Original Research

Changes in Serum Retinol, α-Tocopherol, Vitamin C, Carotenoids, Zinc and Selenium after Micronutrient Supplementation during Alcohol Rehabilitation

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Key words: supplementation, serum, retinol, α-tocopherol, β-carotene, carotenoids, vitamin C, zinc, selenium, alcohol rehabilitation

Objective: To test the effect of a 21-day supplementation with moderate doses of antioxidant nutrients on biochemical indicators of vitamin, carotenoid and trace element levels in alcohol-dependent patients during a program of alcohol rehabilitation.

Design: A randomized double-blind trial was performed comparing two groups receiving daily either a combination of micronutrients (beta-carotene: 6 mg, vitamin C: 120 mg, vitamin E: 30 mg, zinc: 20 mg, selenium: 100 μg) or a placebo.

Subjects: 106 alcohol-dependent patients 20 to 60 years of age without severe liver disease, hospitalized for a 21-day rehabilitation program.

Measure of Outcome: Vitamin C, retinol, α-tocopherol, zeaxanthin/lutein, β-cryptoxanthin, lycopene, α- and β-carotene, zinc and selenium were measured in serum, initially and after supplementation.

Results: (1) In the placebo group, after 21 days of rehabilitation, serum concentrations of vitamin C and all five carotenoids significantly increased, whereas retinol and α-tocopherol concentrations decreased; zinc and selenium levels were unaffected. (2) At the end of the hospital stay, serum indicators were significantly improved in the supplement group as compared to the placebo group for vitamin C, α-tocopherol, β-carotene, zinc and selenium; conversely, lycopene changes were higher in the placebo group than in supplement group. (3) Of the serum antioxidants measured at entrance, only vitamin C was significantly depleted in heavy smokers, and, after the supplementation period, vitamin C was efficiently repleted in this later group.

Conclusion: Our results indicate that a short-term supplementation with physiological doses of antioxidant vitamins, carotenoids and trace elements during alcohol rehabilitation clearly improves micronutrient status indicators. Heavy smokers in particular seem to respond to vitamin C supplementation.

INTRODUCTION

Ethanol metabolism is responsible for generation of reactive oxidant species (ROS) that cause tissue damage and affect a wide range of organ systems [1–3]. Some of its effects are directly due to either ethanol or its metabolites, whereas others are related to nutritional deficiencies associated with alcohol intake. Chronic alcoholism is associated with a high risk of

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Abbreviations: ROS = reactive oxygen species, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = γ-glutamyltransferase, BMI = body mass index, RBP = retinol binding protein, MCV = mean corpuscular volume, Apo = apolipoprotein.

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micronutrient deficiency [4,5]; it may affect nutrient intake, increase the need for specific nutrients (especially when ROS are generated in large amounts) and interfere with their absorption, storage and utilization. Micronutrients such as retinol, α-tocopherol, vitamin C, carotenoids and trace elements (Cu, Zn, Se and Mn) are important factors implicated in the defense against oxidative injury, and a deficit in any one of these elements can result in function impairment of the overall antioxidant system [6–9].

In a previous study [10,11], we observed lower α-tocopherol, vitamin C, carotenoid and selenium status in alcohol-dependent patients in comparison to controls who consumed low amounts of alcohol. After 21 days of alcohol rehabilitation, plasma concentrations of carotenoids increased, whereas vitamin C and retinol concentrations decreased; α-tocopherol and selenium were not altered and remained at low levels in comparison to controls. This fact suggested that the 21-day rehabilitation program was inadequate to reach a micronutrient status comparable to those observed in low drinkers. Therefore, this experiment was designed to explore whether such serum indicators were altered by supplementation with a combination of antioxidant micronutrients (α-tocopherol, vitamin C, β-carotene, zinc and selenium) at physiological doses during alcohol rehabilitation.

