Clinical and biochemical determinants of the extent of liver steatosis in type 2 diabetes mellitus

Alessandra Cosma[a], Diego Cecchet[a], Silvia Gaiani[b], Anna Coracina[a], Paolo Pellizzari[c], Claudio Pizzi[c], Nicola Veronese[a], David Sacerdoti[b] and Paolo Tessari[a]

Objective Nonalcoholic fatty liver disease is very frequent in both type 2 diabetes mellitus (T2DM) and the metabolic syndrome (MS), which share clinical and metabolic characteristics. Whether and to which extent these characteristics can predict the degree of liver steatosis are not entirely clear.

Patients and methods We determined liver fat (divided into four classes) by standard sonographic images, and clinical and biochemical variables, in 60 consecutive patients with T2DM and with features of the MS. We examined both simple and multiple correlations between the degree of liver steatosis and the variables measured.

Results Increased liver fat (defined as > 5% of liver mass) was detected in 88% of the participants. Using simple regression analysis, the class of steatosis correlated positively with BMI, waist, number of factors of the MS, sex (female > male), diastolic blood pressure, insulin resistance, metabolic control, inflammation, C-reactive protein, fibrinogen, and leptin, whereas it correlated negatively with high-density lipoprotein-cholesterol. Using multiple regression analysis, only metabolic control, insulin resistance and/or plasma insulin, and waist, remained correlated significantly with the degree of steatosis. Using an ordered probit statistical model, metabolic control, waist, and insulin concentration predicted the steatosis class in 58% of the cases (≤97% with allowance for one class in either excess or deficit).

Conclusion In patients with T2DM, the extent of liver steatosis is correlated with variables associated with metabolic control and features of the MS. The combination of metabolic control, visceral obesity, and insulin resistance may reasonably predict the degree of liver steatosis in T2DM. Eur J Gastroenterol Hepatol 27:1386–1391

Introduction

Nonalcoholic fatty liver disease is a silent, potentially dangerous condition in both the general population and many metabolic diseases. Its overall prevalence is high (≈10–20%), even greater in some morbid conditions, such as type 2 diabetes mellitus (T2DM), obesity, and hyperlipidemias. These conditions frequently share prominent characteristics of the metabolic syndrome (MS) [1,2], such as abnormal fat deposition, hypertension, dysglycemia, and hypertriglyceridemia, plus other accompanying abnormalities. Nonalcoholic fatty liver disease itself is now considered a feature of the MS [3]. T2DM and MS indeed appear to be closely related diseases, likely associated by cause–effect relationships because of common etiologic factors that may affect the extent of fat accumulation in the liver.

European Journal of Gastroenterology & Hepatology 2015, 27:1386–1391

Keywords: hemoglobin A_{1c}, leptin, liver steatosis, metabolic control, multiple regression analysis, nonalcoholic fatty liver disease, insulin resistance, predictive model, type 2 diabetes mellitus, visceral adiposity

[a]Department of Medicine, Metabolism Division, [b]Department of Medicine, 5th Internal Medicine, University of Padova, Padova and [c]Department of Economics, University Ca' Foscari Venice, Venice, Italy

Correspondence to Paolo Tessari, MD, Department of Medicine, Metabolism Division, Policlinico Universitario, via Giustiniani 2, 35128 Padova, Italy

Tel: +39 049 821 1748; fax: +39 049 875 4179; e-mail: paolo.tessari@unipd.it

Received 21 April 2015 **Accepted** 23 July 2015

Although insulin resistance is considered the primary cause of liver steatosis [4], subtle differences exist in the role of other risk factors present in different conditions. In non-obese individuals without diabetes, steatosis is more strongly associated with markers of oxidative stress and endothelial dysfunction than with the classic ATP III criteria for the MS [5]. In the population of patients with diabetes of the Diabetes Heart Study, associations between liver steatosis (expressed as a continuous variable) and visceral and subcutaneous fat, plasma lipids, and inflammatory indexes were reported [6]. In the general population, the extent of liver fat predicted both the prevalence [7] and the incidence [8] of the MS, and, conversely, the number of factors of the MS was associated with the degree of liver fat [8]. The presence of liver steatosis increased the association of the MS with diabetes and atherosclerosis [9]. Therefore, either one of these factors may have an impact on the extent of liver steatosis [10]. The combination of these factors in a predictive model could be useful to indirectly determine the extent of liver steatosis in the clinical setting.

