

## BENEFICIAL EFFECTS OF OMEGA-3 FATTY ACIDS IN NONALCOHOLIC FATTY LIVER DISEASE, IN CHILDHOOD OBESITY

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### Abstract

Nonalcoholic fatty liver disease (NAFLD) is increasing and is strongly associated with abdominal obesity, dyslipidemia and insulin resistance. To date, there are no proven effective therapies that halt NAFLD progression or improve prognosis in children.

The aim of this study was to determine the effects of Omega-3 fatty acids (enriched in vitamins) treatment in NAFLD, in obese children.

Thirty obese children (10-16 years old) and thirty lean children (10-16 years old) were involved. Each day, for three months, obese children took Omega-3 fatty acids (docosahexaenoic acid, DHA 130 mg and eicosapentaenoic acid, EPA 25 mg) and vitamins (A 200 µg, D 1.25 µg, E 2.5 mg and C 30 mg). The antropometric markers, lipid profile, inflammatory markers (CRP, ESR, fibrinogen, leptin, ceruloplasmin, albumin/globulin ratio), insulin resistance marker (HOMA-IR), plasminogen activator inhibitor 1 (PAI-1) and liver tests (albumin, bilirubin, protrombin time, ALT, AST) were measured before and after treatment. Ultrasounds were used for NAFLD diagnosis.

In obese children *versus* lean subjects all the measured parameters were modified. In obese children, after treatment, lower levels for waist circumference ( $p<0.05$ ), total cholesterol ( $p<0.02$ ), triglycerides ( $p<0.01$ ), PAI-1 ( $p<0.05$ ), ALT and AST activities ( $p<0.02$ ), HOMA-IR ( $p<0.005$ ), bilirulin ( $p<0.02$ ) were measured. After treatment, all the above inflammatory markers ( $p<0.05$ ) were reduced, while albumin ( $p<0.05$ ) and calcium ( $p<0.05$ ) were increased.

In conclusion, in obese children, treatment with Omega-3 fatty acids, associated with low doses of lipid soluble vitamins and 30 mg of vitamin C, has strong beneficial effects in nonalcoholic fatty liver disease.

### Rezumat

Ficatul gras nonalcoolic (NAFLD) are o incidență crescută și este asociat cu obezitatea abdominală, dislipidemia și rezistența la insulină. În prezent, nu există o terapie dovedită pentru stoparea evoluției NAFLD sau pentru îmbunătățirea prognosticului la copii.

Scopul acestui studiu este de a determina efectele acizilor grași Omega-3 (asociați cu vitamine), în tratamentul NAFLD, la copiii obezi.

Au fost recrutați treizeci de copii obezi (10-16 ani) și treizeci de copii normoponderali (10-16 ani). În fiecare zi, timp de 3 luni de zile, copiii obezi au primit acizi grași Omega-3 (acid docosahexaenoic, DHA 130 mg și acid eicosapentaenoic, EPA 25 mg) și vitamine (A 200 μg, D 1, 25 μg, E 2, 5 mg and C 30 mg). Markerii antropometrici, profilul lipidic, markerii inflamatorii (PCR, VSH, fibrinogen, leptina, ceruloplasmina, raportul albumină/globuline), markerul de rezistență la insulină (HOMA-IR), PAI-1 (inhibitorul activatorului plasminogenului-1) și testele hepatice (albumina, bilirubina, timpul de protrombină, ALT, AST) au fost determinate înainte și după tratament. Ecografia hepatică a fost utilizată pentru diagnosticarea NAFLD.

La copiii obezi *versus* subiecții normoponderali, toți parametrii determinați au fost modificați. La copiii obezi, după tratament, s-au înregistrat valori mai mici pentru circumferența taliei ( $p<0.05$ ), colesterolul total ( $p<0.02$ ), trigliceride ( $p<0.01$ ), PAI-1 ( $p<0.05$ ), pentru activitățile ALT și AST ( $p<0.02$ ), HOMA-IR ( $p<0.005$ ), bilirubină ( $p<0.02$ ). După tratament, toți markerii inflamatorii de mai sus ( $p<0.05$ ) au fost reduși, în timp ce, albumina ( $p<0.05$ ) și calciul total ( $p<0.05$ ) au fost crescute.

În concluzie, la copiii obezi, tratamentul cu acizi grași Omega-3, asociați cu doze mici de vitamine liposolubile și cu 30 mg de vitamină C, are efecte benefice importante în ficatul gras nonalcoolic.