MATERIALS AND METHODS

Population and Study Design

Participants in the current study were 118 alcohol-dependent patients hospitalized in the Service de Médecine L of the Centre Hospitalier Universitaire of Nancy (France) for a 21-day rehabilitation program (89 males and 29 females). To be eligible, they had to show no clinical or biological evidence of severe liver disease, no encephalopathy, no ascite, no pancreatitis, plasma albumin $>$30 g/L, bilirubin $<$50 μmol/L, Quick time $>$65%, aspartate aminotransferase (AST) $<$300 U/L and alanine aminotransferase (ALT) $<$300 U/L. Liver biopsies were not performed for ethical reasons. All patients consumed excessive quantities of alcohol until hospitalization (more than 100 g/day expressed as pure alcohol) and were dependent on alcohol according to DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, 3rd ed, revised) dependence criteria [12].

Abstinence and treatment compliance were checked by regular interviews. In addition, during the rehabilitation program, patients had to stay in the hospital department and no alcohol beverage was allowed to enter the hospital area. However, in case of hospital exit during withdrawal, alveolar alcohol levels were controlled at the entrance by using a breath analyzer. In addition, the patients did not take supplements containing any of the studied micronutrients. During the hospital stay, four main meals were served (breakfast, lunch, snack and dinner). Food composition of these meals was chosen in accordance to the French recommended dietary allowances [13]; specifically, energy content was between 2400 and 2700 Kcal/day.

All patients underwent a complete medical examination and a biological screening at entrance and after three weeks of rehabilitation. A detailed standardized interview aimed especially at drinking history and tobacco and drug consumption was performed by a trained interviewer.

The study design was double-blind, placebo-controlled. Participants were stratified by gender and randomly assigned to one of the two treatment groups by using block randomization (n = 10). Group members received one capsule per day for a period of 21 days. The supplement group received 120 mg of vitamin C, 6 mg of β-carotene (1000 retinol equivalents), 30 mg of α-tocopherol, 20 mg of zinc and 100 μg of selenium. Zinc gluconate, α-tocopherol, β-carotene and ascorbic acid were synthetic; selenium was derived from enriched yeast.

The other group received a placebo capsule. We used the supplement and placebo capsules prepared especially for the SU.VI.MAX study [14]. Informed consent was obtained from subjects, and the research protocol was approved by the "Comité consultatif de protection des personnes dans la recherche biomédicale de Lorraine" and was in agreement with the Helsinki Declaration.

In each group, six subjects withdrew from the trial before the end. All withdrawals were due to non-compliance with the rehabilitation program or with micronutrient treatment; finally, statistical analyses were performed on a placebo group of 53 subjects (38 males and 15 females) and a supplement group of 53 subjects (42 males and 11 females). Moreover, some values for vitamin C, zinc and selenium concentration were missing because of transport problems of samples.

Vitamin, carotenoid and trace element status and measurements of biological indexes were determined at baseline and after 21 days of supplementation. Venous blood samples were collected from fasting individuals in trace-element free BD vacutainer tubes (Vacutainer Tube, Becton Dickinson, Grenoble, France) between 7:30 and 8:30 a.m. Blood samples were centrifuged at 1500 *g* for 15 minutes at 4°C and resulting serum aliquots were promptly frozen at −80°C until analysis. For vitamin C, serum samples were stabilized by adding metaphosphoric acid (5%, mass(m)/volume(v)) immediately after separation from the cells (1:2, v/v).

Biological Measurements

Serum cholesterol, triglycerides and activities of GGT, ALT and AST were measured by using standard enzymatic methods. Albumin and bilirubin were determined by using colorimetric methods. Apolipoprotein A-I, retinol binding protein (RBP) and transthyretin concentrations were quantified by using BNA nephelometer and reagents from Dade Behring (Marburg, Germany).

