CLINICAL STUDY

Association of proinsulin and hepatic steatosis in a random, population-based sample

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Abstract

Objective: Proinsulin may represent a predictive marker for assessing insulin resistance and reduced β -cell function. The objective of this study was to investigate the association between hepatic steatosis, proinsulin and other parameters in a random, population-based sample.

Design: Cross-sectional study, conducted in south-western Germany.

Methods: Upper abdominal ultrasound examinations were performed in 343 subjects (147 females, 196 males; average age 40.0 ± 11.5 years). Proinsulin, the proinsulin-to-insulin ratio and other laboratory parameters were determined, and the BMI, waist-to-hip ratio (WHR) and other anthropometric data were documented.

Results: Hepatic steatosis was observed in 80 subjects (23.3%; 29.6%, males; 15.0%, females). Multivariate analysis showed an association with hepatic steatosis for male gender ($P=0.0212$), advancing age ($P=0.0241$), elevated BMI ($P<0.0001$), elevated WHR ($P=0.0024$), alanine aminotransferase ($P=0.0046$), proinsulin ($P=0.0403$) and proinsulin-to-insulin ratio ($P=0.0116$).

Conclusions: There is an association between elevated proinsulin concentrations and hepatic steatosis.

European Journal of Endocrinology 168 195–202

Introduction

Obesity is one of the fastest growing health problems worldwide (1, 2). In Europe, the prevalence of obesity has tripled since 1980 (3). Obesity is closely associated with non-alcoholic fatty liver disease (NAFLD), which is the most common chronic liver disease, affecting nearly 30% of the population in Western industrial nations (4, 5, 6, 7, 8, 9, 10). NAFLD as a clinical entity can be further subdivided histologically into a form characterised by simple hepatic steatosis and another in which the steatosis is complicated by non-specific inflammation; both, however, constitute benign forms of the disorder. In its more malignant form, the disorder presents as non-alcoholic steatohepatitis (NASH). Histologically, these patients exhibit both inflammation and liver damage with the potential for progression toward fibrosis, cirrhosis or hepatocellular carcinoma (11, 12). There is an association between hepatic steatosis and the development of insulin resistance and type 2

diabetes mellitus (13, 14). In fact, patients with NAFLD have a threefold increased risk of developing type 2 diabetes mellitus (15). In addition, the disorder is associated with other components of the metabolic syndrome (4, 12, 16, 17, 18, 19).

Pfutzner *et al.* (20) measured intact proinsulin as a means of assessing for insulin resistance. As insulin resistance worsens, proinsulin secretion increases secondary to the pancreas' reduced capacity to convert this prohormone precursor to insulin. Thus, elevated proinsulin concentrations may provide evidence of abnormal β -cell function and, hence, serve as a predictor for the development of diabetes (20, 21, 22). Proinsulin has also been shown to be a reliable marker for rapidly progressing type 2 diabetes mellitus (22). In addition, proinsulin inhibits fibrinolysis and together with insulin resistance contributes to the risk of cardiovascular disease (23, 24). Objective of this study was to investigate the association between hepatic steatosis, proinsulin and other parameters in a random population-based sample.

Materials and methods

Study population

The Echinococcus Multilocularis and Internal Diseases in Leutkirch (EMIL) Study is a population-based cross-sectional study conducted from November to December 2002 in Leutkirch, a town in south-western Germany. Of 4000 randomly selected inhabitants, a total of 2445 subjects aged 10–65 years were included in the study (participation rate, 62.8%) (25). Following application of the exclusion criteria, 343 subjects were included in the present analysis. Exclusion criteria were: subjects <18 years; seropositivity for hepatitis B or C; history of haemochromatosis; subjects fasting <8 h at the time of ultrasound examination; and elevated alcohol consumption (women: >20 g/day; men: >40 g/day). Also excluded were subjects with incomplete laboratory results (Fig. 1). Two of the 343 subjects reported type 2 diabetes mellitus.

The study was conducted in conformity with the principles of the Helsinki Declaration and Good Clinical Practice recommendations and was approved by the Ethics Commission of the State Medical Council of Baden-Württemberg. All study participants provided their informed written consent.

Examination methods

Patient history, including demographics, leisure activities, past medical history, family medical history, medication history as well as nicotine and alcohol use and nutritional habits were documented using a standardised interview.

