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BRIEF ARTICLE

Changes of liver fat content and transaminases in obese children after 12-mo nutritional intervention

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Abstract

AIM: To assess a relationship between longitudinal changes in liver fat content and biochemical parameters in obese children after 1-year nutritional intervention.

METHODS: Forty-six obese children, 21 males and 25 females, aged 6-14 years, underwent metabolic measurements, liver ultrasonography (US) and chemical-shift magnetic resonance imaging (MRI) examinations at baseline and after 1-year nutritional intervention. A child was defined obese if her/his body mass index (BMI) was above the age- and sex-adjusted BMI Cole's curve passing through the cut-off of 30 kg/m^2 at 18 years. BMI *Z* scores were calculated and adjusted for age and gender by using the Cole's LMS-method and Italian reference data. Biochemistry included serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Abdominal US and chemical-shift MRI were performed according to a randomized sequence. The same radiologist performed US by a GE Logiq 9 (General Electric Healthcare Medical Systems, Milwaukee, WI, United States) using a 3.5-MHz convex array transducer. Liver echogenicity was evaluated independently on videotape by 3 radiologists unaware of the child and MRI outcomes, and a consensus was established. Another experienced radiologist, unaware of the child and US data, performed the abdominal chemical-shift MRI with a 1-t system NT-Intera (Philips Medical Systems, Best, The Netherlands) and a phased-array coil. Liver fat fraction (FF) on MRI was judged elevated when greater than 9%. A FF > 18% was considered expressing more severe cases of fatty liver according to Fishbein. A nutritional-behavioral intervention was recommended to promote a normocaloric balanced diet and active lifestyle based on the Italian guidelines for treatment of childhood obesity.

RESULTS: Compared to baseline, at the end of intervention children showed lower intakes of energy (mean ± SD: 2549 ± 1238 Kcal *vs* 1770 ± 622 Kcal, $P < 0.0001$), total fat (90 ± 47 g *vs* 52 ± 23 g, $P < 0.0001$), carbohydrates (356 ± 174 g *vs* 241 ± 111 g, $P = 0.001$), and protein (99 ± 48 g *vs* 75 ± 23 g, $P = 0.006$) intakes. Prevalence of FF ⩾ 9% declined from 34.8% to 8.7% ($P < 0.01$), with a mean reduction of 7.8% (95%CI: 5.0-10.6). At baseline, FF was associated with liver biochemical parameters (maximum $P < 0.001$). At the end of the intervention association was found with AST ($P = 0.017$). Change of FF was associated with change in AST ($P = 0.027$) and ALT ($P = 0.024$). Rate of increased liver echogenicity declined from 45.6% to 21.7% ($P < 0.0001$). Liver echogenicity was associated with ALT at baseline only ($P < 0.001$). An age- and sex-adjusted multiple regression analysis showed that FF

change was independently associated with change in serum AST (adjusted regression coefficient 0.348, P = 0.048).

CONCLUSION: The results suggest that in obese children longitudinal changes in liver fat content based on MRI may be associated with change in serum transaminases suggesting novelty in monitoring nonalcoholic fatty liver disease.

Key words: Nonalcoholic fatty liver disease; Childhood obesity; Serum transaminases; Magnetic resonance imaging; Nutritional intervention

Core tip: In our study we demonstrate that in obese children longitudinal change in liver fat content evaluated on magnetic resonance imaging is associated with change in serum transaminases and more weakly also with changes in triglyceridemia and apolipoproteins, after a nutritional intervention based on normocaloric balanced diet. Furthemore this is the first study purposely designed to evaluate in obese children whether any relationship may exist of longitudinal changes in liver fat content with changes in liver biochemical parameters These findings may suggest novelty in clinical monitoring nonalcoholic fatty liver disease severity in childhood obesity.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) ranges from fat in the liver to advanced fibrosis and cirrhosis[1]. Obesity is a condition frequently associated with NAFLD both in children and adults[1-3]. The overall prevalence of fatty liver based on histological diagnosis in the pediatric population has been estimated to be around 13%[1]. The highest rate of fatty liver was seen in obese children (32%-50%)[4,5]. In children, NAFLD is becoming more frequently diagnosed with increasing in rates of obesity[5,6], and might indicate a possible metabolic outcome of obesity[5]. Therefore, accurate diagnosis of disease as well monitoring of the patients over time remains a major challenge for pediatricians taking care of the growing number of children with NAFLD[7]. Liver biopsy is the gold standard for establishing diagnosis and severity of NAFLD but it may not be done repeatedly in children. Accordingly, non invasive approaches are important for the clinician. Ultrasonography (US) and chemical-shift magnetic resonance imaging (MRI), fast to perform, may be useful to detect liver fat content and monitoring NAFLD[8-14]. On US, fatty liver produces a diffuse increased echogenicity with posterior beam attenuation[8]. MRI is able to quantify the fat content accurately and identify changes over time in children with NAFLD[9-15]. Indeed, studies proved that assessment by MRI agrees better than US with the diagnosis of steatosis based on biopsy[10,12-14]. While, it is still debatable whether in obese children US may be valuable in identifying high hepatic fat accumulation[8], it may show poor ability in identifying lower fat content compared with MRI[9,12,13].

