

Randomized Placebo-Controlled Trial of Ursodeoxycholic Acid With Vitamin E in Nonalcoholic Steatohepatitis

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Background & Aims: Nonalcoholic steatohepatitis (NASH) is a frequent liver disease that can progress to cirrhosis and for which there is no recognized therapy. UDCA and vitamin E have been considered separately as therapeutic options and have not been shown to be effective. This study tested their combination. **Methods:** Patients with elevated aminotransferase levels and drinking less than 40 g alcohol/week with biopsy-proven NASH were randomly assigned to receive UDCA 12–15 mg · kg⁻¹ · day⁻¹ with vitamin E 400 IU twice a day (UDCA/Vit E), UDCA with placebo (UDCA/P), or placebo/placebo (P/P). After 2 years, they underwent a second liver biopsy. Biopsy specimens were collected, blinded, and scored by a single liver pathologist. **Results:** Forty eight patients were included, 15 in the UDCA/Vit E group, 18 in the UDCA/P group, and 15 in the P/P group; 8 patients dropped out, none because of side effects. Baseline parameters were not significantly different between the 3 groups. Body mass index remained unchanged during the study. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels diminished significantly in the UDCA/Vit E group. Neither the AST nor the ALT levels improved in the P/P group and only the ALT levels in the UDCA/P group. Histologically, the activity index was unchanged at the end of the study in the P/P and UDCA/P groups, but it was significantly better in the UDCA/Vit E group, mostly as a result of regression of steatosis. **Conclusions:** Two years of treatment with UDCA in combination with vitamin E improved laboratory values and hepatic steatosis of patients with NASH. Larger trials are warranted.

Nonalcoholic steatohepatitis (NASH) is one of the most prevalent liver diseases. It is estimated to affect nearly 1% of the population. With the surge in obesity and insulin resistance, its incidence is expected to rise. NASH has the potential to progress to end-stage liver disease; 70% of the cases of cryptogenic cirrhosis present characteristics of NASH.¹ The diagnosis of NASH is associated with higher mortality than the diagnosis of nonalcoholic fatty liver.² So far, no treatment has demonstrated efficacy in patients with this disease.³ Numerous studies have documented beneficial effects of ursodeoxycholic acid (UDCA) in other liver diseases, particularly in cholestatic disorders.⁴ A 1-year open-label trial suggested that UDCA could improve liver enzymes and the histologic grade of steatosis in patients with NASH.⁵ However, a subsequent large trial did not

find that the patients who received UDCA had better liver enzyme levels or histology at the end of the 2-year treatment period than the patients who were randomized to receive placebo.⁶

Oxidative stress is postulated to play a central role in the pathogenesis of NASH. Lipid peroxidation stimulates cytokine production, leading to inflammation and activation of hepatic stellate cells, which enhance fibrosis. In patients at risk of NASH, lower serum concentrations of the lipophilic antioxidant vitamin E have been found.⁷ Vitamin E (300 IU every day) given for 1 year in an open-label trial improved serum alanine aminotransferase (ALT) levels as well as the histologic lesions.⁸ In a placebo-controlled study it was used at a higher dosage (1000 IU every day) in combination with vitamin C for 6 months. The fibrosis score improved significantly at the end of the 6-month treatment period, but this improvement was similar to that in the placebo group.⁹

Therefore, we designed a study testing whether the combination of vitamin E and UDCA could be beneficial to patients with NASH. This is a multicenter prospective, randomized, double-blind, placebo-controlled trial assessing the effects of this drug combination on serum aminotransferase levels and histologic lesions.

Methods

Study Design

Patients 18–75 years of age with a persistent elevation of serum ALT levels of at least 1.5 times the upper limit of normal for at least 6 months and a weekly alcohol consumption of less than 40 g confirmed by the patient and eventually by someone close to the patient were eligible if they had a liver biopsy performed no more than 6 months before inclusion showing macrovesicular steatosis of more than 10% of the hepatocytes, hepatocellular injury (ballooning, dropout), and lobular inflammation. Patients were excluded for any of the following reasons: any laboratory (serologies for hepatitis B virus (HBV) and hepatitis C virus (HCV), abnormal transferrin

Abbreviations used in this paper: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ -glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; P, placebo; UDCA, ursodeoxycholic acid.