Anthropometric data

Body height, body weight, and hip and waist circumference were measured. The BMI and the waist-to-hip

ratio (WHR) were calculated according to the recommendations of the World Health Organization (26).

Laboratory testing

About 25 ml whole blood was obtained from each study participant through phlebotomy of the cubital vein. Subjects' insulin concentration was determined using a RIA (Immunotech, Beckman Coulter Company, Brea, USA; Insulin (e) IRMA kit, Canada); for proinsulin, a immunoenzymometric assay (ZenTech s.a. 4031, Angleur, Belgium) was used. For this assay, the intra-assay CV was 4.3% for 6.97 pmol/l and 7.4% for 60.3 pmol/l; the interassay CV was 6.8% for 7.32 pmol/l and 5.5% for 64.7 pmol/l. In testing for proinsulin, no cross-reactions with insulin or C-peptide were observed. Random glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transferase (GGT), alkaline phosphatase (AP), C-reactive protein (CRP), albumin, cholesterol, HDL, transferrin and ferritin were measured using the Dimension XL unit (Dade Behring, Inc., Newark, DE 19714, USA). Fibrinogen and ceruloplasmin were measured using the Siemens Dade Behring BII Nephelometer (Siemens Healthcare Diagnostics GmbH; Ludwig-Erhard-Straße 12; 65760 Eschborn, Germany). The concentration of LDL was calculated using the Friedewald formula: $\text{LDL-cholesterol} = \text{total cholesterol} - \text{HDL} - (\text{triglycerides} \times 0.45)$ (27).

The Institute of Clinical Chemistry of the University Hospital of Ulm defines the normal reference range for the concentration of proinsulin to be <9.4 pmol/l (28). Participants' pre-examination fasting periods were documented historically. Participants with reported fasting times <8 h were excluded from the study. Regular precision controls were performed to assure proper functioning of all laboratory equipment.

Ultrasound examination and criteria for hepatic steatosis

Ultrasound examinations were performed under standardised conditions by specially trained examiners using four identical HDI 5000 diagnostic ultrasound units (ATL Ultrasound, Philips Medical Systems, Bothell, WA, USA). Unclear or pathological findings were reviewed by an experienced supervisor (>4000 ultrasonographic examinations per year). As far as possible, identical settings were maintained for all units. Findings were documented using a standardised recording questionnaire. Examinations included assessment of the liver, gallbladder, kidneys and spleen. The liver was assessed with respect to size, the presence of focal lesions and evidence of hepatic steatosis. The diagnosis of hepatic steatosis was made on the basis of criteria established by Saverymuttu *et al.* (29), Hamaguchi *et al.* (30) and Charatcharoenwitthaya & Lindor (31). The hepatic parenchyma was compared with the renal

Figure 1 Flow of the subjects across the study.

parenchyma under consideration of the dorsal echo attenuation, visualisation of the diaphragm and ability to assess the intrahepatic vessels. The degree of steatosis was assigned to classes of 'none' (grade 0), 'mild' (grade I), 'moderate' (grade II) and 'severe' (grade III).

Statistical analysis

Statistical calculations were performed using the SAS 9.2 statistics software package (SAS Institute, Inc., Cary, NC, USA). Data were first analysed descriptively. Mean and s.d. were determined for continuous variables. Categorical data were presented with absolute and relative frequencies. In order to detect differences between subjects with and without hepatic steatosis, the Wilcoxon rank-sum test was used for continuous variables, while, for categorical variables, the χ^2 test or, when the number of cases was too small, Fisher's exact test were used.

In a further analysis, proinsulin values were divided into three groups of equal size (tertiles) for subjects with and without hepatic steatosis. The ranges of the three respective tertiles for subjects without hepatic steatosis were as follows: first tertile: 3.26–9.11 pmol/l; second tertile: 9.13–10.8 pmol/l; third tertile: 10.9–47.1 pmol/l respectively. For subjects with hepatic steatosis, the ranges of the three respective tertiles were as follows: first tertile: 4.09–11.0 pmol/l; second tertile: 11.20–14.0 pmol/l; third tertile: 14.1–35.5 pmol/l. In order to demonstrate differences between the three groups, the Kruskal–Wallis test for constant variables and the χ^2 test for categorical variables were used. Bivariate logistic regression was performed to assess the association between hepatic steatosis and proinsulin as well as other factors, including age, BMI, WHR, gender, ALT, AST and GGT, which have been reported in the literature to influence the development of hepatic steatosis. Also studied were insulin and the proinsulin-to-insulin ratio. Variables that showed an association with hepatic steatosis in the bivariate analysis ($\alpha=0.20$) were included in the multivariate analysis. Stepwise logistic regression was used for the multivariate analyses. Statistical significance was set at $\alpha=0.10$. For bi- and multivariate analyses, laboratory parameters were standardised (z-transformed) in order to determine the effect on the adjusted odds ratio (OR) by increase of one s.d. Area under the receiver operating characteristic curve (ROC-AUC) analysis was used to evaluate the diagnostic utility of those laboratory parameters that showed an association in the multivariate analysis. The DeLong and Clarke-Pearson approach was used to compare the ROC curves of different models.

