PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Independent association between low serum amylase and non-alcoholic fatty liver disease in asymptomatic adults: A cross-sectional observational study
AUTHORS	Nakajima, Kei; Oshida, Haruki; Muneyuki, Toshitaka; Saito, Masafumi; Hori, Yumiko; Fuchugami, Hiroshi; Kakei, Masafumi; Munakata, Hiromi

VERSION 1 - REVIEW

REVIEWER	Dr Quentin M. Anstee BSc, MB BS, PhD, MRCP(UK) Senior Lecturer & Honorary Consultant Hepatologist, Institute of Cellular Medicine, Newcastle University, UK.
REVIEW RETURNED	20-Nov-2012

GENERAL COMMENTS	SUMMARY Non-Alcoholic Fatty Liver Disease (NAFLD) is strongly associated with obesity, type 2 diabetes and other features of the metabolic syndrome. Low levels of serum amylase have previously been shown to be associated with obesity and the metabolic syndrome. In this study the authors seek to determine that NAFLD is associated with low serum amylase too. To do this they quantify NAFLD by ultrasound and measure serum amylase levels on a cohort of apparently healthy 1,475 moderate drinkers undergoing routine annual medical check-ups. Hepatitis B infection (but not hepatitis C or autoimmune disease, etc) were excluded and patients with extreme abnormalities in ALT/ALP were excluded. Multivariate analysis demonstrated that low serum amylase was independently associated with a semi-quantitative increasing level of ultrasound determined steatosis. The authors concluded that this may be a useful test to identify NAFLD. COMMENTS The manuscript is well written with a generally good standard of English throughout although a couple of minor points are noted below. The study is an observational cross-sectional cohort study and so does not offer any mechanistic insights or explanation for the association between NAFLD and amylase levels. Figures are well presented. I have the following points to make: 1. I agree with the authors that NAFLD is becoming an increasingly important cause of liver disease however it should be remembered that there is increasing evidence that it is the subset of NAFLD patients that have inflammatory steatohepatitis (NASH) rather than

	simple steatosis that are at risk of hepatic and cardiovascular morbidity and mortality (Ekstedt et al). Furthermore, the degree of steatosis falls as disease progresses to advance fibrosis/cirrhosis and so lower levels of steatosis are seen in the most advanced disease. As such, the value of a test that may identify steatosis is questionable. This point should be addressed in the discussion. 2. The current study uses ultrasound as the modality to quantify degree of steatosis. This technique and has been shown to be both insensitive at levels of steatosis <30% and high subjective with inter- and intra-observer inconsistency. It is certainly not quantitative and so an analysis based on graded severity of ultrasound detected steatosis is not robust. Whilst I appreciate that histological assessment would not be appropriate in this cohort, MRI/MRS is the only radiological technique that has been shown to provide useful quantitative data. 3. Exclusion criteria for other liver diseases are incomplete. Hepatitis C has not been excluded and the authors do not explicitly state that autoimmune (AIH, PBC, etc) and metabolic diseases (haemochromatosis, etc) have been excluded. Whilst they do exclude patients with ALT levels <150 IU/ml, most patients with chronic liver disease actually have much more modest elevations of ALT and so would still be included in the study. 4. It is difficult to see how the use of this test will change practice without additional analysis showing the sensitivity/specificity of defined amylase thresholds as an independent predictor of NAFLD presence in the study cohort and a separate validation set. Given that the cohort is of a reasonable size, I wonder if an analysis with the cohort split into two groups (e.g. 1000 and 475 patients) could be performed to address this? MINOR POINTS 1. Typo: Page 10, line 45. 'increased significantly WITH as serum amylase...' 2. When referring to alcohol intake of patients,the current wording suggests that they consume alcohol regularly. Do the authors actually mean that they do not consume excess alcohol regularly? This should be reworded. CONCLUSION The paper is of some interest to a clinical readership and suggests a further biochemical test that may indicate presence of NAFLD however clinical utility is questionable and it should be remembered that the premise that ultrasound detected/quantified NAFLD is the basis for the analysis in the study is flawed.