A reversed-phase high performance liquid chromatography

(HPLC) method was used for the simultaneous determination of retinol, alpha-tocopherol and carotenoids in serum [15]. Retinol acetate, α-tocopherol acetate and echinenone were used as internal standards for retinol, α-tocopherol and carotenoid determination, respectively. Analytes were isolated by liquid-liquid extraction and concentrated by evaporation. The extracts were chromatographed on a C18, 250 × 3 mm Nucleosil 5-μm column (Macherey Nagel, Duren, Germany) by using a solvent system comprising methanol, acetonitrile and tetrahydrofuran (75:20:5, v/v/v) and detected by a UV-visible diode array detector (model Gold LC-168, Beckman Coulter, Fullerton, CA, USA) programmed at 290 nm (α-tocopherol and α-tocopherol acetate), 325 nm (retinol and retinol acetate) and 450 nm (carotenoids). Lutein and zeaxanthin could not be separated by this method; therefore, their concentration values were presented together. Retinol, retinol acetate, α-tocopherol, α-tocopherol acetate and β-carotene standards were obtained from Fluka (Buchs, Switzerland). Zeaxanthin and β-cryptoxanthin were given gratis by Hoffman-Laroche (Basle, Switzerland). Lycopene and echinenone were purchased from CaroteNature (Lupsingen, Switzerland).

Total vitamin C was evaluated on serum after mixed with metaphosphoric acid (5%) by HPLC method with fluorescence detection [16].

Serum zinc concentrations were determined [17] by flame atomic absorption spectrometry (Perkin Elmer 560, Norwalk, CT) using a fivefold dilution of serum in hydrochloric acid (0.1 M) and external calibration. Serum selenium concentrations were determined on a Perkin Elmer 5100 (Norwalk, CT) equipped with an HGA 600 furnace, an EDL lamp and Zeeman background correction [18]. Serum was diluted fivefold in a solution containing nitric acid (0.1 M) and Triton X 100 (0.2%). Ten microliters of this dilution were injected in a platform pyrolytically coated graphite furnace together with 5 μL of a 3 g/L platinum solution. Simple standard addition calibration was used for the determination. Trace elements normal range serum (Utak, Valencia, CA, USA) was used as internal quality control. The expected values were 1.4 μmol/L for selenium and 14 μmol/L for zinc. The obtained values, expressed as mean ± standard deviation (coefficient of variation), were 1.32 ± 0.07 μmol/L (5.0%) and 13.12 ± 0.33 μmol/L (2.5%), respectively, for selenium and zinc.

Statistical Methods

Results were expressed as mean ± SD or as stated. Statistical comparisons were performed by using two-sided *t* tests and analysis of variance for repeated values from BMDP statistical software (Los Angeles). Values for GGT, AST and ALT activities and serum triglycerides, vitamin C and carotenoids were log-transformed. Both crude and adjusted values for cholesterol and triglyceride levels were used for fat-soluble micronutrients: retinol, α-tocopherol and carotenoids. The interaction of alcohol intake level was studied in ANOVA after categorization of the sample into two groups by using the cutoff of 165 g/day of pure alcohol: 54 patients with alcohol intake <165 g/day, 52 with alcohol intake >165 g/day. An identical procedure was used to study interaction of smoking, by separating subjects into three groups according to cigarette consumption: less than 11 cigarettes/day (non- and moderate smokers: n = 14), between 11 and 29 cigarettes/day (intermediate smokers: n = 42) and more than 29 cigarettes/day (heavy smokers: n = 50). For all comparisons, a p value $\leq$0.05 was considered to be statistically significant.

RESULTS

At baseline, the remaining subjects in the treatment and in the placebo group were similar in term of age, gender ratio, body mass index (BMI), tobacco consumption and alcohol intake as well as biological characteristics: GGT, AST, and ALT activities, mean corpuscular volume (MCV), Quick time, bilirubin, cholesterol, triglyceride, apo A-I, albumin, RBP and transthyretin concentrations (Table 1). In each group, 48 patients were smokers and consumed in average 29 ± 13 cigarettes/day. After the 21-day rehabilitation program GGT, AST and ALT activities, MCV, bilirubin, cholesterol, apo A-I, RBP and transthyretin concentrations significantly decreased, whereas BMI significantly increased. Variations were quite similar in both groups; interaction terms with placebo/treatment status being nonsignificant in ANOVA for repeated values. Unfortunately, smoking status and tobacco consumption were not determined at the end of hospital stay. Nevertheless, we can make the assumption that as in another similar study, smoking did not vary or even could increase after the three weeks of rehabilitation [10].

