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Alimentary Pharmacology & Therapeutics

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SUMMARY

Background
Nonalcoholic fatty liver disease (NAFLD) is a significant health problem for which there is no universally accepted pharmacological treatment. The combination of weight loss and antioxidant drugs to ameliorate insulin resistance and improve steatosis, inflammation and fibrosis provides the rational for therapeutic trials.

Aim
To evaluate the efficacy and safety of a nutritional supplement Viusid in association with diet and exercise for NAFLD.

Methods
A randomized, controlled and parallel-group trial was conducted at a tertiary care academic centre (National Institute of Gastroenterology, Havana, Cuba). We randomly assigned 60 patients with liver biopsy-proven NAFLD to 6 months of treatment with a hypocaloric diet plus aerobic exercise daily and three Viusid sachets daily or a hypocaloric diet and exercise. Endpoints were improvement in the NAFLD activity score (NAS), fibrosis and normalization of serum aminotransferase levels.

Results
A significant improvement in steatosis, necroinflammation and fibrosis was seen in each group of treatment ($P < 0.01$ for each feature). The Viusid group, as compared with the control group, significantly reduced the mean of NAS [from 4.18 to 0.54 points in the Viusid group vs. 4.45 to 2.2 points in the control group ($P < 0.001$)]. On between-group comparison, Viusid was found to be associated with a significantly greater improvement in steatosis ($P < 0.001$), ballooning ($P = 0.002$) and lobular inflammation ($P = 0.025$), but not in fibrosis ($P = 0.07$). Viusid was well tolerated.

Conclusions
Our results indicate that treatment with diet and exercise leads to a notable improvement in the histological features of NAFLD; however, the administration of Viusid intensifies the improvements of histological findings, especially of steatosis and inflammation.

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INTRODUCTION

With the increasing prevalence of obesity, diabetes and the metabolic syndrome in the general population,[1, 2] nonalcoholic fatty liver disease (NAFLD) has become the main cause of chronic liver disease worldwide.[3–5] The histological features of NAFLD range from fat alone (simple steatosis) to fat plus inflammation with or without fibrosis (steatohepatitis). Nonalcoholic steatohepatitis (NASH) is a more severe subtype of NAFLD, which may progress to cirrhosis and hepatocellular carcinoma.[6–8] Thus, treatment of NASH is to halt the progression of the disease. Pharmacological agents such as insulin sensitizers,[9–15] antioxidant agents[12, 16–19] or lipid-lowering agents[20, 21] have been tested in clinical trials; however, there is no conclusive evidence to support their use in clinical practice. Thiazolidinediones (TZDs) have shown promise in the treatment of NASH; however, their favourable effects on liver histology and liver biochemistries disappear on their discontinuation, suggesting that long-term treatment is needed to maintain therapeutic benefits.[15] This is a potentially significant issue; recent studies have questioned the long-term safety of TZDs (especially rosiglitazone).[22] A recent study raised the possibility that TZDs alone without lifestyle modification may not be as effective.[14] In the pathogenesis of NAFLD, lipid peroxidation and overabundance of reactive oxygen species are key mediators in the progression from relatively stable hepatic steatosis to potentially progressive steatohepatitis.[23] This provides a rationale for studies using antioxidant agents; however, trials involving these agents have not clearly demonstrated their potential benefits on histological (steatosis, inflammation and fibrosis) and metabolic (insulin resistance) endpoints, in part limited by small sample size, lack of dependable endpoints and poor control on lifestyle modification such as diet, weight loss and exercise.[12, 16–19] Evidence of the efficacy of diet and exercise in patients with NAFLD is surprisingly scant. However, as this kind of lifestyle modification is comparatively safe, inexpensive and has other health benefits, it could be proposed as first-line treatment for obese patients with NAFLD,[24] despite the limited evidence to support its efficacy.[25, 26] There is an obvious need for the continuous development of new treatment strategies for NASH. Thus, the addition of an antioxidant agent to weight loss through diet and exercise could increase the beneficial effects on histology, particularly in patients with poor diet compliance. Viusid (Catalysis, S.L., Madrid, Spain), a nutritional supplement, has different molecules (ascorbic acid, zinc and glycyrrhizic acid) with recognized antioxidant properties (Table 1).[27–30] Its different chemical compounds are activated through a molecular activation principle that strongly increases their biological activity without modifying their physical structure. Recently, Vilar Gomez *et al.*[31] evaluated the efficacy and safety of Viusid in combination with interferon alpha-2b and ribavirin in patients with chronic hepatitis C. The authors reported that the addition of Viusid to the conventional interferon/ribavirin therapy was associated with significant histological and biochemical improvements, especially in patients without sustained virological response. They suggested that the Viusid-related effect on histological features, especially fibrosis, appears to be associated with hepatoprotective or antioxidant properties. All of these effects could improve the histological pattern of NAFLD, especially inflammation and fibrosis in an attempt to halt disease progression. The adjuvant benefit of Viusid added to weight loss through a hypocaloric diet and exercise for patients with NAFLD has not been evaluated in clinical trials. Therefore, a randomized controlled study was conducted to evaluate whether the addition of Viusid to a hypocaloric diet and exercise would be associated with a greater histological improvement compared with diet and exercise in patients with NAFLD.