REVIEWER	Dr Matthew Armstrong Wellcome Trust Clinical Research Fellow/Registrar in Hepatology NIHR liver biomedical research unit, University of Birmingham (United Kingdom)
REVIEW RETURNED	21-Nov-2012

THE STUDY	My main concerns surround the actual relevance and application of the research question in primary care and the definition/categorisation of the severity of NAFLD.
GENERAL COMMENTS	Nakajima and colleagues have undertaken an extensive study to

	investigate whether serum amylase could be a useful marker of NAFLD in the community. The study is well-written and is a good sample size. However, I have the following comments: Major: 1. Even though the research question is clear and apparent, im not convinced by its level of importance and how useful this would be in primary care. 25% of general populations are estimated to have NAFLD, yet the most important question that still remains difficult to answer is which patients have severe steatohepatitis +/- advanced fibrosis in the community. Amylase is rarely (if ever) undertaken in primary care, and I would not advocate it being introduced for this purpose in this setting 2. The authors themselves elude to the fact that the prevalence of fibrosis is likely to be low in their cohort, due to the low BMI and low prevalence of diabetes. However, the fact that amylase is lower in patients with low non-invasive fibrosis scores, indicates that using a low serum amylase to identify NAFLD patient may result in missing out the most important patients. 3. Their definition of NAFLD is lacking for the impact of a journal such as the BMJ. The lack HCV Ab (prevalence 1.5-2.3% in japan, Lancet Infect dis), ferritin, autoantibodies, albeit likely to account for less than 50 patients, is a limitation. 4. The severity grouping of NAFLD is misleading. Severe NAFLD represents advanced fibrosis, and not excess fat accumulation. Defining NAFLD severity purely on the grounds of USS is misleading and inaccurate. Also there is no mention of validating the sonographers scores (inter-user variability?). How many patients had gallstones on USS (plus was this an exclusion criteria)? How many individuals had an enlarged spleen? I can fully appreciate that liver biopsy in this setting is not feasible or ethical, but other studies (i.e. Wong et al, GUT) have utilised MRS and fibroscan in a cohort size approaching 1000 – to enable the reader to fully understand the spectrum of NAFLD in their cohort. 5. The relationship between amylase and fat accumulation is interesting, but mechanisms of why there is an inverse relationship between amylase and fatty liver should have been investigated. Minor comments: 1. With the normal reference range for serum amylase in most laboratories being 40-140 U/L, why were patients with an amylase <30 excluded? 2. How was alcohol consumption approximated? Validated questionnaire? 3. How many patients screened were excluded and on what grounds? Was the study population representative of the Japanese population 4. How many radiographers performed the USS and what was their inter-variability? How many years experience 5. I have concerns regarding the severity categories for fatty liver on USS. This definition of mild, moderate and severe based on diaphragm and portal vein visualization I am not aware of. How does their technique correlate with %steatosis on biopsy in previous studies that have used this definition. 6. APRI should not be used to define fibrosis in NAFLD, as it was designed for viral hepatitis and has not been well validated in NAFLD 7. In the statistical analysis – why was amylase categorized and not analysed as a continuous variable 8. There is no accurate assessment of insulin sensitivity (i.e. HOMA-

	IR on fasting bloods)

VERSION 1 – AUTHOR RESPONSE

1 Reviewer: Dr Quentin M.

1. I agree with the authors that NAFLD is becoming an increasingly important cause of liver disease however it should be remembered that there is increasing evidence that it is the subset of NAFLD patients that have inflammatory steatohepatitis (NASH) rather than simple steatosis that are at risk of hepatic and cardiovascular morbidity and mortality (Ekstedt et al). Furthermore, the degree of steatosis falls as disease progresses to advance fibrosis/cirrhosis and so lower levels of steatosis are seen in the most advanced disease. As such, the value of a test that may identify steatosis is questionable. This point should be addressed in the discussion.

Response
We are aware that the degree of steatosis decreases as the disease progresses to advance fibrosis/cirrhosis and that ordinary ultrasound methods are poorly able to detect the presence of fibrosis/cirrhosis. Therefore, we have added the following sentence in the Methods:
"Therefore, the grade of NAFLD in this study probably reflects overall hepatic fat accumulation, rather than the severity of fibrosis."

We have also added the following sentences in the Discussion:
"In earlier studies, ultrasonography had a sensitivity of 60–94% and a specificity of 66–95% for detecting fatty liver [24,49]. However, its sensitivity is reduced in subjects with a small amount of fat (< 30%), such as those with mild NAFLD or advanced fibrosis, [14]. To improve the accuracy of detecting and grading of NAFLD, the use of other imaging modalities might be needed, such as magnetic resonance imaging and magnetic resonance spectroscopy, which were reported to provide useful quantitative data in earlier studies [50,51]."