At entrance in the study, no significant difference was observed between treatment and placebo groups for vitamin, carotenoid and trace element serum indicators (Table 2). The concentrations of β-carotene, selenium and zinc were low as compared to French reference values [19]. In the placebo group after the 21 days of rehabilitation, serum concentrations of vitamin C, zeaxanthin/lutein, β-cryptoxanthin, lycopene, α- and β-carotene significantly increased, whereas retinol and α-tocopherol concentrations decreased; zinc, selenium levels and retinol/RBP ratio were unaffected. For fat-soluble nutrients, adjustment for cholesterol and triglyceride concentrations did not affect extents of difference and levels of significance.

At the end of the hospital stay, serum indicators were significantly improved in the treatment group as compared to the placebo group for vitamin C, α-tocopherol, β-carotene, zinc and selenium ($p \leq 0.05$ to $p \leq 0.001$); conversely, lycopene increases were higher in placebo group than in supplement group. Changes over time observed for retinol, zeaxanthin/lutein, β-cryptoxanthin and α-carotene concentrations were quite similar and nonsignificant between the two groups. Taking into account cholesterol and

Table 1. Characteristics of the Subjects before and after Three Weeks of Alcohol Rehabilitation (n = 53 in both Placebo and Supplement Groups)[a]

		Before Rehabilitation	After Rehabilitation	Time effect	
				Change	*p*-value
Age (year)	Placebo	39.9 ± 10.6			
	Supplement	41.3 ± 11.4			
Gender ratio (% females)	Placebo	28.3			
	Supplement	20.8			
Tobacco consumption (cigarettes/day)	Placebo	26.1 ± 14.4	ND		
	Supplement	26.6 ± 15.6	ND		
Alcohol intake (g/day)	Placebo	201 ± 185	0		
	Supplement	187 ± 111	0		
BMI (kg/m^2)	Placebo	22.4 ± 3.4	22.9 ± 3.4	+0.5	***
	Supplement	23.4 ± 4.0	23.8 ± 3.8	+0.4	
GGT activity[b] (U/L)	Placebo	211 ± 349	62 ± 67	−149	***
	Supplement	290 ± 408	85 ± 122	−205	
AST activity[b] (U/L)	Placebo	49.5 ± 51.7	28.1 ± 29.0	−21.4	***
	Supplement	45.1 ± 35.3	24.8 ± 13.8	−20.3	
ALT activity[b] (U/L)	Placebo	61.4 ± 61.1	24.0 ± 19.1	−37.4	***
	Supplement	59.5 ± 60.7	22.1 ± 12.8	−37.4	
Mean corpuscular volume (fL)	Placebo	98.4 ± 7.4	96.8 ± 6.4	−1.6	***
	Supplement	99.0 ± 6.5	97.6 ± 5.8	−1.4	
Quick time (%)	Placebo	98.2 ± 4.8	97.7 ± 5.7	−0.5	—
	Supplement	97.6 ± 4.8	98.1 ± 4.2	+0.5	
Bilirubin (μmol/L)	Placebo	13.5 ± 7.3	6.1 ± 2.4	−7.4	***
	Supplement	12.9 ± 5.6	6.8 ± 2.2	−6.1	
Cholesterol (mmol/L)	Placebo	5.63 ± 1.12	5.36 ± 1.14	−0.27	***
	Supplement	5.75 ± 1.42	5.21 ± 1.02	−0.54	
Triglycerides[b] (mmol/L)	Placebo	1.53 ± 1.40	1.41 ± 0.74	−0.12	—
	Supplement	1.29 ± 0.74	1.31 ± 0.69	+0.02	
Apo A-I (g/L)	Placebo	1.61 ± 0.37	1.20 ± 0.30	−0.41	***
	Supplement	1.71 ± 0.36	1.24 ± 0.20	−0.47	
Albumin (g/L)	Placebo	39.7 ± 3.5	40.4 ± 3.6	+0.7	—
	Supplement	40.1 ± 3.3	39.8 ± 3.7	−0.3	
Retinol binding protein (mg/L)	Placebo	58.2 ± 18.7	41.5 ± 10.7	−16.7	***
	Supplement	59.4 ± 19.1	42.6 ± 12.7	−16.8	
Transthyretin (mg/L)	Placebo	297 ± 70	245 ± 49	−52	***
	Supplement	297 ± 69	237 ± 52	−60	

