

Prolonged n-3 polyunsaturated fatty acid supplementation ameliorates hepatic steatosis in patients with non-alcoholic fatty liver disease: a pilot study

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SUMMARY

Background

Recent studies suggest a role of n-3 long-chain polyunsaturated fatty acids (n-3 PUFA) as peroxisome proliferator-activated receptor- α ligands in improving non-alcoholic fatty liver disease (NAFLD) in rodents. However, data in humans are still lacking.

Aim

To evaluate the efficacy of prolonged PUFA supplementation in patients with NAFLD.

Methods

Fifty-six patients with NAFLD were enrolled. Among the overall eligible patients, 42 assumed n-3 PUFA 1-g capsule daily for 12 months, whereas 14 refused the treatment and were analysed as controls. All patients underwent haematochemical and ultrasound follow-up.

Results

Polyunsaturated fatty acid supplementation significantly decreased serum aspartate transaminase ($P = 0.003$), alanine transaminase ($P = 0.002$), γ -glutamyl transpeptidase ($P = 0.03$), triglycerides ($P = 0.02$) and fasting glucose ($P = 0.02$) in comparison with controls. Circulating arachidonate and n-6/n-3 fatty acid ratio was reduced ($P = 0.0002$, and $P = 0.0001$ respectively) in treated patients. Moreover, ultrasonography demonstrated improvement of liver echotexture after PUFA ($P = 0.0001$), and increase of Doppler perfusion index ($P = 0.001$), whereas no significant changes occurred in controls.

Conclusions

Supplementation with n-3 PUFA improves biochemical, ultrasonographic and haemodynamic features of liver steatosis. Our study supports the efficacy of n-3 PUFA as a new therapeutic approach in the treatment of NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a world-wide diffuse condition due to alimentary, environment and genetic factors in patients with mild or absent alcohol ingestion.¹ It shows an increasing importance because of prevalence, being responsible of most cryptogenic chronic liver diseases in many countries,²⁻⁵ and because of its possible progression to non-alcoholic steatohepatitis (NASH), liver cirrhosis and hepatocellular carcinoma.^{4, 6-10}

Although needle liver biopsy is considered the 'gold standard' for NAFLD diagnosis,¹¹ patients often decline it; moreover, it is not yet clear when to propose such diagnostic tool for the clinical setting of NAFLD.¹² As a further matter of doubt, histological features of NAFLD cannot be distinguished from those of alcohol-induced liver injury,¹ and standardized diagnostic criteria are still lacking.¹³ Although Brunt *et al.*¹⁴ have recently developed an interesting scoring system, the sampling error may cause diagnostic inaccuracies in fatty liver¹⁵ that mainly depend on the size of biopsy,¹⁶ similar to that established in chronic hepatitis C.¹⁷ Therefore, the use of different diagnostic approaches, such as imaging investigations, is emerging in the diagnosis of NAFLD. In particular ultrasonography, a well-tolerated investigation, which reaches a sensitivity of 89% and specificity of 93% in detecting fatty liver, and a sensitivity of 77% and specificity of 89% in assessing liver fibrosis.¹⁸

Several therapeutic approaches have been proposed for NAFLD in correlation with its association with the metabolic syndrome and insulin resistance.¹⁹ Some studies pointed out that insulin resistance may be accompanied by a change in the composition of fatty acids in serum and tissues, with deficiency of n-3 polyunsaturated fatty acids (n-3 PUFA).^{20, 21} Such long-chain fatty acids, present in fish oil and derived from α -linolenic acid, are involved in eicosanoid biosynthesis and interact with some nuclear receptor proteins, thereby influencing the transcription of regulatory genes.^{22, 23} Actually, they are natural ligands of peroxisome proliferator-activated receptor α (PPAR α), a group of nuclear receptors which modulate lipid metabolism in hepatocytes.²⁴ Low levels of circulating n-3 PUFA, with a consequent increase of n-6/n-3 fatty acid ratio, impair PPAR α activity in the liver. This phenomenon is associated with a higher hepatic uptake of circulating free fatty acids, a decrease of hepatocyte microsomal ω -oxidation, peroxisomal and

mitochondrial β -oxidation, a reduced synthesis of fatty acid-transport proteins (namely *very low density lipoproteins*), and an up-regulation of lipogenic transcription factors (namely *sterol regulatory element binding protein-1*, *SREBP-1*; *stimulatory protein-1* and *nuclear factor-Y*).^{22, 25, 26} Previous experimental studies have shown that diets enriched with n-3 PUFA increase insulin sensitivity in rats,²⁷ reduce intra-hepatic triglyceride content and ameliorate steatohepatitis both in mice²⁸ and rats.²⁹ However, studies on the possible beneficial effects of n-3 PUFA treatment in patients with NAFLD are still lacking.