In this study, we measured, in T2DM patients with features of the MS [11], the degree of hepatic steatosis (determined by standard sonographic imaging, and divided into four classes), and clinical and biochemical variables, with the aim of determining simple and multiple correlations between these variables and liver fat. Thereafter, we tested the suitability of an ordered probit model to predict the extent of liver fat from the variables measured.

Patients and methods

Sixty consecutive white T2DM patients (male/female ratio: 25/35) were enrolled by a single clinician during their routine visit at the Diabetes Centre at the Azienda Ospedaliera of Padova (Padova, Italy). All data were handled anonymously. The study complied with the 1983 Helsinki Declaration, as amended in 2008, as well as with local regulations for clinical studies. A detailed familial and clinical history had been collected previously for clinical purposes in each patient, including history of diabetes, cardiovascular (CV) diseases, obesity, autoimmune diseases, personal alcohol intake, smoking habits, weekly physical activity, past and present pathologies, and drug assumptions. The presence of diabetic microvascular as well as macrovascular complications was ascertained from each patient's medical records. Fifty patients were treated with oral hypoglycaemic agents, 29 of them with metformin. All except seven patients were also treated with variable combinations of antihypertensive agents, whereas 24 were treated for hyperlipidemia. Five patients had a positive history for ischemic heart disease and two for cerebrovascular disease. No patient was positive for hepatitis C virus infection. Fifty-four patients were nonsmokers (34 had never smoked, 20 were former smokers, but stopped smoking at least 3 years before the study), and six patients were current smokers.

Anthropometric variables (weight, height, BMI, waist, and hip), blood pressure (the average of two measurements to the nearest ± 2 mmHg value after 10 min in the recumbent position), and heart rate were recorded. Following an overnight fast, a blood sample (≈50 ml) was collected for measurements of cell counts, concentrations of plasma glucose (by reflectometer), insulin, and C-peptide (by ELISA), blood hemoglobin A_{1c} (HbA_{1c}) (by HPLC), total and high-density lipoprotein (HDL)-cholesterol, triglycerides (by enzymatic colorimetric methods), total and fractionated bilirubin, albumin, alkaline phosphatase (by colorimetric methods), alanine transaminase, aspartate transaminase, ceruloplasmin, ferritin, transferrin, and immunoglobulins (by standard centralized laboratory methods). Plasma inflammatory cytokines (s-ICAM, s-VCAM, tumor necrosis factor-α, and interleukin-6) and leptin were determined using ELISA methods (Biosource International, Camarillo, California, USA). Plasma thrombomodulin was also measured by ELISA (Diagnostica Stago, Asniéres-sur-Seine, France). High-sensitive (hs) C-reactive protein and fibrinogen (Behring Nephelometer Analyzer; Dade-Behring, Marburg, Germany) concentrations were measured by nephelometry. Markers of A, B, and C viral hepatitis were determined by indirect immunofluorescence. The albumin to creatinine ratio was measured in a spot urine sample. The insulin-resistance index [homeostatic model assessment (HOMA)] was calculated according to Matthews *et al.* [12].

The degree of liver steatosis was grouped into four classes following the ultrasound (US) classification [i.e. from class 0 (no steatosis) to classes 1–3 with increasing fat content] on the basis of the evaluation of (i) liver brightness relative to that of the kidney; (ii) attenuation of the sonographic beam; and (iii) disappearance of vessel wall [13]. An HDI 5000 US equipment (Philips Medical Systems, Bothell, Washington, USA) and a broad-bandwidth phased array transducer (2–5 MHz) were used. All images were obtained with the same presetting of the sonographic equipment – that is, imaging probe, gain, focus, and depth range. The liver ultrasonography was performed in all patients by a single radiologist.

Statistical analysis

Results were expressed as mean ± standard error.

The statistical analysis was carried out using the 'R' program [14]. The Kruskal–Wallis test was used to simultaneously evaluate the equality among the four groups when the normality hypothesis (by the Shapiro–Wilk normality test) [15] was not satisfied. A P value of less than 0.05 was considered statistically significant.

To calculate the prediction of the degree of steatosis from measured variables, we used an 'ordered probit' statistical model [16]. This is a regression model for ordered data: by considering the association among some independent variables, the model describes the likelihood for a patient to fall within a specific class of steatosis.