MATERIALS AND METHODS

Patients

We recruited 60 patients with histological diagnosis of steatohepatitis (minimal histological criteria for steatohepatitis included steatosis involving at least 5% of hepatocytes and lobular inflammation with or without fibrosis) at a tertiary care academic centre (National Institute of Gastroenterology, Havana, Cuba). Inclusion criteria included male and female patients of 18–70 years of age, absence of significant alcohol

Table 1. Ingredients of Viusid

Malic acid	0.666 g	Ascorbic acid	0.020 g
Glycyrrhizic acid	0.033 g	Folic acid	66 μg
Glucosamine	0.666 g	Cyanocobalamine	0.3 μg
Arginine	0.666 g	Zinc sulphate	0.005 g
Glycine	0.333 g	Pyrodoxal	0.6 mg
Calcium pantothenate	0.002 g		

consumption (weekly ethanol consumption of <20 g) and ability to provide informed consent. Exclusion criteria included presence of any other form of liver disease, positive screening for viral hepatitis B and C, pregnancy or lactation, decompensated cirrhosis, presence of secondary causes of NAFLD such as medications that induce steatosis (corticosteroids, oestrogens, methotrexate, amiodarone, tamoxifen and calcium channel blockers), gastrointestinal bypass surgery, pharmacological treatment with some potential benefit on NAFLD, including ursodeoxycholic acid, vitamin E, betaine, pioglitazone, rosiglitazone, metformin, pentoxifylline or gemfibrozil, use of cholesterol-lowering statin drugs within the 6-month period before enrolment, fasting glucose levels <250 mg/dL (13.3 mmol/L), contraindication to liver biopsy, severe or morbid obesity (body mass index ≥35 kg/m^2), refusal to participate in the study, concomitant disease with reduced life expectancy, severe psychiatric conditions and drug dependence. Patients with hepatic steatosis only were not recruited for the trial.

Ethics

The study was conducted in compliance with the Declaration of Helsinki and approved by the ethics committee and the institutional review board of the National Institute of Gastroenterology. All patients provided written informed consent for participation. The trial had been registered at ClinicalTrials.gov (NCT00509418).

Study design

After initial evaluation, all patients who met the eligibility criteria were consecutively enrolled in the study. They were randomly assigned to receive: hypocaloric diet plus aerobic exercise for 40 min daily and three Viusid oral sachets (50 g) daily (n = 30), or the same hypocaloric diet and exercise daily without Viusid (n = 30), for 24 weeks. Viusid was provided by Catalysis, S.L. (Madrid, Spain).

Randomization was conducted by blocks of 4 (block randomization). It was performed by a health worker experienced in randomization techniques who was not involved in the evaluation or treatment of the participants. The physicians, study coordinators and patients did not have access to the randomization scheme.

All patients were evaluated at baseline and at monthly intervals by an experienced dietitian who strongly instructed the subjects to reduce their caloric intake by 500 kcal/day (achieving a weight loss of approximately 500 g/week).

The dietary pattern was proportionally distributed in carbohydrates 64%, fat 22% with <10% of saturated fatty acids of total daily calories and protein 14% (low-fat diet). All patients were encouraged to avoid the intake of simple sugars and consume higher amounts of fruit, vegetables and whole grains.

All subjects were also recommended to walk or to jog 40 min/day at least 5 days/week, and they were monitored by a short self-report questionnaire for the measurement of habitual physical activity.[32]

Clinical assessment, including body weight and waist circumference measures, liver tests, lipid profile, fasting plasma insulin and glucose, and uric acid determinations, along with compliance with the study medication (verified through sachet count) and adverse events, were determined at baseline and/or at monthly intervals during the 24 weeks of the study.

