CORRESPONDENCE

Vitamin E Therapy in Patients With NASH

To the Editor:

We read with great interest the recent article by Kugelmas et al.,[1] reporting plasma cytokine levels in patients with NASH and the effect of diet and vitamin E on this disease. They found that plasma tumor necrosis factor (TNF), interleukin 8, and interleukin 6 concentrations were significantly elevated compared with control values, and only plasma interleukin 6 levels significantly decreased with diet therapy. This article further reported that vitamin E therapy did not independently influence the biochemical data and plasma cytokine levels in patients with NASH. We previously investigated the plasma transforming growth factor-β1 (TGF-β1) level and the efficacy of vitamin E in patients with NASH.[2] We enrolled 12 patients with NASH and 10 with fatty liver. All patients were given dietary instruction for 6 months, and thereafter vitamin E (α-tocopherol acetate, Juvera™, Eisai Pharmaceutical Co., Tokyo, Japan, 300 mg/day; 100 mg is equivalent to 100 IU) was given for 1 year. In consequence, the plasma TGF-β1 level in patients with NASH (37 $\pm$ 5 ng/ml) was significantly higher than that in patients with fatty liver (10 $\pm$ 4 ng/ml) and healthy subjects (9 $\pm$ 5 ng/ml). Moreover, this elevated plasma TGF-β1 level decreased after a 1-year vitamin E treatment, together with the improvement of biochemical markers and hepatic pathological findings (Table 1). In 5 out of 9 NSAH patients in whom liver biopsy was performed after vitamin E treatment, inflammation and fibrosis were improved. On the other hand, diet therapy improved biochemical data only in patients with fatty liver, but not in those with NASH. We conducted our previous study to assess the efficacy of a long-term vitamin E treatment (a 1-year vitamin E administration after a 6-month diet). However, in the studies performed by Kugelmas et al.,[1] vitamin E was given for only 12 weeks and the patients were given diet therapy at the same time. In fact, Kugelmas et al.[1] mentioned that longer study duration might have shown the efficacy of vitamin E on NASH. However, we had already reported the efficacy of a 12-month treatment of vitamin E on NASH. Moreover, long-term treatment with vitamin E for patients with NASH was tried for obese children and Japanese adults, and the beneficial effect of this vitamin has already been confirmed.[3,4] In addition, the NIH plans to conduct randomized controlled trials of insulin-sensitizing agents and vitamin E therapy for NASH.[5] Although the role of tumor necrosis factor is currently debated in the literature,[6,7] elevated blood level of TGF-β has recently been ascertained.[8] The role of TGF-β1 in hepatic inflammation and fibrosis has been well documented in both animal and clinical studies.[9,10] Therefore, for better understanding of the cytokine network in NASH, we believe that the investigation of plasma TGF-β1 is essential, as well as long-term observation of vitamin E treatment.

Table 1. Effect of Vitamin E on Serum Alanine Transaminase (ALT) and Plasma TGF-β1 Levels in Patients With NASH

	Before Treatment	After Diet	After Vitamin E
ALT (IU/L)	171 $\pm$ 4	161 $\pm$ 14	37 $\pm$ 4
TGF-β1 (ng/ml)	37 $\pm$ 5	36 $\pm$ 6	9 $\pm$ 6

Modified from Hasegawa et al.[2]

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More on Vitamin E Therapy

To the Editor:

Antioxidants are considered a promising tool for obese individuals with nonalcoholic steatohepatitis (NASH) who are unable to lose weight. However, a beneficial effect of these drugs needs still to be proven, since it has hitherto been inferred only from few pilot studies.[1] Therefore we read with interest the paper of Kugelmas et al.,[2] who evaluated in patients with NASH the effects of diet and exercise ± antioxidant vitamin E (800 IU daily) on cytokines profile and liver function tests. Regarding the latter aspect, they concluded that this dose of vitamin E provided no apparent added benefit upon serum transaminase levels. In our opinion, this conclusion did not take into sufficient consideration the eclipsing of vitamin E effects by patients' unpredictable extent of lifestyle modifications and/or adherence to drug prescription itself.