Children with NAFLD are usually asymptomatic and come to clinical attention because of elevated liver enzymes or fatty liver seen in incidentally observed elevated serum aminotransferases[6]. While concordance between serum aminotransferases and US in identifying fatty liver[12,16,17] may be low, it has been proved for MRI[12,18]. Despite the potential clinical and practical relevance, there is lack of studies in the current literature assessing the relationship of longitudinal change of liver fat content with liver biochemical parameters in pediatric age.

The aim of the present study was to assess whether any association may exist of longitudinal changes in liver fat content with change in liver biochemical parameters in obese children who underwent a 1-year nutritional intervention.

MATERIALS AND METHODS

Fifty children, aged 6-14 years, were consecutively recruited at the Pediatric Department of the San Paolo Hospital, Milan, Italy, between July 1, 2010 and June 31, 2012 according to the following eligibility criteria. Inclusion criteria were: age > 6 years, obesity, and white parents. Exclusion criteria were: having syndromic, organic and hormonal conditions besides obesity that may predispose to liver disease, including infectious hepatitis B and C, alpha-1-antitrypsin deficiency, medications affecting liver metabolism, diabetes, any alcohol consumption. Children aged < 6 years were not included as it may be difficult to perform accurately MRI for lack of compliance. Obesity was defined according to the International Obesity Task Force[19].

The parents of eligible children or their legal guardian received detailed explanation about the aim of the study, and signed a consent form. The Hospital Ethics Committee approved the study protocol and gave ethical clearance.

Anthropometric measurements, nutritional and metabolic examinations, US and MRI assessments were performed within 3 ± 1 d before starting intervention (baseline) and one year (± 5 d) after (end of intervention).

Anthropometry and clinical data

Anthropometrical evaluation included measurements of weight and height. The body mass index (BMI) was calculated from the ratio of weight to height^2 (kg/m^2).

A child was defined obese if her/his BMI was above the age- and sex-adjusted BMI Cole's curve passing through the cut-off of 30 kg/m^2 at 18 years[19]. BMI *Z* scores were calculated and adjusted for age and gender by using the Cole's LMS-method[20] and Italian reference data[21]. A medical history was additionally collected at baseline from parents by a standardized questionnaire at a personal interview conducted by the same pediatrician.

Biochemistry

Fasting blood samples were taken at 8 h ± 30 min am Flavoured glucose at a dose of 1.75 g/kg body weight (up to a maximum of 75 g) was then given orally, and additional blood samples were taken for measurements of plasma glucose at 120 min. Blood samples were collected and analysed immediately at the Hospital Department of Biochemistry. Total cholesterol (TC), high-density-lipoprotein cholesterol (HDL-C) and triacylglycerol (TG) plasma levels were measured using a dry multiplayer enzymatic method (Ectachem DT-60; Eastman Kodak Co., Rochester, NY). Low-density-lipoprotein cholesterol (LDL-C) serum levels were calculated according to the Friedewald formula [LDL-C = TC - (HDL-C + TG/5)][22]. Apolipoprotein A1 and Apolipoprotein B were measured using a Modular analyzer (BNII, Dade Behring; Marburg, Germany). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (γGT) were measured by a Hitachi 917 Analyzer and Roche Diagnostics reagents (both Mannheim, Germany).