2. The current study uses ultrasound as the modality to quantify degree of steatosis. This technique and has been shown to be both insensitive at levels of steatosis <30% and high subjective with inter- and intra-observer inconsistency. It is certainly not quantitative and so an analysis based on graded severity of ultrasound detected steatosis is not robust. Whilst I appreciate that histological assessment would not be appropriate in this cohort, MRI/MRS is the only radiological technique that has been shown to provide useful quantitative data.

Response
We fully agree with this comment. Therefore, we have added the following limitation in the Discussion:
"To improve the accuracy of detecting and grading of NAFLD, the use of other imaging modalities might be needed, such as magnetic resonance imaging and magnetic resonance spectroscopy, which were reported to provide useful quantitative data in earlier studies [50,51]."

3. Exclusion criteria for other liver diseases are incomplete. Hepatitis C has not been excluded and the authors do not explicitly state that autoimmune (AIH, PBC, etc) and metabolic diseases (haemochromatosis, etc) have been excluded. Whilst they do exclude patients with ALT levels <150 IU/ml, most patients with chronic liver disease actually have much more modest elevations of ALT and so would still be included in the study.

Response
We agree with this comment. To help explain the situation, we have added the following limitation to

the Discussion:
“It is also possible that individuals with primary biliary cirrhosis, autoimmune hepatitis or other forms of liver dysfunction (e.g., haemochromatosis and Wilson disease) were included in the present study, even though we excluded subjects with elevated hepatic enzymes (≥ 150 IU/ml). However, the prevalence of these diseases is quite low in the general population [55-57].”

4. It is difficult to see how the use of this test will change practice without additional analysis showing the sensitivity/specificity of defined amylase thresholds as an independent predictor of NAFLD presence in the study cohort and a separate validation set. Given that the cohort is of a reasonable size, I wonder if an analysis with the cohort split into two groups (e.g. 1000 and 475 patients) could be performed to address this?

Response
We conducted sensitivity/specificity analyses using the receiver operator characteristic curve method. We found that a serum amylase level of 68 mg/dl (77% sensitivity/71% specificity using Youden index) was the optimal cutoff for overt NAFLD with an area under the curve of 0.63 (P=0.0001). However, we think that showing the cutoff for overt NAFLD is likely to encourage clinicians to measure serum amylase in primary care. Considering the comments of the second reviewer, we did not add the results of these sensitivity/specificity analyses to the manuscript. At this time, we believe that serum amylase should not be routinely measured in clinical practice until it has been validated and its clinical relevance has been confirmed.

To prevent readers from misunderstanding our findings, we have changed the conclusion in the Abstract, as follows:
“LSA may be a valuable marker for moderate or severe NAFLD” to “LSA may be associated with moderate or severe NAFLD”

MINOR POINTS
1. Typo: Page 10, line 45. 'increased significantly WITH as serum amylase...'

Response
We have deleted “with” from this sentence.

2. When referring to alcohol intake of patients, the current wording suggests that they consume alcohol regularly. Do the authors actually mean that they do not consume excess alcohol regularly? This should be reworded.

Response
In response to this comment, we have revised this description of alcohol consumption, as follows:
“Subjects completed a questionnaire recording lifestyle factors, including habitual alcohol consumption, which was classified in terms of the frequency (none, occasional, and daily) and the amount of ethanol consumed per day (< 20 g, 20–39 g, 40–59 g, or ≥ 60 g). Subjects who habitually consumed ≥ 20 g ethanol per day were excluded from the study.”

Theoretically, the subjects included in this study are unlikely to consume excess alcohol on a regular basis.

