis, and one with hepatic carcinoma metastases; these accounted for 1.9% of this group. The systemic identification and exclusion of subjects with any possible secondary cause of steatosis from the diagnosis of NAFLD may have caused an underestimation of the prevalence of NAFLD, which may in some cases have been a coexistent pathology. It is recognized that the cutoffs used to define exclusions to the diagnosis of NAFLD are, to some extent, arbitrary and the prevalence will depend upon the precise definition used.

The majority of exclusions from the diagnosis of NAFLD were on the basis of excess alcohol intake. It is recognized that estimation of alcohol consumption can be unreliable, and in particular that subjects and investigators may underestimate intake. This, in turn, can lead to overestimation of the prevalence of NAFLD. The prevalence of alcohol intake ≥ 14 units/week as a secondary cause for steatosis was relatively low in our study population (14.6%) when compared with a similar age band in the general population of England, in which the prevalence of alcohol intake ≥ 21 units/week for men and ≥ 14 units/week for women has been quoted as 23% and 10%, respectively (26). While this may represent an underestimation of intake, it is also possible that it reflects genuinely lower alcohol consumption in a frailer population in which use of multiple prescription medications is not uncommon.

Subjects in this study were all aged 61–76 years at the time of examination and were predominantly Caucasian. Previous studies have shown that the prevalence of NAFLD increases with age (although we did not find evidence of that within our age range), and therefore it is possible that the prevalence of NAFLD in our study population was greater than that of the general diabetic population. The results may be less applicable to populations in other countries, particularly those in which the prevalence of other causes of liver disease (such as alcoholic liver disease and viral hepatitis) is markedly different to that in the U.K.

It has previously been shown that NAFLD is associated with features of the

metabolic syndrome within the general population, and the same was true in this population of people with type 2 diabetes. The association of a shorter duration of diabetes with liver disease has been described before. One possible explanation is that the greater degree of hyperinsulinemia in early type 2 diabetes drives uptake of free fatty acids by hepatocytes. Unexpectedly, the use of metformin was associated with the presence of NAFLD, independently of BMI and glycemic control. Previous studies of the use of metformin in NAFLD have shown a positive or neutral effect in individuals (6), and it therefore seems unlikely that there is a causative link between metformin use and NAFLD here. It is possible that those participants who were on metformin had other risk factors for NAFLD that were not accounted for in the present analysis, such as inflammatory markers. Furthermore, it is possible that results were confounded by indication, i.e., participants may have been previously prescribed metformin in an attempt to treat hepatic steatosis. Finally, it is possible that the significant association between metformin and steatosis was a consequence of a type 1 statistical error.

It is acknowledged that while data on most variables were gathered concomitantly with hepatic ultrasound examination, BMI was calculated 1 year previously during the baseline examination. It is therefore possible that the results of the regression analysis are less robust than they would have been with fully contemporaneous data collection. However, changes in BMI over 1 year would in most cases have been small and randomly distributed throughout the study population, and this should not have substantially affected the results of analysis.

Another limitation of this study is that subjects did not have a liver biopsy and histological examination, the gold standard technique for identifying steatosis; performance of this invasive procedure would have been neither feasible nor ethical in a population study of this magnitude. It was recognized that some participants with a normal ultrasound scan could have undiagnosed hepatic fibrosis and thus be at the severe end of the spectrum of NAFLD. Of note, any misclassified cases would tend to underestimate the prevalence of NAFLD and reduce rather than magnify any differences in clinical associations between groups. Furthermore, reanalysis excluding those participants with a normal liver

ultrasound scan but other evidence of possible fibrosis revealed similar results to the main analysis. A further limitation is that only approximately one-fifth of patients invited to attend the baseline clinic from the Lothian Diabetes Register did so. Significantly, however, analysis revealed that this population was representative of that invited in terms of duration of diabetes, HbA_{1c}, and treatment with insulin.

In conclusion, in assessing the prevalence of NAFLD in people with type 2 diabetes, this study has advantages of robust ultrasound gradings, systematic exclusion of other causes for liver disease, and a relatively large, unselected population of older individuals. In the future, it is possible that the association of liver disease with other features of the metabolic syndrome could be used to target screening for NAFLD. Further research is required to ascertain whether the risk of progression of NAFLD to cirrhosis in patients with type 2 diabetes in the clinical setting is as high as that predicted—if so, this would provide further impetus toward early diagnosis so that they might be eligible for entry into clinical trials, screening for complications, and ultimately new therapies.

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R.M.W. designed the study, collected and analyzed data, and wrote the manuscript. J.F.P. designed the study, analyzed data, and edited the manuscript. S.G. designed the study, collected data, and edited the manuscript. E.P. designed the study, collected data, and edited the manuscript. L.D.N. collected data and edited the manuscript. P.C.H. designed

the study and edited the manuscript. B.M.F. edited the manuscript. L.A.F.V.L. collected data and edited the manuscript. G.I.J. designed the study and edited the manuscript. R.M.R. designed the study and edited the manuscript. M.W.J.S. was principal investigator, designed the study, analyzed data, and edited the manuscript.

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References

- Ryan CK, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl* 2002;8:1114–1122
- Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002;346:1221–1231
- Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;132:112–117
- Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999;30:1356–1362
- El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004;126:460–468
- Bugianesi E, Gentilcore E, Manini R, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005;100:1082–1090
- Belfort R, Harrison SA, Brown K, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355:2297–2307
- Gupte P, Amarapurkar D, Agal S, et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 2004;19:854–858
- Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 2007;30:1212–1218
- Fabbrini E, Conte C, Magkos F. Methods for assessing intrahepatic fat content and steatosis. *Curr Opin Clin Nutr Metab Care* 2009;12:474–481
- Price JF, Reynolds RM, Mitchell RJ, et al. The Edinburgh Type 2 Diabetes Study: study protocol. *BMC Endocr Disord* 2008;8:18
- Marioni RE, Strachan MW, Reynolds RM, et al. Association between raised inflammatory markers and cognitive decline in elderly people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes* 2010;59:710–713
- Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. *Clin Radiol* 1991;43:26–31
- Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? *Eur J Gastroenterol Hepatol* 2003;15:539–543
- Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002;123:1705–1725
- Williamson RM, Perry E, Glancy S, et al. The use of ultrasound to diagnose hepatic steatosis in type 2 diabetes: intra- and interobserver variability and comparison with magnetic resonance spectroscopy. *Clin Radiol* 2011;66:434–439
- Longo R, Pollesello P, Ricci C, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. *J Magn Reson Imaging* 1995;5:281–285
- Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40:1387–1395
- Fishbein M, Castro F, Cheruku S, et al. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. *J Clin Gastroenterol* 2005;39:619–625
- Fishbein MH, Gardner KG, Potter CJ, Schmalbrock P, Smith MA. Introduction of fast MR imaging in the assessment of hepatic steatosis. *Magn Reson Imaging* 1997;15:287–293
- Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT Alcohol Consumption Questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. *Arch Intern Med* 1998;158:1789–1795
- Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003;37:1202–1219
- Trombini P, Piperno A. Ferritin, metabolic syndrome and NAFLD: elective attractions and dangerous liaisons. *J Hepatol* 2007;46:549–552
- Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929–938
- Crosignani A, Battezzati PM, Invernizzi P, Selmi C, Prina E, Podda M. Clinical features and management of primary biliary cirrhosis. *World J Gastroenterol* 2008;14:3313–3327
- The Information Centre for Health and Social Care. Statistics on alcohol: England, 2010 [article online], 2010. Available from www.ic.nhs.uk/pubs/alcohol10. Accessed 8 April 2011