Enteral feeding enriched with carotenoids normalizes the carotenoid status and reduces oxidative stress in long-term enterally fed patients

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Summary

Background & aims: Circulating carotenoid levels decrease progressively in patients receiving long-term enteral tube feeding with carotenoid-free formulas. Low dietary intake and low blood levels of carotenoids are associated with a higher risk of morbidity and mortality from chronic diseases. The aim of this study was to examine the effects of a low dose carotenoid mixture (3-mg/1500 kcal) for 3 months on serum carotenoid levels and oxidative stress in patients receiving long-term enteral nutrition as the sole source of nutrition.

Methods: This randomized, double blind, controlled study compared patients receiving enteral nutrition with carotenoids (N = 26) and without carotenoids (control group; N = 25).

Results: Patients on long-term enteral nutrition had low baseline serum carotenoid levels. Three months of enteral feeding enriched with carotenoids significantly (P < 0.01) increased serum carotenoid levels compared with the control group. Oxidative stress as measured by NF-kB levels was decreased at 3 months compared with the control group (P < 0.05). No significant changes in MDA levels were observed during the study period in either group.

Conclusions: This study demonstrated that enteral nutrition containing small amounts of carotenoids (3-mg/1500 kcal) in patients requiring long-term enteral feeding normalizes serum carotenoid levels to the lower end of the range found in

KEYWORDS

Enteral nutrition; Enteral tube feeding; Carotenoids; Oxidative stress; Nuclear Factor-kappaB

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age-matched controls. The NF-κB data indicate a reduction in oxidative stress in these patients. Therefore, the use of formulas containing a mixture of carotenoids should be recommended for long-term enteral nutrition.

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Introduction

During the last 20 years, the use of nutritional support at home has expanded in many industrialized countries. The British Artificial Nutrition Survey (BANS) recently demonstrated a considerable increase in enteral tube feeding and parenteral nutrition in both hospitals and in the community, with the largest growth for enteral tube feeding in the community, which includes nursing homes. The average increase in the prevalence of adult patients on home enteral tube feeding during 2003 has been estimated to be 8%. The use of home enteral tube feeding continues to increase in elderly and disabled patients. The most common route of access is by gastrostomy. Enteral feeding should meet the specific nutritional requirements of the group of patients with acute or chronic disease and should provide, at least, a comparable intake of health promoting micronutrients as recommended for the same group of people without the disease.

Carotenoids are ingredients of a normal diet, which are naturally occurring yellow, orange or red pigments found in fruit and vegetables. Carotenoids occur as carotenes such as \( \beta \)-, \( \alpha \)- and \( \gamma \)-carotene and lycopene, and their oxygenated derivatives (the xanthophylls) such as \( \beta \)-cryptoxanthin, lutein and zeaxanthin. The carotenoids \( \beta \)-carotene, \( \alpha \)-carotene and \( \beta \)-cryptoxanthin are precursors of vitamin A. Blood carotenoid concentrations typically reflect dietary carotenoid intake and are sensitive markers of changes in intake. In healthy subjects fed for two weeks on a low carotenoid diet (\(< 0.4 \text{ mg carotenoids per day}\)), plasma concentrations of carotenoids decreased to \(< 60\% \text{ of baseline}\). Subsequent carotenoid supplementation with a fruit and vegetable concentrate in these subjects restored plasma carotenoid levels within one week. It is, therefore, not surprising that circulating carotenoid levels progressively decrease in patients receiving enteral feeding as their sole source of nutrition. Plasma carotenoid levels may become undetectable in this group of patients. These findings are relevant to the clinical setting where patients often receive enteral formulas for a significant period of time.

Until recently, standard enteral formulas did not contain carotenoids, despite the principle that the composition of enteral formulas should mimic normal dietary intake where feasible and appropriate. Until now no studies have examined the effects of a mixture of carotenoids fed at levels consistent with a normal dietary intake.

Prospective observational studies have found inverse associations between dietary intake reflected by blood levels of carotenoids and the risk of chronic diseases. Moreover, several studies have demonstrated that low dietary intake and low plasma levels of carotenoids are associated with increased mortality. These findings suggest that the consumption of carotenoids at levels in the range of those found in a normal healthy diet may provide significant health benefits and be associated with reduced risk of chronic diseases and mortality.