Liver biopsy was performed at baseline and at 24 weeks of treatment and read by a single pathologist. The NAFLD activity score (NAS) was used to evaluate histological features of active injury that are potentially reversible in the short term.[33] The score is defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3) and ballooning (0–2); thus ranging from 0 to 8. Fibrosis was graded 0–4 (0, none; 1, sinusoidal or periportal; 2, sinusoidal and periportal; 3, bridging fibrosis; 4, cirrhosis). Biopsy specimens were examined by a single pathologist who was unaware of the patients' clinical and biochemical data, treatment assignment and liver biopsy sequence. A sample length of at least 20 mm was considered a prerequisite for adequate specimen evaluation. The pathologist had an intraobserver agreement between two readings from good to excellent with K statistics ranging from 0.74 to 0.96.

Primary outcomes (histology)

- Improvement in the NAS at 24 weeks of treatment as compared with baseline score.
- Improvement in fibrosis score at 24 weeks of treatment as compared with baseline score.

Secondary outcome (aminotransferase)

- Biochemical improvements: defined as improvement in aminotransferase levels at 24 weeks.

Statistical methods

The baseline characteristics were summarized in percentage for categorical variables and as means ± s.d., median values and their ranges for continuous variables. The chi-square test was applied to categorical variables. The two-sample *t*-test was used to compare means, and the Mann–Whitney *U*-test if they were not normally distributed. Outcome measurements included all patients who were randomized and received at least one dose of study medication (intention-to-treat analysis). The Wilcoxon signed-rank tests were used to compare changes between the baseline and post-treatment histological scores (primary endpoint), and the Wilcoxon rank-sum tests were used for treatment group comparisons. All patients with assessable baseline biopsy specimens were included in the analysis. Comparison of serum biochemical and metabolic parameters, and anthropometry (secondary endpoint) before and after treatment was performed using the Wilcoxon signed-rank tests. Spearman's rank correlation coefficient was used as a measure of association. Weighted kappa coefficients were calculated to examine the intraobserver agreement between two histological scorings. The safety analysis included all treated patients who had at least one safety evaluation after baseline.

The results reported in the literature related to nutritional approach and histological improvements are extremely variable, depending on diet and exercise compliance and on the amount of weight loss. We estimated an average of 39% of histological improvement. The study was designed to have a statistical power of 80% to detect an absolute difference of 39% in the rates of histological improvement (69% in the group with Viusid vs. 30% in the control group). Considering a type I error of 0.05 and a type II error of 0.20, 30 patients per arm were needed to reach statistical significance.

All confidence intervals, significance tests and resulting *P*-values were two-sided, with an alpha level of 0.05.

Statistical analyses were performed using SPSS Inc. for Windows, release 13, Chicago, IL.

RESULTS

A total of 60 patients were recruited at a tertiary centre (National Institute of Gastroenterology, Havana, Cuba) between February and March 2007. The flow of participants through the trial is presented in Figure 1. All randomized patients received at least one dose of the study medication; 11 subjects did not complete the study: six patients (three in each group) declined for personal reasons and five subjects (two assigned to Viusid and three assigned to the control group) were lost during the follow-up period. No patient received co-interventions during the trial that could have affected the outcomes.

Baseline characteristics were comparable across the two groups (Table 2). The two groups had evidence of insulin resistance at baseline as suggested by high fasting insulin and HOMA score means. No patient was diagnosed as diabetic during the trial.

Histological response (primary endpoint)

The histological analysis was based on 60 patients with assessable baseline liver biopsy specimens. A total of 42 patients (70%) had paired liver biopsies (20 and 22 patients in the control and Viusid groups respectively). The median of the liver specimens was 24 mm (range: 20–54). There were significant improvements from baseline to 6 months in steatosis, lobular inflammation, ballooning, fibrosis and NAS in each group of treatment (Table 3). When compared with baseline, the administration of Viusid markedly reduced the mean NAS score [4.18–0.54 points with a mean improvement of 3.64 (95% CI, 3.2–4) for Viusid vs. 4.45–2.2 points with a mean improvement of 2.25 (95% CI, 1.7–2.8) for diet/exercise ($P < 0.001$)] on post-treatment liver biopsy. Similarly, significant changes in the degree of steatosis ($P < 0.001$), ballooning ($P = 0.002$) and lobular inflammation ($P = 0.025$), but not of fibrosis ($P = 0.07$), occurred in the patients assigned to Viusid as compared with control group. Of the 60 patients enrolled, 27 had a NAS of ≥5 (13 and 14 patients in the control and Viusid groups respectively). Among the patients with a NAS of more than 5, the highest reduction in the mean change from the baseline NAS score was reported in patients treated with Viusid [5.30 ± 0.65 to 1.66 ± 0.57 points with a mean reduction of 3.64 ($P < 0.001$)] as compared with control group [5.61 ± 0.63 to 2.66 ± 0.66 with a mean reduction of 2.95 ($P < 0.001$)], with an absolute difference of 0.69 points and a 95% confidence interval for the difference of 0.32–1.05 ($P < 0.001$).

