Diabetologia (2008) 51:444–450
DOI 10.1007/s00125-007-0897-4

ARTICLE

Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients

G. Targher · L. Bertolini · S. Rodella · G. Zoppini · G. Lippi · C. Day · M. Muggeo

Received: 25 September 2007 / Accepted: 12 November 2007 / Published online: 6 December 2007

Abstract
Aims/hypothesis Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiovascular disease in type 2 diabetes. Currently, there is a lack of information on associations between NAFLD and microvascular complications of diabetes. We assessed the associations between NAFLD and both chronic kidney disease (CKD) and retinopathy in a large cohort of type 2 diabetic individuals using a cross-sectional design.

Methods Prevalence rates of retinopathy (by ophthalmoscopy) and CKD (defined as overt proteinuria and/or estimated GFR ≤60 ml min^{-1} 1.73 m^{-2}) were assessed in 2,103 type 2 diabetic individuals who were free of diagnosed cardiovascular disease and viral hepatitis. NAFLD was ascertained by patient history, blood sampling and liver ultrasound.
Results NAFLD patients had higher ($p<0.001$) age- and sex-adjusted prevalence rates of both non-proliferative (39 vs 34%) and proliferative/laser-treated retinopathy (11 vs 5%), and CKD (15 vs 9%) than counterparts without NAFLD. In logistic regression analysis, NAFLD was associated with increased rates of CKD (odds ratio 1.87; 95% CI 1.3–4.1, $p=0.020$) and proliferative/laser-treated retinopathy (odds ratio 1.75; 1.1–3.7, $p=0.031$) independently of age, sex, BMI, waist circumference, hypertension, diabetes duration, HbA_{1c}, lipids, smoking status and medications use.
Conclusions/interpretation Our findings suggest that NAFLD is associated with an increased prevalence of CKD and proliferative/laser-treated retinopathy in type 2 diabetic individuals independently of numerous baseline confounding factors. Further studies are required to confirm the reproducibility of these results and to evaluate whether NAFLD contributes to the development or progression of CKD and retinopathy.

Keywords Chronic kidney disease · CKD · Liver disease · Liver fat · Metabolic syndrome · Microvascular complications · NAFLD · Non-alcoholic fatty liver disease · Retinopathy · Type 2 diabetes

Abbreviations

ACR	albumin/creatinine ratio
ALT	alanine aminotransferase
CKD	chronic kidney disease

G. Targher · L. Bertolini
Department of Internal Medicine,
Sacro Cuore Hospital,
Negrar (VR), Italy

S. Rodella
Department of Radiology,
Sacro Cuore Hospital,
Negrar (VR), Italy

G. Targher (✉) · G. Zoppini · M. Muggeo
Division of Endocrinology,
Department of Biomedical and Surgical Sciences,
University of Verona, Ospedale Civile Maggiore,
Piazzale Stefani, 1,
37126 Verona, Italy
e-mail: giovanni.targher@univr.it

G. Lippi
Section of Clinical Chemistry,
Department of Biomedical and Morphological Sciences,
University of Verona,
Verona, Italy

C. Day
Institute of Cellular Medicine, Newcastle University,
Newcastle upon Tyne, UK

CVD	cardiovascular disease
GGT	γ-glutamyltransferase
MDRD	modification of diet in renal disease
NAFLD	non-alcoholic fatty liver disease
OR	odds ratio

Introduction

Non-alcoholic fatty liver disease (NAFLD), in its whole spectrum of disease ranging from simple steatosis to steatohepatitis and cirrhosis, is the most common cause of abnormal liver function tests among adults in Western countries [1–4]. The prevalence of NAFLD has been reported to be in the 15 to 30% range in the general population in various countries [5–7] and is almost certainly increasing. Accordingly, a huge number of individuals are at risk of developing advanced chronic liver disease.

Compared with non-diabetic individuals, people with type 2 diabetes appear to be at increased risk of developing NAFLD and certainly have a higher risk of developing fibrosis and cirrhosis [1–4]. It has been reported that ~70 to 75% of type 2 diabetic patients have some form of NAFLD [8, 9].