The aim of the present study was to evaluate the effect of prolonged n-3 PUFA supplementation in patients with NAFLD by means of clinical examination, blood tests and liver ultrasonography.

MATERIALS AND METHODS

Patients

Consecutive patients with NAFLD admitted to our Department were enrolled in the study. Ultrasonography features of fatty liver, namely bright liver echotexture, was the first criterion of inclusion. A nutritional and alcohol investigation was performed to record alimentary habits. The study was approved by the local ethical committee and conducted in accordance with the terms of the Helsinki declaration. All subjects gave written informed consent to take part in the pilot study.

Exclusion criteria were: history of alcohol intake >30-g daily, the use of drugs known to be associated with liver steatosis, undernutrition, chronic viral hepatitis and chronic liver disease of other aetiologies (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, hereditary haemochromatosis, Wilson's disease, α_1 antitrypsin deficiency and celiac disease). Moreover, patients younger than 18 years, pregnant and nursing women were excluded.

Design of the study

All the selected patients were planned for oral administration of n-3 PUFA ethyl ester (eicosapentaenoic acid and docosahexaenoic acid in the ratio of 0.9/1.5 respectively), 1-g capsule per day for 12 months. The product was packed in labelled and numbered boxes. Compliance was checked by counting the returned empty boxes. Some patients who had met the inclusion criteria but refused the treatment were monitored

as controls without therapy. Concern about side effects and assumption to ingest an experimental drug, despite investigators' assurances, were given as the reasons for non-participation to the treatment.

A food-frequency questionnaire of 109 food items, previously validated³⁰ and updated in 2003, was used as screening nutritional test. Moreover, alcohol intake of patients was recorded by a graduated frequency questionnaire, which was administered during the screening nutritional visit, according to the World Health Organization guidelines.³¹

A medical examination with measurement of body mass index (BMI), blood tests and liver ultrasonography was performed at baseline and at the end of treatment. The laboratory evaluation included serum aspartate transaminase (AST), alanine transaminase (ALT), γ -glutamyl transpeptidase (GGT), triglycerides and fasting glucose. Arachidonate plasma level and long-chain fatty acid n-6/n-3 ratio was also tested. Blood samples were taken, centrifuged and immediately frozen at -80°C until assessments. Circulating levels of arachidonate and PUFA were calculated by means of a gas-chromatographic technique coupled with a selected ion-monitoring equipment (Agilent Technologies, Cernusco sul Naviglio, Milan, Italy). B-mode ultrasound and duplex Doppler investigation were performed by the same expert operator by using a high-quality ultrasound device equipped with a multifrequency Convex array transducer (Voluson 530 DMT; Kretz Technik AG, Zipf, Austria). Either 3.5 or 7.5 MHz ultrasound frequency was used for either liver scanning or the detection of vascular parameters respectively. The studied patients were fasted for at least 12 h before ultrasound scanning. Intra-operator variability was assessed by performing a second ultrasonography after 7 days in 12 patients: the coefficient of variation was $<5\%$ both for B-mode and echo-Doppler findings. In the B-mode imaging evaluation, liver echotexture was scored on a four-grade scale by comparing it with the right kidney cortical echogenicity:^{18, 32, 33}

- Grade 0: steatosis absent.
- Grade 1: mild steatosis (lightly and homogeneously increased liver echotexture, with patent intra-hepatic vascular pattern; posterior attenuation absent).
- Grade 2: moderate steatosis (moderate increase of liver echotexture; partial dimming of the vessels; early posterior attenuation).
- Grade 3: severe steatosis (diffuse increase of liver echogenicity, with no longer visible intra-hepatic vessels; heavy posterior attenuation).