Results

The clinical and biochemical characteristics of the T2DM patients studied are reported in Table 1. Fifty-six patients fully complied with the ATP III criteria for the MS [11], three fulfilled two criteria (hypertension and diabetes), and one only had diabetes. The overall prevalence of steatosis was 88% (34% mild, 34% moderate, and 20% severe).

Simple and multiple correlations

Significant direct, simple correlations (Table 2) were found between the steatosis class, and either sex (female > male), BMI, waist, hip (but not with the waist/hip ratio), the number of features of MS, diastolic blood pressure, plasma

Table 1. Clinical and biochemical characteristics of the 60 type 2 diabetes mellitus patients studied

BMI (kg/m^2)	32.5 ± 0.7
Waist (cm)	106.8 ± 1.5
Waist/hip ratio	0.97 ± 0.01
PAS (mmHg)	146 ± 2
PAD (mmHg)	88 ± 1
Total cholesterol (mmol/l)	4.86 ± 0.08
HDL-cholesterol (mmol/l)	1.43 ± 0.05
LDL-cholesterol (mmol/l)	2.70 ± 0.08
Triglycerides (mmol/l)	1.57 ± 0.09
AST/ALT < 1 (% of patients)	55
Insulin (pmol/l)	95 ± 8
Plasma glucose (mmol/l)	9.22 ± 0.28
HOMA	5.6 ± 0.6
HbA_{1c} (%) (normal values: 4–5.9%)	7.7 ± 0.2
Leptin (μg/l)	22.5 ± 2.2
hsPCR (mg/l)	4.83 ± 0.64
Ferritin (μg/l)	205 ± 28
TNF-α (ng/l)	7.07 ± 0.57
Average IMT (mm)	0.88 ± 0.03
Maximum IMT (mm)	1.20 ± 0.04
Presence of plaques (%)	58
FMD (%)	5.02 ± 0.23

Data are expressed as mean ± SE.
ALT, alanine transaminase; AST, aspartate transaminase; FMD, flow-mediated vasodilation; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; hsPCR, high-sensitive PCR; HOMA, homeostatic model assessment (index of insulin resistance); IMT, intima–media thickness; LDL, low-density lipoprotein; PAD, diastolic pressure; PAS, systolic pressure; TNF-α, tumor necrosis factor α.

Table 2. Simple linear correlations between the degree of liver steatosis (independent variable) and clinical and metabolic variables

	No steatosis ($N=7$)	Mild steatosis ($N=20$)	Moderate steatosis ($N=20$)	Severe steatosis ($N=13$)	P value
Age (years)	67±1	65±1	65±2	64±2	0.421
Sex (male/female)	4/3	11/9	8/12	2/11	0.031
BMI (kg/m^2)	28.6±1.8	30.7±1.3	33.8±1.2	36.4±1.3	<0.001
Waist (cm)	94.9±4.2	104.7±1.9	108.6±2.4	115.8±2.4	<0.001
Hip (cm)	97.4±5.5	108.6±1.9	113.8±4.4	115.8±2.4	<0.001
Waist/hip	0.99±0.05	0.98±0.01	0.96±0.01	0.96±0.02	0.513
MS factors (N)	2.6±0.3	3.3±0.2	3.5±0.2	3.8±0.2	0.001
PAS (mmHg)	144±9	149±4	146±3	142±3	0.339
PAD (mmHg)	84±2	87±2	90±2	92±3	0.007
Glucose (mmol/l)	8.0±0.7	9.1±0.5	9.1±0.5	10.3±0.8	0.044
Insulin (pmol/l)	52±14	78±10	106±11	142±25	<0.001
HOMA	2.5±0.7	3.9±0.5	6.3±0.8	9.9±2.3	<0.001
HbA_{1c} (%)	6.9±0.2	7.5±0.2	7.9±0.2	8.5±0.5	<0.001
Diabetes duration (years)	13.0±4.8	9.6±1.9	10.6±1.9	9.9±2.4	0.770
AST/ALT	1.29±0.15	0.91±0.05	0.85±0.04	1.04±0.11	0.326
Total cholesterol (mmol/l)	4.9±0.8	4.8±0.2	4.8±0.1	5.0±0.2	0.662
HDL-cholesterol (mmol/l)	1.7±0.3	1.5±0.1	1.4±0.1	1.4±0.1	0.040
TG (mmol/l)	1.2±0.1	1.5±0.1	1.6±0.2	1.7±0.2	0.081
LDL-cholesterol (mmol/l)	2.6±0.2	2.7±0.1	2.7±0.1	2.9±0.2	0.667
Leptin (µ/l)[a]	20.4±9.9	17.3±2.7	26.8±3.5	28.9±4.7	0.006
WBC (×10^3/µl)	5.53±0.7	6.39±0.37	6.78±0.33	6.87±0.41	0.025
Fibrinogen (g/l)	3.09±0.33	3.29±0.17	3.90±0.24	3.97±0.27	0.021
Ferritin (µg/l)	100±27	238±42	169±26	273±100	0.995
hsPCR (mg/l)	2.73±0.49	2.95±0.65	5.70±1.27	7.19±1.94	0.009
TNF-α (ng/l)	5.61±1.96	6.30±0.96	7.40±0.96	9.84±1.24	0.098
FMD (%)	5.10±0.89	4.97±0.46	4.73±0.40	5.25±0.17	0.543
IMT (average) (mm)	0.82±0.08	0.93±0.05	0.85±0.05	0.85±0.06	0.760
IMT (maximum) (mm)	1.14±0.13	1.20±0.07	1.20±0.07	1.30±0.06	0.391
Carotid plaques (%)	43	70	60	69	0.644