[a] Results are expressed as mean ± standard deviation.
[b] ANOVA on log transformed values.
— NS.
*** $p < 0.001$; rehabilitation effect for both groups in ANOVA for repeated values.
Differences in change between placebo and supplement groups were non significant for all variables.
ND = not determined.

triglyceride concentration did not alter significance of differences in fat-soluble nutrient levels.

ANOVA including the level of alcoholization at entrance (as grouping factor: more and less 165 g/day of pure alcohol) did not show any significant interaction of this factor in supplement related variation of the studied serum indicators (data not shown). Of the serum antioxidants measured at entrance, only vitamin C was significantly related to smoking status in both placebo ($p \leq 0.05$) and supplement groups ($p \leq 0.001$) (Fig. 1); serum vitamin C concentrations were higher in non- and moderate smokers (fewer than 11 cigarettes/day) than in heavy smokers (more than 29 cigarettes/day). In the supplement group after rehabilitation, vitamin C levels were no longer related to cigarette consumption, contrary to that who was observed in the placebo group ($p \leq 0.05$). After the rehabilitation period, vitamin C increased markedly in the supplemented group of intermediate and heavy smokers, the plasma concentration showed a significant 150% to 240% increase after supplementation in comparison to a 10% to 17% increase in placebo subgroups of similar smoking status.

DISCUSSION

In a such population of alcohol-dependent patients one has often great difficulty excluding subjects with liver impairment which could impact with specific effect on alcohol metabolism.

Table 2. Serum Concentrations of Vitamins, Carotenoids and Trace Elements Before and After Three Weeks of Alcohol Rehabilitation (n = 53 in both Placebo and Supplement Groups)[a]

		Before Rehabilitation	After Rehabilitation	Change over Time	Time Effect[d]	Treatment Effect[e]
Vitamin C[b,c] (μmol/L)	Placebo	42.6 ± 33.8	46.0 ± 29.3	+3.4	*	***
	Supplement	33.6 ± 31.4	75.2 ± 22.8	+41.6		
Retinol (μmol/L)	Placebo	2.38 ± 0.88	1.71 ± 0.57	−0.67	***	—
	Supplement	2.47 ± 0.86	1.73 ± 0.69	−0.74	°°°	~
Retinol/RBP ratio (μmol/μmol)	Placebo	0.85 ± 0.14	0.86 ± 0.14	+0.01	—	—
	Supplement	0.87 ± 0.12	0.86 ± 0.24	−0.01	~	~
α-Tocopherol (μmol/L)	Placebo	27.1 ± 6.9	24.4 ± 6.6	−2.7	**	***
	Supplement	25.6 ± 7.8	28.1 ± 8.6	+2.5	°°°	°°°
Zeaxanthin/Lutein[b] (μmol/L)	Placebo	0.21 ± 0.15	0.39 ± 0.18	+0.18	***	—
	Supplement	0.18 ± 0.13	0.35 ± 0.22	+0.17	°°°	~
β-Cryptoxanthin[b] (μmol/L)	Placebo	0.04 ± 0.06	0.09 ± 0.07	+0.05	***	—
	Supplement	0.03 ± 0.06	0.07 ± 0.11	+0.04	°°°	~
Lycopene[b] (μmol/L)	Placebo	0.23 ± 0.25	0.29 ± 0.214	+0.06	**	*
	Supplement	0.28 ± 0.26	0.28 ± 0.248	+0.00	°°°	°
α-Carotene[b] (μmol/L)	Placebo	0.04 ± 0.05	0.15 ± 0.08	+0.11	***	—
	Supplement	0.03 ± 0.05	0.11 ± 0.08	+0.08	°°°	~
β-Carotene[b] (μmol/L)	Placebo	0.15 ± 0.11	0.42 ± 0.16	+0.27	***	***
	Supplement	0.11 ± 0.11	0.76 ± 0.54	+0.65	°°°	°°°
Zinc (μmol/L)	Placebo	10.7 ± 1.4	10.6 ± 1.6	−0.1	—	**
	Supplement	10.7 ± 1.9	11.6 ± 2.0	+0.9		
Selenium (μmol/L)	Placebo	0.74 ± 0.18	0.71 ± 0.15	−0.03	—	***
	Supplement	0.74 ± 0.17	1.11 ± 0.17	+0.37		