Duplex Doppler ultrasound allowed a quantitative measurement of fatty storage on the basis of Doppler perfusion index (DPI; i.e. the ratio between hepatic artery blood flow and the total liver blood flow), according to a previous study.³⁴ In that paper, patients with histological findings of liver steatosis underwent Doppler ultrasound and morphological data were compared with DPI in a multivariate analysis. The histological grade of fatty liver was inversely associated with DPI, showing that DPI may be used as a non-invasive tool for monitoring NAFLD and its response to therapy.

Statistical analysis

Sample size was calculated by considering that group sizes of 10 and 40 achieve 100% power to detect a difference of 0.05 U between a 12-month change of DPI of 0.00 in the control group and one of 0.05 in the PUFA group, assuming an uniform distribution and a common s.d. of 0.03, at a level of alpha of 0.05 with a two-sided Mann-Whitney test. Variables were given as median and interquartile range because of skewed distributions. Comparison between the controls and treated patients was performed with the exact Mann-Whitney test for continuous variables and with the Fisher's exact test for ordinal variables. The Fisher's exact test was also used to compare, in both groups, the number of subjects with elevated AST, ALT, GGT, triglyceride and glucose serum levels at 12th month vs. baseline, and to test whether PUFA treatment was associated with the 12-month probability of transition from fatty liver to normal liver or from a higher to a lower degree of liver steatosis. Intra-group comparisons of arachidonate plasma level and of n-6/n-3 ratio were performed with the Wilcoxon-signed rank test. Moreover, DPI modifications were assessed by duplex Doppler ultrasound at the end of follow-up both in the control and in the PUFA group, and comparison to baseline findings was accomplished by the paired Student's *t*-test. A *P*-value <0.05 was considered significant. Statistical analysis was performed using StatXact 6 (Cytel, Cambridge, MA, USA).

RESULTS

Patients' characteristics

We enrolled 56 patients with NAFLD: 42 underwent n-3 PUFA supplementation (23 males and 19 females;

| | Control group (n = 14) | PUFA group (n = 42) | P-value |
|----------------------------|---------------------------|------------------------|---------|
| Gender (male/female ratio) | 9/5 | 23/19 | 0.1 |
| Age (years) | | | |
| Median (IQR) | 58 (14) | 62 (12) | 0.2 |
| Range | 31–75 | 32–77 | |
| Mean (s.d.) | 56 (12) | 58 (10) | |
| Weight (kg) | | | |
| Median (IQR) | 83 (13) | 76 (18) | 0.3 |
| Range | 64–99 | 57–102 | |
| Mean (s.d.) | 83 (10) | 79 (15) | |
| Stature (m) | | | |
| Median (IQR) | 1.73 (0.11) | 1.67 (0.12) | 0.05 |
| Range | 1.60–1.83 | 1.49–1.83 | |
| Mean (s.d.) | 1.72 (0.07) | 1.66 (0.09) | |
| BMI (kg/m ²) | | | |
| Median (IQR) | 27.9 (4.0) | 29.6 (8.1) | 0.7 |
| Range | 22.8–34.3 | 23–38.6 | |
| Mean (s.d.) | 28.2 (4) | 28.6 (4.7) | |
| DPI | | | |
| Median (IQR) | 0.12 (0.04) | 0.12 (0.09) | 0.8 |
| Range | 0.04–0.19 | 0.06–0.24 | |
| Mean (s.d.) | 0.12 (0.04) | 0.13 (0.05) | |
| AST (IU/L) | | | |
| Median (IQR) | 27 (14) | 23 (8) | 0.1 |
| Range | 12–55 | 14–48 | |
| Mean (s.d.) | 31 (12) | 23 (8) | |
| ALT (IU/L) | | | |
| Median (IQR) | 38 (29) | 34 (22) | 0.3 |
| Range | 15–68 | 12–83 | |
| Mean (s.d.) | 39 (19) | 32 (17) | |
| GGT (IU/L) | | | |
| Median (IQR) | 46 (40) | 37 (38) | 0.3 |
| Range | 26–159 | 19–287 | |
| Mean (s.d.) | 59 (37) | 52 (54) | |
| Triglycerides (mg/dL) | | | |
| Median (IQR) | 168 (83) | 186 (102) | 0.4 |
| Range | 81–261 | 75–446 | |
| Mean (s.d.) | 174 (80) | 180 (63) | |
| Fasting glucose (mg/dL) | | | |
| Median (IQR) | 100 (33) | 99 (29) | 0.5 |
| Range | 83–115 | 82–119 | |
| Mean (s.d.) | 110 (28) | 104 (25) | |

