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# ORIGINAL ARTICLE

# Predictors of non-alcoholic fatty liver disease in obese children

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**Objective:** To evaluate predictors of non-alcoholic fatty liver disease (NAFLD) in obese children.
**Design:** Cross-sectional study.
**Subjects:** Two hundred and sixty-eight obese children not consuming alcohol and without hepatitis B or C were consecutively studied at an auxology clinic.
**Measurements:** Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, uric acid, glucose, glucose during oral glucose tolerance testing (OGTT), insulin, insulin during OGTT, insulin resistance as estimated by homeostasis model assessment (HOMA), C-reactive protein (CRP), and systolic and diastolic blood pressure were measured. Fatty liver was diagnosed by ultrasonography using standard criteria. Univariable and multivariable logistic regression was used to evaluate predictors of NAFLD. All predictors except gender and pubertal status were modeled as continuous variables.
**Results:** NAFLD was detected in 44% of obese children. At univariable analysis, male gender, *Z*-score of body mass index (BMI) (*Z*-BMI), ALT, AST, GGT, triglycerides, uric acid, glucose, glucose during OGTT, insulin, insulin during OGTT, HOMA, CRP and systolic blood pressure were predictors of NAFLD, whereas HDL-cholesterol and late-pubertal status were predictors of the normal liver. At multivariable analysis, however, only *Z*-BMI, ALT, uric acid, glucose during OGTT and insulin during OGTT were independent predictors of NAFLD.
**Conclusion:** *Z*-BMI, ALT, uric acid, glucose during OGTT and insulin during OGTT are independent predictors of NAFLD in Italian obese children, with most of the prediction explained by ALT and *Z*-BMI.
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**Keywords:** non-alcoholic fatty liver disease; obesity; children; adolescents; prevalence; risk factors

## Introduction

Non-alcoholic fatty liver disease (NAFLD) has been reported recently as the most frequent liver disease in children (Lavine and Schwimmer, 2004). Although simple steatosis has a benign prognosis, non-alcoholic steatohepatitis (NASH), with advanced histopathology, may progress to cirrhosis (Molleston *et al*., 2002; Brunt, 2004).

Pooling data from studies performed mainly in tertiary medical centers, the prevalence of NAFLD in obese children has been reported to range from 20 to 77% (Chan *et al*., 2004). Because alcohol consumption and hepatic viral infections are uncommon at this age (Bedogni *et al*., 2004), fatty liver in children is due predominantly to NAFLD.

Obesity, hyperglycemia and hypertriglyceridemia are the most important risk factors for NAFLD in adults, and insulin resistance is hypothesized to play a central role in its pathogenesis (Marchesini *et al*., 2003; Neuschwander-Tetri and Caldwell, 2003; Bedogni *et al*., 2005). Owing to this evidence, NAFLD is presently considered a manifestation of the metabolic syndrome (Marchesini *et al*., 2003; Neuschwander-Tetri and Caldwell, 2003). There is increasing

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evidence that obesity, hyperglycemia and insulin resistance are risk factors for NAFLD also in children (Kawasaki *et al*., 1997; Schwimmer *et al*., 2003, 2005; Chan *et al*., 2004). In epidemiologic studies, insulin resistance is evaluated most commonly using the homeostasis model assessment (HOMA) method (Bonora *et al*., 2000; Wallace *et al*., 2004), but measurement of insulin during oral glucose tolerance testing (OGTT) is considered an optimal means of evaluating insulin resistance (Matsuda and DeFronzo, 1999; Iughetti *et al*., 2004).

C-reactive protein (CRP) is frequently elevated in subjects with the metabolic syndrome (Brea *et al*., 2005). Moreover, CRP is an independent predictor of NAFLD in adults (Park *et al*., 2004) and is higher in children with liver steatosis and elevated serum alanine aminotransferase (ALT) than in children with normal liver and serum ALT (Mandato *et al*., 2005). Serum uric acid is elevated in most subjects with the metabolic syndrome, and has been proposed as an independent predictor of NAFLD in adults (Lonardo *et al*., 2002).