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saturation, low α_1 -antitrypsin, antinuclear antibodies superior to 1:80, antimitochondrial antibodies) or histologic findings suggestive of another liver disease, decompensated cirrhosis, serious disease limiting life expectancy, pregnancy and lactation, treatment with a drug known to induce NASH (amiodarone, calcium channel blocker, tamoxifen), and oral anticoagulation. The study was approved by the appropriate ethical committees and conducted in compliance with the Declaration of Helsinki.

Capsules containing UDCA 250 mg and placebo capsules were provided by Falk Pharma GmH (Freiburg, Germany). Tablets containing vitamin E (natural d-tocopherol) 400 IU and placebo tablets were provided by Antistress AG (Rapperswil, Switzerland). The medication was delivered by the pharmacy of the lead center (Inselspital, Bern) and shipped regularly to the patients, depending on their requirements. The pharmacy established before the start of the study a list randomly assigning each patient to 1 of the 3 arms of the study: UDCA 12–15 mg \cdot kg⁻¹ \cdot day⁻¹ with vitamin E 400 IU twice a day (UDCA/Vit E), UDCA 12–15 mg \cdot kg⁻¹ \cdot day⁻¹ with placebo (UDCA/P), and placebo/placebo (P/P). The patients as well as their physicians were blinded to the treatment until completion of the whole study. Patients were informed about the potential benefits of regularly exercising and, if they were overweight, about those of weight loss. However, the patients were not actively asked to change their lifestyle or to change their diet.

The objective of this randomized controlled trial was to investigate the effects of UDCA with vitamin E on liver histology and serum aminotransferase levels, because the hypothesis tested was that this combination might improve NASH. Therefore, liver histology and serum aminotransferase levels were defined as the primary end points of the study. It was assumed that no amelioration would be observed in the group of patients assigned to placebo. With 3 groups, choosing an effect size f of 0.4 for both end points, a power of 0.8, and setting α at .05, 21 patients should be enrolled in each group for a total of 63 in the study (Software Power and Precision; Biostat, Englewood, NJ). With a dropout rate of 20%, the study was designed to enroll 76 patients. It was decided that the study would enroll patients for a period no longer than 4 years.

At 1 year and at 2 years, patients had a follow-up visit comprising a physical examination, weight and blood pressure recording, and determinations of serum ALT, aspartate aminotransferase (AST), alkaline phosphatase, γ -glutamyltransferase (GGT), bilirubin, albumin, fasting blood glucose, triglycerides, and cholesterol were repeated. At the end of the 2 years of treatment, the patients had a second percutaneous liver biopsy.

At the end of the study, all the slides of the liver biopsies were collected and blinded to the patient, sequence of the biopsy, and assigned arm. The biopsies were then scored by a single pathologist (A.Z.) according to the scoring system proposed by Promrat et al.¹⁰ This system attributes a score from 0 (normal) to 4 to 6 histologic features of NASH (steatosis, hepatocellular injury, parenchymal inflammation, portal inflammation, fibrosis, and presence of Mallory bodies).

Statistical Analysis

The Kruskal-Wallis analysis of variance test was used to compare values between the 3 groups. The Wilcoxon matched pairs test was used for comparisons with treatment groups. For categorical parameters, the percentage distribution was ana-

lyzed with the χ^2 test. Differences at $P < .05$ were considered statistically significant.

Results

Between January 1, 1999 and December 31, 2002, 48 patients from 7 different Swiss centers were included in the study. The demographic characteristics (age, gender, body mass index [BMI], ongoing therapy for arterial hypertension, and diabetes mellitus) were not significantly different between the 3 groups (Table 1). The mean serum levels of ALT and AST were elevated in the 3 groups at baseline and not significantly different. None of the 6 histologic parameters was significantly different among the 3 groups (Table 2). It was not possible to retrieve the slides of the initial liver biopsy of 1 patient in each group, but none of these 3 patients completed the study. Eight patients dropped out, 3 in the UDCA/Vit E arm, 3 in the UDCA/P arm, and 2 in the P/P arm (Figure 1). Importantly, no patient dropped out because of side effects.