Abdominal US and chemical-shift MRI

Abdominal US and chemical-shift MRI were performed according to a randomized sequence. The same radiologist (AR) performed US by a GE Logiq 9 (General Electric Healthcare Medical Systems, Milwaukee, WI, United States) using a 3.5 MHz convex array transducer. Liver echogenicity was evaluated independently on videotape by 3 radiologists unaware of the child and MRI outcomes, and a consensus was established. Absolute agreement among judgments before discussion occurred in 94% of cases at baseline, and in 93.5% of cases at the end of intervention. Echogenicity was graded as follows: grade 0, normal echogenicity; grade 1, slight increase in liver echogenicity, slight exaggeration of liver and kidney echo discrepancy, and relative preservation of echoes from the walls of the portal vein; grade 2, loss of echoes from the walls of the portal veins, particularly from the peripheral branches, greater posterior beam attenuation, and greater discrepancy between hepatic and renal echoes; grade 3, greater reduction in beam penetration, loss of echoes from most of the portal vein wall, including the main branches, and large discrepancy between hepatic and renal echoes[23].

Another experienced radiologist (CP), unaware of the child and US data, performed the abdominal chemical-shift MRI with a 1-T system NT-Intera (Philips Medical Systems, Best, The Netherlands) and a phased-array coil. For each child, axial T1-weighted gradient echo images both in-phase [15/6.90 (repetition time msec/echo time msec), flip angle of 25°] and out-of-phase [15/3.45 (repetition time msec/echo time msec), flip angle of 25°] were obtained. The echo time is the time from the application of the radio frequency pulse to peak of the signal. Section thickness was 10 mm, field of view was 375 mm, and matrix was 256 pixels. Scan duration was 1 m/23 s for each in-phase or out-of-phase image. Respiratory compensation was used (scan duration for respiration, 1.6 s). Twenty contiguous transverse images in-phase and out-of-phase were obtained. Two operators not involved in the study and unaware of the patient's disease independently selected the slice showing the best evidence of liver parenchyma to be considered for examination and the region of interest (ROI). Three ROI with the same area (80-100 mm^2) were drawn on the corresponding in-phase and out-of-phase images. Two regions were drawn in the right liver, one in the left liver. For each region, the signal intensity was measured on both in-phase and out-of-phase images and the mean pixel signal intensity data were recorded for further calculation. The liver fat fraction (FF) was calculated from the mean pixel signal intensity data using the formula: FF = [SI (in-phase) - SI (out-of-phase)]/2SI (in-phase). The hepatic FF was considered to be normal at a value less than 9% and non-normal as FF $\geqslant$ 9%[10,11]. A FF > 18% was considered expressing more severe cases of fatty liver according to Fishbein[24]. Additionally the cut off of 5% for FF was used according to studies conducted on obese children, *e.g.* [14].

Nutritional-behavioral intervention and dietary habits

A nutritional-behavioral intervention was recommended to promote a normocaloric balanced diet and active lifestyle based on the Italian guidelines for treatment of childhood obesity[25]. Diet was normocaloric (daily caloric intake by sex and age) and consisted of carbohydrate (55%-60%, < 10% high glycemic index carbohydrate), fat (25%-30%, < 10% saturated fatty acids, polyunsaturated up to 10%, monounsaturated up to 15%), protein (12%-15%), fiber [range: age (year) plus 5 - age (year) plus 10, g][25]. Children and parents or the legal guardians underwent 1-h nutritional counselling by the same experienced dietician. Written guidelines were given to the parents and children, including general nutritional advices, food choice lists, selected week-menu, recommended average servings for principal food categories according to age and sex. Additionally, recommendations were given to engage in a moderate daily exercise program (30-45 min/d aerobic physical exercise), tailored to individual preferences. Dietary habits of children were assessed at baseline and at the end of intervention by means of an age-adjusted Food Frequency Questionnaire (FFQ) made up of 116 items and designed according to Block[26]. The same experienced dietician, unaware of the obesity status of children, interviewed mothers for approximately 50 min and each meal was analyzed to find out which food was eaten and how often. Usual portion sizes were esti-

Table 1 Body mass index -*z* score, fat fraction, liver echogenicity, liver biochemical parameters and lipid profile of the studied population at baseline and at the end of intervention, values are mean (SD) + and median or number of observation (%)