**Keywords:** non-alcoholic fatty liver disease (NAFLD), childhood obesity, Omega-3 fatty acids, treatment

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in children and adolescents in the United States, and most probably also in the rest of the industrialized world [1].

With the epidemic of childhood obesity, nonalcoholic fatty liver disease (NAFLD) has become an important problem [2]. The prevalence of (NAFLD) ranges from at least 3% in children overall to about 50% in obese children [3].

The mildest form is simple steatosis in which triglycerides accumulate within hepatocytes. A more advanced form of NAFLD, non-alcoholic steatohepatitis, includes inflammation and liver cell injury, progressive to cryptogenic cirrhosis [4].

NAFLD should be suspected in all of the overweight or obese children and adolescents older than 3 years with increased waist circumference especially if there is a NAFLD history in relatives. The typical presentation, however, is in children aged 10 years and older. The first diagnostic step in these children should be abdominal ultrasound and liver function tests, followed by exclusion of other liver diseases. Overweight/obese children with normal ultrasonographic imaging and

normal liver function tests should still be monitored due to the poor sensitivity of these tests at a single assessment [1].

Insulin resistance seems to be the main pathophysiologic culprit in NAFLD [5].

Young individuals with fatty liver are more insulin resistant and present with a higher prevalence of metabolic abnormalities than do individuals without intrahepatic fat accumulation [6].

JNK (cJun NH<sub>2</sub>-terminal kinase) activation, induced by various stimuli such as: obesity induced inflammation, free fatty acids (FFAs), oxidative stress, or endoplasmic reticulum (ER) stress, mediates insulin resistance and subsequent hepatic steatosis. JNK activation plays a pivotal role in the inflammatory pathway. So, another important aspect of NAFLD is chronic hepatic inflammation, which is a key event for the progression of the disease.

It is known that Omega-3 fatty acid, DHA is a non-specific inhibitor of JNK and so it reduces inflammation [7].

Other important effects of Omega-3 fatty acids are: potential cardiovascular benefits (antidysrhythmic, antiatherogenic, antithrombotic, anti-inflammatory and endothelial protective effects, systolic and diastolic blood pressure lowering effects) and potential noncardiovascular benefits (prevents depression and diabetes mellitus) [8].

To date, there are no proven effective therapies that halt NAFLD progression or improve prognosis in children [9].

The aim of this study was to determine the effects of Omega-3 fatty acids (enriched in vitamins) treatment in NAFLD, in obese children.

## Materials and Methods

**Study Subjects.** A total of 30 children (aged 10-16 years old), overweight and obese children were enrolled in this study, taking into account the criterion of increased echogenicity in the liver, as noted on ultrasounds. The children had the body mass index (BMI), measured as kg/m<sup>2</sup> between 85th percentile and 95th percentile (overweight children) or above the 95th percentile (obese children). Drug hepatotoxicity and genetic/metabolic diseases that can cause fatty liver, such as Wilson's disease and cystic fibrosis, were excluded. Other causes of chronic hepatitis, such as chronic viral hepatitis were also excluded, but none of the subjects met these exclusion criteria. So, the obese group, formed by 30 subjects, was compared with age and gender matched lean children (n=30). All subjects were nonsmokers. Obese children took Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) and vitamins (A 200 µg, D 1.25 µg, E 2.5 mg and

C 30 mg) each day, for three months. These drugs were supplied by Queisser Pharma Dopple Herz. Blood samples were collected before and after treatment for different measurements. The study protocol was approved by the Ethical Commission of Emergency Hospital for Children "Grigore Alexandrescu", Bucharest and a written informed consent was obtained from each of the parents.

**Analytical methods.** Blood samples were taken after an overnight fast. Routine blood tests: fasting serum glucose, serum lipids (total cholesterol, HDL-cholesterol, triglycerides), serum creatinine, ALT and AST activities were measured in the clinical laboratory, by colorimetric methods (Vitros 950 AT Ortho Clinical Diagnostic Inc., Rochester-USA). Plasma insulin assay was performed using a commercially available ELISA human insulin kit (DRG instruments GmbH, Marburg, Germany, Catalog no. EIA 2935). The homeostatic model assessment (HOMA), used to quantify insulin resistance was calculated as the product of the patient's blood glucose and insulin serum levels after fasting, divided by a constant value [10]. LDL-cholesterol was calculated according to the Friedewald equation [11]. Enzyme-linked immunosorbent assays (ELISA) methods were used for: fibrinogen (ELISA kit, Abnova GmbH, Germany, Catalog no. KA0475), leptin (EIA-2395 kit, DRG Instruments GmbH, Germany), plasminogen activator inhibitor-1 (PAI-1 antigen ELISA reagent kit, no. TC11070; Technoclone GmbH, Vienna, Austria) and C-Reactive Protein (CRP) (ELISA kit no. STA-392, Cell Biolabs, Inc., USA). The plasma ceruloplasmin level determination was based on the oxidize activity of the protein towards p-phenylenediamine [12].