There is now growing evidence that NAFLD, especially in the type 2 diabetic population, may be linked to an increased risk of developing cardiovascular disease (CVD) independently of other known risk factors [9–13]. It suggests that the identification of NAFLD in patients with type 2 diabetes may help predict CVD risk, with important management implications. Identifying people with NAFLD would highlight a subgroup of type 2 diabetic patients who should be targeted with more intensive therapy to decrease their risk of future CVD events.

To our knowledge, there is currently a lack of information on associations between NAFLD and microvascular chronic complications such as retinopathy and chronic kidney disease (CKD) in people with type 2 diabetes. Both of these chronic complications represent a major problem for patients and healthcare systems. Moreover, the possible impact of NAFLD on risk of these complications deserves particular attention in view of the implications for screening/surveillance strategies in the growing number of NAFLD patients.

We have assessed whether NAFLD, as diagnosed by ultrasound, the most widely used imaging test for detecting hepatic steatosis, is associated with an increased risk of prevalent retinopathy and CKD in a large cohort of type 2 diabetic adults using a cross-sectional design.

Methods

Participants Study participants were recruited from the Valpolicella Heart Diabetes Study cohort, a prospective observational study designed primarily to evaluate associations between type 2 diabetes and incidence of chronic vascular complications. Details on the study design and recruitment methods have been published elsewhere [11, 12]. Briefly, we enrolled all of the outpatients with type 2 diabetes (n=2,103; 66.3% of the whole sample of patients who attended the clinic) who regularly attended our clinic in the period January to December 2000 and who did not have any clinical evidence of cancer, cirrhosis or other secondary causes of chronic liver disease (alcohol abuse, viral hepatitis, autoimmune hepatitis, hepato-toxic medications such as amiodarone, glucocorticoids, barbiturates, tamoxifen and other chemotherapics) and were also free of diagnosed CVD (angina, myocardial infarction, ischaemic stroke, recurrent transient ischaemic attacks, lower extremity amputation, claudication, carotid stenosis >60% or prior coronary/peripheral revascularisation procedures as ascertained by patient history and examination, chart review, electrocardiograms and echo–Doppler scanning of carotid and lower limb arteries) [11, 12]. This cohort of patients was comparable for main demographic variables with the whole sample of patients who regularly attended our clinic. All participants gave their informed consent. The local ethics committee approved the study protocol.

Clinical measurements and laboratory procedures BMI was calculated by dividing weight in kilograms by height in metres squared. Waist circumference was measured at the level of the umbilicus. Blood pressure was assessed in triplicate with a standard mercury manometer. Information on daily alcohol consumption, smoking status and current use of medications was obtained from all participants by questionnaire [11]. Most participants were abstainers (76.6%) or drank minimally (alcohol consumption <20 g/day; 13.2% of total); only 10.2% (n=215) of participants drank moderately (alcohol consumption ranging from 20 to 60 g/day).

Venous blood was drawn in the morning after an overnight fast. Serum liver enzymes, creatinine and other biochemical blood measurements were determined by standard laboratory procedures (DAX 96; Bayer Diagnostics, Milan, Italy). Most participants had serum liver enzymes within the reference ranges in our laboratory, which for aspartate aminotransferase, alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT) activities were 10 to 35 U/l for women and 10 to 50 U/l for men, respectively. It is known that diabetic patients with NAFLD can have raised or normal serum liver enzymes; aminotransferases fluctuate over time and they may be in the reference range, irrespective of steatosis, when a single measurement is used [1–3]. No participants had seropositivity for viral hepatitis B and C. LDL-cholesterol was calculated by Friedewald's equation [i.e. (total cholesterol – HDL-cholesterol – triacylglycerol)/5]. HbA_{1c} was measured using a high-performance liquid