All tests were two-tailed. Statistical significance was set at $\alpha=0.05$. The *P* value was given to four decimal places, while the OR and 95% confidence interval (CI) were given to three decimal places.

Results

Complete clinical profiles, anthropometric and ultrasonographic data and laboratory findings were available for 343 subjects (147 women, 42.9%; 196 men, 57.1%; average age 40.0 ± 11.5 years). Ultrasonographic evidence of hepatic steatosis was documented in 80 subjects (23.3%). NAFLD was more common in men (29.6%, $n=58$) than in women (15.0%, $n=22$). The difference in the frequency of NAFLD between men and women was statistically significant ($P=0.0044$). Subjects with hepatic steatosis have, compared with persons without steatosis, higher proinsulin concentrations ($P<0.0001$; Table 1).

Proinsulin concentrations were further studied in subjects with hepatic steatosis: proinsulin values for subjects with and without evidence of hepatic steatosis were grouped in tertiles and analysed. There was a statistically significant difference for WHR and insulin among subjects with hepatic steatosis. There was also a statistically significant difference for the parameters WHR and insulin in the group of subjects without evidence of hepatic steatosis (Table 2).

Bivariate analysis showed a difference between men and women with respect to hepatic steatosis

Table 1 Demographics and other characteristics of subjects with and without hepatic steatosis. These data are presented as the mean $\pm$ s.d. or number of cases and percentage.

	Hepatic steatosis		<i>P</i> value ^a
	Not diagnosed (<i>n</i> =263)	Diagnosed (<i>n</i> =80)	
Demographics			
Sex <i>n</i> (%)			
Female	125 (47.5)	22 (27.5)	0.0015
Male	138 (52.5)	58 (72.5)	
Age (years)	37.1 ± 11.1	45.2 ± 10.7	<0.0001
BMI (kg/m ²)	24.0 ± 3.6	29.7 ± 5	<0.0001
WHR	0.8 ± 0.1	0.9 ± 0.1	<0.0001
Laboratory parameters			
Insulin (μ U/ml)	6.4 ± 4.9	10.3 ± 7.9	<0.0001
Proinsulin (pmol/l)	10.6 ± 3.8	13.4 ± 4.5	<0.0001
Proinsulin–insulin ratio	2.2 ± 1.3	1.7 ± 0.8	0.0004
ALT (U/l)	14.0 ± 6.3	20.8 ± 9.2	<0.0001
AST (U/l)	8.9 ± 2.6	10.7 ± 3.4	<0.0001
GGT (U/l)	11.4 ± 11.7	19.7 ± 13.5	<0.0001
AP (U/l)	78.4 ± 20.5	87 ± 18.8	<0.0001
CRP (mg/l)	2.1 ± 3.8	3.4 ± 4.6	<0.0001
Albumin (g/l)	41.4 ± 4.2	41.6 ± 4	0.8549
Fibrinogen (g/l)	3.1 ± 0.7	3.4 ± 0.7	0.0007
Ferritin (μ g/l)	101.2 ± 131.5	177.6 ± 188	<0.0001
Transferrin (g/l)	2.6 ± 0.5	2.7 ± 0.5	0.0408

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AP, alkaline phosphatase; CRP, C-reactive protein; GGT, γ -glutamyl transferase; WHR, waist-to-hip ratio.

^aDifferences in continuous data were assessed using Wilcoxon's rank-sum test; differences in categorical data were assessed using the χ^2 test or Fisher's exact test.

Significant values are in bold type.