On this subject, we recently compared in a single-blind, randomized study the effect of vitamin E (α-acetate tocopherol, 400 IU/day) versus placebo on transaminase values and ultrasonographic bright liver in 28 children with obesity-related nonalcoholic fatty liver disease (NAFLD) treated with low-calorie diet and exercise.[3] Baseline liver biopsy was available only in 10 patients. In line with Kugelmas et al., variations in transaminase levels and percentage of patients who normalized transaminase values were comparable in the two groups. However, looking at the subgroup of patients adherent to vitamin E intake (as shown by a twofold increase of vitamin serum levels) but unable to lose weight, we found that all of them normalized transaminase levels. Changes of their transaminase values were comparable to those obtained in children of the placebo group who lost weight (differences between means of baseline alanine aminotransferase values and alanine aminotransferase values after 2 months of treatment were −26 U/L and −31 U/L, respectively; $P = 0.54$). However, vitamin E was not effective upon ultrasonographic liver brightness. Our results are in keeping with data from two uncontrolled pilot studies in adolescents[4] and adults[5] with NASH, where comparable doses of vitamin E had been used. Interestingly, in our study also, compliance to vitamin E itself was variable: Only 10/14 patients reached serum values compatible with a correct taking of the drug.

Compliance to vitamin E therapy, based on blood levels, unfortunately could not be evaluated in the Kugelmas et al. paper. However, we believe that reevaluation of their results after stratifying the two patient groups on the basis of their veritable compliance to a diet leading to substantial weight reduction might better define the distinct effects of lifestyle modification and vitamin E supplementation on liver function tests.

Regarding the Kugelmas et al. conclusion about the need of pilot trials to evaluate effectiveness of higher doses of vitamin E, one should consider that the amount of the drug chosen in their study is comparable to that successfully prescribed by others in NASH/NAFLD.[3–5] It corresponds to several times (on average, 50 times) the vitamin E daily requirements, and it has been considered adequate to expect an antioxidant effect in controlled studies regarding other oxidative stress-related diseases.[6–7]

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Reply:

We appreciate the comments of Yoneda and coworkers and Vajro and coworkers. Over 15 years ago, our laboratory first described elevated serum IL-1 levels in alcoholic hepatitis patients, and subsequently we reported increased monocyte production of tumor necrosis factor alpha in these patients.[1,2] It is now well documented that dysregulated cytokine metabolism plays a pivotal role in the development of liver injury and many of the metabolic/systemic complications of alcoholic hepatitis.[3–5] The major goal of our study in nonalcoholic steatohepatitis (NASH) patients was to determine whether they had similarly elevated serum concentrations of proinflammatory/acute-phase cytokines and increased monocyte production of these cytokines.[6] Next, we wanted to determine whether these patients had increased serum hyaluronic acid levels, a marker of sinusoidal endothelial cell dysfunction and fibrosis. Lastly, we wanted to determine the possible beneficial effects of short-term weight loss and vitamin E supplementation.

Hasegawa, Yoneda, and coworkers report their findings on vitamin E supplementation on transforming growth factor beta (TGF-β) levels in NASH patients.[7] We certainly agree that TGF-β is an important profibrotic cytokine that requires further evaluation in NASH patients. It is also clear in *in vitro* and in animal studies that vitamin E is highly effective at blocking activation of stellate cells and fibrosis and blocking inflammation and liver injury.[8,9] However, the efficacy of vitamin E in humans in a variety of inflammatory states has been much more controversial. A classic example is the multiple cardiovascular studies in which vitamin E alone or in combination with other antioxidants has been extensively evaluated. There are a host of atherosclerosis studies with "alphabet soup" names such as HOPE, CHAOS, etc., that have yielded conflicting data, with most of the larger studies finding limited or no beneficial effects of vitamin supplementation.[10] A major issue is whether the vitamin E dose administered has been appropriate. If one is trying to decrease oxidative stress then vitamin E

should be given in a dose sufficient to decrease oxidative stress, as assessed by markers such as urinary isoprostanes, or to reduce activation of redox sensitive transcription factors such as nuclear factor-κB (NFκB).[10] Thus, our strong opinion is that appropriate antioxidant-dose-finding studies need to be performed before large clinical trials are initiated. Reviewing the study by Hasegawa, Yoneda, and coworkers, one could argue that NASH patients improved their TGF-β levels merely as a beneficial effect of diet and weight loss, and the effect was not seen immediately, but only after a one-year period of time that included vitamin E therapy. Their study was neither randomized nor placebo-controlled, and it would be very premature to say that 300 IU of vitamin E is appropriate therapy for NASH or for downregulating TGF-β levels. There also could be racial/ethnic differences in how Japanese respond to vitamin E therapy versus Americans.