chromatography analyser (HA-8140; Menarini Diagnostics, Florence, Italy); the upper limit of normal for the laboratory was 5.9%. Because a number of factors such as age, ethnicity and sex can influence serum creatinine concentrations, the level of kidney function was defined by estimated GFR using the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) study [14]. The MDRD formula is as follows: estimated GFR = $186.3 \times (\text{serum creatinine}^{-1.154}) \times (\text{age}^{-0.203}) \times 1.212$ (if black) $\times 0.742$ (if female). Urinary AER was measured from an early morning urine sample as the albumin/creatinine ratio (ACR) by an immuno-nephelometric method; microalbuminuria and macro-albuminuria (overt proteinuria) were defined as ACR >2.5 and >30 mg/mmol for men and ACR >3.5 and >30 mg/mmol for women, respectively [15]. For this study, CKD was defined as overt proteinuria and/or estimated GFR ≤60 ml min^{-1} 1.73 m^{-2} [14]. Both of these outcome measures were confirmed in all participants in a least two consecutive occasions (within 3–6 months after the initial examination).

At baseline, a single ophthalmologist, who was blinded to clinical patients' characteristics, diagnosed retinopathy by fundoscopy after pupillary dilation, according to a clinical disease severity scale [16, 17]. The intra-observer variability for the estimation of non-proliferative retinopathy was within 4%. Retinopathy was classified into three categories as follows: absent, non-proliferative (i.e. microaneurysms, intra-retinal haemorrhages and/or hard exudates) and proliferative/laser-treated retinopathy (new vessel formation, fibrous proliferations, vitreous haemorrhages or previous laser coagulation therapy). Proliferative retinopathy was confirmed by fundus fluorescein angiography in all participants [17]. The presence of sensory neuropathy (by biothesiometer) was not extensively recorded among the study participants.

Hepatic ultrasonography scanning was performed in all participants by an experienced radiologist, who was blinded to participants' details. The diagnosis of hepatic steatosis was made on the basis of characteristic sonographic features, i.e. evidence of diffuse hyper-echogenicity of liver relative to kidneys, ultrasound beam attenuation and poor visualisation of intra-hepatic structures [1–3]. Liver ultrasonography has a sensitivity of ~90% and a specificity of ~95% in detecting moderate and severe steatosis, but its sensitivity is reduced when hepatic fat infiltration upon liver biopsy is less than 33% [18]. A semi-quantitative sonographic scoring for the degree of hepatic steatosis was not available in our study. The intra-observer variability for the ultrasound diagnosis of hepatic steatosis was within 3% [19].

Statistical analysis Data are means±SD unless otherwise indicated. Skewed variables (serum triacylglycerol and creatinine concentrations) were logarithmically transformed to improve normality prior to analysis. Statistical analyses included the unpaired *t* test and the χ^2 test (for categorical variables). The independence of the associations of variables with prevalent retinopathy and CKD, included as the dependent variables, was assessed by multivariable logistic regression analyses and expressed as odds ratios (OR). In these analyses, men and women were combined and first-order interaction terms for sex × NAFLD interactions on risk for retinopathy and CKD were examined. Because the interactions were not statistically significant, sex-pooled multivariable logistic regression analysis was used to assess the independence of the association of NAFLD with retinopathy or CKD, respectively. Retinopathy was categorised as absent, non-proliferative or proliferative/laser-treated, whereas CKD was defined as presence of overt proteinuria and/or estimated GFR ≤60 ml min^{-1} 1.73 m^{-2} [14]. We performed two multivariable logistic regression models: (1) model 1, in which ultrasound-diagnosed NAFLD, sex, age, BMI, waist circumference, smoking status, LDL-cholesterol, triacylglycerol, HbA_{1c}, diabetes duration and medications use (hypoglycaemic, lipid-lowering, anti-hypertensive or anti-platelet drugs) were included as covariates; and (2) model 2, which additionally adjusted for hypertension (blood pressure ≥130/85 mmHg or being treated for hypertension) and presence of CKD or retinopathy, respectively. In light of the well-known association between alcohol drinking and liver injury [1–3], we repeated the analyses described above after excluding participants who were light to moderate drinkers (n=492, 23.4%). Values at $p<0.05$ were considered to be statistically significant.