In none of the 3 groups the BMI changed significantly during the 2 years, and the mean BMI remained similar between the 3 groups until completion of the study. BMI at baseline was 31.1 ± 7.4 , 29.7 ± 4.3 , and 29.5 ± 4.7 kg/m² in the UDCA/Vit E, UDCA/P, and P/P groups, respectively. BMI at 1 year was 30.1 ± 7.6 , 29.7 ± 3.8 , and 29.8 ± 2.3 kg/m² in the UDCA/Vit E, UDCA/P, and P/P groups, respectively. BMI at 2 years was 31.1 ± 6.5 , 30.1 ± 4.6 , and 31.0 ± 3.7 kg/m² in the UDCA/Vit E, UDCA/P, and P/P groups, respectively.

Regarding the biochemical response, there was a significant decrease in the mean serum ALT levels in the UDCA/Vit E arm at 1 year and at 2 years. Moreover, the mean ALT serum levels reached normal range (Figure 2A). There was also a significant decrease in the ALT levels in the UDCA/P arm, but these values did not reach normal range. The number of patients with normal ALT at 1 year and at 2 years was 10 and 8 in the UDCA/Vit E group, 5 and 5 in the UDCA/P group, and 4 and 3 in the P/P group. The improvement of the serum ALT levels was larger in the UDCA/Vit E arm than in the UDCA/P arm, and it was significantly larger than the change measured in the P/P arm (Figure 2B). The ALT changes were not significantly different in the UDCA/P arm in comparison to the P/P arm. There was also a significant decrease in the mean serum levels of AST in the UDCA/Vit E group at 1 year and at 2 years in comparison to the baseline measurements (Figure 2C). Moreover, these values reached normal range. There was a slight decrease of the serum AST values in the UDCA/P arm that was not statistically significant. The number of patients with normal AST at 1 year and at 2 years was 10 and 11 in the UDCA/Vit E group, 7 and 8 in the UDCA/P group, and 5 and 4 in the P/P group. The changes of serum AST levels were statistically significant in the UDCA/Vit E arm in comparison to the P/P arm (Figure 2D). This was not the case in the UDCA/P arm.

Thirty-two patients had a second liver biopsy. Eight patients did not have a second liver biopsy, 2 in the UDCA/Vit E arm, 4 in the UDCA/P arm, and 2 in the P/P arm. Each of the 6 features of NASH had a better score at the end of treatment in the combination therapy group, yet for only one, steatosis, the improvement was statistically significant. The other 5 features did not improve significantly (Figure 3A). None of the 6 itemized scores were significantly better in

Table 1. Baseline Values

Variable (N)	UDCA + Vit E (15)	UDCA + P (18)	P + P (15)	P value
Age (y)				.66
Mean \pm SD	46 \pm 14	47 \pm 12	44 \pm 14	
Median (range)	47 (21–74)	51 (22–63)	49 (23–71)	
Gender (%)				.53
Male	10 (67%)	13 (72%)	8 (53%)	
Female	5 (33%)	5 (28%)	7 (47%)	
Diabetes mellitus				.55
N (%)	4 (27%)	4 (22%)	2 (13%)	
Ongoing therapy for high blood pressure				.52
N (%)	5 (33%)	5 (28%)	7 (47%)	
Weight (kg)				.64
Mean \pm SD	90 \pm 7	89 \pm 4	83 \pm 4	
Median (range)	78 (65–145)	84 (61–122)	83 (59–115)	
BMI (kg/m ²)				.93
Mean \pm SD	31 \pm 7	30 \pm 4	30 \pm 5	
Median (range)	31 (22–51)	29 (23–38)	31 (22–38)	
AST (10–41 IU/L)				.68
Mean \pm SD	66 \pm 36	63 \pm 38	50 \pm 14	
Median (range)	51 (31–125)	53 (2–179)	46 (34–90)	
ALT (10–41 IU/L)				.19
Mean \pm SD	88 \pm 42	112 \pm 64	76 \pm 43	
Median (range)	84 (21–74)	91 (62–276)	71 (60–187)	
Alkaline phosphatase (36–108 IU/L)				.40
Mean \pm SD	84 \pm 28	106 \pm 51	94 \pm 54	
Median (range)	77 (47–137)	99 (62–247)	85 (47–255)	
γ -glutamyltransferase (11–64 IU/L)				.22
Mean \pm SD	95 \pm 92	150 \pm 143	114 \pm 95	
Median (range)	49 (18–316)	90 (28–606)	79 (29–329)	
Bilirubin (3–26 μ mol/L)				.78
Mean \pm SD	13 \pm 6	12 \pm 6	11 \pm 4	
Median (range)	12 (6–24)	10 (4–23)	10 (6–21)	

SD, standard deviation.