Serum ALT has been proposed as a marker of NAFLD in epidemiologic studies after the exclusion of hepatic viral infections and metabolic diseases (Clark *et al*., 2003; Yu and Keeffe, 2003; Schwimmer *et al*., 2005). However, utilizing elevated serum ALT as a marker of NAFLD results in the omission of 46% cases of diseases in the general adult population (Bedogni *et al*., 2005). The accuracy of serum ALT for detecting NAFLD in obese children has not undergone a formal evaluation.

We tested whether insulin resistance, CRP and serum uric acid are independent predictors of NAFLD, and evaluated the accuracy of serum ALT as a marker of NAFLD in obese children.

## Methods

*Subjects*

Two hundred and sixty-eight children were studied consecutively at the Auxology Division of the Istituto Auxologico Italiano (Piancavallo, Verbania, Italy). Eligibility criteria were: (1) age $\geqslant 6$ and $\leqslant 20$ years; (2) body mass index (BMI) $>$90th percentile for gender and age using the Italian reference data (Cacciari *et al*., 2002); (3) secondary obesity; (4) absence of any drug treatment; (5) abstinence from alcohol; (6) absence of serological markers of hepatitis B (HBV) and hepatitis C (HCV).

*Methods*

Liver ultrasonography was performed by the same operator implementing standard criteria (Saverymuttu *et al*., 1986; Joseph *et al*., 1991). Mild steatosis was defined as slightly increased liver echogenicity with normal vessels and absent posterior attenuation; moderate steatosis as moderately increased liver echogenicity with partial dimming of vessels and early posterior attenuation; and severe steatosis as diffusely increased liver echogenicity with absence of visible vessels and heavy posterior attenuation. NAFLD was operationally defined as any degree of fatty liver in the absence of HBV and HCV infection and alcohol intake. Normal liver was defined as the absence of fatty liver. Pubertal status was classified as pre-pubertal (stage 1), early pubertal (stages 2 and 3) or late pubertal (stages 4 and 5) according to Tanner (1990). Because there were few pre-pubertal subjects ($n=13$), pre-pubertal and early pubertal children were pooled ($n=94$) and compared to late pubertal children ($n=174$). Alcohol consumption was determined by interview with the children and/or their parents. Hepatitis B surface antigen (HBsAg) and antibodies against hepatitis C virus (HCV) were measured to rule out hepatitis B and C (Bellentani *et al*., 1999). Weight and stature were measured following the anthropometric standardization reference manual (Lohman *et al*., 1990). BMI was calculated as weight (kg)/stature (m)$^2$. *Z*-scores of weight (*Z*-weight), stature (*Z*-stature) and BMI (*Z*-BMI) were calculated from Italian reference data using the LMS method (Cacciari *et al*., 2002). Serum ALT, aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, uric acid and glucose were measured using standard laboratory methods and insulin was measured using a chemiluminescent immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). Insulin resistance was estimated using the HOMA method (Wallace *et al*., 2004). CRP was measured using an immunoturbidimetric assay (CRP RX, Roche Diagnostics, Indianapolis, IN, USA). Systolic and diastolic blood pressures were measured following standard procedures (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004). Glucose and insulin were measured at 0, 30, 60, 90 and 120 min during an OGTT performed with 1.75 g of glucose kg of weight (up to 75 g). The areas under the curve (AUC) of insulin and glucose during OGTT were calculated using the trapezoid rule and divided by 10 000. The study protocol was approved by the local Ethics Committee and parental consent was obtained.

*Statistical analysis*

Values of continuous variables are given as medians and interquartile ranges (IQR) because of skewed distributions. Between-group comparisons of continuous variables were performed with the Mann—Whitney test and those of ordinal variables with Fisher' s exact test. Logistic regression was used to evaluate the association between potential predictors and NAFLD, coded as present vs absent. Besides gender and pubertal status, all predictors were evaluated as continuous variables. ALT, AST, GGT, cholesterol, HDL, LDL, triglycerides, glucose, insulin, and systolic and diastolic blood pressure were divided by 10 before use in the logistic regression models. Insulin, HOMA and insulin-OGTT were