Data are expressed as mean±SE.
ALT, alanine transaminase; AST, aspartate transaminase; FMD, flow-mediated vasodilation; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; hsPCR, high-sensitive PCR; IMT, intima–media thickness; LDL, low-density lipoprotein; MS, metabolic syndrome; PAD, diastolic pressure; PAS, systolic pressure; TG, triglyceride; TNF-α, tumor necrosis factor α; WBC, white blood cell.
[a]Analysis carried out on log values.

glucose, insulin (also shown in Fig. 1), HOMA, HbA_{1c}, leptin, white blood cell count, fibrinogen, or hsPCR. An inverse correlation was found between the class of steatosis and HDL-cholesterol.

Using multiple regression analysis, by setting the class of liver steatosis as a dependent variable, and combinations (in group of three) of metabolic and anthropometric variables as independent variables, only HbA_{1c}, HOMA (or insulin) and BMI (or waist) were independently and positively correlated with the degree of hepatic steatosis (Table 3). By including in the analysis, as a fourth independent variable, the number of MS-associated factors and using either BMI or waist as indexes of adiposity, only HbA_{1c} remained a significant predictor of the class of steatosis (P values 0.017 and 0.007, respectively), whereas insulin and HOMA showed borderline significance (P value between 0.07 and 0.1). Following the further addition as an independent variable, of either sex, fibrinogen, hsPCR, or leptin (as log value), only HOMA ($P=0.035$) and BMI ($P=0.033$) remained statistically significantly associated with the steatosis class.

Table 3. Multiple regression analysis between the degree of steatosis (dependent variable) and hemoglobin A_{1c}, waist (or BMI), and insulinemia (or homeostatic model assessment) as independent variables (and after correction for sex)

Independent variables	P
HbA_{1c}	0.031*
BMI	0.083
HOMA	0.021*
HbA_{1c}	0.029*
Waist	0.015*
HOMA	0.064
HbA_{1c}	0.013*
BMI	0.082
Insulin	0.013*
HbA_{1c}	0.017*
Waist	0.023*
Insulin	0.042*

HbA_{1c}, hemoglobin A_{1c}; HOMA, homeostatic model assessment.
*Significant associations.

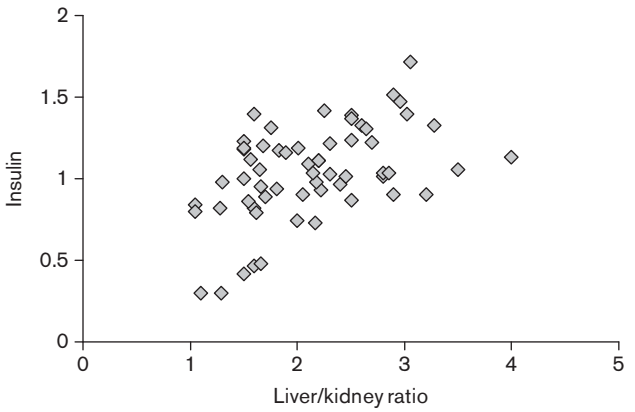

Fig. 1. Direct correlation between the estimate of liver fat (measured with echography and expressed as the liver to kidney ratio) and plasma insulin concentration (pmol/l, log 10 transformation). The reported r and P values refer to the simple correlation. $r=0.523$; $P<0.001$.