The source of variation between the control group and the obese subjects was assessed by the unpaired Student t-test. Correlations were calculated using Pearson function.

### **Results and Discussion**

Non alcoholic fatty liver disease (NAFLD) is associated with obesity, diabetes mellitus and metabolic syndrome [13,14,15]. In other words, hyperlipidemia with hyperinsulinemia and insulin resistance in overweight and obese children with fatty liver may be important signs of liver dysfunction in childhood NAFLD, irrespective of serum aminotransferases. In overweight or obese children with hyperlipidemia or insulin resistance, evaluation for NAFLD is warranted [16].

Data results are shown in tables I and II.

**Table I**  
Clinical data of the enrolled subjects

Parameters	N group Control group (n=30)	O group Obese children before treatment (n=30)	TO group Obese children after treatment (n=30)	p	p''
Age ( years)	13.2± 4.9	11.88 ± 4.37	11.88±4.37	Ns	Ns
Sex (F/M)	15/10	18/12	18/12	Ns	Ns
Weight (kg)	44.82 ± 10	55.86 ±22.56	53.62 ±20.74	<0.001	Ns
Height (m)	1.60± 0.11	1.42 ± 0.23	1.46 ± 0.22	Ns	Ns
BMI (kg/m <sup>2</sup> )	18.81 ± 1.7	25.63 ± 3.09	23.82±3.03	<0.001	Ns
WC (cm)	67.4 ± 6.6	90.36± 12.99	80.95 ± 10.60	<0.001	p<0.05
SBP mmHg	97.3 ± 5.2	138.61 ± 12.46	118.33 ± 10.94	<0.001	<0.001
DBP mmHg	59.25 ± 2.6	71.38± 6.37	56.66 ± 4.92	<0.001	<0.001

BMI represent the body mass index; WC-waist circumference, SBP-systolic blood pressure, DBP-dyastolic blood Pressure; p value represents the t-test result of comparison obese *versus* control groups and p'' value represents the t-test result of comparison obese children before treatment *versus* obese children after treatment

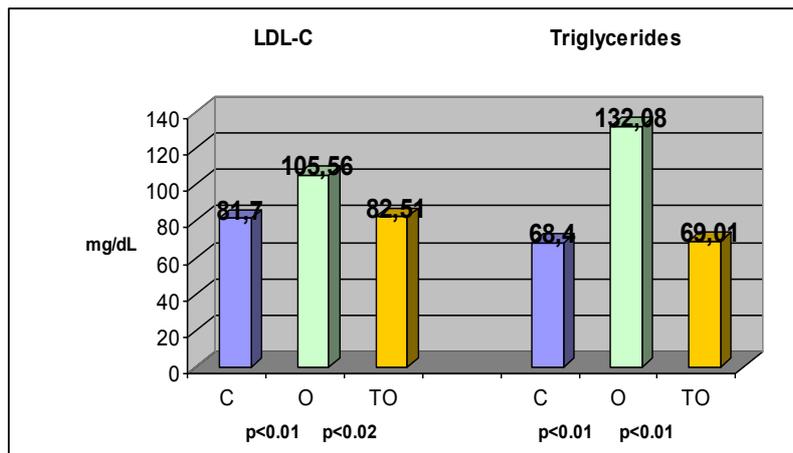
**Table II**  
The values for plasma markers, before and after treatment