log-transformed using natural logarithms to achieve linearity of logits. Significant predictors of NAFLD at univariable analysis were evaluated in three separate multivariable logistic regression models differing only for how glucose and insulin were evaluated. Model 1 employed gender, pubertal status, *Z*-BMI, ALT, AST, GGT, HDL-cholesterol, triglycerides, uric acid, glucose, insulin, CRP and systolic blood pressure as predictors; model 2 employed gender, pubertal status, *Z*-BMI, ALT, AST, GGT, HDL-cholesterol, triglycerides, uric acid, HOMA, CRP and systolic blood pressure as predictors; model 3 employed gender, pubertal status, *Z*-BMI, ALT, AST, GGT, HDL-cholesterol, triglycerides, uric acid, glucose-OGTT, insulin-OGTT, CRP and systolic blood pressure as predictors. Multivariable fractional polynomials were used to test whether linearity of logits could be improved by power transformation of predictors in

**Table 1** Measurements of the children

| | *Normal liver* (n = *149*) | *NAFLD* (n = *119*) | P* |
|---|---|---|---|
| Age (years) | 14.9 (3.3) [8.1–18.5] | 14.6 (3.5) [7.7–18.4] | 0.262 |
| Gender (male/female, *n*) | 49/100 | 69/50 | <0.0001 |
| Pubertal status (pre + early/late, *n*) | 42/107 | 52/67 | 0.008 |
| Weight (kg) | 87.8 (23.2) [50.3–154.3] | 98.9 (29.9) [55.7–175.5] | <0.0001 |
| *Z*-weight (SDS) | 2.38 (0.92) [0.35–4.19] | 2.82 (0.78) [1.20–4.66] | <0.0001 |
| Stature (m) | 1.60 (0.12) [1.37–1.97] | 1.62 (0.13) [1.27–1.82] | 0.6238 |
| *Z*-stature (SDS) | 0.21 (1.54) [−2.84–3.56] | 0.23 (1.56) [−2.35–2.99] | 0.717 |
| BMI ($kg\,m^{2-1}$) | 34.2 (6.1) [25.7–49.6] | 37.9 (8.7) [27.8–58.6] | <0.0001 |
| *Z*-BMI (SDS) | 2.5 (0.7) [1.4–3.8] | 2.9 (0.6) [1.7–4.5] | <0.0001 |
| ALT ($U\,l^{-1}$) | 19 (12) [7–94] | 36 (33) [7–245] | <0.0001 |
| AST ($U\,l^{-1}$) | 20 (7) [10–50] | 26 (15) [12–92] | <0.0001 |
| GGT ($U\,l^{-1}$) | 15 (7) [2–100] | 20 (12) [6–114] | <0.0001 |
| Cholesterol ($mg\,dl^{-1}$) | 160 (43) [74–263] | 164 (36) [75–258] | 0.7307 |
| HDL-cholesterol ($mg\,dl^{-1}$) | 48 (15) [28–113] | 43 (13) [24–80] | 0.0011 |
| LDL-cholesterol ($mg\,dl^{-1}$) | 102 (41) [19–199] | 110 (35) [21–207] | 0.1963 |
| Triglycerides ($mg\,dl^{-1}$) | 78 (39) [9–208] | 91 (62) [37–247] | 0.0135 |
| Uric acid ($mg\,dl^{-1}$) | 5.9 (1.6) [3.7–8–7] | 6.6 (1.7) [3.2–10.6] | <0.0001 |
| Glucose ($mg\,dl^{-1}$) | 77 (10) [60–93] | 70 (11) [54–120] | 0.0092 |
| Glucose OGTT ($AUC\,10000^{-1}$) | 1.27 (0.25) [0.84–1.98] | 1.34 (0.27) [0.96–2.70] | 0.0002 |
| Insulin ($mU\,L^{-1}$) | 9 (6) [2–73] | 13 (10) [3–82] | <0.0001 |
| Insulin OGTT/($AUC\,10000^{-1}$) | 0.65 (0.41) [0.26–2.40] | 0.89 (0.56) [0.29–2.54] | <0.0001 |
| HOMA | 1.8 (1.3) [0.3–16.0] | 2.6 (1.8) [0.6–14.2] | <0.0001 |
| CRP ($mg\,dL^{-1}$) | 0.3 (0.5) [0.1–8.5] | 0.5 (0.8) [0.1–6.2] | 0.0036 |
| Systolic blood pressure (mm Hg) | 120 (10) [95–170] | 125 (15) [100–170] | 0.0350 |
| Diastolic blood pressure (mm Hg) | 80 (10) [50–100] | 80 (10) [40–110] | 0.6972 |