Prediction model

The patients' allocation into each of the four steatosis classes, on the basis of the ordered probit model, is reported in the panels of Fig. 2 using different combinations (i.e. models) of variables. The number of patients correctly classified by the statistical model into each class of steatosis versus that determined by the direct echographic measurement is reported in the 'gray' boxes intercepted by the main diagonal. The number of patients in whom the statistical model underestimated steatosis by one or more classes is reported above and to the right of this line, whereas those in whom the model led to overestimation by one or more classes is reported below and to the left of this line.

Using as dependent variables HbA_{1c}, waist and insulinemia, the probability of an exact correspondence between the model and the US data was 57.6% (Fig. 2a). By allowing a 1-grade error, the correspondence was 96.5%, with an overall error of 39% (19% because of overestimation and 20% because of underestimation).

Using as dependent variables HbA_{1c}, number of factors of the MS, and the leptin/BMI ratio, the probability of an exact correspondence between the model and the US data was 57.4% (Fig. 2b). By allowing a 1-grade error, the correspondence was 92.5%, with an overall error of 35% (15% because of overestimation and 20% because of underestimation).

Using as dependent variables HbA_{1c}, number of factors of the MS, waist, BMI, HOMA, and the leptin/BMI ratio, the probability of an exact correspondence between the model and the US data was 63.3% (Fig. 2c). By allowing a 1-grade error, the correspondence was 94%, with an overall error of 30% (16% because of overestimation and 14% because of underestimation).

Finally, using as dependent variables sex, HbA_{1c}, number of factors of the MS, and insulinemia, the probability of an exact correspondence between the model and the US data was 542% (Fig. 2d). By allowing a 1-grade error, the correspondence was 95%, with an overall error of 41% (17% because of overestimation and 24% because of underestimation).

Discussion

In this study, we investigated the relationships between clinical and metabolic parameters, commonly associated with features of diabetes and the MS, and the degree of liver steatosis (divided into four classes, and evaluated using a noninvasive, ultrasonic technique) in 60 T2DM patients, most of them (≈93%) complying with the criteria

(a)
Model 1

Statstical model \ Echography	0	1	2	3
0	2	0	0	0
1	5	15	7	1
2	0	4	11	5
3	0	1	2	6

(b)
Model 2

Statstical model \ Echography	0	1	2	3
0	3	1	0	0
1	2	11	5	1
2	2	4	13	5
3	0	1	2	4

(c)
Model 3

Statstical model \ Echography	0	1	2	3
0	4	0	0	0
1	0	10	4	1
2	2	6	12	3
3	0	0	2	5

(d)
Model 4

Statstical model \ Echography	0	1	2	3
0	2	1	0	0
1	5	13	7	1
2	0	4	11	6
3	0	2	1	5

Fig. 2. Capability of the statistical model(s) to predict the class (0 = absence; 1 = low; 2 = medium; and 3 = high, as indicated on the top horizontal line) of liver steatosis compared with echography. The number of cases identified by both the model and echography is shown in the gray boxes. The numbers of cases reported in boxes either immediately above or below the gray boxes are those with 1-error degree of class prediction (i.e. either overestimation or underestimation by 1 class, respectively). (a) Model 1: calculated using hemoglobin A_{1c} (HbA_{1c}), waist, and insulinemia as dependent variables. (b) Model 2: calculated using HbA_{1c}, number of factors of the metabolic syndrome, and the ratio between waist and insulinemia as dependent variables. (c) Model 3: calculated using HbA_{1c}, number of factors of the metabolic syndrome, waist, BMI, homeostatic model assessment, and the leptin/BMI ratio as dependent variables. (d) Model 4: calculated using sex, HbA_{1c}, number of factors of the metabolic syndrome, and insulinemia as dependent variables.

of the MS according to ATP III [11] (Table 1). Using simple regression analysis, we found that the degree of liver steatosis was associated positively with obesity, the number of coexisting MS factors, diastolic blood pressure, a poorer metabolic control, lower HDL-cholesterol, and inflammatory markers. Using multiple regression analysis, only HOMA (or insulin concentrations), visceral obesity, and HbA_{1c} remained significantly and independently associated with steatosis. Therefore, from our observations, metabolic control, insulin resistance, and (visceral) adiposity emerged as the strongest factors associated with the degree of liver steatosis in T2DM.