Parameters	N group Control group (n=30)	O group Obese children before treatment (n=30)	TO group Obese children after treatment (n=30)	p	p''
PAI-1ng/mL	18.44±2.70	58.11± 33.31	30.02±12.25	<0.002	<0.05
PT s	0.9 ±0.09	1.04±0.03	1.09±0.03	Ns	Ns
Ca total mg/dL	9.33±0.47	9.09±0.66	9.48±0.41	Ns	<0.05
AST(U/L)	19.11±2.56	24.08±7.68	17.5±5.74	<0.05	<0.02
HOMA-IR	2.98± 0.34	4.89 ±2.40	2.54±1.15	<0.001	<0.001
Glycemia mg/dL	85.2 ± 3.4	89.33±16.83	68.5±4.27	Ns	<0.001
ALT(U/L)	13.07 ± 2	27.25± 21.57	14.4±5.86	<0.01	<0.02
Creatinine mg/dL	0.80±0.15	0.70±0.13	0.66±0.10	Ns	Ns
Bilirubin mg/dL	0.55±0.29	0.45 ±0.17	0.38 ±0.13	Ns	<0.02
Albumin g/L	60.72±2.51	53.70±5.73	57.07±1.60	<0.02	<0.05
Albumin/globulin ratio	1.26±0.12	1.13±0.20	1.23±0.38	<0.01	<0.05
Leptin ng/mL	2.16±1.3	17.51± 9.26	13.26±3.48	<0.001	<0.05
ESR mm/h	8.5±1.24	17.66±12.67	9.66±2.10	<0.01	<0.05
Fibrinogen g/L	3.23±0.12	3.51± 1.16	2.93±0.63	Ns	<0.05
CRP mg/L	0.95±0.5	6.99±1.24	6.0±0.10	<0.04	<0.05
Ceruloplasmin mg/dL	29.82 ± 3	30.66±34.03	25.58 ±3.47	Ns	<0.05
Cholesterol mg/dL	134.46± 16	172.66± 32.73	144.85± 22.61	<0.01	p<0.02
Triglycerides mg/dL	68.4±28.7	132.08±84.26	69.01±20.32	<0.01	<0.01
HDL-C mg/dL	53.37±12.98	45.66±14.03	48.5±13.85	<0.01	<0.04
LDL-C mg/dL	81.7 ± 7.7	105.56±29.08	82.54±21.38	<0.04	<0.04

p value represents the t-test result of comparison obese *versus* control groups and p'' value represents the t-test result of comparison obese children before treatment *versus* obese children after treatment; PAI-1, plasminogen activator inhibitor-1; ESR, erythrocyte sedimentation ratio, HOMA-IR, homeostatic model assessment-insulin resistance; PT, protrombin time; Ca total – total calcium levels; ALT – alanin transaminase/aminotransferase; AST – aspartat transaminase; CRP – C reactive protein; HDL – high density lipoprotein; LDL – low density lipoprotein

In our study, the obese children had dyslipidemia, characterised by high triglyceridemia and low HDL-C *versus* lean children. It is known that this type of dyslipidemia increases the risk of atherosclerosis because high triglycerides levels are processed into small, dense LDL and small, less

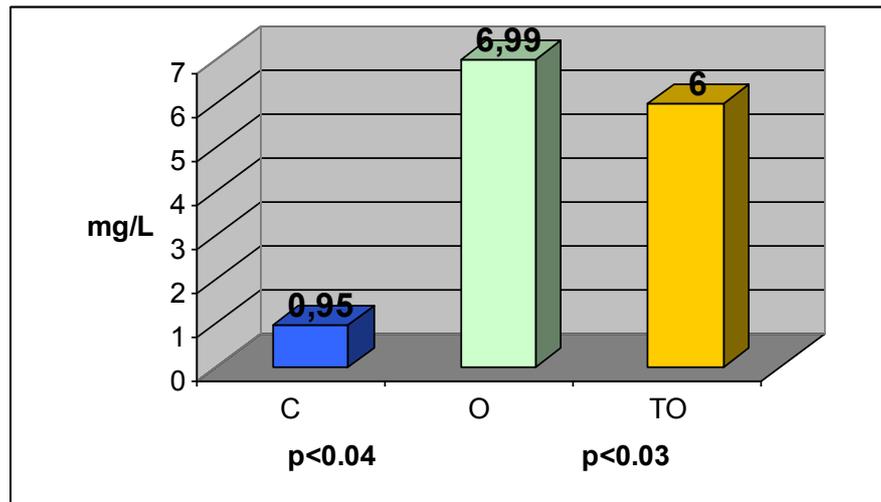
stable HDL [17]. We calculated positive correlations between triglycerides and transaminases ( $r=0.40$  for AST and  $r=0.66$  for ALT,  $p<0.05$ ). Both transaminases ALT and AST were significantly increased in obese children *versus* control, even the values were in normal range. After treatment, the lipid profile was improved: triglycerides were strongly lowered and total cholesterol and LDL-C were decreased (Figure 1). Also, transaminases activities and waist circumference were significantly decreased after treatment. These results are important because the values of transaminases activities together with waist circumference are included in different algorithms used to predict liver fibrosis [18].