Table 2 Demographics and other characteristics of subjects with and without hepatic steatosis according to proinsulin tertiles. These data are presented as the mean $\pm$ s.d. or number of cases and percentage. Differences in continuous data were assessed using Kruskal–Wallis test; differences in categorical data were assessed using the χ^2 test or Fisher's exact test.

	Subjects without hepatic steatosis, mean (s.d.)			Subjects with hepatic steatosis, mean (s.d.)		
	Tertile 1 (n=87)	Tertile 2 (n=88)	Tertile 3 (n=88)	Tertile 1 (n=27)	Tertile 2 (n=26)	Tertile 3 (n=27)
Demographics						
Gender n (%)						
Female	44 (50.6)	45 (51.1)	36 (40.9)	9 (33.3)	5 (19.2)	8 (29.6)
Male	43 (49.4)	43 (48.9)	52 (59.1)	18 (66.7)	21 (80.8)	19 (70.4)
Age (years)	35.7 (8.8)	38.5 (11.9)	37.0 (12.2)	43.0 (11.7)	45.0 (10.6)	47.6 (9.8)
BMI (kg/m ²)	23.5 (3.3)	23.7 (3.5)	24.8 (3.8)*	29.2 (4.0)	28.8 (5.9)	31.1 (4.9)
WHR	0.8 (0.1)	0.8 (0.1)	0.8 (0.1) [†]	0.9 (0.1)	0.9 (0.0)	0.9 (0.1)*
Laboratory parameters						
ALT (U/l)	13.2 (4.6)	13.0 (5.7)	15.8 (7.8) [†]	21.0 (11.0)	20.8 (8.0)	20.6 (8.4)
AST (U/l)	8.7 (2.5)	8.7 (2.2)	9.2 (2.9)	11.1 (3.3)	11.0 (3.4)	10.0 (3.5)
GGT (U/l)	11.4 (16.4)	10.0 (6.4)	12.8 (9.9)*	17.9 (11.5)	20.3 (16.2)	21.1 (12.8)
AP (U/l)	73.0 (17.2)	78.4 (18.9)	83.6 (23.6) [†]	83.1 (15.2)	86.7 (18.6)	91.4 (21.7)
Insulin (μ U/ml)	5.4 (3.6)	5.4 (2.6)	8.3 (6.9) [†]	9.4 (11.4)	9.0 (4.2)	12.4 (5.7) [†]
CRP (mg/l)	1.9 (3.1)	2.2 (5.0)	2.2 (3.0)	2.3 (2.6)	3.7 (5.1)	4.2 (5.5)
Albumin (g/l)	41.0 (3.7)	41.2 (3.6)	42.0 (5.0)	41.6 (4.5)	42.8 (3.9)	40.3 (3.3)
Fibrinogen (g/l)	3.1 (0.6)	3.1 (0.6)	3.2 (0.8)	3.2 (0.7)	3.6 (0.8)	3.4 (0.5)
Ferritin (μ g/l)	81.2 (81.9)	88.4 (92.6)	138.8 (187.6)*	194.4 (164.2)	194.3 (258.2)	144.8 (120.9)
Transferrin (g/l)	2.7 (0.6)	2.4 (0.5)	2.6 (0.5)*	2.6 (0.4)	2.7 (0.4)	2.7 (0.5)
LDL (mmol/l)	3.2 (0.9)	3.3 (0.9)	3.4 (1.1)	3.8 (1.0)	3.8 (1.0)	3.7 (0.8)
HDL (mmol/l)	1.6 (0.4)	1.6 (0.3)	1.5 (0.4)	1.4 (0.4)	1.3 (0.3)	1.2 (0.2)
Triglyceride (mmol/l)	1.0 (0.6)	0.9 (0.4)	1.2 (0.9) [†]	1.7 (1.1)	2.2 (1.4)	2.1 (1.4)
Sonographic findings						
Hepatic steatosis n (%)						
No	87 (100)	88 (100)	88 (100)	—	—	—
Grade I	—	—	—	15 (55.6)	12 (46.2)	12 (44.4)
Grade II/III	—	—	—	12 (44.4)	14 (53.9)	15 (55.6)
Drinking habits						
Alcohol consumption n (%)						
Former consumers	2 (2.3)	2 (2.3)	5 (5.7)	1 (3.7)	0 (0)	2 (7.4)
0 g	34 (39.1)	36 (40.9)	35 (39.8)	7 (25.9)	11 (42.3)	13 (48.2)
1–20 g/day	30 (34.5)	27 (30.7)	28 (31.8)	10 (37.0)	12 (46.2)	10 (37.0)
21–40 g/day	15 (17.2)	14 (15.9)	14 (15.9)	9 (33.3)	3 (11.5)	2 (7.4)
> 40 g/day	6 (6.9)	9 (10.2)	6 (6.8)	—	—	—

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; CRP, C-reactive protein; GGT, γ -glutamyl transferase; WHR, waist-to-hip ratio. * $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$.