Table 1. Baseline characteristics of patients compared between control and PUFA groups

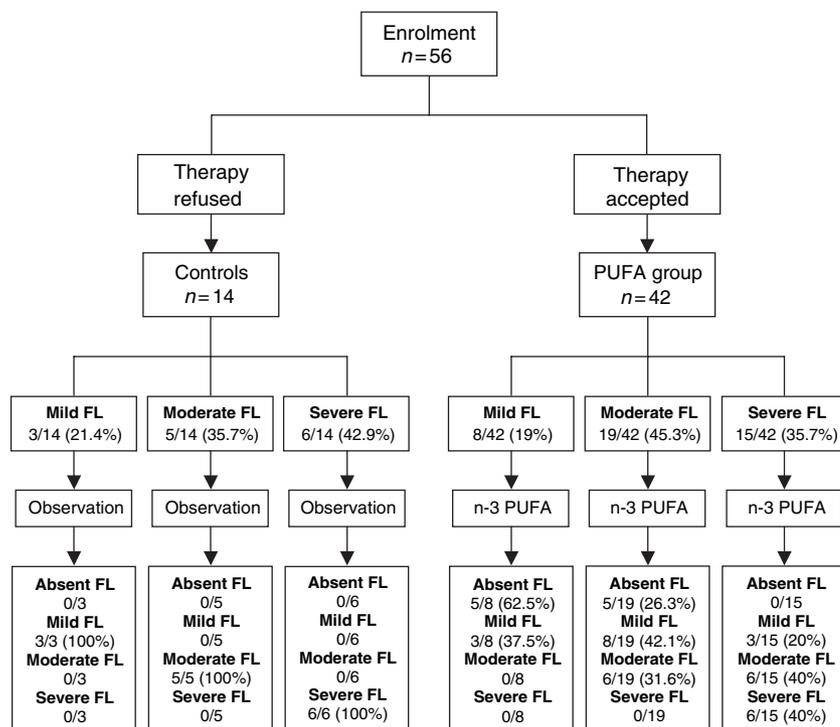
Variables used for statistical analysis are expressed as median (IQR).

PUFA, polyunsaturated fatty acid; BMI, body mass index; DPI, Doppler perfusion index; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ -glutamyl transpeptidase; IQR, interquartile range.

Laboratory normal values are: AST, 5–40 IU/L; ALT, 5–40 IU/L; GGT, 10–40 IU/L; triglycerides, 50–170 mg/dL; fasting glucose, 65–110 mg/dL.

P-values were calculated by the Mann-Whitney test (for continuous variables) and the Fisher's exact test (for gender).

Figure 1. Patient stratification of the control and polyunsaturated fatty acid (PUFA) group according to the baseline echotexture and ultrasonography changes of fatty liver (FL) after 12-month monitoring (observation) or treatment (n-3 PUFA).



mean age 58 years, range 32–77 years), whereas 14 spontaneously refused the treatment and were used as controls (nine males and five females; mean age 56 years, range 31–75 years). Both the control and treated subjects were consecutively enrolled during the same 6-month period of time.

The baseline characteristics of patients are given in Table 1. The two groups were well matched for all demographic, nutritional and clinical features. Moreover, ultrasound stages of steatosis were paired between the control and PUFA groups ($P = 1.0$) (Figure 1).

Efficacy of n-3 PUFA

The changes after 12 months are given in Table 2. The median change in BMI was 0.0 kg/m² in both groups, meaning that no significant variations occurred during the 12-month observation. DPI significantly decreased in the control group, and increased in the PUFA group. AST increased in the control group and remained stable in the PUFA group, whereas ALT and GGT increased in the control group and decreased in the treated patients. Triglycerides increased slightly in controls and decreased substantially in patients submitted to supplementation; however, the inter-individual variability in these changes was high, as shown by

large values of interquartile ranges. Similar to triglycerides, but to a lesser degree, fasting glucose serum level increased in the control group and decreased in the PUFA group. Improvements were also shown when considering the lower number of patients with elevated laboratory parameters after 12 months of supplementation in comparison with the beginning, whereas no significant changes resulted in the controls (Table 3).

