

Efficacy of Lifestyle Changes in Subjects with Non-Alcoholic Liver Steatosis and Metabolic Syndrome May Be Improved with an Antioxidant Nutraceutical: A Controlled Clinical Study

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Abstract

Introduction The prevalence of liver steatosis is particularly high in subjects with signs of the metabolic syndrome, and current therapeutic guidelines mostly rely on lifestyle changes alone, which rarely achieve significant objective improvements. In the present study, we evaluated the possibility of monitoring objective improvements in these subjects, before and after a dietary regimen.

Methods Overall, 78 subjects with metabolic syndrome and ultrasound confirmation of liver steatosis were included in an open, controlled study; all of these subjects were treated for 90 days with the standard regimen of diet and exercise. One group of 43 subjects (Group A) also received a Eurosil 85[®]-based nutraceutical (silymarin + vitamin E) as a dietary adjunct, whereas the remaining 35 subjects (Group B) represented the control group. Changes from baseline values were recorded in biometric, biochemical, and ultrasound data. For assessments and monitoring of liver steatosis, two indexes were utilised—Hepatic Steatosis Index (HSI) and Lipid Accumulation Product (LAP) index.

Results The absolute changes from baseline were significantly higher in Group A in biometric parameters (reduction of abdominal circumference, Body Mass Index, ultrasound measurement of right liver lobe) and in both the HSI and LAP indexes. Both treatments were well tolerated.

Conclusion The results observed suggest that the use of a Eurosil 85[®]-based nutraceutical as a dietary adjunct with antioxidant properties potentially favours the efficacy of the dietary regimen alone and may possibly improve the

subjects' motivation to sustain such lifestyle changes over time.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD), a condition ranging from uncomplicated liver steatosis to steatohepatitis (NASH) and potentially to end-stage diseases such as cirrhosis or hepatocarcinoma [1], is the most common cause of liver disease worldwide [2, 3]. The prevalence of liver steatosis in Italy has been reported to be within the 35–40 % range in the general population [4–6], with sharp increases in overweight and obese subjects (67 and 91 %, respectively [7]). Whereas the concomitant presence of liver steatosis, obesity and insulin resistance is a common clinical finding, there is still ongoing research to elucidate the pathogenetic mechanisms involved [8, 9]; for example, whether NAFLD is a cause or consequence of insulin resistance [10]. However, accumulating evidence confirms not only a correlation between NAFLD and the metabolic syndrome [8, 11, 12] but also the association of NAFLD with an increased cardiovascular risk [13]. Intrahepatic oxidative stress is also known to be one of the critical factors that may determine progression of NAFLD to more severe conditions involving liver inflammation and fibrosis [12]. Therefore, from the standpoint of the primary care physician, monitoring the presence of liver steatosis in subjects with signs of metabolic syndrome (particularly abdominal obesity, elevated triglycerides and abnormal fasting glycaemia) should be normal clinical practice, with the aim of taking preventive measures against the development of more severe stages of NAFLD. Since the usual biochemical parameters (alanine transaminase [ALT], aspartate transaminase [AST], γ -glutamyl transpeptidase

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(γ GT) may be within normal ranges in patients with uncomplicated steatosis, their use as a screening test would probably result in underestimation of the presence of NAFLD, whereas imaging techniques such as liver ultrasonography (USG) are a potentially more sensitive tool for diagnosis of liver steatosis [3, 14]. According to recent guidelines [3], the therapeutic approach to liver steatosis is mostly focused on lifestyle interventions, including weight reduction, dietary modification and aerobic physical exercise [3]. A weight loss of at least 7 % has been shown to improve steatosis and also to reduce parameters of necroinflammation [15]. However, actual long-term compliance of subjects with NAFLD to lifestyle intervention protocols is usually reported to be unsatisfactory. Reinforcing the 'motivation to change' in these subjects seems to be a critical factor, and strategies of targeted behavioural counselling, to provide these subjects not only with motivation but also with tools to monitor improvements and therefore to sustain the lifestyle changes, have been proposed for evaluation in clinical trials [16]. In a similar perspective, with the aim of improving the subjective and objective results of lifestyle changes in subjects with liver steatosis, and therefore to possibly reinforce their motivation to compliance, the present study has observed the effects of a nutraceutical product as an adjunct to the standard diet and exercise regimen. Since silymarin has consistently been shown to improve parameters related to insulin resistance [17–20], we have observed the efficacy and tolerability of a nutraceutical based on a new formulation of silymarin which provides, at the recommended daily posology, a high dosage of the active ingredient silibinin, together with vitamin E. The clinical use of oral formulations containing silymarin or silibinin has been documented in several clinical trials conducted in patients with ASH or NASH [17–20]. Vitamin E, when used at a high dosage (800 IU), has also been shown to improve several inflammatory parameters in patients with NASH [21]. The specific aim of the present study was to observe whether, in subjects with a less advanced stage of NAFLD (steatosis), a moderate regimen of diet and exercise, plus a combination of a relatively low dosage of vitamin E (60 IU) and a new formulation of silymarin (by its antioxidant activities) could provide additional clinical improvements.