[a] Results are expressed as mean ± standard deviation.
[b] ANOVA on log transformed values.
[c] n = 50 in placebo group and n = 43 in supplement group.
[d] time effect in placebo group, ANOVA for repeated values.
[e] differences in change between placebo and supplement groups, *t* test.
— = NS, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; test on crude values.
~ = NS, ° $p \leq 0.05$, °° $p \leq 0.01$, °°° $p \leq 0.001$: test on adjusted values for serum cholesterol and triglyceride concentrations.

Our group of alcohol abusers was carefully controlled and withdrawal was monitored. Liver biopsy was not performed; however, subjects were selected according to severe criteria, and biological markers of alcohol consumption (GGT and MCV), hepatocellular injury (AST and ALT) and protein synthesis (albumin, transthyretin and RBP) were measured.

In comparison to reference data obtained in low drinkers [20,21], the levels of biological markers of alcohol consumption and hepatocellular injury at entrance (GGT, mean corpuscular volume, AST and ALT) were higher in our group of alcohol-dependent patients, while albumin concentration was lower, and no difference was observed for bilirubin. Mean values for transthyretin and RBP were within reference values. In our study group, the enzyme markers of liver cell membrane disruption (AST and ALT) were normalized after three weeks of abstinence, contrary to GGT activity and MCV. Generally, in abstinent patients without liver disease, AST and ALT activities return to normal after three weeks, GGT activity after six to eight weeks and MCV after three months [22]. We found that albumin concentration was normal in all patients at entrance and did not increase significantly during withdrawal. Surprisingly, concentrations of transthyretin and RBP significantly decreased with alcohol abstinence. This phenomenon has already been noticed [23,24] and could be due to decreased secretion or escape into the circulation.

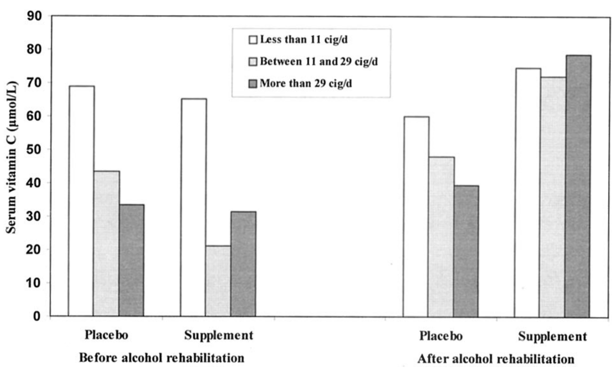

Fig. 1. Serum concentration of vitamin C before and after alcohol rehabilitation in both supplement and placebo groups according to cigarette consumption. cig/d = cigarettes a day.

During the rehabilitation period of 21 days, we observed in the placebo group an increase in serum levels of the five carotenoids and a decrease of retinol and its specific carriers (transthyretin and RBP); the ratio retinol/RBP were unaffected.