Results

Among the 2,103 study participants, 284 (13.5%) had CKD defined as either overt proteinuria or estimated GFR ≤60 ml min^{-1} 1.73 m^{-2} (205 of whom had estimated GFR ≤60 ml min^{-1} 1.73 m^{-2} without overt proteinuria) and 398 (18.9%) participants had isolated microalbuminuria, whereas 987 (46.9%) participants had retinopathy (798 of whom had non-proliferative retinopathy and 189 had proliferative/laser-treated retinopathy). The prevalence rates of non-proliferative and proliferative/laser-treated retinopathy and CKD were essentially similar between men and women.

The baseline characteristics of participants stratified by NAFLD status are shown in Table 1. Persons with NAFLD were older, more centrally obese, more likely to be male and had both longer diabetes duration and greater frequency of hypertension than those without NAFLD. They also had lower estimated GFR and HDL-cholesterol, and higher triacylglycerol, HbA_{1c} and liver enzymes (although the vast

Table 1 Clinical and biochemical characteristics of the diabetic cohort by NAFLD status

Variable	Without NAFLD (n=682)	With NAFLD (n=1,421)	p value
Sex (% men)	59	63	0.001
Age (years)	57±3	59±4	0.001
BMI (kg/m^2)	26±4	27±3	0.001
Waist circumference (cm)	92±11	95±13	0.001
Duration of diabetes (years)	12±3	14±3	0.001
Oral hypoglycaemic drugs (%)	60	63	0.30
Insulin only (%)	15	20	0.001
Anti-hypertensive users (%)	61	68	0.001
Aspirin users (%)	45	49	0.20
Lipid-lowering users (%)	32	35	0.50
Current smokers (%)	22	23	0.70
Systolic blood pressure (mmHg)	126±12	129±14	0.001
Diastolic blood pressure (mmHg)	80±11	82±13	0.001
HbA_{1c} (%)	6.9±1.0	7.2±0.9	0.001
Triacylglycerol (mmol/l)	0.92 (0.4–2.5)	1.31 (0.6–3.6)	0.001
HDL-cholesterol (mmol/l)	1.44±0.4	1.39±0.3	0.001
LDL-cholesterol (mmol/l)	3.35±0.5	3.34±0.4	0.70
e-GFR (ml min^{-1} 1.73 m^{-2})	96±31	91±32	0.001
AST (U/l)	18±4	23±9	0.001
ALT (U/l)	20±4	25±11	0.001
GGT (U/l)	20±5	27±10	0.001
Elevated ALT[a] (%)	0	12	0.001
Non-proliferative retinopathy (%)	34	39	0.001
Proliferative/laser-treated retinopathy (%)	5	11	0.001
Microalbuminuria alone (%)	15	20	0.001
CKD (%)	9	15	0.001

Cohort size: n=2,103
Data are means±SD or frequencies, except for skewed variables (triacylglycerol), which are expressed as medians and range values
Differences were assessed by the unpaired t test (for normally distributed variables) and by the χ^2 test (for categorical variables)
[a] Men >50 U/l; women >35 U/l
AST, aspartate aminotransferase; e-GFR, estimated GFR

majority of NAFLD patients, i.e. ~88%, had serum ALT activity concentrations within the reference range). Moreover, the proportion using insulin or anti-hypertensive drugs was higher in NAFLD patients, whereas the proportion using aspirin, statins or fibrates was essentially similar in both groups. Smoking status and LDL-cholesterol did not significantly differ between the groups.

Notably, as shown in Table 1, participants with NAFLD had higher age- and sex-adjusted prevalence rates of non-proliferative and proliferative/laser-treated retinopathy, and higher isolated microalbuminuria and CKD than their counterparts without NAFLD.