Data are given as medians, interquartile ranges (round brackets), and minimum and maximum values (square brackets).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; CR*P*, C-reactive protein; GGT, gamma-glutamyl-transferase; HDL-cholesterol, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; LDL, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; SDS, standard deviation scores; *Z*-weight, *Z*-score of weight; *Z*-stature, *Z*-score of stature; Z-BMI, z-score of BMI.

multivariable models. No transformation improved linearity so that all variables were modeled with a power of 1. The Hosmer–Lemeshow statistic was used to assess the goodness-of-fit of the models (Hosmer and Lemeshow, 2000). Probabilities obtained by logistic regression were used to draw ROC curves using the DeLong, DeLong and Clarke–Pearson method and the area under the ROC curves (AUC) was used to assess the accuracy of the models (Zhou *et al*., 2002). Statistical significance was set to a value of $P<0.05$ for all tests. Statistical analysis was performed using STATA 9.2 (STATA Corporation, College Station, TX, USA).

## Results

*Measurements of children with and without NAFLD*

Forty-four percent of the 268 children ($n=119$) had NAFLD. Among children with NAFLD, 35 had mild, 69 had moderate and 15 had severe steatosis. Table 1 gives the measurements of the children with and without NAFLD.

Age was similar in children with and without NAFLD ($P=0.262$). NAFLD was more frequent in males than in females (58 vs 33%, $P<0.0001$) and less frequent in late pubertal than in pre-pubertal and early pubertal children (38 vs 55%, $P=0.008$). *Z*-BMI ($P<0.0001$), ALT ($P<0.0001$), AST ($P<0.0001$), GGT ($P<0.0001$), HDL-cholesterol ($P=0.0011$), triglycerides ($P=0.0135$), uric acid ($P<0.0001$), glucose ($P=0.0092$), glucose-OGTT ($P=0.0002$), insulin ($P<0.0001$), insulin-OGTT ($P<0.0001$), HOMA ($P<0.0001$), CRP ($P=0.0036$) and systolic blood pressure ($P=0.0350$) were higher in children with NAFLD than in those without NAFLD. Cholesterol ($P=0.7307$), LDL-cholesterol ($P=0.1963$) and diastolic blood pressure ($P=0.6972$) did not differ between children with and without NAFLD.

**Table 2** Univariable analysis of predictors for NAFLD in obese children

| | *OR (95% CI) [p-LR]* |
|---|---|
| Male gender | 2.81 (1.71–4.64) [<0.0001] |
| Late puberty | 0.51 (0.31–0.84) [0.009] |
| Z-BMI (SDS) | 4.97 (2.89–8.56) [<0.0001] |
| ALT ($U\,l^{-1}$/10) | 1.56 (1.33–1.83) [<0.0001] |
| AST ($U\,l^{-1}$/10) | 2.22 (1.62–3.05) [<0.0001] |
| GGT ($U\,l^{-1}$/10) | 2.02 (1.48–2.76) [<0.0001] |
| HDL ($mg\,dl^{-1}$/10) | 0.70 (0.56–0.88) [0.002] |
| Triglycerides ($mg\,dl^{-1}$/10) | 1.09 (1.03–1.15) [0.005] |
| Uric acid ($mg\,dl^{-1}$) | 1.73 (1.39–2.16) [<0.0001] |
| Glucose ($mg\,dl^{-1}$/10) | 1.62 (1.19–2.22) [0.002] |
| Glucose OGTT (AUC/10000) | 12.35 (3.56–42.80) [<0.0001] |
| Insulin [ln($mU\,L^{-1}$/10)] | 3.51 (2.14–5.75) [<0.0001] |
| Insulin OGTT [ln(AUC/10000)] | 3.68 (2.02–6.71) [<0.0001] |
| HOMA (ln) | 3.48 (2.17–5.59) [<0.0001] |
| CRP ($mg\,dl^{-1}$) | 1.43 (1.12–1.82) [0.005] |
| Systolic blood pressure (mm Hg/10) | 1.23 (1.01–1.50) [0.039] |

Abbreviations: 95% CI = 95% confidence interval; OR, odds ratio; *P*-LR, value of *P* associated with the likelihood ratio test; other abbreviations as in Table 1.