These results are in line with those previously published by our team [11]. As with Lecomte *et al.* [10] and Girre *et al.* [25], we did not observe any significant alteration in selenium concentrations; zinc levels also were not affected by short term abstinence. Moreover, serum α-tocopherol levels were reduced, and vitamin C rose moderately at the end of hospital stay, contrary to previous results from our group [10]. For fat-soluble nutrients, adjustment for cholesterol and triglyceride concentrations did not affect extents of difference and levels of significance. In this study, no dietary intake data was collected before or after the rehabilitation program; however, as previously shown, changes in serum nutritional indicators should be independent of food intake alteration. In a previous paper [11], we showed that carotene and vitamin A intakes were lower in alcoholics before abstinence than in low drinkers; however, the differences were of limited— or did not reach—statistical significance. After abstinence, we observed a significant increased of the amounts of retinol and carotene ingested. In this later study, carotenoid levels increased in plasma after withdrawal, and this effect was not modified after adjustment for confounding factors such as nutritional intake, smoking or plasma lipids. This suggested a specific effect of withdrawal on plasma carotenoid levels. In this later study [10], the withdrawal period led to a drop of serum vitamin C level although the nutritional intake was not significantly modified.

At the end of the trial, we observed in the whole group higher serum concentrations of vitamin C, α-tocopherol, β-carotene, zinc and selenium in participants who received multi-supplement during the 21-day rehabilitation than in the placebo group. Since the present study had a double-blind design, diet intake of the two groups of alcohol abusers should not differ significantly and should not interfere with the effect of micronutrient supplementation.

Such improvement in vitamin E, β-carotene, zinc and selenium status after supplementation with physiological amounts of vitamins, β-carotene and trace elements was previously described in healthy individuals [19]. Serum selenium concentrations reached reference range for French adults [19], whereas serum zinc and α-tocopherol concentrations remained low. Difficulty in increasing serum zinc concentrations after supplementation have been previously described [19]. Moreover, our results about retinol level are in agreement with those of previous studies [19,26–28]; administration of provitamin A such as β-carotene did not alter rehabilitation-related decrease in retinol concentration.

Changes in lycopene levels were significantly lower in alcohol-dependent patients taking the multi-supplement containing β-carotene than in placebo group; the same trend observed for α-carotene was non significant. These two hydrocarbon carotenoids have physical and chemical characteristics similar to those of β-carotene, and literature concerning the effect of supplemental β-carotene on concentrations of these carotenoids is controversial. An elevated plasma α-carotene and lycopene have been described [29] while other studies showed a significant increase only in plasma α-carotene levels [27,30–32], which is assumed to be, at least in part, due to contamination of the supplement. Some studies have reported a negative effect of β-carotene supplementation on the bioavailability of lycopene [33].

The role of alcohol in vitamin C status is complex and probably involves behavioral as well as metabolic influence. Alcohol consumption may reduce the intake and bioavailability of vitamin C [34,35] and increase urinary excretion [36,37], as well as the requirement due to increased free radical activity associated with ethanol metabolism [1,38]. During alcohol rehabilitation, inverse processes probably occur and could explain the large response to vitamin C intake.

In addition, prior to supplementation, our data confirm that cigarette smoking is associated with decreased plasma vitamin C levels [39–41]. In the literature, this association is independent of factors which affected plasma ascorbic levels, such as alcohol and vitamin intake [41], and all observations suggest that smoking directly lowers plasma vitamin C levels by mechanisms which do not depend on dietary vitamin C intake, such as impaired vitamin C absorption or decreased turnover [42,43]. After the supplementation period, vitamin C increased markedly in the supplemented group of intermediate and heavy smokers; the plasma concentration showed a significant 1.5-fold to 2.4-fold increase after supplementation. Most likely, at least part of the better response among smokers could be attributed to their lower vitamin C concentration at baseline. Thus, smokers in general seem to benefit more from vitamin C supplementation than do nonsmokers. Additionally, perhaps smokers have a higher homeostatic requirement, thereby contributing to a higher degree of supplement bioavailability in this group [44].

In conclusion, our data indicate that short-term supplementation (21 days) with moderate doses of antioxidant vitamins, β-carotene and trace elements during an alcohol rehabilitation program clearly improves micronutrient status. Of the serum antioxidants measured, vitamin C is the only one depleted by smoking, and depleted vitamin C concentrations can be normalized by moderate supplementation in heavy smokers.

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