2 Reviewer: Dr Matthew Armstrong

Major:
1. Even though the research question is clear and apparent, im not convinced by its level of importance and how useful this would be in primary care. 25% of general populations are estimated to have NAFLD, yet the most important question that still remains difficult to Response is which patients have severe steatohepatitis +/- advanced fibrosis in the community. Amylase is rarely (if ever) undertaken in primary care, and I would not advocate it being introduced for this purpose in this setting

Response
We agree with this comment. Therefore, we have added the following limitation to the Discussion:
“Finally, the clinical relevance of measuring serum amylase remains unclear. The current results suggest that LSA is likely to detect overt NAFLD, but not NASH, which is the most important hepatic disorder. However, from the cardiometabolic perspective, even simple steatosis may be important, particularly in nonobese people, because steatosis may be more strongly associated with insulin resistance than is obesity [17,19,20]. Currently, serum amylase is rarely considered in clinical practice, except in certain situations, such as suspected pancreatitis. Therefore, numerous clinical and animal studies are needed to validate and confirm the clinical relevance of measuring serum amylase before it can be introduced into primary care for the detection of cardiometabolic diseases and NAFLD.”

2. The authors themselves elude to the fact that the prevalence of fibrosis is likely to be low in their cohort, due to the low BMI and low prevalence of diabetes. However, the fact that amylase is lower in patients with low non-invasive fibrosis scores, indicates that using a low serum amylase to identify NAFLD patient may result in missing out the most important patients.

Response
From a liver perspective, it is possible that low serum amylase may miss patients with fibrosis. However, from a cardiometabolic perspective, even simple steatosis may be important, particularly in nonobese people, because steatosis may be more strongly associated with insulin resistance than is obesity.

In addition, the estimated prevalence of NASH is quite low in our study population, particularly if we consider the overall prevalence of NASH in Japan. Therefore, we have added the following limitation to the Discussion:
“In fact, the estimated prevalence of NASH in this study is quite low when we compare it with prevalence of NASH in a nationwide study in Japan. It was reported that approximately 20–25% of patients with diabetes had NAFLD, a population in which the prevalence of NASH might be 30–40% [48]. Therefore, the estimated prevalence of NASH might be less than 1% of all of the subjects in this study.”

Therefore, different outcomes might be expected if the same analysis is conducted in a different population, such as patients with type 2 diabetes or metabolic syndrome.

3. Their definition of NAFLD is lacking for the impact of a journal such as the BMJ. The lack HCV Ab (prevalence 1.5-2.3% in japan, Lancet Infect dis), ferritin, autoantibodies, albeit likely to account for less than 50 patients, is a limitation.

Response
In accordance with this comment, we have updated the prevalence rate from 0.49–0.98% in Japanese blood donors to 1.5–2.3% in Japan. We have also added the following limitation to the Discussion:

“However, the prevalence of hepatitis C infection was reported to be 1.5–2.3% in Japan [52]. Low prevalence rates of hepatitis C (< 1.5%) were also recently reported in Asian-Pacific, tropical Latin American, and North American countries [53].”

Regarding autoantibodies, we have added the following sentences:
“It is also possible that individuals with primary biliary cirrhosis, autoimmune hepatitis or other forms of liver dysfunction (e.g., haemochromatosis and Wilson disease) were included in the present study, even though we excluded subjects with elevated hepatic enzymes (≥ 150 IU/ml). However, the prevalence of these diseases is quite low in the general population [55-57].”

4. The severity grouping of NAFLD is misleading. Severe NAFLD represents advanced fibrosis, and not excess fat accumulation. Defining NAFLD severity purely on the grounds of USS is misleading and inaccurate. Also there is no mention of validating the sonographers scores (inter-user variability?). How many patients had gallstones on USS (plus was this an exclusion criteria)? How many individuals had an enlarged spleen? I can fully appreciate that liver biopsy in this setting is not feasible or ethical, but other studies (i.e. Wong et al, GUT) have utilised MRS and fibroscan in a cohort size approaching 1000 – to enable the reader to fully understand the spectrum of NAFLD in their cohort.

Response
Unfortunately, the sonographers’ scores are unknown, so we cannot assess inter-user variability. However, all of the sonographers are medically registered.

Subjects with gallstones, cholecystectomy, or splenomegaly were included in this study, and the proportions of subjects with these features have been added to Table 1. We have also added the following text to the Methods and Results:

Methods
“We also examined the findings of gallstones, cholecystectomy, and splenomegaly, which was defined as a spleen index (calculated as the long dimension × short dimension on splenotomogram) ≥ 30 [26,27].”

Results
“There were no significant differences in the prevalence rates of gallstones, cholecystectomy, or splenomegaly between quartiles, possibly because of the small numbers of subjects with these features, although the rates of cholecystectomy and splenomegaly were higher in Q4 compared with the other quartiles.”