2 Methods

2.1 Patients and Clinical Assessments

From September to December 2013, a total of 80 consecutive subjects in generally good health, who had visited their primary care doctor for digestive disturbances and had

been referred to our Echography Service in order to perform USG to determine the presence and stage of liver steatosis and to measure the size of the right liver lobe [21, 22], were proposed to be included in an open, controlled, observational clinical study. The study design was quite simple. All subjects with at least grade 1 steatosis and concomitant presence of metabolic syndrome were prescribed a standard Mediterranean diet to follow for 3 months, since good efficacy has been reported with this kind of diet in NAFLD patients [23]; in particular, 1 month of a fibre-rich, moderately ipocaloric diet with preferential use of food with medium or low glycaemic index, followed by 2 months of an isocaloric diet with similar food balance. In addition, subjects were encouraged to take regular physical exercise (e.g. 15 min of brisk walking/day). A subgroup of subjects, with matching biometric parameters and physical conditions, was also prescribed to take, as a dietary adjunct, two tablets/day of a nutraceutical product containing (in each tablet) 210 mg of Eurosil 85[®], a new silymarin formulation with approximately 60 % (e.g. 125 mg) of silibinin and 30 IU of vitamin E (Legalon E, Rottapharm Madaus, Monza, Italy). To ensure compliance, all patients of the latter group were immediately given a supply of 200 tablets of nutraceutical product (sufficient for the treatment period). All subjects were also given a leaflet where the initial slightly ipocaloric diet and the subsequent maintenance diet were clearly detailed. They were also scheduled for a control visit and re-assessment of all biometric, biochemical parameters and USG tests after 90 days. The inclusion criteria were confirmation of liver steatosis (at least grade 1), abdominal circumference >94 cm in men or >80 cm in women, triglyceride levels (TGL) >150 mg/dL (1.7 mmol/L), and fasting glycaemia >100 mg/dL (5.6 mmol/L). Exclusion criteria were ongoing acute or chronic hepatitis, presence of other diagnosed liver diseases, signs of biliary obstruction, daily alcohol intake >30 mg, concomitant use of antioxidant products such as silymarin, ademethionine and glutathione, uncontrolled diabetes mellitus and/or major change in antidiabetic therapy within the previous 3 months. These conditions were ruled out at screening visit by anamnesis, physical examination and routine laboratory analyses. Body Mass Index (BMI), abdominal circumference, blood pressure, glycaemia, glycated haemoglobin, triglyceride and cholesterol (total, low-density lipoprotein, high-density lipoprotein) levels, ALT, AST, and γ GT were recorded, together with the standard anamnestic data, as baseline values for all patients. The Hepatic Steatosis Index (HSI) and the Lipid Accumulation Product (LAP) index were also calculated, as these are simple tools for the assessment of liver steatosis. The HSI was calculated using the formula $[(8 \times \text{ALT}/\text{AST}) + \text{BMI} (+2 \text{ if female}) (+2 \text{ if with diabetes mellitus})]$, and the LAP was calculated using the

formula [abdominal circumference in cm (-65 if male or -58 if female) \times TGL in mmol/L] [24, 25].

2.2 Ultrasonography

All subjects were examined by the same doctor, in order to minimise individual variability, by means of an Aplio diagnostic ultrasound system (Toshiba Medical Systems, Tokyo, Japan) with convex multifrequency probe/transducer 3–5 MHz. Ascending oblique subcostal scans of the right lobe were performed; for the left lobe, longitudinal scans were taken (during inspiratory apnea) at the point in which the cava vein connects to the right atrium. The usual assessment (grades 0–3) of steatosis was adopted, where grade 0 represents normal echogenicity, non-different from renal cortex echogenicity with homogeneous texture and therefore no steatosis; grade 1 occurs when the liver echogenicity is minimally increased with reference to the renal cortex, with slightly rougher texture; grade 2 occurs when the liver echogenicity reduces deeper ultrasound projection and clear distinctions of portal vein branches; and grade 3 occurs when the marked increase in liver echogenicity, with non-homogenous texture, obscures the vascular walls and diaphragmatic outline. A lateral left position, in inspiratory apnea, was then adopted to assess the liver size; ascending subcostal longitudinal scans were performed [22].