Table 2. Baseline Histology

Variable (N)	UDCA + Vit E (14)	UDCA + P (17)	P + P (14)	P value
Steatosis (0–4)				.83
Mean \pm SD	2.6 \pm 1.1	2.9 \pm 0.8	3.0 \pm 0.7	
Median (range)	3 (1–4)	3 (2–4)	3 (2–4)	
Hepatocellular injury (0–4)				.88
Mean \pm SD	1.8 \pm 1.1	1.6 \pm 0.9	1.8 \pm 1.0	
Median (range)	1 (1–4)	1 (1–4)	1 (1–4)	
Parenchymal inflammation (0–4)				.67
Mean \pm SD	1.4 \pm 1.0	1.5 \pm 0.9	1.2 \pm 0.6	
Median (range)	1 (0–4)	1 (0–3)	1 (0–2)	
Activity index ^a				.75
Mean \pm SD	5.8 \pm 2.0	6.1 \pm 1.6	6.0 \pm 1.2	
Median (range)	6 (2–9)	6 (4–8)	6 (3–8)	
Portal inflammation (0–4)				.31
Mean \pm SD	2.0 \pm 1.5	1.8 \pm 1.3	0.9 \pm 1.2	
Median (range)	1.5 (0–4)	1 (0–4)	0 (0–3)	
Mallory bodies (0–4)				.40
Mean \pm SD	0.3 \pm 0.5	0.4 \pm 0.7	0.9 \pm 1.0	
Median (range)	0 (0–1)	0 (0–2)	0.5 (0–3)	
Fibrosis (0–4)				.29
Mean \pm SD	1.8 \pm 1.5	1.4 \pm 1.3	1.0 \pm 1.0	
Median (range)	2 (0–4)	1 (0–3)	1 (0–3)	

SD, standard deviation.

^aActivity index is the sum of the stages for steatosis, hepatocellular injury, and parenchymal inflammation.

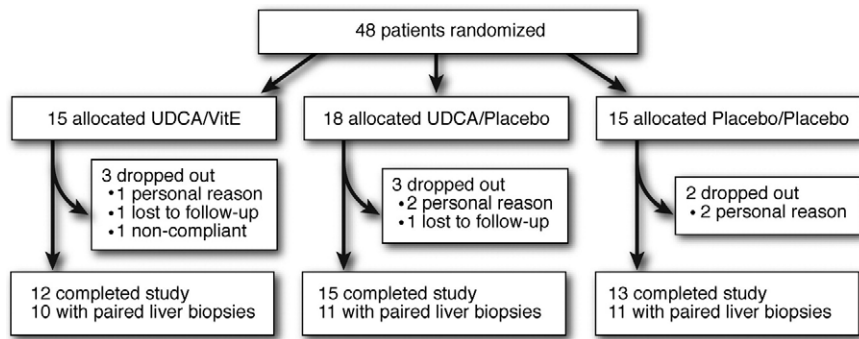


Figure 1. Flow of participants through the study. No patients dropped out as a result of side effects.

the UDCA/P arm (Figure 3B), and except for steatosis, they were all slightly worse at the end of the study in the P/P arm (Figure 3C). The activity index, which takes into account steatosis, hepatocellular injury, and parenchymal inflammation, was significantly better in the UDCA/Vit E arm at the end of the study than its value at the beginning, modestly lower in the UDCA/P arm, and unchanged in the P/P arm (Figure 3D). Improvement of the activity index in the UDCA/

Vit E arm was essentially due to regression of steatosis (Figure 4).