Biochemical response (secondary endpoint)

Statistically significant improvements in serum alanine aminotransferase (ALT) and aspartate aminotransferase

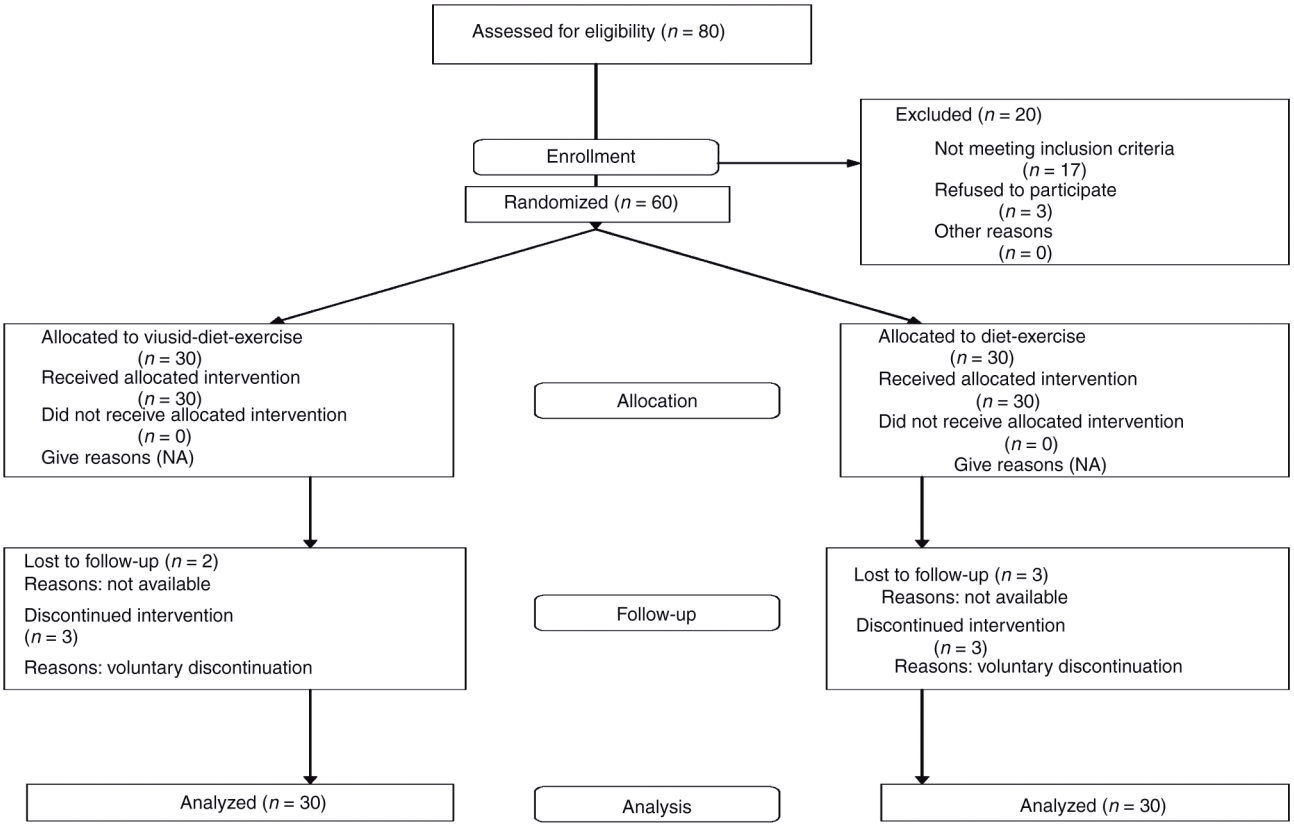

Figure 1. Flow of participants through the study.

(AST) levels were observed in both groups of treatment in comparison with pre-treatment values; however, no significant difference was observed between groups (Table 4). Uric acid levels slightly decreased by 12% (from 325 to 286 mmol/L, $P = 0.04$) in patients who received Viusid and diet/exercise, as compared with 6% (from 348 to 328 mmol/L, $P = 0.38$) in subjects assigned to diet/exercise (Table 4). Similarly, triglycerides levels significantly decreased (32% from baseline, $P = 0.01$) in the patients who received Viusid in comparison with unchanged levels in the patients treated with diet/exercise (Table 4). Normalization of triglycerides levels correlated with a reduction of the degree of hepatic steatosis ($r = 0.45$, $P = 0.03$) in the patients assigned to Viusid, but poorly correlated ($r = 0.33$, $P = 0.15$) in subjects treated with diet/exercise.