Variable	Baseline (n = 46)	End of intervention (n = 46)	[1]*P* value
BMI Z score	2.3 (0.4); 2.1	1.90 (0.6); 1.9	< 0.001
Fat fraction (%)	7.8 (13.3)	0.01 (8.3)	< 0.001
FF ⩾ 5%	21 (45.7)	5 (10.9)	< 0.001
FF ⩾ 9%	16 (34.8)	4 (8.7)	< 0.001
Liver echogenicity (%)			
0	25 (54.3)	36 (78.3)	< 0.0001
1	14 (30.4)	9 (19.6)	
2	6 (13.0)	1 (2.2)	
3	1 (2.2)	0 (0.0)	
ALT (UI/L)	34.8 (12.0); 29.0	23.8 (8.70); 22.50	< 0.001
AST (UI/L)	30.7 (10.1); 29.0	25.9 (7.50); 26.00	< 0.001
γGT (UI/L)	15.7 (5.2); 15.0	13.1 (3.80); 12.00	< 0.001
Total cholesterol (mmol/L)	4.39 (0.70); 4.48	4.30 (0.76); 4.23	0.211
HDL cholesterol (mmol/L)	1.22 (0.24); 1.23	1.21 (0.22); 1.24	0.916
LDL cholesterol (mmol/L)	2.64 (0.59); 2.60	2.60 (0.67); 2.56	0.306
Triglycerides (mmol/L)	1.31 (0.62); 1.20	0.99 (0.46); 0.94	< 0.001
Apo A1 (g/L)	1.24 (0.21); 1.21	1.28 (0.21); 1.26	0.122
Apo B (g/L)	0.73 (0.17); 0.70	0.66 (0.15); 0.67	0.022

[1]Adjusted for age, sex and duration of obesity; [2]Student's *t* test for paired data or Wilcoxon signed-rank test. Normal range[32]: ALT (5-45 UI/L) for age 1-19 years; AST (15-55 UI/L) for age 1-9 years and (5-45 UI/L) for age 10-19 years; gammaGT (5-32 U/I) for age 4 mo-9 years and (5-24 UI/L) for age 10-15 years. SI convertion factors: to convert cholesterol, divide values by 0.0259; to convert triglycerides, divide values by 0.0113; to convert Apo A1 and Apo B values by 0.01. BMI: Body mass index; FF: Fat fraction; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; γGT: Gamma glutamyltransferase; HDL: High-density-lipoprotein; LDL: Low-density-lipoprotein.

mated using household measures, the weight of purchase (*e.g.*, pasta) or unit (*e.g.*, fruit juice). A 24-h recall on the day prior to the hospital admission was further recorded at the end of the interview to standardize the usual serving size.

Medical examinations were scheduled every 3 mo during the intervention period. At each visit, parents of children or their legal guardians filled out a FFQ and physical activity recall to evaluate adherence to lifestyle recommendations. Quantification and analysis of the energy intake and nutrient composition were performed with an ad hoc PC software program developed at our department and based on the Food and Nutrient Data Base issued by the National Institute of Nutrition[27].

Sample size

The sample size was determined to detect a minimum effect size of 0.5 for a correlation between change in FF and change in liver biochemical parameters. Admitting a type I error level of 0.05 with a power of 95%, and allowing for a drop out up to 10%, 43 children needed to be recruited.

Statistical analysis

Data are expressed as mean (SD) and median or number of observations (percentage). Intra-subject comparisons were performed by the Student's *t* test for paired data or the Wilcoxon test, as appropriate. The association of liver fat fraction with BMI *Z* score, lipid profile, serum aminotransferases and gamma glutamyltransferase was assessed by Spearman's correlation coefficient. When fat fraction was dichotomized (cut off 9%) Student's *t* test for unpaired data or the Mann-Whitney *U* test were used. A multiple adjusted stepwise regression model was fitted for change of FF, including as covariates change in liver biochemical parameters and lipid profile, and additional potential confounders (age, sex, duration of obesity, change in BMI *Z* score). All values of $P < 0.05$ were considered to indicate statistical significance (two-tailed test). The statistical package For social sciences (SPSS) package version 19.0 for Windows (SPSS, Chicago, IL) was used for the statistical analysis.