Discussion

This double-blind, randomized, placebo-controlled study found that UDCA 12–15 mg · kg⁻¹ · day⁻¹ in combination with vitamin E 400 IU twice daily improves serum amino-

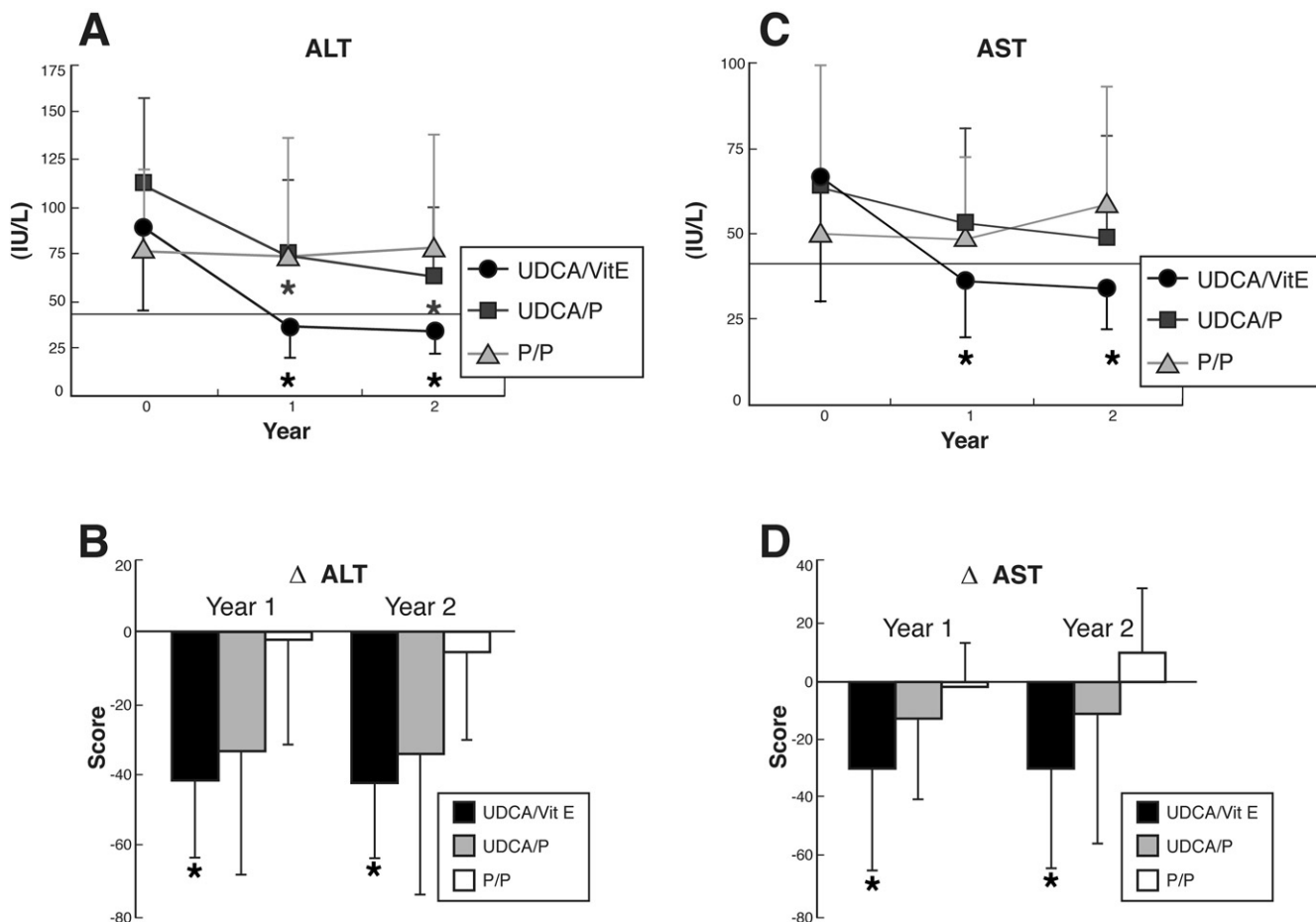


Figure 2. Effects on serum aminotransferase levels. (A) Evolution of ALT during the study period. Asterisks indicate a difference in comparison to value at inclusion ($P < .05$). The horizontal line represents the upper normal limit. (B) Changes from baseline in ALT levels at 1 year and at end of the study. Asterisks indicate a difference in comparison to the changes measured in the P/P group ($P < .05$). (C) Evolution of AST during the study period. Asterisks indicate a difference in comparison to value at inclusion ($P < .05$). The horizontal line represents the upper normal limit. (D) Changes from baseline in AST levels at 1 year and at end of the study. Asterisks indicate difference in comparison to the changes measured in the P/P group ($P < .05$). Values represent means \pm standard deviations.