Metabolic response

In both groups, fasting plasma insulin and insulin sensitivity (homeostatic model assessment of insulin resistance, HOMA-IR), were markedly reduced in comparison with pre-treatment values (Table 4). Fasting plasma insulin was reduced by 28% ($P = 0.01$) and 41% ($P = 0.01$) in subjects treated with Viusid and diet/exercise and diet/exercise only respectively. Similarly, insulin resistance was significantly reduced by 42% ($P = 0.01$) and 35% ($P = 0.01$) in the patients who received Viusid and diet/exercise and diet/exercise only respectively (Table 4).

Anthropometric response

Dietary intervention and physical activity had an important effect on anthropometric parameters in the two groups of treatment, irrespective to Viusid administration. At 6 months of treatment, weight, waist circumference and body mass index were considerably reduced in comparison with baseline values (Table 4). Body weight was reduced by a mean of 10% ($P < 0.001$) and 12% ($P < 0.001$) of the initial weight in the subjects treated with dietary intervention/exercise alone or combined with Viusid respectively, as compared with pre-treatment values. Similar results

Table 2. Baseline characteristics

Variable	Viusid, diet and exercise (n = 30)	Diet and exercise (n = 30)	P-value
Age (years)	45 ± 10	49 ± 10	0.13
Male gender, n (%)	18 (60)	16 (53)	0.79
Weight (kg)	83.5 ± 15	82.4 ± 13	0.71
Body mass index (kg/m^2)	29.8 ± 5	31.5 ± 4	0.14
Waist (inches)	39.1 ± 4	40.5 ± 3.2	0.14
ALT (IU/l)	43 ± 28	46 ± 29	0.92
AST (IU/l)	37 ± 23	44 ± 23	0.30
Fasting plasma glucose (mmol/L)	4.7 ± 0.9	4.9 ± 0.9	0.81
Cholesterol (mmol/L)	5.2 ± 1	5.3 ± 1.7	0.66
Triglycerides (mmol/L)	1.9 ± 0.8	1.8 ± 0.9	0.54
HDL-C (mmol/L)	0.84 ± 0.2	0.70 ± 0.3	0.34
Uric acid (mmol/L)	325 ± 110	348 ± 85	0.36
Fasting plasma insulin (μU/mL)	18 ± 12	21.6 ± 15	0.18
Insulin sensitivity HOMA (%S)	3.6 ± 1.2	4.8 ± 1.3	0.25
NAS*, n (%)			
3–4	16 (53)	17 (57)	0.91
>5	14 (47)	13 (43)	0.90

Plus or minus values are means ± SD. For all laboratory measures, for continuous demographics and anthropometric: P-value Mann–Whitney U-test. Proportions: percentage, P-value chi-square. To convert mmol/L of glucose to mg/dL, multiply by 18. To convert mmol/L of triglycerides to mg/dL, multiply by 89. To convert mmol/L of cholesterol to mg/dL, multiply by 38.7. To convert mmol/L of HDL to mg/dL, multiply by 39.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol.

* NAS indicates NAFLD activity score. NAS between 3 and 4 correspond with a diagnosis of probable NASH, and NAS of ≥5 correspond with diagnosis of NASH.

were observed for waist circumference, which was reduced by a mean of 10% ($P < 0.001$) of the initial waist circumference in each treated group. There was a significant correlation between weight ($r = 0.565$, $P < 0.0001$) and waist circumference loss ($r = 0.518$, $P < 0.0001$) and histological improvements (Figure 2a,b).

Dietary intake and physical activity assessment

Table 5 shows the dietary recall data. All subjects who completed the study and were assigned to Viusid had a mean weight loss of 9.9 kg at 6 months, which corresponds to a reduction in daily energy intake of approximately 410 kcal. The participants who completed the study and were allocated to the control group had a mean weight loss of 8.8 kg at 6 months, which corresponds to a reduction in daily energy intake of approximately 370 kcal. However, the difference between groups was not statistically significant.

The group assigned to low-fat diet and Viusid reduced the mean protein intake by 1% of daily calories, increased the mean carbohydrate intake by 13% of daily calories and reduced the mean fat intake by 12% of daily calories relative to baseline. The group assigned to low-fat diet alone increased the mean protein intake by 1% of daily calories, increased the mean carbohydrate intake by 12% of daily calories and reduced the mean fat intake by 12% of daily calories relative to baseline; however, the difference between groups was not statistically significant.

Reported physical activity was similar among the two groups of treatment. The mean scores of the indices of physical activity were 3.3 and 3.1 in the patients assigned to the Viusid and the control groups respectively.