**Figure 1**

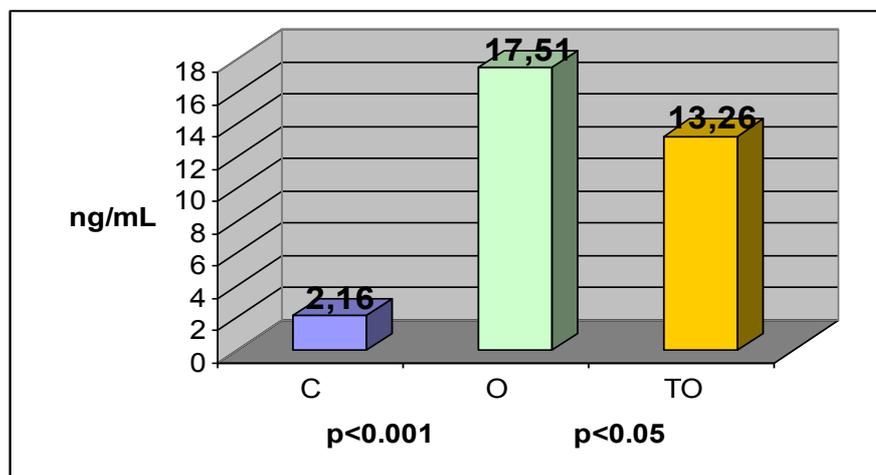
Plasma LDL-C and triglycerides in obese children with NAFLD, before and after treatment and normal ponderal children (control).

High waist circumference is equivalent to central obesity or visceral adipose tissue which is fundamental to the proinflammatory state. In addition to the production of proinflammatory cytokines, visceral adipose tissue produces the adipocytokine leptin. In the present study, the inflammatory markers (leptin, CRP, ESR, fibrinogen, ceruloplasmin, albumin/globulin ratio) had higher levels in obese children *versus* lean children and the values were reduced by the treatment. Leptin has been associated with increased steatosis in NAFLD and there is also evidence that leptin directly promotes hepatic fibrogenesis [18]. In figures 2 and 3 it can be noticed the beneficial effect of the treatment, by lowering leptin and CRP. In table II, the decreased values for ESR, fibrinogen, ceruloplasmin, albumin/globulin ratio obtained after treatment, are shown.



**Figure 2**

Plasma CRP values in obese children with NAFLD, before and after treatment.



**Figure 3**

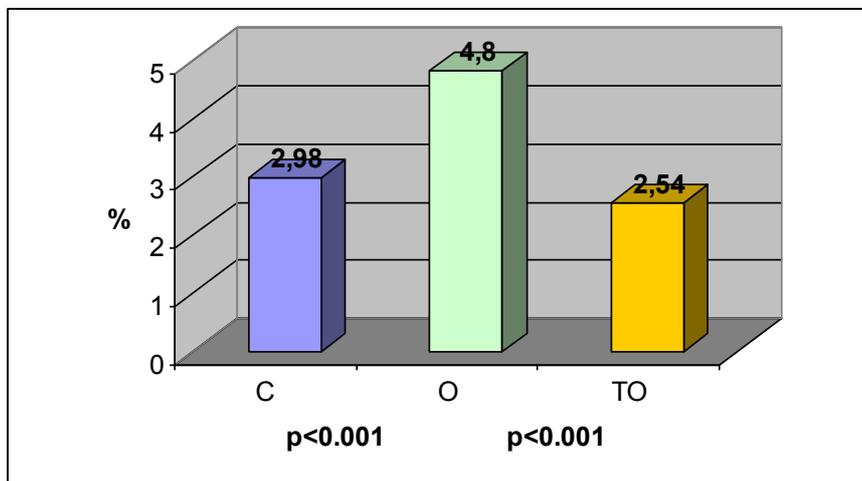
Plasma leptin values in obese children with NAFLD, before and after treatment and lean children (control group)

It is known that the proinflammatory status present in obesity is an important culprit for insulin resistance. When insulin resistance develops, free fatty acids are inappropriately shifted to nonadipose tissues, including the liver. Insulin resistance increases free fatty acid (FFA) flux to the liver by decreased inhibition of lipolysis and also increased *de novo* lipogenesis [5]. FFAs and their lipotoxic intermediates have been implicated in hepatocellular injury by promoting inflammation, endoplasmic reticulum

stress, mitochondrial dysfunction and oxidative stress. As a result of these processes, hepatocytes begin to die and release inflammatory cytokines and reactive oxygen species which then further fuel an already upregulated obesity-related inflammatory environment [18].