In univariate logistic regression analysis (Table 2), NAFLD was associated with increased prevalence rates of retinopathy or CKD (p<0.001). Male sex, older age, hypertension, HbA_{1c}, diabetes duration, diabetes treatment (insulin), estimated GFR (inversely), triacylglycerol, LDL-cholesterol, liver enzymes (ALT and GGT), smoking (only for nephropathy) were also significantly associated (p< 0.001 for all) with increased rates of retinopathy or CKD (not shown). Presence of CKD was also closely associated with a greater prevalence of proliferative/laser-treated retinopathy (p<0.001).

In multivariate logistic regression analysis, the significant association of NAFLD with non-proliferative (p<0.01) and proliferative/laser-treated (p<0.001) retinopathy was only slightly weakened after adjustment for age, sex, BMI, waist circumference, smoking, LDL-cholesterol, triacylglycerol, HbA_{1c}, diabetes duration and medications use (Table 2). After additional adjustment for hypertension and CKD (Table 2), the association between NAFLD and non-proliferative retinopathy became not significant, whereas the prevalence rate of proliferative/laser-treated retinopathy remained approximately twofold greater among those with NAFLD (p=0.031).

Similarly, the association between NAFLD and CKD (Table 2) was little affected by adjustment for age, sex, BMI, waist circumference, smoking, LDL-cholesterol, triacylglycerol, HbA_{1c}, diabetes duration and medications (p<0.001; model 1). The prevalence rate of CKD remained markedly

Table 2 Univariate and multivariate logistic regression analyses showing associations of NAFLD with prevalent retinopathy and chronic kidney disease among type 2 diabetic patients

Variable	Univariate	Multivariate model 1	Multivariate model 2
Non-proliferative retinopathy			
OR	1.6	1.5	1.19
95% CI	1.1–2.3	1.03–2.2	0.8–1.7
p value	0.001	0.01	0.50
Proliferative/laser-treated retinopathy			
OR	2.2	2.0	1.75
95% CI	1.2–4.2	1.1–4.2	1.1–3.7
p value	0.001	0.001	0.031
Chronic kidney disease			
OR	2.4	2.2	1.87
95% CI	1.6–4.7	1.3–4.5	1.3–4.1
p value	0.001	0.001	0.020

Cohort size: n=2,103

Model 1: adjustment for age, sex, BMI, waist circumference, smoking, LDL-cholesterol, triacylglycerol, HbA_{1c}, diabetes duration and medications use

Model 2: further adjustment for hypertension and chronic kidney disease (for the first and second variable of the table) or for hypertension and proliferative/laser-treated retinopathy (for the last variable of the table)

greater in patients with NAFLD after further adjustment for hypertension and proliferative/laser-treated retinopathy (p= 0.020; model 2). The results did not substantially change after exclusion of participants with isolated microalbuminuria (p=0.031; model 2; not shown).

All statistical analyses yielded qualitatively similar results in participants with microalbuminuria alone, but the association between NAFLD and isolated microalbuminuria became not significant after multiple adjustments for the confounders included in model 2.

Almost identical results were obtained in regression models that also adjusted for serum ALT or GGT activity concentrations (not shown). In univariate regression analysis, both serum ALT and GGT concentrations were significantly associated with CKD (OR 1.5, 95% CI 1.1–3.4 for ALT; and 1.4, 95% CI 1.1–3.0 for GGT, respectively) and with proliferative/laser-treated retinopathy (1.3, 1.1–2.8 for ALT; and 1.5, 1.1–3.3 for GGT, respectively). However, neither ALT nor GGT were associated with retinopathy and CKD after controlling for age, sex, diabetes duration, HbA_{1c}, medications use and components of the metabolic syndrome.

Exclusion of participants who were light to moderate drinkers did not alter the independent associations between NAFLD and increased rates of CKD (OR 2.01, 95% CI 1.2–4.8) and of proliferative/laser-treated retinopathy (OR 1.72, 1.1–4.9), respectively.

Discussion

This is the first study specifically aimed at assessing the associations of ultrasound-diagnosed NAFLD with retinopathy and CKD in a large cohort of type 2 diabetic individuals.