We also agree with Varjro and coworkers that compliance is always an issue in clinical studies. We did not measure vitamin E levels in our patients. However, pill counts were performed, and all patients had over 80% compliance. Moreover, patients were contacted by telephone on a weekly basis and seen in clinic every three to six weeks. The study was performed as part of a master's degree thesis (Beverly Vivian), and was done on a National Institutes of Health–funded General Clinical Research Center. Thus, adherence to both diet and exercise, and medication compliance would be much more likely in this study than in standard medical practice, in our opinion. We agree with both investigators that antioxidants have great theoretic potential in NASH. However, we feel that it is important to scientifically determine the appropriate dose of vitamin E and whether vitamin E should potentially be given with other antioxidants such as glutathione prodrugs to achieve maximal benefit.

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A Role for the 2-Oxoglutarate Carrier in Glutathione Transport Into Hepatocyte Mitochondria?

To the Editor:

A recent report in HEPATOLOGY proposed 2-oxoglutarate as a glutathione (GSH) carrier.[1] The authors' evidence is based on the competition and kinetics of 2-oxoglutarate transport in isolated rat liver mitochondria. However, they show V_{max} (1.9 ± 0.1 nmol/mg prot/25 seconds) and Km (3.1 ± 0.3 mM) values for 2-oxoglutarate transport into mitochondria that are not in agreement with those previously reported (*e.g.*, Bisaccia et al.[2] and references therein). Loading the mitochondria with internal substrate is necessary for measuring transport in metabolite exchange systems such as the 2-oxoglutarate/malate antiport (*e.g.*, Dawson et al.[3]). In fact, isolating hepatocyte rat mitochondria as the authors indicated and incubating them in their standard incubation medium for 20 minutes at 0°C in the presence of 10 mM malate, we found that (1) mitochondrial malate content (measured fluorimetrically[4]) increased from 356 ± 45 μM (nonloaded controls, n = 6) up to 1065 ± 87 μM (n = 7; $P < .01$); (2) that V_{max} and Km values for 2-oxoglutarate transport into mitochondria were 4.1 ± 0.3 nmol/mg prot/25 seconds and 0.9 ± 0.2 mM, respectively (n = 6); (3) that rates of 2-oxoglutarate transport into mitochondria (0.7 ± 0.1 nmol/mg prot/25 seconds) were similar in the presence or in the absence of externally added GSH (0.1–5 mM; n = 4 in each case; external 2-oxoglutarate concentration was 0.25 mM, its approximate physiologic cytosolic content in hepatocytes from fed rats[5]); and that (4) phenylsuccinate (1 mM), an inhibitor of the 2-oxoglutarate carrier, decreased 2-oxoglutarate transport into mitochondria by 61% ± 8 % (n = 5), but did not affect significantly control rates of GSH uptake (1.4 ± 0.3 nmol/mg prot/25 seconds in the presence of 10 mM external GSH [n = 6], a rate similar to that found by Mastersson et al.[6] in liver mitochondria; 2-oxoglutarate and GSH transport rates were measured as indicated in ref. 1, but using malate-loaded mitochondria). These data, in agreement with our observations in tumor cells,[7] do not support the evidence of Coll et al.[1] for 2-oxoglutarate as a GSH carrier. The original work by Mastersson et al.[6] showed that at external GSH levels <1 mM, GSH is transported into the mitochondrial matrix by a high-affinity component (Km, approximately 60 μM; V_{max}, approximately 0.5 nmol/mg prot/minute), which is saturated at levels of 1 to 2 mM and stimulated by ATP. Another component has lower affinity (Km, approximately 5.4 mM; V_{max}, approximately 5.9 nmol/mg prot/minute) and is stimulated by ATP and ADP. These kinetic parameters do not correspond with those calculated for the 2-oxoglutarate transport (see above).