RESULTS

Forty-six children (92%), 21 males and 25 females, completed the intervention and had all US, MRI and blood tests performed. No difference was observed among children who completed the study or not, for any variable at baseline (minimum $P > 0.130$). No focal liver lesions were observed in any child. At recruitment, mean (SD; median) of age, duration of obesity was, respectively, 10.1 (2.4; 10.0) years, 4.4 (2.8; 3.4) years. Plasma glucose was 868 (6.3; 86.0) mg/L at fasting and 993 (17.3; 99.5) mg/L at 120 min at baseline, and 856 (6.6; 85.0) mg/L at fasting and 977 (14.2; 98.0) mg/L at 120 min at the end. Compared to baseline at the end of intervention children showed lower intakes of energy (mean ± SD: 2549 ± 1238 Kcal *vs* 1770 ± 622 Kcal, $P < 0.0001$), total fat (90 ± 47 g *vs* 52 ± 23 g, $P < 0.0001$), carbohydrates (356 ± 174 g *vs* 241 ± 111 g, $P = 0.001$), and protein (99 ± 48 g *vs* 75 ± 23 g, $P = 0.006$) intakes. Children spent 2.3 h (1.6 h)/d of physical activity at baseline and 2.8 h (1.8 h)/d at the end of intervention ($P = 0.174$).

Table 1 reports the main characteristics and clinical, liver and lipid profile. After intervention there was a reduction in BMI *Z* score (mean reduction 0.32, 95%CI: 0.21-0.43), triglycerides (0.33, 0.15-0.50) mmol/L, ALT (11.1, 5.6-16.5) mg/dL, AST (4.8, 2.1-7.4) mg/dL, γGT (2.5, 1.5-3.6) U/L and apolipoprotein B (7.2, 1.1-13.2) mg/dL. Rate of increased liver echogenicity declined from 45.6% to 21.7% ($P < 0.0001$). On MRI, rate of liver FF ⩾ 9% declined from 34.8% to 8.7% ($P < 0.001$), and from 45.7% to 10.97% ($P < 0.001$) at cut off of 5% for FF, with a mean FF (95%CI) reduction of 7.8 (5.0-10.6)%. In children who exhibited FF ⩾ 9% at baseline ($n = 16$) (maximum FF 43%), FF declined from a (mean ± SD: 22.6% ± 11.5% *vs* 6.2% ± 10.5%, $P < 0.0001$), and in 12 was lower than 9%.

At baseline fat fraction was associated with liver biochemical parameters, while at the end of intervention association was found of FF with transaminases. Change of FF was associated with changes in AST and ALT.

Table 2 Spearman correlation coefficent (*P* value) of fat fraction and liver echogenicity with liver function parameters

	AST	ALT	γGT
Baseline			
Fat fraction	0.634 (< 0.0001)	0.456 (< 0.001)	0.469 (< 0.0001)
Liver echogenicity	0.282 (0.0530)	0.437 (< 0.001)	0.232 (0.0750)
End of treatment			
Fat fraction	0.351 (0.0170)	0.297 (0.056)	0.279 (0.0630)
Liver echogenicity	0.260 (0.1030)	0.242 (0.070)	0.116 (0.2540)
Change (end of treatment-baseline)			
Fat fraction	0.326 (0.0270)	0.331 (0.024)	0.271 (0.0720)
Liver echogenicity	0.225 (0.0820)	0.260 (0.063)	0.204 (0.1900)

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: Gamma glutamyltransferase.

Liver echogenicity was associated with ALT at baseline only (Table 2). Fat fraction was also associated with triglycerides (Spearman correlation coefficient, *r* baseline, *r* = 0.516, *P* < 0.0001; end of intervention, *r* = 0.329, *P* = 0.031; change, *r* = 0.517, *P* < 0.0001) and Apo B (baseline, *r* = 0.391, *P* = 0.008; change, *r* = 0.398, *P* = 0.024), but not with cholesterol or Apo A1 (minimum *P* = 0.114). No association of liver echogenicity was found with triglycerides or cholesterol or apolipoproteins (minimum *P* = 0.063). No association of BMI *Z* score was found with FF or liver echogenicity (minimum *P* = 0.517).

An age and sex adjusted multiple stepwise regression model showed that FF change was independently and positively associated with serum AST level change (adjusted regression coefficient 0.348, *P* = 0.048).