Patients treated with PUFA for 12 months showed a significant decrease of arachidonate plasma level: 267 (241) vs. 296 (247) $\mu\text{g/mL}$ [end of treatment vs. baseline respectively; median (interquartile range), $P = 0.0002$]. Range of values was 52–498 vs. 87–598 $\mu\text{g/mL}$, and mean values (s.d.) were 260 (151) vs. 298 (152) $\mu\text{g/mL}$. Moreover, circulating n-6/n-3 ratio was significantly reduced after the treatment with PUFA: 7 (5) vs. 13 (7) [end of treatment vs. baseline; median (interquartile range), $P = 0.0001$]. Range of values was 3–24 vs. 9–28, and mean values (s.d.) were 11 (5) vs. 16 (5). Conversely, no significant differences of these parameters were evident in the control group at time 12 months vs. baseline [median (interquartile range): 292 (255) vs. 289 (231) $\mu\text{g/mL}$, $P = 0.7$], for arachidonate plasma levels, and 13 (4) vs. 12 (9) ($P = 0.3$), for n-6/n-3 ratio. Range of values at 12th month vs. baseline in the controls was 202–552 vs. 192–568 $\mu\text{g/mL}$, and mean values (s.d.) were 329 (114) vs. 322

Table 2. Between group comparison of changes after 12-month observation

| | Control group (<i>n</i> = 14) | PUFA group (<i>n</i> = 42) | <i>P</i> -value |
|--------------------------------|-----------------------------------|--------------------------------|-----------------|
| BMI (kg/m²) | | | |
| Median (IQR) | 0.00 (1.6) | 0.01 (1.1) | 0.7 |
| Range | 0.00–3.5 | 0.00–3.0 | |
| Mean (s.d.) | 0.21 (0.79) | 0.00 (1.0) | |
| DPI | | | |
| Median (IQR) | –0.01 (0.03) | 0.07 (0.09) | 0.001 |
| Range | 0.00–0.06 | 0.00–0.16 | |
| Mean (s.d.) | –0.01 (0.02) | 0.08 (0.06) | |
| AST (IU/L) | | | |
| Median (IQR) | 4 (8) | 0 (4) | 0.003 |
| Range | 2–16 | 0–13 | |
| Mean (s.d.) | 4 (6) | –2 (4) | |
| ALT (IU/L) | | | |
| Median (IQR) | 4 (8) | –4 (5) | 0.002 |
| Range | 0–12 | 0–53 | |
| Mean (s.d.) | 3 (5) | –4 (8) | |
| GGT (IU/L) | | | |
| Median (IQR) | 4 (15) | –3 (7) | 0.03 |
| Range | 2–27 | 0–45 | |
| Mean (s.d.) | 4 (12) | –4 (13) | |
| Triglycerides (mg/dL) | | | |
| Median (IQR) | 4 (36) | –25 (83) | 0.02 |
| Range | 8–63 | 0–376 | |
| Mean (s.d.) | 9 (27) | –46 (88) | |
| Fasting glucose (mg/dL) | | | |
| Median (IQR) | 4 (13) | –5 (17) | 0.02 |
| Range | 2–15 | 2–36 | |
| Mean (s.d.) | 4 (9) | –6 (14) | |

Values used for statistical analysis are expressed as median (IQR).

PUFA, polyunsaturated fatty acid; BMI, body mass index; DPI, Doppler perfusion index; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ -glutamyl transpeptidase; IQR, interquartile range.

P-values were calculated by the Mann–Whitney test.

(110) $\mu\text{g/mL}$, for arachidonate plasma level, while range was 9–25 vs. 11–25, and mean values (s.d.) were 14 (5) vs. 14 (4), for n-6/n-3 ratio.