Our major finding was that NAFLD, as diagnosed by patient history, blood sampling and characteristic sonographic features, is closely associated with increased prevalence rates of CKD and proliferative/laser-treated retinopathy in a type 2 diabetic population. Notably, these associations appear to be independent of a broad spectrum of baseline confounding factors, such as traditional cardiovascular risk factors, diabetes duration, glycaemic control, components of the metabolic syndrome and medications use (statins, aspirin, hypoglycaemic and anti-hypertensive drugs). Additionally, our patients were free of diagnosed CVD and cirrhosis; the evaluation of patients with such complications would almost certainly have confounded interpretation of the data. Our findings are corroborated by a recent prospective study of 10,337 apparently healthy men followed for ~3.5 years, demonstrating that mildly elevated serum GGT activity concentrations (as surrogate markers of NAFLD) are associated with an increased incidence of CKD (defined as overt proteinuria and/or estimated GFR <60 ml min^{-1} 1.73 m^{-2}) independently of age, BMI, metabolic syndrome features, insulin resistance as estimated by the homeostasis model assessment, C-reactive protein, cigarette smoking and daily alcohol consumption [20].

Another major finding of our study was that more than four-fifths (~88%) of NAFLD patients had serum ALT activity concentrations within the reference range. This finding provides further evidence that ALT and other serum liver enzymes appear to be insensitive markers for NAFLD, especially in the type 2 diabetic population. Indeed, it is well known that the full histological spectrum of NAFLD may be present among patients with 'normal' serum liver enzymes, which therefore cannot be reliably used to exclude the presence of advanced NAFLD [1–4]. Our finding further supports the notion that the 'normal' reference values for serum liver enzymes currently used to exclude NAFLD need to be revised [2, 21, 22].

Clearly, we must be cautious in making any causal inference, given the cross-sectional design of the study. The underlying biological mechanisms by which NAFLD might contribute to the development or progression of CKD and retinopathy are poorly understood. The most obvious explanation for our findings is that the greater prevalence of CKD and retinopathy in NAFLD patients simply reflects the coexistence of underlying known risk factors. However, since in our study NAFLD was associated with CKD and proliferative/laser-treated retinopathy independently of a broad spectrum of established risk factors, it is conceivable that NAFLD may confer an excess risk over and above the

risk expected as a result of the underlying known risk factors. This suggests that NAFLD is not merely a marker of vascular complications of diabetes, but may also be involved in their progression. The possible molecular mediators linking NAFLD, retinopathy and CKD may include the release of some pathogenic mediators from the liver, including the following: elevated advanced glycated end-products, increased reactive oxygen species, elevated C-reactive protein, plasminogen activator inhibitor-1, IL-6, TNF-α, transforming growth factor-β1 and other pro-inflammatory cytokines. Importantly, several studies have shown that these potential mediators of vascular and/or renal injury are markedly higher in obese and/or diabetic patients with NAFLD than in those without [23–32]. Consistent with the hypothesis that liver inflammation (or other liver-derived factors) in NAFLD may play a direct role in the development or progression of CKD, Cheng et al. have shown that in a large cohort of Chinese type 2 diabetic patients, those with chronic hepatitis B virus infection were more likely to develop end-stage renal disease than those not infected with hepatitis B virus [33]. Finally, NAFLD may worsen whole-body insulin resistance and hyperglycaemia [2–4, 13], which may in turn contribute to the progression of retinopathy and CKD [27, 34–36]. This notion is supported by the observation, in this study, that HbA_{1c} was significantly higher in patients with NAFLD than in those without.

The potential implications of our findings for patient care are that in people with type 2 diabetes the casual detection of NAFLD during ultrasound examination should alert clinicians to the coexistence of chronic vascular complications warranting evaluation and treatment as much as the risk of advancing liver disease.