Moreover at baseline acanthosis nigricans, a skin features associated to hyperinsulinemia, was present in 15.4% of the evaluated children and decline to 10.1% after intervention.

Lastly, Table 3 compares liver biochemical and lipid profile when considering the FF dichotomized at 9%. At baseline aminotransferases, γGT and triglycerides were higher in children with liver FF ⩾ 9%. At the end of intervention, aminotransferases and total cholesterol levels were higher in children with FF ⩾ 9%. Mean difference (95%CI) of BMI *Z* score and duration of obesity between children with FF ⩾ 9% *vs* FF < 9% was respectively 0.15 (-0.12; 0.42) and 1.1 (-0.69; 2.87), at baseline. Out of 16 children with FF ⩾ 9%, 8 (50%) showed FF > 18%. Mean (SD) and median of AST, ALT and γGT between children with FF > 18% *vs* 9% < FF < 18% was respectively 44.6 (12.7), 42.0 *vs* 31.9 (8.5), 29.0 (*P* = 0.035); 63.6 (28.5), 55.0 *vs* 37.4 (22.7), 30.5 (*P* = 0.027); 20.2 (5.0), 21.5 *vs* 16.1 (3.7), 16.0 (*P* = 0.063) at baseline. At baseline only ALT values were elevated in children with FF > 18%. After intervention the ALT mean values ranged from 63.6 (28.5), 55 to 32.7 (14.2), 29.0 (*P* < 0.012), value that is in the normal range. In particular out of children with FF > 18% (*n* = 8) 87.5% (*n* = 7) showed elevated ALT values and declined to 25% (*n* = 2) after intervention. Similar results were found when considering a cut off of 5% for FF.

DISCUSSION

This is the first study purposely designed to evaluate in obese children whether any relationship may exist of longitudinal changes in liver fat content with changes in liver biochemical parameters after a nutritional intervention based on normocaloric balanced diet. The study based assessment of liver fat content on MRI, that has been recognized performing better than US for evaluation of liver fat[13] in the general population, as well in obese adolescents[16] and children with NAFLD[14,15].

The overall prevalence of elevated (⩾ 9%) liver fat fraction at baseline was 34.8%, that is within the range (32%-40%) estimated in recent studies conducting in obese children and using MRI[4,14,15]. At the end of intervention, the liver FF declined in three-quarter of children, and was lower than 9% in 91.3% of children with baseline FF ⩾ 9%. Similar findings have been reported in other studies conducted in obese children with NAFLD[14,15]. In this study, children showed after intervention a decrease in BMI *Z* score of 13.9%, that may be not unexpected given the decline occurred in dietary intake. Others studies estimated at the end of nutritional interventions a BMI *Z* score reduction ranging from 12.7%[15] to about 40%[14]. It should be noted that the present study may not fully reflect across-country experience. Indeed even in the most successful weight control program for children in the US, the success rate of weight loss may differ[28]. It cannot be excluded that genetic and environmental factors, different compliance to nutritional intervention may contribute, at least in part, to this difference. Therefore the results of the present study should be contextualized.

Nonalcoholic fatty liver disease, as a component of the metabolic syndrome[1], is usually suggested by finding elevated serum hepatobiliary enzymes (mostly ALT and GGT)[12,18,24]. Serum ALT activity is a widely available and inexpensive test for the screening and initial evaluation of NAFLD[29]. The sensitivity of this biochemical marker, however, remains low because a number pediatric patients may present ALT values in the normal range[29]. Moreover, given the clinical relevance of identifying children with steatohepatitis (NASH), several studies have described biomarkers associated with pediatric NASH[29,30]. Patton *et al*[30], evaluating clinical relation of histopathology with transaminases in pediatric NAFLD, found that AST was superior to ALT in distinguishing NAFLD patterns, while other studies showed ALT performing better[12,15,24]. Indeed, it is now widely accepted that the degree of ALT elevation does not correlate with the presence or severity of histological findings of NAFLD[29]. For this reason in the present study we considered not only the transaminases value at baseline and at the end of treatment but also the change.