*Univariable analysis of NAFLD predictors*

Table 2 gives the results of the univariable analysis of NAFLD predictors.

Confirming the results of between-group comparisons (cp. Table 1), age ($P=0.183$), cholesterol ($P=0.713$), LDL-cholesterol ($P=0.215$) and diastolic blood pressure ($P=0.531$) were not associated with NAFLD (likelihood ratio test; data not shown). NAFLD was 2.8 times more likely in males and 0.5 times less likely in late-pubertal than in pre-pubertal and early-pubertal children. An increase of 1 standard deviation score (SDS) of *Z*-BMI was associated with a fivefold increase in the odds of NAFLD. An increase of $10\,U\,l^{-1}$ of ALT, AST and GGT increased the odds of NAFLD of 56, 122 and 102%, respectively. An increase of $10\,mg\,dl^{-1}$ of triglycerides was associated with a 9% increase and one of HDL with a 30% decrease in the odds of NAFLD. The odds ratio (OR) of NAFLD associated with an increase of $1\,mg\,dl^{-1}$ of uric acid was 1.73 and the corresponding value for CRP was 1.43. An increase of $10\,mg\,dl^{-1}$ of glucose was associated with a 62% increase and one of log-transformed insulin and with a 251% increase in the odds of NAFLD. An increase of 1 unit of log-transformed HOMA was similarly associated with a 248% increase in the odds of NAFLD. An increase of 1 unit of glucose-OGTT had the highest OR (12.35) and was a stronger predictor of NAFLD than one of log-transformed insulin-OGTT (OR = 3.68). Lastly, an increase of 10 units of systolic blood pressure increased the odds of NAFLD of 23%. The accuracy of univariable predictions, as determine by ROC–AUC, is given in Figure 1.

*Multivariable analysis of NAFLD predictors*

Significant predictors of NAFLD at univariable analysis (cp. Table 2) were evaluated in three logistic regression models differing for how insulin and glucose were evaluated: fasting glucose and insulin (model 1) vs HOMA (model 2) vs insulin-OGTT and glucose-OGTT (model 3) (see Methods). In models 1 and 2, only *Z*-BMI, ALT and uric acid were identified as significant predictors (data not shown); in model 3, glucose-OGTT and insulin-OGTT were significant together with *Z*-BMI, ALT and uric acid (Table 3).

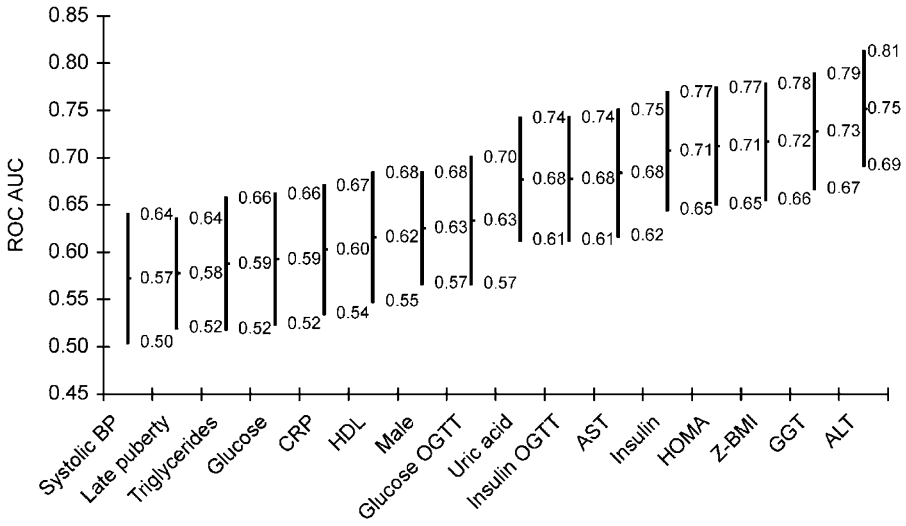


**Figure 1** Accuracy of univariable predictors of NAFLD in obese children. Values are areas under the receiver operating characteristic curve (ROC–AUC) with 95% intervals; abbreviations as in Tables 1 and 2.