2.3 Statistics

Continuous variables were assessed by means of mixed adjusted models by the baseline measure as a covariate. Differences were also assessed using a *t* test for the main outcomes. Categorical variables were compared using Fisher's exact test. The analysis was performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) and the level of significance was established at 0.05 (two-sided).

3 Results

Results are shown in Table 1 (as means of the absolute changes from respective baselines); the statistical differences are shown between the changes observed in Group A (subjects treated with the nutraceutical as an adjunct to diet) and in the control group (Group B; treatment with diet only). Baseline values of the two groups were not significantly different. BMI and abdominal circumference did not change in Group B, whereas a significant reduction ($p = 0.022$ and $p = 0.028$, respectively) was found in Group A. In the latter group the average USG-measured size of the right liver lobe decreased from baseline (17.24 cm) to the final visit at 90 days (-0.96 cm), while

in Group B the decrease was smaller (-0.41 cm), with significant difference ($p = 0.044$) between groups. ALT and AST values were within the normal limit at baseline; even if no differences between the two groups were found in their changes from baseline, the calculation of HSI (which includes BMI and the presence of diabetes mellitus) highlighted a significant difference in reduction in Group A ($p = 0.0134$). Similarly, even if the reduction in triglyceride level showed only a higher reduction trend in Group A, using the calculation of the LAP index (which also considers the abdominal circumference), a significant ($p < 0.001$) difference is achieved. No adverse events have been reported.

4 Discussion

The role of dietary intervention in the management of NAFLD is well-established and the so-called 'lifestyle change' approach still represents, in the absence of other conditions, the basic recommendation for subjects with liver steatosis [3]. However, published evidence, in accordance with usual clinical practice, reports that the efficacy of this approach is often diminished by poor implementation and reduced adherence to the prescribed regimen of diet and exercise [16]. The results of the present 3-month observational study, conducted with non-diabetic subjects diagnosed with liver steatosis and also characterised by the presence of insulin resistance and other signs of metabolic syndrome, confirm that lifestyle intervention alone may not be sufficient to achieve significant objective improvements which may be important to favour the necessary long-term adherence to such a regimen. In subjects with similar clinical features, it has recently been reported that significant improvements in Bright Liver Score are achieved only after 6 months of adherence to a Mediterranean diet together with regular physical exercise [26]. For these reasons, the significantly improved results that we have observed in the parallel group (in which subjects were instructed not only to follow the dietary regimen but also to assume a dietary adjunct with a specific liver antioxidant rationale), may represent an example of a more integrated approach that could achieve clinical improvements within a shorter timeframe (3 months) and possibly motivate a sustained adherence to the lifestyle change. Since transaminases are usually within normal limits, we feel that the use of an AST/ALT ratio and validated derived indexes such as fatty liver index, HIS and LAP may be helpful in assessing the severity of the liver steatosis, thereby representing an additional simple and non-expensive diagnostic tool. Recent studies have found that when compared with gold-standard methods for the detection and quantification of liver fat content

Table 1 Demographic, biometric, ultrasonographic, biochemical data (mean \pm SD), as well as Hepatic Steatosis Index (HSI) and Lipid Accumulation Product (LAP) index, measured at baseline and after 90 days of treatment with a nutraceutical as an adjunct to diet (Group A) or with diet-only (Group B)