In the group of patients supplemented with PUFA, ultrasonography disclosed a regression of bright liver at the end of treatment, with some patients showing a normal echotexture (Figure 1). In particular, after supplementation there were 10/42 (23.8%) patients without steatosis, 14/42 (33.3%) with mild steatosis, 12/42 (28.6%) with moderate steatosis and 6/42 (14.3%) with

severe steatosis. Conversely, the number of patients with fatty liver and the degree of steatosis was unchanged in the control group (Figure 1). Sixty-four per cent of patients in the PUFA group had a regression from liver steatosis to normal liver or from a greater to a lower degree of steatosis vs. 0% in the control group ($P = 0.0001$). As a proof of haemodynamic improvement, possibly due to lipid removal from the liver, a significant increase of DPI was observed at the end of treatment with n-3 PUFA, whereas no significant modifications occurred in the control group (Figure 2).

Safety of n-3 PUFA

No dropout was recorded in the group of patients treated with PUFA. No adverse events were recorded in anyone.

DISCUSSION

Non-alcoholic fatty liver disease and NASH are chronic liver diseases that may lead to liver impairment and cirrhosis.⁴ Thus, several therapeutic proposals have been tested till now, but a validated and unique approach does not exist yet.^{1, 13} This is the first study where the efficacy of prolonged n-3 PUFA intake is tested in humans.

A current problem of many clinical and therapeutic investigations is the evaluation of the outcome. In particular, routine haematochemical tests are helpful in patients with NAFLD,³ but not sufficient to assay the real effectiveness of the treatment. Actually, changes of different serum factors may not be always paralleled by variations in liver conditions. On the other hand, needle liver biopsy provides the most conclusive evaluation for both disease diagnosis and follow-up, but it is impossible to submit all the patients to liver biopsy because of its invasiveness and possible complications.³⁵

Beyond needle liver biopsy, the 'gold standard' to distinguish between NAFLD and NASH, ultrasonography is commonly used to make a diagnosis of NAFLD and for monitoring patients with fatty liver.³⁶ In fact, it is the cheapest and best tolerated imaging technique,³⁵ and an adequate use of B-mode investigation allows semiquantitative assay of hepatic fat content not only in severe and moderate steatosis, but also in mild cases.³³ A further tool may be represented by duplex Doppler ultrasound and DPI analysis, which

Table 3. Rate of patients with haematochemical parameters above the upper normal range (elevated parameters) in the control ($n = 14$) and PUFA ($n = 42$) groups at 12th month (T12) vs. baseline (T0)

| Elevated parameters | Control group | | | PUFA group | | |
|---------------------|---------------|-----------|-----------------|------------|-----------|-----------------|
| | T0 | T12 | <i>P</i> -value | T0 | T12 | <i>P</i> -value |
| AST | 5 (35.7) | 6 (42.9) | 1.0 | 5 (11.9) | 3 (7.1) | 0.7 |
| ALT | 8 (57.1) | 7 (50) | 1.0 | 26 (61.9) | 7 (16.7) | 0.0001 |
| GGT | 10 (71.4) | 11 (78.6) | 1.0 | 25 (59.5) | 15 (35.7) | 0.04 |
| Triglycerides | 10 (71.4) | 10 (71.4) | 0.6 | 29 (69) | 12 (28.6) | 0.0001 |
| Fasting glucose | 7 (50) | 10 (71.4) | 0.4 | 22 (52.4) | 16 (38.1) | 0.2 |

Data are given as number of patients (percentage).

PUFA, polyunsaturated fatty acid; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ -glutamyl transpeptidase.

P-values were calculated using the Fisher's exact test.

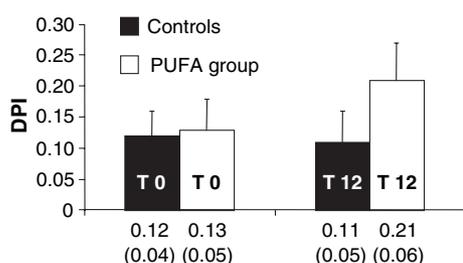


Figure 2. Changes of Doppler perfusion index (DPI) after 12 months (T12) vs. baseline (T0) [statistical analysis by the Student's *t*-test: $P = 0.8$ in the control, and $P = 0.001$ in the polyunsaturated fatty acid (PUFA) group]. The values under the columns are mean (s.d.).

provides a quantitative, non-invasive evaluation of NAFLD by measuring hepatic artery and portal vein blood flows.³⁴ We have previously shown that DPI varies according to the severity of liver disease: it resulted lower than 0.20 in NAFLD and in chronic hepatitis of various aetiologies, whereas it was higher than 0.30 in compensated liver cirrhosis; a pair group of healthy volunteers enrolled in the same period as control group maintained a normal DPI range of 0.20–0.30 resulting from mean values \pm 2s.d.³⁷