($P = 0.0018$). Men had a 2.4-fold increased risk for hepatic steatosis (OR = 2.388; CI = 1.382–4.127). With respect to age, subjects aged 51–65 years had an 11.0-fold risk for hepatic steatosis ($P < 0.0001$). The risk of steatosis increased 1.2-fold for each increase by one s.d. in the proinsulin concentration. BMI and WHR also showed an association with increased proinsulin levels (Table 3).

At multivariate analysis, the parameters gender, age, BMI, WHR, ALT, proinsulin and proinsulin-to-insulin ratio showed a statistically significant association with hepatic steatosis. An increase of one s.d. in the proinsulin concentration was associated with a 1.4-fold increase in the risk of hepatic steatosis ($P = 0.0403$; Table 4).

With respect to the diagnosis of hepatic steatosis, ALT shows a larger area under the ROC curve (AUC = 0.7648) than does proinsulin (AUC = 0.7503; Table 5). The AUC of the model with all covariables (AUC = 0.9101) identified at model selection is

significantly larger than the AUC of the model without proinsulin and ALT (AUC = 0.8971). When the model with all covariables except proinsulin (AUC = 0.9091) was compared with the model containing all covariables (AUC = 0.9101), however, there was no statistically significant difference ($P = 0.7337$). Similarly, there was no statistically significant difference when comparing the model containing all covariables (AUC = 0.9091) with the model containing all covariables except ALT (AUC = 0.9049, $P = 0.4243$; Table 5).

Discussion

In this study, NAFLD was associated with proinsulin concentrations: subjects with higher proinsulin concentrations had an increased risk of developing hepatic steatosis. To our knowledge, this is the first population-based study investigating the association between hepatic steatosis and proinsulin. To date, research has mainly sought to elucidate the association between

Table 3 Relationship between variables and hepatic steatosis. Potential predictors for hepatic steatosis were evaluated by bivariate logistic regression analysis.

	Hepatic steatosis (n)		OR (95% CI)	P value ^a
	No	Yes		
Sex				
Female	125	22	Ref.	
Male	138	58	2.388 (1.382–4.127)	0.0018
Age (years)				
18–30	70	5	Ref.	
31–40	105	24	3.200 (1.166–8.785)	0.0240
41–50	51	22	6.039 (2.143–17.016)	0.0007
51–65	37	29	10.973 (3.920–30.714)	<0.0001
BMI				
<25	175	8	Ref.	
25–29	71	42	12.937 (5.786–28.928)	<0.0001
≥30	17	30	38.593 (15.300–91.349)	<0.0001
WHR				
Normal	195	20	Ref.	
Elevated	68	60	8.603 (4.834–15.311)	<0.0001
Proinsulin			2.064 (1.506–2.829)	<0.0001
Insulin			2.029 (1.473–2.797)	<0.0001
Proinsulin/ insulin ratio			0.545 (0.384–0.772)	0.0006
ALT			2.386 (1.785–3.190)	<0.0001
AST			1.806 (1.407–2.319)	<0.0001
GGT			2.021 (1.484–2.752)	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; WHR, waist-to-hip ratio.

^aBivariate logistic regression.

Significant values are in bold type.

proinsulin and coronary artery disease (CAD), metabolic syndrome and type 2 diabetes mellitus (20, 21, 23, 24, 32, 33).

A comparison of subjects positive for hepatic steatosis with elevated vs normal proinsulin levels showed no significant differences with respect to age, BMI, WHR, gender, severity of steatosis or other laboratory parameters. In agreement with findings in the literature, a statistically significant association between hepatic steatosis and proinsulin as well as other factors, including male gender, advancing age, elevated BMI and WHR and ALT concentration was found (12, 34, 35, 36). It is possible that the elevated ALT concentrations observed in subjects with hepatic steatosis may be associated with the pathomechanism underlying the development of type 2 diabetes mellitus and could be used as a predictor for type 2 diabetes mellitus (37).