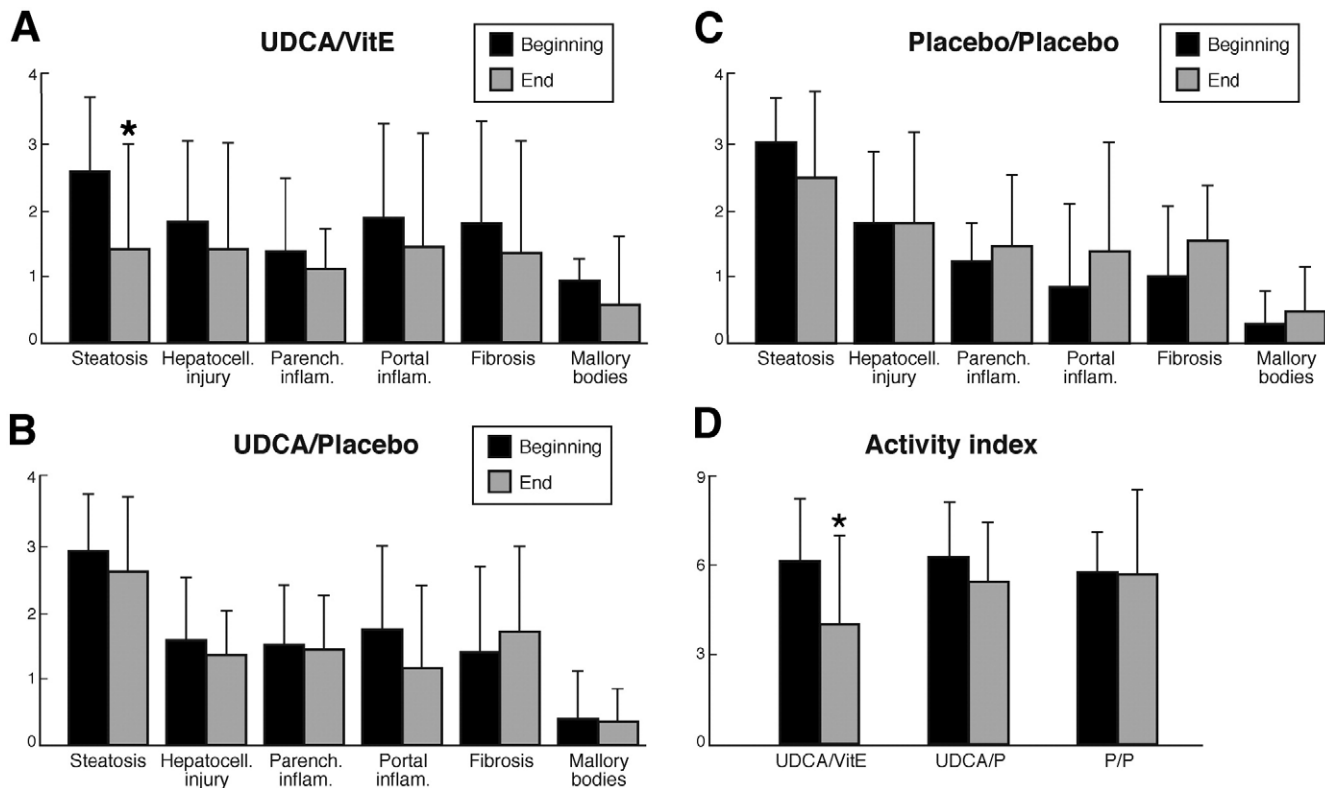


Figure 3. Effects on liver histology. (A) Scores for 6 histologic features of nonalcoholic steatosis (steatosis, hepatocellular injury, parenchymal inflammation, portal inflammation, fibrosis, and presence of Mallory bodies) at entry in the study and at the end of the 2 years of treatment with UDCA in combination with vitamin E. Asterisk indicates a significant difference in comparison to value at inclusion. (B) Scores for the biopsies of the patients who received UDCA and placebo. (C) Scores for the biopsies of the patients who received placebo only. (D) Changes in the activity index, which represents steatosis, hepatocellular injury, and parenchymal inflammation, at entry and at completion of the study for the 3 groups. Asterisk indicates a significant change in the combination group. Values are means \pm standard deviations.

transferase levels and liver histology of patients with NASH. This combination had beneficial effects, which could not be reached with UDCA monotherapy. With this therapeutic association the average of the serum AST and ALT levels reached normal range, and their decrease was significant. This was not the case in the P/P group or in the UDCA monotherapy group. The histology improved significantly only in patients assigned to the combined therapy. Patients who took UDCA and vitamin E had less steatosis and a lower activity index at the end of the 2 years of treatment. No such changes occurred in patients who took UDCA alone or placebo.