Our study has several strengths, including the large number of participants, the complete nature of the dataset, the ability to adjust for multiple confounders and the ultrasound diagnosis of NAFLD in all participants. However, despite the comprehensive nature of the dataset, there are some potential limitations to our study. First, the cross-sectional study design precludes the establishment of causal or temporal relations between NAFLD, CKD and retinopathy. Prospective studies will be required to sort out the time sequence of events. Second, we used estimated GFR rather than more precise measures of kidney function, like iothalamate clearance. Third, the diagnosis of non-proliferative retinopathy was based only on fundoscopy (whereas that of proliferative retinopathy was confirmed by fundus fluorescein angiography), so it is possible that subtle retinopathy changes may have been missed. However, the possibility that subtle retinopathy changes might have gone partly unnoticed would have weakened, rather than strengthening our findings. Fourth, although our results were adjusted for components of the metabolic syndrome, a condition closely associated with insulin resistance, we did not directly measure insulin resistance by euglycaemic clamp in our population. Finally, the NAFLD diagnosis was based on ultrasound imaging (without a semi-quantitative scoring for the degree of steatosis) and exclusion of other secondary causes of chronic liver disease, but was not confirmed by liver biopsy. It is known that none of the radiological features can distinguish between non-alcoholic steatohepatitis and other forms of NAFLD, and that only liver biopsy can assess the severity of damage and the prognosis [1–4]. However, liver biopsy would be impossible to perform routinely in a large epidemiological study. Moreover, liver ultrasound is by far the commonest way of diagnosing NAFLD in clinical practice [1–4]; it has a good sensitivity and specificity in detecting moderate and severe steatosis, but this sensitivity is reduced when hepatic fat infiltration upon biopsy is less than 33% [18]. Thus, although some non-differential misclassification of NAFLD on the basis of ultrasound is likely (some of the control persons could have underlying NAFLD, despite normal serum liver enzymes and a negative ultrasound), this limitation would serve to attenuate the magnitude of our effect measures towards null; thus, our results can probably be considered as conservative estimates of the relationship between NAFLD, CKD and retinopathy.

In conclusion, our findings suggest that NAFLD is associated with an increased prevalence of proliferative/laser-treated retinopathy and CKD in a large cohort of type 2 diabetic individuals. This association appears to be independent of a broad spectrum of baseline confounding variables, such as traditional cardiovascular risk factors, diabetes duration, glycaemic control, metabolic syndrome components and medications use (i.e. hypoglycaemic, lipid-lowering, anti-hypertensive and anti-platelet drugs). These cross-sectional findings, although not definitive, are sufficiently provocative to warrant further study. Future prospective studies could, for example, determine whether NAFLD actually predicts the development or progression of retinopathy and CKD in type 2 diabetes.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Adams LA, Angulo P, Lindor KD (2005) Nonalcoholic fatty liver disease. CMAJ 172:899–905
2. Day CP (2006) Nonalcoholic fatty liver disease: current concepts and management strategies. Clin Med 6:19–25
3. McCullough AJ (2006) Pathophysiology of non-alcoholic steatohepatitis. J Clin Gastroenterol 40 (Suppl 1):S17–S29
4. Marchesini G, Marzocchi R, Agostini F, Bugianesi E (2005) Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol 16:421–427
5. Clark JM, Brancati FL, Diehl AM (2003) The prevalence and aetiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 98:960–967

6. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S (2005) Prevalence of and risk factors for non-alcoholic fatty liver disease: the Dionysos Nutrition and Liver Study. Hepatology 42:44–52
7. Browning JD, Szczepaniak LS, Dobbins R et al (2004) Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40:1387–1395
8. Medina J, Fernandez-Salazar LI, Garcia-Buey L, Moreno-Otero R (2004) Approach to the pathogenesis and treatment of non-alcoholic steatohepatitis. Diabetes Care 27:2057–2066
9. Targher G, Bertolini L, Padovani R et al. (2007) Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 30:1212–1218
10. Targher G, Bertolini L, Padovani R et al (2006) Increased prevalence of cardiovascular disease among type 2 diabetic patients with non-alcoholic fatty liver disease. Diabet Med 23:403–409
11. Targher G, Bertolini L, Poli F et al (2005) Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. Diabetes 54:3541–3546
12. Targher G, Bertolini L, Rodella S et al (2007) Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care 30:2119–2121
13. Targher G, Arcaro G (2007) Nonalcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis 191:235–240
14. Levey AS, Coresh J, Balk E et al; National Kidney Foundation (2003) National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 139:137–147
15. American Diabetes Association (2007) Standards of medical care in diabetes—2007. Diabetes Care 30 (Suppl 1):S4–S41
16. Wilkinson CP, Ferris FL, Klein RE et al; Global Diabetic Retinopathy Project Group (2003) Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 110:1677–1682
17. Targher G, Bertolini L, Zoppini G, Lippi G, Zenari L (2007) Diabetic retinopathy predicts cardiovascular mortality in type 2 diabetic men and women: response to Juutilainen et al. [Letter]. Diabetes Care 30:e51
18. Saadeh S, Younossi ZM, Remer EM et al (2002) The utility of radiological imaging in non-alcoholic fatty liver disease. Gastroenterology 123:745–750
19. Targher G, Bertolini L, Padovani R, Zenari L, Zoppini G, Falezza G (2004) Relation of non-alcoholic hepatic steatosis to early carotid atherosclerosis in healthy men. Role of visceral fat accumulation. Diabetes Care 27:1498–1500
20. Ryu S, Chang Y, Kim DI, Kim WS, Suh BS (2007) Gamma-glutamyltransferase as a predictor of chronic kidney disease in nonhypertensive and nondiabetic Korean men. Clin Chem 53:71–77
21. Ratziu V, Imbert-Bismut F, Messous D, Poynard T (2004) The elusiveness of "normal" ALT in fatty liver. Hepatology 39:1172
22. Belfort R, Harrison SA, Brown K et al (2006) A placebo-controlled trial of pioglitazone in subjects with non-alcoholic steatohepatitis. N Engl J Med 355:2297–2307
23. Alessi MC, Bastelica D, Mavri A et al (2003) Plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation. Arterioscler Thromb Vasc Biol 23:1262–1268
24. Targher G, Bertolini L, Scala L et al (2007) Plasma PAI-1 levels are increased in patients with non-alcoholic steatohepatitis. Diabetes Care 30:e31–e32
25. Targher G (2006) Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. J Hepatol 45:879–881
26. Targher G, Bertolini L, Padovani R, Rodella S, Arcaro G, Day C (2007) Differences and similarities in early atherosclerosis between patients with non-alcoholic steatohepatitis and chronic hepatitis B and C. J Hepatol 46:1126–1132
27. Brownlee M (2005) The pathobiology of diabetic complications. Diabetes 54:1615–1625
28. Horiuchi S (2002) The liver is the main site for metabolism of circulating advanced glycation end products. J Hepatol 36:123–125
29. Abiru S, Migita K, Maeda Y et al (2006) Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. Liver Int 26:39–45
30. Holt HB, Wild SH, Wood PJ et al (2006) Non-esterified fatty acid concentrations are independently associated with hepatic steatosis in obese subjects. Diabetologia 49:141–148
31. Chalasani N, Deeg MA, Crabb DW (2004) Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with non-alcoholic steatohepatitis. Am J Gastroenterol 99:1497–1502
32. Albano E, Mottaran E, Vidali M et al (2005) Immune response towards lipid peroxidation products as a predictor of progression of non-alcoholic fatty liver disease to advanced fibrosis. Gut 54:987–993
33. Chen AYS, Kong APS, Wong VWS et al (2006) Chronic hepatitis B viral infection independently predicts renal outcome in type 2 diabetic patients. Diabetologia 49:1777–1784
34. Groop PH, Forsblom C, Thomas MC (2005) Mechanisms of disease: pathway-selective insulin resistance and microvascular complications of diabetes. Nat Clin Pract Endocrinol Metab 1:100–110
35. Sarafidis PA, Ruilope LM (2006) Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. Am J Nephrol 26:232–244
36. Marshall SM, Flyvbjerg A (2006) Prevention and early detection of vascular complications of diabetes. BMJ 333:475–480