The proinsulin-to-insulin ratio is lower in subjects with hepatic steatosis than in those without hepatic steatosis. An elevated proinsulin-to-insulin ratio might thus protect subjects from development of hepatic steatosis (OR=0.545). There have been no previous reports of an association between the proinsulin-to-insulin ratio and hepatic steatosis. According to Hanley *et al.*, there are many contradictory reports regarding an association between overweight and the proinsulin-to-insulin ratio. It is possible that non-diabetic β-cells compensate for the increased insulin demand by increasing insulin secretion. Because the

secretion of proinsulin does not increase, a reduced proinsulin-to-insulin ratio is the result (38).

Considering proinsulin levels without regard to the presence of hepatic steatosis, findings published by Hanley *et al.* (38) reveal a significant correlation between proinsulin, WHR and body fat. Bryhni *et al.* reported an association between increasing age and elevated proinsulin concentrations. Bryhni *et al.* postulate that, with advancing age, there is a decrease in the ability of the β-cells to convert proinsulin to insulin, leading to elevated proinsulin levels. Thus, proinsulin would appear to be an early marker for β-cell dysfunction (21, 39). The study by Strawbridge *et al.* (33) identified new variants of the proinsulin gene; a predisposition for type 2 diabetes mellitus may result from alleles associated with both elevation and reduction in proinsulin concentrations.

Division of subjects into three equal groups (tertiles) based on their proinsulin concentrations revealed no statistically significant differences either for subjects with hepatic steatosis or for those without evidence supporting this diagnosis. In both groups, however, WHR and insulin values showed significant differences within the proinsulin tertiles.

When the group of subjects with ultrasonographic evidence of hepatic steatosis is examined independently of proinsulin levels, subjects with this disorder exhibit higher insulin levels. Similarly, studies by Sung *et al.* (12) and Ardigo *et al.* (36) show that higher fasting insulin concentrations are associated with an increased deposit of intrahepatic fat. There are, however,

Table 4 Stepwise logistic regression analysis to assess the independent predictors of hepatic steatosis. Multiple logistic regression analysis was performed using hepatic steatosis as the dependent variable and proinsulin levels and other variables as the independent variables. For the analysis, constant parameters were z-transformed.

	OR (95% CI)	P value ^a
Gender		
Female	Ref.	
Male	2.598 (1.153–5.851)	0.0212
Age		
18–30	Ref.	
31–40	2.260 (0.646–7.905)	0.2019
41–50	3.370 (0.892–12.727)	0.0732
51–65	4.668 (1.224–17.797)	0.0241
BMI		
<25	Ref.	
25–29	5.954 (2.425–14.619)	<0.0001
≥30	12.244 (4.063–36.895)	<0.0001
WHR		
Normal	Ref.	
Elevated	3.292 (1.525–7.109)	0.0024
ALT	1.709 (1.180–2.474)	0.0046
Proinsulin	1.423 (1.016–1.993)	0.0403
Proinsulin/insulin ratio	0.577 (0.377–0.884)	0.0116

ALT, alanine aminotransferase; CI, confidence interval; Ref, references; WHR, waist-to-hip ratio.

^aMultiple logistic regression.

Significant values are in bold type.

Table 5 Area under the receiver operating characteristic (ROC) curve for detection of steatosis hepatitis.

	AUC	95% CI	P value
Proinsulin	0.7503	0.6913–0.8093	0.7172
ALT	0.7648	0.7062–0.8234	
Main	0.8857	0.8477–0.9237	0.0037
Main + ALT + proinsulin	0.9101	0.8759–0.9444	
Main + proinsulin	0.9049	0.8667–0.9431	0.5996
Main + ALT	0.9091	0.8751–0.9430	
Main + proinsulin	0.9049	0.8667–0.9431	0.4243
Main + ALT + proinsulin	0.9101	0.8759–0.9444	
Main + ALT	0.9091	0.8751–0.9430	0.7337
Main + ALT + proinsulin	0.9101	0.8759–0.9444	
Main	0.8857	0.8477–0.9237	0.0026
Main + ALT	0.9091	0.8751–0.9430	
Main	0.8857	0.8477–0.9237	0.0266
Main + proinsulin	0.9049	0.8667–0.9431	

Main, all covariables (BMI, age, WHR and sex) except proinsulin and ALT; ALT, alanine aminotransferase; AUC, area under the curve; WHR, waist-to-hip ratio. Significant values are in bold type.

also genetic variants that influence intrahepatic fat accumulation (40, 41). According to Speliotes *et al.* (40), NAFLD is genetically determined or hereditary in 26–27% of cases. Certain variants may even exert influence over glycaemic metabolic processes.