Both compounds have been previously investigated in clinical trials with results that could not prove their efficacy. After an open-label pilot study suggested that UDCA improves liver enzymes and steatosis in patients with NASH,⁵ a larger randomized study found improvements that were not significantly different from those recorded in the placebo-controlled arm, leading the investigators to conclude that UDCA is actually not better than placebo.⁶ In contrast, in the present study, the patients in the placebo group experienced no amelioration of their serum aminotransferase levels, and their histologic lesions tended to worsen. The dose of UDCA was the same and the duration of the treatment identical in both trials. The reason for this difference is unclear. It might reflect a different degree of lifestyle modifications in the patients enrolled in the placebo group; they were not actively engaged to exercise and to watch

their body weight in the present trial in contrast to the other protocol. Performing more physical activities and weight reduction, even modest, can improve nonalcoholic fatty liver disease.^{11,12}

Regarding vitamin E, preliminary clinical studies reported that it can improve the aminotransferase levels¹³ and also decrease histologic lesions.^{8,14} In a 6-month study comparing vitamin E (400 IU/day) monotherapy to its combination with pioglitazone (30 mg/d), Sanyal et al¹⁴ reported that vitamin E in monotherapy improved aminotransferase levels and steatosis. Hasegawa et al⁸ found that a year of vitamin E (300 mg/day) not only improved these end points but also the inflammation and the fibrosis. However, other studies found limited effects of vitamin E; adding it to lifestyle modifications (low fat diet, exercise) was not beneficial,¹⁵ and it was less potent than metformin.¹³ Our trial is the first to test UDCA in combination with vitamin E. The study testing pioglitazone in combination with vitamin E reported an improvement of ballooning, pericellular fibrosis, and presence of Mallory bodies that were not observed in the vitamin E monotherapy group.¹⁴ However, 1 of the 10 patients who received pioglitazone experienced hepatotoxicity. Vitamin E and UDCA, the compounds tested in the present trial, are safe, and their combination was well-tolerated; no patients dropped out as a result of side effects.

The possible mechanisms of action of each of these drugs can provide an explanation for their efficacy in combination.