	Group A		Group B		Change from baseline		
	Baseline	90 days	Baseline	90 days	Group A	Group B	Statistical difference between groups
<i>N</i>	43	43	35	35			
Age (years)	56.63 \pm 12.79		55.40 \pm 13.63				
Gender (M/F)	18/25	18/25	24/11	24/11			
BMI	31.8 \pm 4.80	31.1 \pm 4.85	29.7 \pm 3.66	29.7 \pm 3.79	-0.71	-0.004	<i>p</i> = 0.022
Abdominal circumference	109.3 \pm 11.6	104.53 \pm 11.18	104.5 \pm 8.9	102.74 \pm 9.00	-4.81	-1.78	<i>p</i> = 0.028
Right liver lobe (cm)	17.24 \pm 2.11	16.28 \pm 2.22	16.64 \pm 1.42	16.23 \pm 1.70	-0.96	-0.41	<i>p</i> = 0.044
Fasting plasma glucose (mg/dL)	109.74 \pm 25.35	115.75 \pm 28.19	106.62 \pm 28.70	107.11 \pm 15.42	6.01	0.49	NS
Hb A1c %	4.36 \pm 2.18	4.62 \pm 0.73	4.38 \pm 2.22	4.89 \pm 1.07	0.26	0.51	<i>p</i> < 0.05
ALT (IU/L)	33.08 \pm 26.97	28.02 \pm 22.27	25.11 \pm 15.56	24.76 \pm 14.59	-5.06	0.35	NS
AST (IU/L)	23.78 \pm 14.40	22.49 \pm 12.45	20.13 \pm 6.18	21.37 \pm 11.29	-1.29	1.24	NS
γ Gt (IU/L)	43.83 \pm 52.27	33.33 \pm 21.59	27.98 \pm 14.14	28.60 \pm 14.66	-10.50	0.62	NS
Triglycerides (mg/dL)	182.33 \pm 90.68	133.11 \pm 67.24	156.09 \pm 61.65	137.01 \pm 63.26	-49.22	-19.08	NS
Total cholesterol (mg/dL)	216.78 \pm 52.80	205.98 \pm 45.67	209.27 \pm 39.59	208.55 \pm 31.37	-10.8	-0.73	NS
LDL-C (mg/dL)	117.37 \pm 34.09	113.58 \pm 28.39	117.82 \pm 29.54	118.38 \pm 29.62	-3.79	0.56	NS
HDL-C (mg/dL)	57.25 \pm 18.78	56.22 \pm 12.48	55.67 \pm 14.92	57.63 \pm 15.27	-1.02	2.00	NS
HSI	43.62 \pm 5.49	41.76 \pm 5.59	40.20 \pm 5.23	40.00 \pm 5.41	-1.85	-0.19	<i>p</i> = 0.0134
LAP	97.77 \pm 48.50	64.06 \pm 33.03	75.43 \pm 40.89	62.28 \pm 33.94	-33.72	-13.16	<i>p</i> < 0.001

(e.g. liver biopsy and proton magnetic resonance spectroscopy), the steatosis biomarkers are not comparatively reliable to quantify liver steatosis, particularly in the presence of fibrosis and inflammation [27, 28]. However, in subjects with relatively mild symptoms, when the use of invasive or expensive clinical tests are not usually recommended, the surrogate biomarkers may still be helpful in monitoring the efficacy of lifestyle changes and possible adjunct treatments. In this study, the choice of the silymarin-based nutraceutical has been based on the documented safety, even at a high dosage [29], and the scientific evidence of the pharmacokinetic and pharmacologic properties of silymarin. Once absorbed from the gastrointestinal tract, silibinin (the main component of silymarin) undergoes liver metabolism. Due to enterohepatic cycling [29], it subsequently concentrates in the liver where it may exert the antioxidant properties that have been extensively documented both in vitro and in vivo [30, 31], and the improvement in parameters of insulin resistance that have also been documented [17–19]. On the other hand, the role of insulin resistance and intrahepatic oxidative stress in the pathogenesis of NAFLD and in the progression of disease severity has been repeatedly highlighted [10, 12]. At the recommended posology of two capsules/day, the nutraceutical provides 420 mg of Eurosil 85[®], a silymarin

preparation containing 60 % (e.g. approximately 250 mg) of silibinin and 60 IU of vitamin E.

Our study has several limitations: the relatively small numerosity, the trend towards higher biometric parameters that we found in the group treated with both diet and a nutraceutical, and the difficulty in confirming compliance to the prescribed diet and exercise regimen. Moreover, the USG assessments have not been performed by a 'blind' third party, and the decrease in the HSI and LAP indexes that we have observed in the 3-month period are still relatively small. However, with regard to the USG assessments, all have been performed by a single investigator, who took maximal care in following a well-standardised procedure. With regard to the small effect size, this was sufficient to provide the treating primary care doctors with some objective evidence by which they could more effectively encourage subjects to continue the treatments.

5 Conclusion

The present non-interventional study suggests that, in subjects with liver steatosis and metabolic syndrome, who are prescribed a recommended diet and exercise regimen to

follow, the use of a nutraceutical with specific antioxidant properties as a dietary adjunct may improve the efficacy of those lifestyle changes. We suggest the need for a larger, randomised clinical trial to provide further insight into the current evidence.

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Conflict of interest None.

Compliance with ethics guidelines For the present, spontaneous, observational study, authorisation by the Ethical Commission of the Clinic Center was granted, and the principles of the Helsinki Declaration of 1975, as revised in 2000 and 2008, were applied. Informed consent was obtained from all subjects, and the subjects' right to withdraw from the study without giving reason was clearly allowed.

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