Safety analysis

Nausea and diarrhoea were reported in one subject who received Viusid. No laboratory adverse event was

Table 3. Patients' histological characteristics and outcome at 6 months

Variable	Viusid–diet–exercise (n = 30)				Diet–exercise (n = 30)				*P*-value for between-group comparison
	Before treatment	After treatment	Change	*P*-value	Before treatment	After treatment	Change	*P*-value	
Steatosis	2.27 ± 0.6	0.4 ± 0.2	−1.87 ± −0.9	<0.001	2.35 ± 0.6	1.4 ± 0.7	−0.95 ± −0.7	0.001	<0.001
Ballooning	0.91 ± 0.5	0.09 ± 0.01	−0.82 ± −0.7	<0.001	0.95 ± 0.5	0.40 ± 0.2	−0.55 ± −0.5	0.002	0.002
Lobular inflammation	1 ± 0.6	0.05 ± 0.01	−0.95 ± −0.6	<0.001	1.15 ± 0.5	0.40 ± 0.2	−0.75 ± −0.6	0.003	0.025
NAS*	4.18 ± 1.2	0.54 ± 0.1	−3.64 ± −1.2	<0.001	4.45 ± 1.4	2.2 ± 0.7	−2.25 ± −0.2	<0.001	<0.001
Fibrosis	0.95 ± 0.3	0.23 ± 0.01	−0.72 ± −0.4	0.012	1 ± 0.7	0.45 ± 0.2	−0.55 ± −0.3	0.012	0.07

Plus or minus values are means ± SD.
Continuous parameters were analysed using the Wilcoxon signed-ranks test and the Wilcoxon rank-sum tests were used for treatment group comparisons.
* NAS (NAFLD activity score) was defined as the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning (0–2); thus ranging from 0 to 8.

reported with the use of Viusid. There was no incidence of discontinuation or dose modification of Viusid secondary to adverse events.

DISCUSSION

In the current study, we demonstrated that a comprehensive lifestyle modification based on a hypocaloric diet and exercise was found to induce a significant weight loss, and it was associated with marked histological improvements from baseline to 6 months on steatosis, lobular inflammation, ballooning, fibrosis and NAS. However, the addition of Viusid to dietary restriction and exercise significantly reduced NAS by 3.64 points, as compared with 2.25 points reached by lifestyle modification alone. Additionally, the administration of Viusid induced a significant improvement in steatosis, and ballooning, but not in fibrosis, as compared with lifestyle modification alone. Our data strongly suggest that when compared with baseline, lifestyle modification leads to a considerable improvement in serum aminotransferase levels as well as insulin sensitivity. Interestingly, uric acid and triglycerides levels were decreased in subjects who received Viusid, as compared with those who received dietary therapy/exercise. There was a significant correlation between the normalization of triglyceride levels and the improvement in steatosis in the patients assigned to the Viusid group. This finding could be consistent with a remarkable reduction in serum triglyceride levels as well as in steatosis scores, which were seen only in the patients treated with Viusid; however, these are only preliminary observations that should be interpreted carefully and investigated in further studies. Finally, lifestyle modification through increased physical activity and hypocaloric diet led to a clear effect on anthropometric parameters, irrespective of Viusid administration. Viusid was well tolerated and only minor transient adverse events such as nausea and diarrhoea were reported.

The mechanisms responsible for explaining the effect of lifestyle modification through hypocaloric diet and exercise on liver enzymes and histology have not been well studied because of the limited number of trials evaluating the effectiveness of weight loss for NAFLD.[34] However, there are theoretical reasons why a reduced caloric diet, exercise and weight loss might improve NASH. Such lifestyle modification has been shown to improve insulin resistance, hyperlipidaemia, abdominal and body fat, and inflammatory markers