In this study, the obese group, according to the HOMA-IR values and NHANES criteria (The National Health and Nutrition Examination Survey, USA) had insulin resistance [18]. Treatment reduced HOMA-IR values. The improved metabolic profile after treatment may be due both to Omega-3 fatty acids and vitamins effects (Figure 4).

Vitamin E has been most widely studied. Being fat-soluble, vitamin E can stabilize mitochondrial function and is theorized to inhibit lipid peroxidation and subsequent free radical reactions. Smaller, nonrandomized trials have found that vitamin E improves biochemical markers of liver inflammation. However, in one of the largest randomized controlled trials (with 45 patients), patients taking vitamin E showed improvement in their fibrosis scores. Vitamin E may be an effective adjunctive therapy [13,19].



**Figure 4**

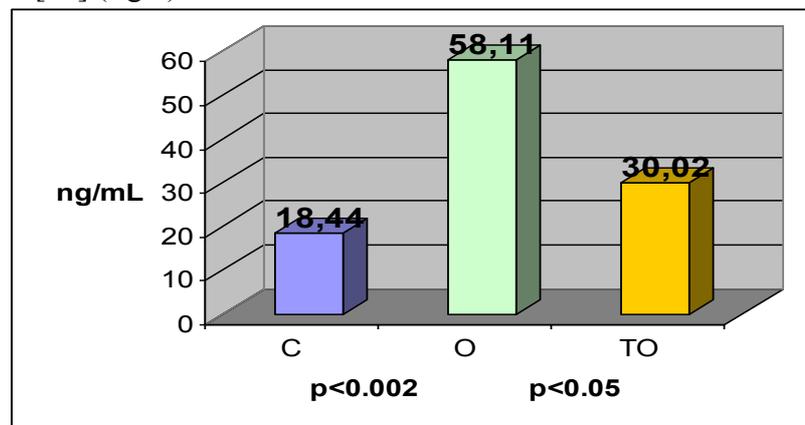
Plasma HOMA-IR in obese children with NAFLD, before and after treatment and control group.

NAFLD not only complicates obesity, but also perpetuates its metabolic consequences [20]. Several studies indicate that NAFLD, especially in its necro-inflammatory form (NASH), is associated with a systemic proinflammatory/prothrombotic state, independently of shared metabolic risk factors. This suggests that NAFLD/NASH is not simply a marker of the proinflammatory/prothrombotic state in the metabolic syndrome but is actively involved in its pathogenesis, possibly through the

systemic release of proinflammatory and procoagulant factors (C-reactive protein, plasminogen activator inhibitor-1, interleukin-6, fibrinogen, and other proinflammatory cytokines) from the steatotic liver [21]. In cultured human hepatocytes, exposure to fatty acids activates oxidative stress and production of prothrombotic markers and decreases expression of insulin receptors [22].

Several studies demonstrated that plasma plasminogen activator-1 (PAI-1) and monocyte chemoattractant protein 1 (MCP-1) are increased in severe fibrotic NAFLD [23,24,25,26].

In our study, the obese children had higher levels of PAI-1 *versus* the lean children. The treatment reduced PAI-1 level and thus, the treatment prevented liver fibrosis and cardiovascular complications in the obese children [27] (fig.5).



**Figure 5**

Plasma PAI-1 in obese children with NAFLD, before and after treatment and lean children.

For this study, it is worth to mention that the treatment reduced blood pressure. This result, in relation with the cluster good effects of treatment on dyslipidemia, glycaemia, waist circumference is important and demonstrates that Omega-3 fatty acids can be used to prevent metabolic syndrome.

### **Conclusion**

The treatment with Omega-3 fatty acids (DHA 130mg and EPA 25mg) acts on all the pathological mechanisms involved in NAFLD. The treatment reduced inflammation, improved insulin sensitivity and modulated fibrinolysis.

The mainstay of treatment of NAFLD remains rational weight loss, but this study demonstrates that supplements with Omega-3 fatty acids and low doses of antioxidant lipid soluble vitamins can delay or prevent liver fibrosis.

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