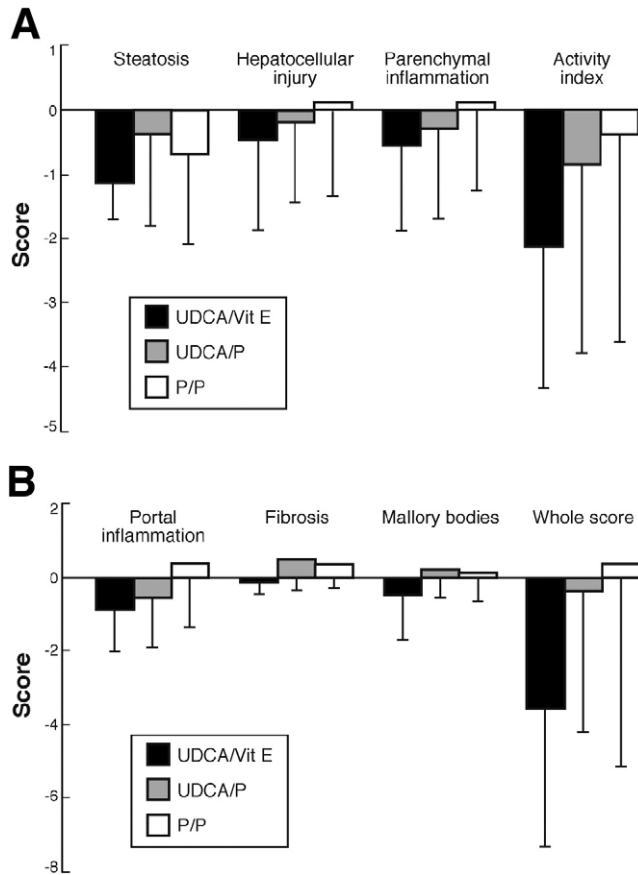


Figure 4. Changes from baseline in liver histology at end of the study. (A) Changes in the score for steatosis, hepatocellular injury, parenchymal inflammation, and activity index. Values represent means \pm standard deviations. Changes for these 4 criteria showed larger improvements in the combination group than in the other 2 groups. However, comparisons of the 3 groups by Kruskal-Wallis analysis of variance found no statistically significant differences. (B) Changes in the score for portal inflammation, fibrosis, Mallory bodies, and in the whole score. Values represent means \pm standard deviations. Changes for these 4 criteria showed larger improvements in the combination group and small worsenings in the P/P group. However, comparisons of the 3 groups by Kruskal-Wallis analysis of variance found no statistically significant differences. The differences for the whole score were not significant ($P = .052$).

Oxidative stress plays an important role in the pathogenesis of NASH.^{16,17} The liver of patients with NASH provides many ingredients to trigger and promote oxidative stress.¹⁸ By definition, patients with NASH present hepatocellular steatosis. They also frequently have hyperglycemia, if not diabetes mellitus,¹⁹ and sometimes excessive intrahepatic iron.^{20,21} These factors promote and aggravate lipid peroxidation. The lipid-soluble vitamin E (α -tocopherol) has powerful free radical-scavenging properties in biologic membrane, and it interrupts the propagation of the peroxidation of polyunsaturated fatty acid. On the other side, UDCA has numerous protective properties including replacement of toxic bile acids, stabilization of cell membranes, and immunomodulatory effects, which are particularly relevant in chronic cholestatic liver diseases.⁴ Mitochondrial dysfunction and apoptosis are prominent features of NASH,^{22,23} and UDCA can inhibit apoptosis through various

mechanisms.²⁴ In particular, UDCA can modulate the mitochondrial membrane potential and the mitochondrial production of reactive oxygen species.^{25,26} It remains to be clarified whether such mechanisms are underlying the positive effects of these 2 drugs given in combination.

Despite the fact that this is one of the few randomized, placebo-controlled studies in the field of NASH, this trial has several shortcomings. The number of patients enrolled is limited; moreover, 8 patients dropped out, and only 32 paired liver biopsies were available for analysis. This number is too small to draw firm conclusions. Sampling variability as a result of uneven distribution of the histologic lesions of NASH is a limitation.²⁷ Nevertheless, a liver biopsy is required to establish the diagnosis of NASH, and hepatic histology remains an important end point because no alternative methods can better measure the effect of a potential therapy. We chose the histologic scoring system proposed by Promrat et al.¹⁰ It has the merit to quantify the relevant findings without excessive complexity. The scoring system proposed by Brunt et al²⁸ in 1999 assesses in a detailed manner the different histologic features of NASH. More recently, the Nonalcoholic Steatohepatitis Research Network validated a histologic scoring system designed to encompass the full spectrum of the lesions of nonalcoholic fatty liver diseases inclusive of those found in pediatric cases.²⁹ In this system, steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis are evaluated semiquantitatively, and 9 additional features are recorded as present or absent. The score we are using has been developed to score NASH in adults and attributes a score to 6 histologic features (steatosis, hepatocellular injury, parenchymal inflammation, portal inflammation, fibrosis, and presence of Mallory bodies). The present study lacks the power to exclude an effect of UDCA ($12\text{--}15\text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) in monotherapy, but these negative results are in line with those of the randomized trial of Lindor et al,⁶ which included 166 patients and tested UDCA at the same dosage and for the same period of time (2 years). These results do not exclude the possibility that UDCA at a higher dosage might be beneficial in this indication. Finally, our study did not include an arm of vitamin E with placebo in place of UDCA and therefore does not provide information on the value of vitamin E in monotherapy in the treatment of NASH.

In conclusion, this prospective, double-blind, placebo-controlled study suggests that the combination of UDCA and vitamin E improves serum levels of ALT and AST as well as the hepatic steatosis of NASH, and that it is superior to UDCA monotherapy. This drug combination, which was well-tolerated, needs to be tested further; in particular, larger clinical trials are warranted.

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