**Table 3** Multivariable analysis of predictors for NAFLD in obese children

| | *OR (95% CI)* | P-*Wald* | *Std. coeff.* |
|---|---|---|---|
| Male gender | 0.65 (0.28–1.48) | 0.303 | −0.1200 |
| Late pubertal status | 0.47 (0.21–1.06) | 0.070 | −0.1963 |
| ZBMI (SDS) | 4.65 (2.26–9.55) | <0.0001 | 0.4788 |
| ALT ($U\,l^{-1}$/10) | 1.69 (1.20–2.37) | 0.002 | 0.8905 |
| AST ($U\,l^{-1}$/10) | 0.79 (0.39–1.63) | 0.529 | −0.1578 |
| GGT ($U\,l^{-1}$/10) | 0.76 (0.54–1.08) | 0.130 | −0.1889 |
| HDL ($mg\,dl^{-1}$/10) | 0.96 (0.72–1.28) | 0.776 | −0.0282 |
| Triglycerides ($mg\,dl^{-1}$/10) | 0.95 (0.87–1.03) | 0.196 | −0.1309 |
| Uric acid ($mg\,dl^{-1}$/10) | 1.45 (1.08–1.96) | 0.015 | 0.2635 |
| Insulin OGTT [ln(AUC/10000)] | 2.14 (1.07–4.27) | 0.031 | 0.2064 |
| Glucose OGTT [ln(AUC/10000)] | 6.63 (1.58–27.84) | 0.010 | 0.2480 |
| Systolic blood pressure (mm Hg/10) | 1.00 (0.77–1.30) | 0.993 | −0.0008 |
| CRP ($mg\,dl^{-1}$) | 1.00 (0.70–1.44) | 0.994 | 0.0006 |

Abbreviations: *P*-Wald = value of *P* associated with the Wald test; Std. coeff. = standardized regression coefficient; other abbreviations as in Tables 1 and 2.

As determined by standardized regression coefficients, ALT was the strongest multivariable predictor of NAFLD. In comparison, *Z*-BMI explained 54% of the variability explained by ALT, uric acid 30%, glucose-OGTT 28% and insulin-OGTT 23%. Model 3 fitted well ($P=0.563$, Hosmer–Lemeshow statistic) and its accuracy (AUC = 0.84, 95% CI 0.79–0.88) was good and significantly higher than that of the univariable *Z*-BMI ($P<0.0001$) and ALT models ($P=0.0011$, Bonferroni's correction; Figure 1).

*Accuracy of ALT as a marker of NAFLD*

Serum ALT was greater than $30\,U\,l^{-1}$ in 104 children (39%) and greater than $40\,U\,l^{-1}$ in 66 children (25%). 76 children with ALT greater than $30\,U\,l^{-1}$ and 49 with ALT greater than $40\,U\,l^{-1}$ had NAFLD ($P<0.0001$ for both). The sensitivity and specificity associated with the prediction of NAFLD from ALT were 64 and 81% at a cutoff of $30\,U\,l^{-1}$ (ROC–AUC = 0.72, 95% CI 0.67–0.77) and 41 and 89% at one of $40\,U\,l^{-1}$ (ROC–AUC = 0.65, 95% CI 0.60–0.70). Use of ALT as continuous variable (Figure 1) improved the accuracy of the estimate as compared to the cut-point of $40\,U\,l^{-1}$ ($P<0.0001$) but not to that of $30\,U\,l^{-1}$ ($P=0.2350$, Bonferroni's correction).

## Discussion

In the present study, 44% of a large sample of obese children studied at an auxology obesity clinic had NAFLD defined as the presence of liver steatosis at ultrasonography in the absence of HBV and HCV infection and alcohol consumption. It should be noted that our criterion for alcohol intake was more restrictive than that commonly employed, that is, less than or equal to $20\,g\,day^{-1}$ (Neuschwander-Tetri and

Caldwell, 2003; Bedogni *et al*., 2005), because the great majority of our children did not drink alcohol. Even if a prevalence of 44% is within the expected range, it is substantially lower than that observed in a recent series of Chinese children with a similar degree of obesity (77%, $P<0.0001$, Fisher's exact test) (Chan *et al*., 2004). This difference is of interest because it may be explained by genetic and/or environmental factors, similarly to what has been hypothesized for adults (Browning *et al*., 2004; Weston *et al*., 2005).