Included these features as confounding factors did not alter the associations between LSA and NAFLD (Tables 2 and 3). Therefore, we did not include these Models considering we have already shown data for 7 models.

We have added the following sentences in the Discussion regarding MRS and fibroscan, which is relatively expensive:
“To improve the accuracy for the detection and grading of fatty liver, magnetic resonance imaging or magnetic resonance spectroscopy is considered as the image technique that has been shown to provide useful quantitative data as several investigators reported in recent years [50,51].”

5. The relationship between amylase and fat accumulation is interesting, but mechanisms of why there is an inverse relationship between amylase and fatty liver should have been investigated.

Response

In response to this comment, we have described two potential mechanisms in the Discussion. The first, involves obesity and insulin resistance while the other involves systemic fat deposition or genetic factors. To improve clarity, we have incorporated this information in the Discussion using the following subheading:

“Potential mechanisms of the inverse relationship between serum amylase and NAFLD”
We have also added the following sentence in the Discussion:
“As another explanation for the inverse relationship between serum amylase and NAFLD…”

Minor comments:
1. With the normal reference range for serum amylase in most laboratories being 40-140 U/L, why were patients with an amylase <30 excluded?

Response
As noted by the reviewer, the normal reference range for serum amylase in most Japanese laboratories is 50–150 U/L, primarily considering the aetiology of pancreatitis. However, we think that normal reference range for serum amylase in the context of metabolic abnormalities has not been evaluated. Therefore, to determine the potential relationship between serum amylase and cardiometabolic features, including NAFLD, we used a wider range of values in the current analysis. In accordance with this comment, we have added the following sentences in the Methods:
“…estimated glomerular filtration rate (eGFR) ≤ 35 ml/min/1.73 m2, serum amylase ≤ 30 IU/l (the lower 5th percentile in an earlier study [12]) or ≥ 200 IU/l based on previous reports [21,22], as well as those suspected of having cancer. In the current analysis, to investigate the potential relationship between serum amylase and cardiometabolic features, including NAFLD, we included subjects with a wider range of serum amylase levels than the current reference ranges.”

2. How was alcohol consumption approximated? Validated questionnaire?

Response
We assessed alcohol consumption with two questions rather than a single question. Alcohol consumption was approximated into four grades. To clarify this, we have added the following sentences to the Methods:
“Subjects completed a questionnaire recording lifestyle factors, including habitual alcohol consumption, which was classified in terms of the frequency (none, occasional, and daily) and the amount of ethanol consumed per day (< 20 g, 20–39 g, 40–59 g, or ≥ 60 g).”

Unfortunately, we are unaware of whether this questionnaire has been validated.

3. How many patients screened were excluded and on what grounds? Was the study population representative of the Japanese population

Response
In response to this comment, we have added the sentence “The exclusion criteria and a flow chart summarizing subject disposition are shown in Supplemental Figure 1.” in the Methods.

We believe that the study population is somewhat representative of the Japanese population because we did not select them with any specific criteria except for the aetiology of NAFLD. However, as described in the Discussion, “In addition, the fact that more than half of the subjects in this study had repeatedly undergone detailed medical check-ups may also contribute to the lower prevalence of NASH, because repeated check-ups may promote consciousness of healthcare and favourable lifestyles.” Therefore, people who regularly undergo these detailed medical check-ups may be more

healthcare conscious, because the detailed medical check-ups conducted as part of this programme take more time and are more expensive than the annual mandatory check-ups.

To better clarify this, we have revised our the description of the check-ups in the Methods:
“We recruited, at random, 2,472 asymptomatic subjects aged 30–79 years who underwent thorough annual medical check-ups, in which the subjects underwent a more extensive array of clinical tests than would be performed in routine check-ups, at the Social Insurance Omiya General Hospital, Saitama, Japan.”

4. How many radiographers performed the USS and what was their inter-variability? How many years experience

Response
A few (no more than five) sonographers performed the USS and most have more than 10 years of clinical experience. Unfortunately, inter-user variability is unknown. Nevertheless, all of sonographers involved in this study are medically registered for practicing in the gastrointestinal field. To become a registered medical sonographer in Japan, sonographers must complete ≥ 3 years of clinical training experience and pass a rigorous test. Considering this comment, we have changed the Methods, as follows:
echography specialists” → “… registered medical sonographers who only work at “Social Insurance Omiya General Hospital. The sonographers were blinded to the subjects’ data.”