GSH, which is required for normal mitochondrial function, is not synthesized within mitochondria, but is taken up from the cytosol.[6]

Recent evidence shows a critical role of mitochondrial GSH in regulating mitochondrion-based cell death mechanisms (*e.g.*, Ortega et al.[7] and references therein). Thus, despite difficulties in the preparation of pure individual carriers and instability in their purified forms, molecular identification of the mitochondrial GSH carrier is indeed an important question that needs further investigation. Glutamate inhibits GSH uptake by mitochondria; however, both the glutamate/hydroxyl transporter and the glutamate/aspartate antiporter do not exhibit multiple components and have Km values of 4 to 6 mM.[6] However, GSH transport through voltage-dependent anion channels seems an interesting possibility.[6,8]

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Reply:

In the preceding letter, Estrela and coworkers challenge the conclusion from our recent study[1] indicating that the 2-oxoglutarate carrier functions as a glutathione (GSH) transporter. Our work showed the functional expression of the 2-oxoglutarate carrier in mitochondria from *Xenopus* oocytes, its sensitivity to altered mitochondrial membrane fluidity, and competition with GSH for transport.[1] Their argument is based on the discrepancy between the kinetic parameters we reported with those previously published[2] and their inability to reproduce our findings. This comparison, however, is misleading, as the work referred to by Estrela et al. describes the kinetics of the 2-oxoglutarate carrier from bovine heart mitochondria reconstituted in liposomes[2] that differed from those of isolated rat-liver mitochondria.[3] Since our findings in *Xenopus* oocytes indicated the ability of the 2-oxoglutarate carrier to transport GSH into mitochondria, we examined the uptake of 2-oxoglutarate in mitochondria from rat liver in exchange with GSH instead of malate; rat-liver mitochondria display a concentration of GSH about 9–12 mmol/L[4]. We would like to clarify that all transport measurements of 2-oxoglutarate as well as GSH in mitochondria from rat liver or *Xenopus* oocytes were done at 25°C, as detailed previously.[5] In attempting to reproduce our findings, it was unfortunate that Estrela et al. examined the transport of 2-oxoglutarate at 0°C, what most likely reflects the binding of 2-oxoglutarate to mitochondria rather than its transport.

In our study[1] we characterized the kinetics of 2-oxoglutarate in relationship with those of GSH in isolated rat-liver mitochondria that were previously reported by Martensson et al.[6] and Colell et al.[7] While these published kinetic parameters of GSH were in mutual agreement and showed two components, the kinetics of 2-oxoglutarate in rat-liver mitochondria exhibited a single Michaelis-Menten component[1] with K_M and V_{max} similar to the high-capacity, low-affinity transport site for GSH.[6,7]

Thus, while the functional expression in oocytes unequivocally demonstrates the role of 2-oxoglutarate carrier as a GSH transporting polypeptide, its sensitivity to appropriate membrane fluidity and kinetics provide compelling evidence that the 2-oxoglutarate carrier may account for the low-affinity transport of GSH. While we agree with Estrela et al. about the critical importance of mitochondrial GSH in controlling the rate of reactive oxygen generation within mitochondria and the fate of cells in response to stress, as shown by our own work[8–10] as well as the work of others,[11,12] the identification of the mitochondrial GSH carrier responsible for the high-affinity transport of GSH clearly will require more work. In this regard, the suggestion by Estrela and coworkers that the mitochondrial transport of GSH may occur through the voltage-dependent anion channel (see their letter), referring to the work of Cummings et al.[13] is unfounded, since these authors demonstrated that voltage-dependent anion channel does not play any role in the mitochondrial transport of GSH.

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The Clinical Profile of Acute Hepatitis A Infection: Is It Really So Severe?

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To the Editor:

The interesting editorial by Siłogren[1] stresses the role of hepatitis A virus (HAV) as a cause of severe hepatitis and fulminant hepatitis, placing renewed focus on the issue of universal vaccination and suggesting that the policy of vaccinating only high-risk groups, which is currently adopted in the United States and in most western countries, be reconsidered.[2,3]