Table 4. Patients' baseline characteristics and outcome at 6 months

Variable	Viusid–diet–exercise (n = 30)			Diet–exercise (n = 30)			P-value for between-group comparison*
	Before treatment	After treatment	P-value	Before treatment	After treatment	P-value	P-value
Weight (kg)	83.5 ± 15	73.6 ± 14	<0.001	82.4 ± 13	73.7 ± 11	<0.001	0.37
Waist (inches)	39.1 ± 4	35 ± 4.2	<0.001	40.5 ± 3.2	36 ± 5	<0.001	0.59
Body mass index (kg/m^2)	29.8 ± 5	26.3 ± 4.8	<0.001	31.5 ± 4	28 ± 4.1	<0.001	0.90
ALT (IU/l)	43 ± 28	23 ± 17	<0.001	46 ± 29	27 ± 17	0.01	0.94
AST (IU/l)	37 ± 23	24 ± 14	0.02	44 ± 23	23 ± 12	<0.001	0.13
Uric acid (mmol/L)	325 ± 110	286 ± 96	0.04	348 ± 85	328 ± 106	0.38	<0.01
Fasting plasma glucose (mmol/L)	4.7 ± 0.9	4.6 ± 0.9	0.95	4.9 ± 0.9	5.2 ± 1.7	0.28	0.84
Cholesterol (mmol/L)	5.2 ± 1	4.5 ± 1.1	0.01	5.3 ± 1.7	4.5 ± 1	0.02	0.77
Triglycerides (mmol/L)	1.9 ± 0.8	1.3 ± 0.5	0.01	1.8 ± 0.9	1.9 ± 1	0.71	0.01
HDL-C (mmol/L)	0.84 ± 0.2	0.85 ± 0.2	0.86	0.70 ± 0.3	0.70 ± 0.3	0.86	0.81
Fasting plasma insulin (μU/mL)	18 ± 12	13 ± 7	0.01	21.6 ± 15	12.7 ± 7	0.01	0.47
Insulin sensitivity, HOMA-IR	3.6 ± 2	2.1 ± 1.2	0.01	4.8 ± 3	3.1 ± 2	0.01	0.39

Plus or minus values are means ± SD. Continuous parameters were analysed using the Wilcoxon signed-ranks test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance.
* P-values are for the comparison between groups (change from baseline).

(IL-6, TNF-alpha). All these factors have been strongly related to metabolic syndrome and NASH.[35–40] Although diet-induced weight loss is recommended as primary treatment for fatty liver, little or no evidence is available for the effects of macronutrient content (carbohydrates, fat and protein) on liver histology in patients with NASH. A paper published recently reviewed the role of different diets in metabolic syndrome and the implications for NAFLD.[41] The authors suggested that intake of diets that are lower in saturated fat (<10%), but normal in carbohydrates (50–60%) and proteins (≈15%), tend to be beneficial for ameliorating features of the metabolic syndrome, including effects on insulin sensitivity, but not on serum triglyceride levels. In the current study, we found that serum triglyceride levels were poorly modified by the intake of a low-fat diet normal in carbohydrates in the patients treated with diet and exercise; however, they were significantly reduced in the patients treated with Viusid. There is no evidence available to support the molecular basis of this mechanism; however, some component of Viusid, such as folic acid, could be linked to the improvement of methylation reactions that play pivotal roles in the function of hepatocytes, including the prevention of triglyceride accumulation in the liver, the production of the antioxidant glutathione and the preservation of cell membrane integrity.[42]

Encouraging effects of Viusid on liver histology have been reported in patients with chronic hepatitis C treated with unmodified interferon and ribavirin.[31] The mechanisms responsible for explaining the effect of Viusid on liver histology remain unknown. Nevertheless, there is a potential hepatoprotective mechanism in the chemical composition of Viusid that could be explained by the recognized anti-inflammatory and antioxidant properties of its different molecules, such as zinc, glycyrrhizin acid, ascorbic acid and folic acid.[17, 27–30, 43]

The main strength of this study was determined by the excellent patients' compliance with lifestyle modification resulting in higher rates of histological improvement and the presence of a concurrent control group allowing adequate comparison between control and experimental groups for outcome measures. A weakness of our study was that patients were treated for 24 weeks only and it remains unclear whether the biochemical and histological improvements can be