In the present study obese children with FF ⩾ 9% showed higher values of aminotransferases than children with FF < 9% at baseline and at the end of intervention. In particular AST mean values were within the normal range both at baseline and at the end of intervention,

Table 3 Liver biochemical parameters and lipid profile of the studied population at baseline and at the end of intervention in relation to liver FF, dichotomized at 9%, alues are mean (SD) + and median

	Baseline (n = 46)		[1]*P* value	End of intervention (n = 46)		[1]*P* value
	FF < 9% (n = 30)	FF ⩾ 9% (n = 16)		FF < 9% (n = 42)	FF ⩾ 9% (n = 4)	
[2]AST (UI/L)	26.70 (5.9); 26.00	38.20 (12.3); 40	0.001	29.10 (8.6); 28.50	34.70 (6.4); 33.00	0.015
[2]ALT (UI/L)	26.50 (7.7); 25.00	50.50 (28.3); 48.50	0.001	30.50 (13.6); 29.00	40.70 (16.1); 40.50	0.015
[2]γGT (UI/L)	14.40 (5.0); 13.00	18.20 (4.8); 17.50	0.018	15.40 (5.3); 14.50	16.00 (3.7); 15.50	0.092
Triglycerides (mmol/L)	1.14 (0.44); 1.10	1.62 (0.80); 1.38	0.013	0.96 (0.44); 0.89	1.36 (0.45); 1.25	0.090
Total cholesterol (mmol/L)	4.34 (0.69); 4.35	4.48 (0.74); 4.54	0.539	4.22 (0.73); 4.18	5.06 (0.62); 4.98	0.034
HDL cholesterol (mmol/L)	1.23 (0.21); 1.24	1.20 (0.29); 1.17	0.716	1.21 (0.20); 1.24	1.23 (0.36); 1.21	0.950
LDL cholesterol (mmol/L)	2.58 (0.61); 2.58	2.76 (0.56); 2.75	0.348	2.54 (0.63); 2.53	3.21 (0.75); 3.03	0.086
Apo A (g/L)	1.21 (0.01); 1.23	1.29 (0.24); 1.20	0.259	1.29 (0.21); 1.27	1.12 (0.13); 1.12	0.199
Apo B (g/L)	0.71 (0.18); 0.68	0.77 (0.15); 0. 70	0.261	0.65 (0.14); 0.02	0.85 (0.13); 0. 86	0.064

[1]Adjusted for age, sex, BMI *Z* score and duration of obesity; [2]Significance of difference between FF groups (Student's *t* test for unpaired data or the Mann-Whitney *U* test). SI convertion factors: to convert cholesterol, divide values by 0.0259; to convert triglycerides, divide values by 0.0113; to convert Apo A1 and Apo B values by 0.01. Normal range[32]: ALT [5-45 UI/L] for age 1-19 years; AST [15-55 UI/L] for age 1-9 years and [5-45 UI/L] for age 10-19 years; gammaGT [5-32 U/I] for age 4 mo-9 years and [5-24 UI/L] for age 10-15 years[32]. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; γGT: Gamma glutamyltransferase; HDL: High-density-lipoprotein; LDL: Low-density-lipoprotein.

while ALT mean values was slight superior to the upper normal value at baseline but normal after one year of intervention. Similar results were found when considering a cut off of 5% for FF.

However, considering more severe cases of fatty liver (17.4%) ALT mean values were elevated and normalized after the intervention. In particular the ALT values normalized in 62.5% of children with FF > 18%. The association of liver biochemical parameters with FF was stronger than with liver echogenicity both at baseline and the end of intervention, in accordance with the literature[8,18]. Change in any liver biochemical parameter was not was associated with change in liver echogenicitys, supporting previous findings[8]. On the contrary, change in AST and ALT was associated with change in liver fat fraction. These results may be expected. Indeed as MRI may agree better than US with the diagnosis of steatosis based on biopsy[10,12-14], MRI may be a reasonable way to follow liver fat in NAFLD.