In Italy, as in other economically developed areas, the epidemiology of HAV infection has changed drastically in the past 10 years, with a decrease in herd immunity and an increase in the number of susceptible adults and thus of symptomatic cases.[4] Nonetheless, in our experience, the overall profile of the disease remains favourable, and fulminant and fatal cases are extremely rare. In particular, at the Domenico Cotugno Infectious-Disease Hospital of Naples, in which all cases of hepatitis in the metropolitan area are hospitalized, independently from their clinical complexity, and which is also the region's reference center for fulminant hepatitis, 1182 cases of HAV infection have been diagnosed in the past five years, based on serological evidence of immunoglobulin M anti-HAV (61% men; mean age, 16 $\pm$ 5 years; 4% of cases superimposed on subjects affected by chronic HBV or HCV infection). Although the clinical picture was serious for nearly one-third of these patients (22% with severe general symptoms; 20% with transaminase levels that were almost 30 times the normal values; 12% with serum bilirubin $>$ 300 mmol/L; 6% with coagulopathy with international normalized ratio $>$ 1.8; and 1.8% with a relapsing or a prolonged course), only 2 of them presented severe complications (*i.e.,* pancreatitis and aplastic anemia), with resolution in both of them, and only 4 patients progressed to fulminant hepatitis.5. One of these patients, a pregnant women, required transplantation, and 3 recovered spontaneously; 1 of the latter 3 was suffering from chronic hepatitis C infection, and another had a history of low-dose acetaminophen intake, although nearly 20% of the patients without complications reported the use of this drug before the appearance of jaundice.

In other words, only 0.3% of the more than 1,000 consecutive cases of HAV infection progressed to fulminant hepatitis, and none of the cases was fatal. These data are consistent with reports from Italy's national hepatitis surveillance system, which indicate that only 1 death has occurred among the 11,063 cases of HAV infection from 1965 to 2000.[6]

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Reply:

I read with interest the letter from Amoroso et al. and appreciate their data, which I believe supports the case for more widespread vaccination against hepatitis A. The authors agree that in Italy as in the United States there has been an increased susceptibility to hepatitis A viral infection because of a decrease of natural immunity in the general population. They note that among 1,182 cases diagnosed at their hospital during the last 5 years, at least one-third (394 individuals) had severe clinical symptoms during the course of the illness, with compli-

cations such as aplastic anemia, coagulopathy, and pancreatitis. Four of their patients progressed to acute fulminant hepatitis (0.33%); one experienced a liver death (a pregnant woman required a liver transplantation). Indeed, these statistics, although reported from a single institution, underscore the need for prevention. Most, if not all, of these cases could have been prevented if the patients had received hepatitis A vaccine. As their 33% severe-symptom occurrence attests, hepatitis A is not a trivial infection. I would also note that surveillance systems are notoriously defective, and they underreport cases, morbidity, and mortality. One wonders about the expense incurred in treating the 394 individuals with severe symptoms—the direct costs of hospitalization and liver transplant, and the indirect costs of lost manpower and diverted resources. In the United States, the annual economic burden of hepatitis A in adolescents and adults was estimated at $488.8 million in a study that took into account the costs of medicines, hospitalization, physician visits, diagnostic studies, therapy for fulminant disease, liver transplantation, loss of income from missed work days, and mortality.[1] The cost-effectiveness of routine immunization against hepatitis A vaccine was evaluated through meticulous review of studies, reviews, editorials, and letters published in 5 major languages (including Italian) between February 1992 and December 2001, and the conclusion was that the use of hepatitis A virus (HAV) vaccine in developed countries had cost-effectiveness comparable to that of other childhood vaccines.[2] Indeed, we have recently witnessed the effects of the current U.S. policy of limited HAV vaccination in the outbreak of more than 600 symptomatic cases of HAV in the states of Georgia, Tennessee, and Pennsylvania. Thus far, 3 patients have died, and thousands of exposed individuals needed immediate passive immunization. None of the cities where the outbreak occurred were located in states that the Centers for Disease Control and Prevention recommends consideration for immunization because of high or intermediate rates of endemic HAV.[3] Therefore, immunization directed to specific groups would not control the infection, in part because about half of the hepatitis A infections occur in patients without known factors. It is impossible to predict who would be at risk of infection with the hepatitis A virus.

As I noted before, the rate of acute liver failure due to HAV has not decreased over the years; in contrast, the acute liver failure cases due to hepatitis B virus infection have substantially decreased, in part due to universal immunization.[4] To suggest that the significant morbidity and mortality due to hepatitis A is an acceptable outcome in Italy or in the U.S. when cost-effective, safe, and highly immunogenic HAV vaccines are available would appear to this author to be the wrong strategy and it deserves urgent reconsideration.

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