Ultrasonography and echo-Doppler are the methods of choice for assessing fatty liver because of their critical reproducibility.¹³ A study by Fowler *et al.*³⁸ showed intra- and inter-observer variability in calculating the DPI, due to physiological haemodynamics of different subjects and technical properties of ultrasound equipment, whereas the phase of respiration and the method for obtaining blood flows from cross-sectional area of vessels did not significantly influence

the assay. We have used ultrasonography and echo-Doppler in patients with NAFLD, a condition where recurrent changes in hepatic microcirculation are reported as a consequence of intra-hepatic fat storage.^{39, 40} Inter-observer variability and variations due to technical peculiarities were strongly reduced by performing investigation under the same operator and ultrasound device. To test intra-observer variability, a group of patients underwent ultrasonography twice within the first 7 days at baseline, and differences were calculated on a test-re-test modality, resulting <5%. Every session was performed with an angle of insonation lower than 60°, to avoid instrumental errors that may occur when the angle is not maintained below this limit.³⁸

As many patients with fatty liver have alterations of body weight and wrong alimentary habits, subjects with unbalanced diet at the screening nutritional test were excluded from the study in order to avoid significant modifications of BMI during the observation and to test the real efficacy of n-3 PUFA supplementation apart from possible benefits of dietary restrictions. Actually, BMI of subjects of both the groups did not change significantly after 1 year of observation.

Liver steatosis has a multifactorial pathogenesis, with a strong correlation with the metabolic syndrome and insulin resistance.^{13, 19, 41} PUFA may ameliorate insulin sensitivity because of their ability to bind to PPAR α .²² This effect has been extensively demonstrated in animal models of fatty liver. Sekiya *et al.*²⁸ obtained the disruption of a lipogenic factor, namely the SREBP-1 gene, with improvement of insulin-dependent metabolism (i.e. reduction of glucose, insulin and free fatty acid serum levels) in *ob/ob* mice

submitted to dietary PUFA supplementation. Levy *et al.*²⁹ found that the 'Quantitative Insulin Sensitivity Check Index' was higher in the fish oil-fed Fisher 344 rats than in the control animals.

In the present study, we found an improvement of fasting glucose level and lipid pattern in treated patients in comparison with controls. Restoration of insulin sensitivity induces triglyceride redistribution, with lipid storage in adipose tissue and consequent decrease of triglyceride serum levels. Ultrasonography examination demonstrated an improvement in liver echotexture after intake of n-3 PUFA, with a significant regression of hepatic brightness associated with a remarkable increase of DPI, which corresponds to better liver haemodynamics.^{34, 37} In addition, these patients showed a significant reduction of circulating fatty acid n-6/n-3 ratio and of arachidonate level, which in turn may promote liver steatosis by impairing eicosanoid-related metabolic functions.²² Finally, ALT serum levels, a possible marker of liver injury derived from triglyceride accumulation,²⁸ were also reduced in the treated patients whereas no significant differences were evident in the control group.

In this pilot study, we used the lowest known dosage of n-3 PUFA, namely 1 g/day. As far as we know there is no evidence about the optimal amount and duration of treatment for NAFLD, as this is the first study performed in humans, we thus cannot exclude that a higher intake could have reached even better results.

This is the first report about n-3 PUFA supplementation performed in humans with NAFLD and its results are backed up by biological plausibility. Although the study was not randomized, the statistical analysis showed homogeneous features of the two groups of patients.

In conclusion, prolonged n-3 PUFA dietary supplementation had a positive effect on fatty liver in patients with NAFLD. The treated patients showed a reduction of triglyceride and ALT serum level and significant benefits on liver ultrasonographic pattern, in terms of regression of bright echotexture and increase of DPI, in comparison with baseline features. The DPI, measured by echo-Doppler, indicated an improvement of liver blood flow as a consequence of reduced intra-hepatic fat accumulation. The results of the present study warrant evaluation of n-3 PUFA in a large randomized, double-blind, placebo-controlled trial using liver histology as an end-point to better determine the potential therapeutic effects of n-3 PUFA in human NAFLD.

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