Age and sex adjusted regression analysis showed that FF change was independently associated with change in serum AST level. This result could be of clinical and practical relevance when monitoring NAFLD in childhood obesity and suggest that more research is necessary to elucidate the liver serologic biomarkers and their changes through intervention with greatest sensitivity and specificity in predicting steatohepatitis. Additionally, it should be noted that at univariate analysis an association was found between change of liver FF with change of triglyceridemia, and apolipoprotein B. Indeed, while association with change in triglyceridemia may well reflect variation in hepatocyte accumulation of triglycerides[3], hallmark of NAFLD, association with Apo B, that represents non-high density lipoprotein cholesterol, including very-low density lipoprotein, may suggest the risk to develop non-alcoholic steatohepatitis[31]. Additionally, longitudinal studies support that NAFLD may be linked with increased risk of cardiovascular disease, independently of classical atherosclerotic risk[1].

A limitation of the present study is the absence of a control group of obese children on free diet. Indeed, may be not fully agreement on ethnicity of recruitment of a such control group considering also that the lack of interventions in the treatment of childhood obesity would not met the current requirements of the Italian Society of Pediatrics[25]. A second limitation is that the gold standard to assess food intake is the 3 d food record, however the Frequency Food Questionnaire is largely used and in the present study was associated with a 24-h recall to standardize the usual serving size. A third limitation is that this study did not plan assessment of fat liver content by liver biopsy, that may be considered unethical in obese "healthy" children, and by proton magnetic resonance spectroscopy, a technique that may show high ability, as well MRI, for the evaluation of hepatic steatosis in general population (Youden's index ranging from 0.647 to 0.842)[9,13], and additionally filters the signal fat-fraction from potential confounding technical and biological noises.

On the whole, within the limitations of this study, one may conclude that in obese children longitudinal change in liver fat content evaluated on MRI is associated with change in serum transaminases and more weakly also with changes in triglyceridemia and apolipoproteins, suggesting novelty in clinical monitoring NAFLD severity in childhood obesity.

Large longitudinal trials with adequate power are desiderable to better investigate the relationship of longitudinal variations of liver fat content with serum liver and lipid profile, to elucidate the liver sierologic biomarkers and their changes with greatest sensitivity and specificity in predicting steatohepatitis and their clinical means in NAFLD management.

COMMENTS

Background

Children with nonalcoholic fatty liver disease (NAFLD) are usually asymptomatic and come to clinical attention because of elevated liver enzymes or fatty liver seen in incidentally observed elevated serum aminotransferases. While concordance between serum aminotransferases and ultrasonography (US) in identifying fatty liver may be low, it has been proved for magnetic resonance

imaging (MRI). Despite the potential clinical and practical relevance, there is lack of studies in the current literature assessing the relationship of longitudinal change of liver fat content with liver biochemical parameters in pediatric age.

Research frontiers

Accurate diagnosis of disease as well monitoring of the patients over time remains a major challenge for pediatricians taking care of the growing number of children with NAFLD. The research hotspot is to elucidate the liver sierologic biomarkers and their changes with greatest sensitivity and specificity in predicting steatohepatitis and their clinical means in NAFLD management.

Innovations and breakthroughs

While concordance between serum aminotransferases and US in identifying fatty liver may be low, it has been proved for MRI. Hovewer there is lack of studies in the current literature assessing the relationship of longitudinal change of liver fat content with liver biochemical parameters. This is the first study purposely designed to evaluate in obese children whether any relationship may exist of longitudinal changes in liver fat content with changes in liver biochemical parameters after a nutritional intervention based on normocaloric balanced diet.

Applications

These results suggest that in obese children longitudinal change in liver fat content evaluated on MRI is associated with change in serum transaminases and more weakly also with changes in triglyceridemia and apolipoproteins, suggesting novelty in clinical monitoring NAFLD severity in childhood obesity.

Terminology

NAFLD ranges from fat in the liver to advanced fibrosis and cirrhosis. Obesity is a condition frequently associated with NAFLD both in children and adults US and chemical-shift MRI, fast to perform, may be useful to detect liver fat content and monitoring NAFLD. Studies proved that assessment by MRI agrees better than US with the diagnosis of steatosis based on biopsy.

Peer review

This is a very interesting study and a commendable piece of work analyzing the impact of a dietary intervention on liver fat content and biochemical parameters in obese children during a 1-year follow-up. The study provides solid evidence by means of metabolic measurements, liver ultrasonography and magnetic resonance imaging that a nutritional-behavioral intervention based on a normocaloric balanced diet and active lifestyle is useful in reducing fatty liver in obese children. Moreover the correlation between MRI and liver enzyme may be of practical value in NAFLD management in childhood.

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