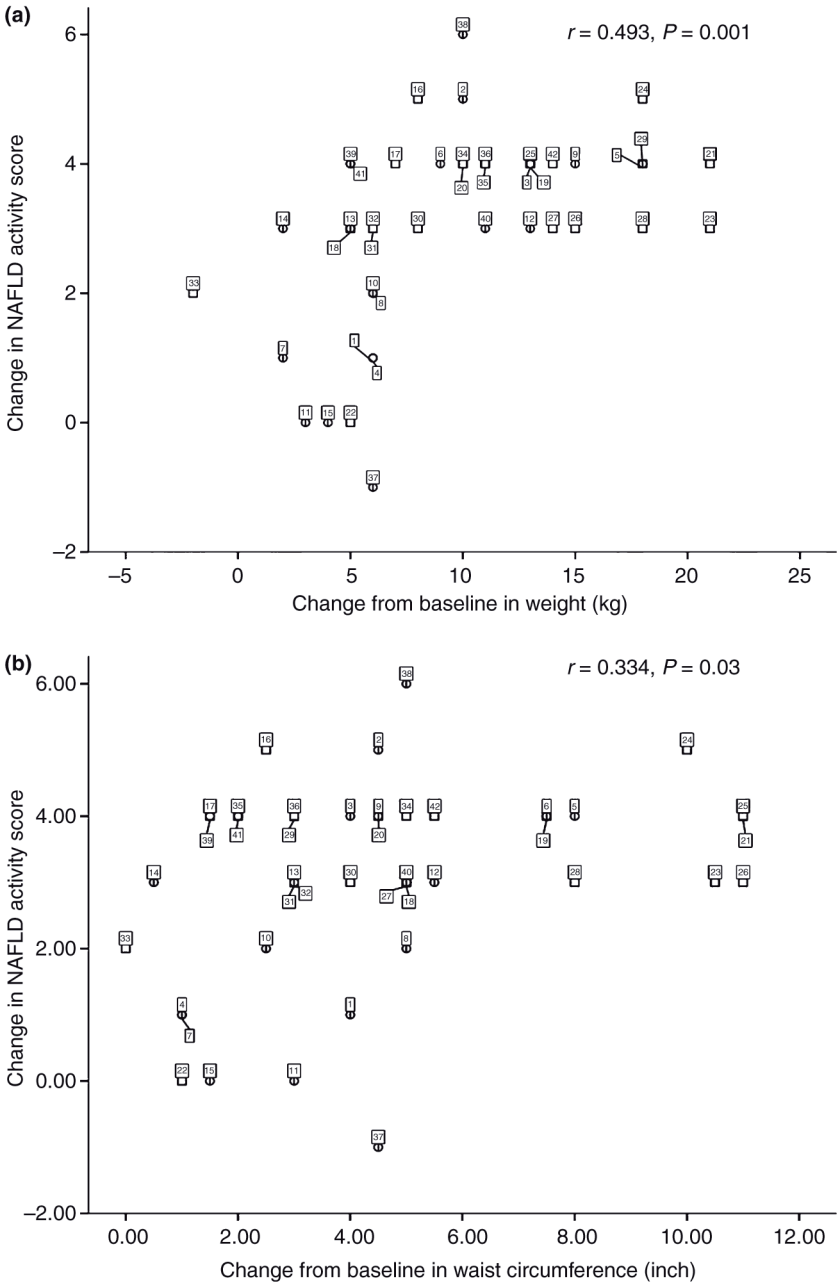

Figure 2. Correlation between change in body weight (a) and waist circumference (b) and change in nonalcoholic fatty liver disease (NAFLD) activity score. In (a) and (b), each circle (control group) or square (Viusid group) represents one or more patients. Circle and square overlapping is possible. Individual data are given with numbers in boxes.

sustained beyond 24 weeks. Continued dietary intervention for long periods could lead to loss of treatment adherence, which might eventually reverse any beneficial effect on hepatic histology. For these reasons, long-term studies that support the continued efficacy of dietary intervention are needed. Furthermore, the effect of Viusid in those patients who do not adhere to recommendations on lifestyle modification remains unknown. Therefore, further studies are needed to evaluate the effectiveness of Viusid in this group of patients. Another limitation of our study was that investigators and patients were not blinded to treatment assignment as no placebo was added to lifestyle modification in the control group.

In conclusion, the study supports the use of lifestyle modification through hypocaloric diet and exercise in patients with NAFLD. However, the benefit may be augmented by Viusid or perhaps other antioxidants in an attempt to prevent disease progression. Additional studies are required to confirm the long-term effect of Viusid in these patients as well as in patients with inadequate compliance with lifestyle modification.

Table 5. Changes in dietary composition between baseline and 6 months for the two groups* (nutrient intake per day)

Variable	Baseline (n = 49)	P-value†	6 months (n = 49)	Absolute change	P-value
Energy (kcal)					
Viusid	2017 ± 1077	0.92	1547 ± 690	−410 ± 501	0.73
Control	1990 ± 1096		1620 ± 737	−370 ± 407	
Protein (% kcal)					
Viusid	18 ± 8	0.60	17 ± 7	−1 ± 5	0.98
Control	17 ± 7		18 ± 8	1 ± 10	
Carbohydrates (% kcal)					
Viusid	50 ± 14	0.77	63 ± 21	13 ± 24	0.86
Control	49 ± 13		61 ± 25	12 ± 22	
Fat (% kcal)					
Viusid	32 ± 12	0.76	20 ± 27	−12 ± 24	0.99
Control	33 ± 14		21 ± 29	−12 ± 27	
Saturated Fat (% kcal)					
Viusid	12 ± 3	0.93	7 ± 14	−5 ± 21	0.86
Control	13 ± 5		7 ± 17	−6 ± 23	

* The values for dietary macronutrient data are given as the mean (±s.d.) percentage of total calories on the basis of dietary recall from the 49 patients who completed the study; missing values were imputed.
† P-values are for between-group comparisons and were calculated with use of the unpaired t-test.

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