5. I have concerns regarding the severity categories for fatty liver on USS. This definition of mild, moderate and severe based on diaphragm and portal vein visualization I am not aware of. How does their technique correlate with %steatosis on biopsy in previous studies that have used this definition.

Response
We used the following articles to establish the definition of mild, moderate, and severe NAFLD based on diaphragm and portal vein visualisation and have cited them in the text:

Schwenzer NF, Springer F, Schraml C, et al. Non-invasive assessment and quantification of liver steatosis by ultrasound, computed tomography and magnetic resonance. J Hepatol 2009;51:433–45.

Shannon A, Alkhouri N, Carter-Kent C, et al. Ultrasonographic quantitative estimation of hepatic steatosis in children With NAFLD. J Pediatr Gastroenterol Nutr 2011;53:190–5.

We have also added the following sentences in the Methods:
“In a previous study, the ultrasonographic steatosis score determined using these grades was highly correlated with the histological grade of steatosis ($r = 0.80$, $P < 0.001$), but not with inflammatory activity ($r = 0.10$) or fibrosis score ($r = 0.19$) [25].”

6. APRI should not be used to define fibrosis in NAFLD, as it was designed for viral hepatitis and has not been well validated in NAFLD

Response
In accordance with this comment, we have deleted the data and sentences concerning APRI.

7. In the statistical analysis – why was amylase categorized and not analysed as a continuous variable

Response
To examine the associations between serum amylase and NAFLD, we used logistic regression

models, which yields odds ratios (OR) and 95% confidential intervals (95% CI), controlling for relevant confounding factors. Although it is possible to include serum amylase as a continuous variable in logistic regression, when the repeated Model 3 in Table 2 using amylase as a continuous variable, the odds ratio of serum amylase for NAFLD was 0.986 (95% CI: 0.982–0.992; P < 0.0001), indicating a significant inverse association. Therefore, we think that many readers will find it difficult to understand such data. Since using a continuous variable generally yields relatively small ORs but a narrow CI, we categorised serum amylase in this study.

8. There is no accurate assessment of insulin sensitivity (i.e. HOMA-IR on fasting bloods)

Response
The reviewer is correct that insulin sensitivity was not assessed in this study. Therefore, we have added the following sentence in the Discussion:
"However, because insulin resistance, as determined by the homeostasis model assessment of insulin resistance for example, was not examined in this study, further large epidemiological studies evaluating insulin resistance are needed to confirm the current hypothesis."

Other revisions
We have added or revised several phrases to improve clarity, as follows:

Introduction
"In this context, we hypothesised that LSA may be associated with NAFLD independently of MetS, type 2 diabetes, and even obesity."

Methods
Fatty liver, which was determined by comparing liver echogenicity with that of the renal cortex [23], was defined as NAFLD. Additionally, the severity of NAFLD was graded into three categories: mild NAFLD, a slight increase in liver echogenicity with normal visualisation of the diaphragm and the portal veins; moderate NAFLD, a moderate increase in liver echogenicity……

Discussion
However, similar to an earlier study [12], HbA1c was not associated with LSA, probably because most subjects in the lowest quartile of serum amylase in this study had no or only mild insulin resistance or mild hyperinsulinaemia, which is likely compensated for and results in euglycaemia or mild hyperglycaemia.

We have also added 15 new references, which are indicated in red font in the reference list.

VERSION 2 – REVIEW

REVIEWER	Dr Matthew Armstrong Centre for Liver Research and NIHR Liver BRU University of Birmingham
REVIEW RETURNED	11-Dec-2012

THE STUDY	The authors have addressed alot of concerns by adding clearer methodology and additional limitations in the discussion.
GENERAL COMMENTS	The authors have extensively addressed all my major concerns. So in that light the paper is robust and scientifically justified. Im still concerned the manuscript will be criticised based on categorising steatosis based on USS alone. However, the fact that the authors have previously validated this technique in a biopsy cohort goes

	some way to addressing this limitation and furthermore all 3 categories of steatosis have lower amylases than the non-fatty population (thus, the categorical emphasis is not that important). I agree with the authors that this now requires further study, and that we should strongly caution against its use by GPs until such studies have taken place. To ensure GPs do not over-interpret these interesting findings i would advocate that the BMJ consider an editorial on this piece of work.