Nearly one-third of patients with NAFLD also suffer from diabetes mellitus (15). Although hepatic steatosis and insulin resistance are associated with one another, the association between hepatic steatosis and the development of type 2 diabetes mellitus remains unclear (13). It has been postulated that insulin resistance is involved in the pathogenesis of this disorder (13, 15). According to Pfutzner *et al.* (20), elevated proinsulin levels can be considered a marker for insulin resistance. Compared with the intravenous glucose tolerance test, proinsulin has a specificity of 100% but only a limited sensitivity of 49% for the diagnosis of insulin resistance (20). Because of the significant association between proinsulin and hepatic steatosis found in this study, together with the fact that proinsulin has today become an important diagnostic tool in the diagnosis of type 2 diabetes mellitus (20, 21, 22, 42), it would appear justified to consider the presence of hepatic steatosis and elevated proinsulin levels to be a marker indicating an increased risk for the development of type 2 diabetes mellitus.

A limitation of this study is the use of ultrasonography to diagnose NAFLD. Compared with histological findings, ultrasonography has a reported sensitivity of 80–95% and a specificity of 90–95% for diagnosing this disorder (36, 43). Ethical and logistical factors, however, precluded the use of liver biopsy in a population-based study. The frequency of hepatic steatosis in our population sample stood at 23.3%, which corresponds with findings reported in comparable studies (4, 11); findings also showed a predilection for males at 29.6%, which also corroborates the findings of other studies (6). Another possible

limitation is the fasting time before obtaining blood samples for insulin and proinsulin levels: only 343 of 2445 subjects reported a fasting time of at least 8 h as required for inclusion in the statistical analysis. In addition, the accuracy of fasting times depends on subjects' self-report.

In conclusion, findings of this study support an association between hepatic steatosis and elevated proinsulin concentrations. Because of the known close association of hepatic steatosis with metabolic syndrome and type 2 diabetes mellitus, proinsulin may represent a simple diagnostic criterion for the identification of patients who are at risk for these conditions. Further studies will be required to fully assess the importance of proinsulin as a diagnostic parameter.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The study was initiated by the government of the state of Baden-Württemberg, Germany. Financial support was provided through the Baden-Württemberg State Health Office of the District Government of Stuttgart, Germany, as well as the Regional Health Office in Ravensburg, Germany. Further support was granted by the administration of the City of Leutkirch, Germany. Sample collection supplies and part of the laboratory testing were supplied by Sarstedt AG and Co, Nürnbrecht, Germany. B O Boehm is supported by the German Research Council (GRK 1041).

Author contribution statement

S Wengert, S Oeztuerk, M M Haenle, W Koenig, A Imhof, B O Boehm and W Kratzer were involved in the design and conduct of the study. S Wengert, S Oeztuerk, M M Haenle, W Koenig, A Imhof, B O Boehm, M Wilhelm and R Mao collected and analysed the data. S Wengert, S Oeztuerk, M M Haenle, B O Boehm, R A Mason and W Kratzer were involved in data interpretation and manuscript writing. All authors read and approved the final version.

Acknowledgements

Members of the EMIL Study group (in alphabetical order): G Adler, A Armsen, H-M Banzhaf, M Bauerdick, U Bertling, B O Boehm, B O Brandner, S O Brockmann, M Deckert, C Dingler, S Eggink, M Fuchs, W Gaus, H Goussis, A Gruenert, M M Haenle, W Hampl, C Haug, B Hay, M-L Huetter, A Imhof, P Kern, P Kimmig, A Kirch, D Klass, W Koenig, W Kratzer, M Kron, B Manfras, K Meitinger, T Mertens, R Oehme, G Pfaff, I Piechotowski, S Reuter, T Romig, A F A von Schmiesing, G Steinbach, M Tourbier, A Voegtler, T Walcher, S Wolff.

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Received 3 April 2012

Revised version received 29 October 2012

Accepted 7 November 2012