These observational data largely agree with previous findings reported in both the general population and in morbid conditions [6–8]. They are also in agreement with theoretical assumptions and/or pathophysiological mechanism(s) underlying the role of poor glycemic control (likely secondary to insulin resistance, poor compliance to therapy, or other reasons), insulin [as a suppressor of very low density lipoproteins secretion, thus enhancing liver triglyceride accumulation] and visceral fat (as a major metabolic conditioner) as possible causes of liver steatosis.

The significant association between hepatic steatosis and HbA_{1c}, which also persisted using various combinations of independent variables in the multivariate analysis (Table 3), is interesting and somehow new. HbA_{1c} was worse in the patients with greater liver fat content (Table 2). These data are in agreement with previous reports showing that HbA_{1c} was greater in T2DM patients with liver steatosis than in those without liver steatosis [17, 18], but not with other studies [19]. The careful allocation of our patients to each class of liver steatosis strongly supports a role of metabolic control as a determinant of the extent of liver fat accumulation in T2DM, likely mediated by insulin resistance.

Although liver steatosis was found to increase the association of either diabetes or the MS with clinical atherosclerosis [9], in our study we did not find any correlation between the degree of liver steatosis and early CV abnormalities and/or risk factors (i.e. intima–media thickness, flow-mediated vasodilation, and carotid plaques) as reported previously [20]. Such an unexpected finding deserves further comments. Adiposity itself, the number of CV risk factors, and/or other metabolic and clinical characteristics, already present in our T2DM patients and commonly associated with the MS, might have obscured the potential impact of the degree of liver steatosis itself on the early signs of atherosclerosis. Alternatively, the relatively old age as well as the long disease duration of our patients, in whom a number of potentially adverse CV factors had been accumulated over the years, might have outweighed the possible role, as a CV risk factor, of liver steatosis *per se*. It would be interesting to observe the patients prospectively, still free from major CV events, to ascertain whether any future event would be linked to the degree of liver steatosis.

Leptin levels were significantly greater, on bivariate analysis, in the two higher classes of steatosis (Table 2). These data confirm previous findings in unselected individuals, reporting an association between liver steatosis and leptin levels [21], and extend this observation to T2DM. From a mechanistic standpoint, the direct relationship between leptin and steatosis may either reflect a pathogenic role of leptin itself in lipotoxicity, or, more likely, the failure of the antisteatogenic action of leptin, suggesting a state of 'peripheral leptin resistance', as reported for obesity [22].

We used a predictive, statistical model using different combinations of variables, with the aim of testing which model could be better associated with the degree of liver steatosis as determined directly by echography. These results are reported in Fig. 2. Model prediction was the best using HbA_{1c}, waist and insulinemia as dependent variables. The accuracy was ~58%, increasing to ~97% by allowing an error of one class (i.e. as either overestimation or underestimation). The other models tested performed slightly worse, but their results were very close to those of the best model (Fig. 2). Therefore, metabolic control, visceral adiposity, and insulin concentration (and/or resistance) in combination appeared to be the best predictors of the class of steatosis. These indexes are relatively easy to measure in clinical practice; therefore, they could provide a useful and immediate tool to estimate indirectly the degree of liver steatosis in these patients.

We underline the fact that our regression model is based on a static cross-section of patients. Therefore, although the degree of steatosis is identified from the covariates, we cannot compute time-related variables as hazard ratios or times at which steatosis appears or worsens.

In conclusion, in this study, we report a number of correlations between features of the MS and the class of liver steatosis in a group of T2DM patients, most of them complying with the MS criteria. The extent of liver fat was associated with indexes of metabolic control, insulin resistance, and visceral adiposity. A simple predictive model of the extent of steatosis, on the basis of these indexes and potentially useful in clinical practice, is proposed.

Acknowledgements

The authors thank Dr Raffaella Marin for measurements of plasma lipids and Dr Cristina Marescotti for measurement of inflammatory cytokines. They are indebted to Dr Stefania Maggi for her helpful advice in the design of the study.

This study was supported by an ex 60% grant from the University of Padova.

Conflicts of interest

There are no conflicts of interest

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