Despite the identification of many predictors of NAFLD at univariable analysis (Table 2), only *Z*-BMI, ALT, uric acid, glucose-OGTT and insulin-OGTT were independent predictors of NAFLD in our obese children. It should be pointed out that the contribution of glucose-OGTT and insulin-OGTT to the prediction of NAFLD was slightly lower than that of uric acid and substantially lower than that of ALT and BMI (Table 3). Using a different analytic approach (ordinal regression analysis with NAFLD severity as the outcome), BMI, ALT and insulin were identified as independent predictors of NAFLD also in a recent cross-sectional study of obese Chinese children (Chan *et al*., 2004). It should be, however pointed out that, according to the results of a longitudinal study performed in obese Japanese children, obesity duration may be better than BMI in predicting liver fibrosis (Kinugasa *et al*., 1984). Moreover, our children with severe liver steatosis and elevated ALT ($n=11$) had higher values of fasting insulin (14 (15) vs 9 (6), median (IQR), $P=0.0057$), insulin during OGTT (0.84 (0.62) vs 0.61 (0.36), $P=0.0103$) and HOMA (2.8 (3.2) vs 1.7 (1.1), $P=0.0040$) than those without liver steatosis and elevated ALT ($n=121$). Although insulin measurement during OGTT is considered the best option to evaluate insulin resistance (Matsuda and DeFronzo, 1999), it is not simple to perform in epidemiologic studies, where fasting insulin and HOMA are more frequently employed (Bonora *et al*., 2000; Wallace *et al*., 2004). It is therefore of some interest that in the present study, an independent contribution of insulin and glucose to NAFLD emerged only when they were modeled as OGTT measurements.

Interestingly, uric acid, suggested as an independent predictor of NAFLD in adults (Lonardo *et al*., 2002), was confirmed to be an independent predictor of NAFLD in our study of obese children. Contrary to studies performed in adults (Park *et al*., 2004), we found no evidence that CRP is an independent predictor of NAFLD in obese children. However, CRP levels tended to be higher in our children with NAFLD and elevated ALT (0.4 (0.6), median (IQR), $n=76$) than in those without NAFLD and normal ALT (0.3 (0.5), $n=121$, $P=0.065$), as found in a case–control study by Mandato *et al*. (2005).

Employing *Z*-BMI, ALT, uric acid, glucose-OGTT and insulin-OGTT as predictors, 84% of cases of NAFLD could be identified in our children. Using the common cutoffs of 30 and 40 $U\,l^{-1}$, the true positive rate of NAFLD as detected by ALT was 64 and 41% and the true negative rate was 81 and 89%, respectively. Thus, similar to what has been shown for adults (Bedogni *et al*., 2005), ALT alone should not be used as a surrogate marker of NAFLD in obese children.

Even if the present study was performed in a large series of obese children, its limitations must be recognized. First, its results cannot be extended to the general population. The lack of a control group of normal-weight children, due mainly to ethical constraints, does not allow to establish whether the identified predictors will work equally well in non-obese children with NAFLD. Second, as we have discussed in detail elsewhere (Bedogni and Bellentani, 2004; Bedogni *et al*., 2005), although ultrasonography is a desirable technique for epidemiologic studies owing to being readily available and non-invasive, it cannot detect a fatty infiltration $<30\%$, thus possibly underestimating the prevalence of fatty liver. More importantly, ultrasonography cannot detect inflammation and fibrosis and thus, cannot be used to establish the severity of the underlying disease (Neuschwander-Tetri and Caldwell, 2003). Thus, the predictors identified in our study should not be considered predictors of NASH. Third, whereas we excluded ALT elevations owing to alcohol intake, viral infections and drug consumption, we did not perform tests to exclude ALT elevations owing to metabolic disorders such as $\alpha$-1-antitrypsin deficiency, Wilson's disease and celiac disease.

In conclusion, (1) in Italian obese children, *Z*-BMI, ALT, uric acid, glucose-OGTT and insulin-OGTT are independent predictors of NAFLD, whereas CRP is not, and (2) ALT alone is not accurate enough to be employed as a marker of NAFLD.

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