Numerous diseases have been associated with an increased oxidative stress. Carotenoids exhibit potent antioxidant activity by radical trapping or singlet oxygen quenching activity and are therefore important for the prevention of lipid peroxidation and inactivation of metabolically generated free radical species. Although the antioxidant role of \( \beta \)-carotene has been widely researched, other carotenoids may have even more important antioxidant functions in the human body. An example is lycopene that has a more potent singlet oxygen quenching capacity than \( \beta \)-carotene. To assess the effects of antioxidants on oxidative stress, the biomarkers malondialdehyde (MDA) and Nuclear Factor-kappaB (NF-κB) are widely used. The detection of MDA is a commonly used method to quantify the degree of lipid peroxidation. NF-κB is a ubiquitous transcription factor that is activated in chronic inflammatory diseases associated with oxidative stress, such as arthritis, sarcoidosis and diabetic complications. Antioxidants, including some of the carotenoids, might prevent oxidative stress induced NF-κB activation and mitigate the feed forward amplification that is seen in numerous chronic diseases.

The purpose of this study was to investigate the effects of enteral formulas supplemented with a low dose carotenoid mixture on serum carotenoid levels and on oxidative stress in patients receiving...
long-term enteral nutrition as a sole source of nutrition.

**Materials and methods**

**Study design**

The study was a randomized, double blind, controlled, parallel study, comparing two groups: patients receiving enteral tube feeding with a formula supplemented with carotenoids (treatment group) and patients receiving enteral tube feeding with a formula without carotenoids (control group). An age-matched group of healthy controls was used to assess serum carotenoid levels in an ambulatory group with a similar age range.

**Patients**

Adult patients receiving enteral tube feeding as the sole source of nutrition for more than three months were identified. The characteristics of the patients under investigation are described in Table 1. Detailed information concerning the purpose and methods used in the study was provided, before written informed consent from the subjects or guardian was obtained. The Ethics Committee of the Tel-Aviv Sourasky Medical Center (Israel) approved the study. Exclusion criteria were: presence of hepatic or renal disease as assessed by an elevation of liver enzymes or renal function, clinical signs of infection and/or inflammation at study entry, confirmed by their physician, smoking, use of a nutritional supplement containing carotenoids, use of <1000 kcal of enteral nutrition per day, intake of more than three servings of fruit, fruit juice (apple and orange juice excluded), fruit mousse or canned fruits per week, intake of more than two servings of apple and orange juice daily, and intake of more than three servings of vegetables, vegetable juice, pureed vegetables or vegetable-based soup/sauce per week or a combination of more than three weekly servings of fruits and vegetables. More than 200 patients were screened and the enrolled patients were randomly

<table>
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<th>Characteristic</th>
<th>Treatment group</th>
<th>Control group</th>
<th>Healthy controls</th>
</tr>
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<tbody>
<tr>
<td>N</td>
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<td>28</td>
<td>13</td>
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<tr>
<td>Age (y)</td>
<td>71.7 ± 23.1</td>
<td>71.1 ± 22.3</td>
<td>77.0 ± 9.7</td>
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<td>Gender (M/F)</td>
<td>6/21</td>
<td>9/19</td>
<td>4/9</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>61.9 ± 12.2</td>
<td>57.8 ± 13.4</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 5.3</td>
<td>23.2 ± 4.4</td>
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</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>7.55 ± 5.47</td>
<td>7.81 ± 6.24</td>
<td>—</td>
</tr>
<tr>
<td>% of patients ≥ 10 mg/l</td>
<td>39</td>
<td>27</td>
<td>—</td>
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<tr>
<td>Duration tube feeding prior to commencing study (months)a</td>
<td>23.3 ± 31.5</td>
<td>35.6 ± 38.0</td>
<td>—</td>
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<tr>
<td>Intake at enrollment (ml/day)</td>
<td>1383 ± 296</td>
<td>1479 ± 279</td>
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</tr>
<tr>
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<tr>
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<td>—</td>
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<td>—</td>
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<td>10</td>
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</tr>
<tr>
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<td>7</td>
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<tr>
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<tr>
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<tr>
<td>Other</td>
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<td>1</td>
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</tr>
</tbody>
</table>

Mean ± SD.

aDocumented data only available for n = 15 and 16 for treatment and control groups, respectively.
assigned to one of the two treatment groups. The intent-to-treat group consisted of 55 patients (see Fig. 1). Four patients were excluded from the final analysis; three patients died during the study period and one patient had a protocol violation. Therefore, the per protocol analysis consisted of 51 patients. Twenty-six patients received standard enteral formulas enriched with carotenoids and the control group of 25 patients received standard enteral formulas without carotenoids. Assessments were performed at baseline (before supplementation with the carotenoid mixture) and again after 3 weeks and 3 months of enteral feeding.

**Nutritional intervention**

This study consisted of two study groups: a group of patients given an enteral formula supplemented with carotenoids (Nutrison Standard or Nutrison Multi Fibre, Nutricia, Zoetermeer, The Netherlands); and another group of patients given an enteral formula without carotenoids (identical to above products, but with no added carotenoids). The carotenoid mixture consisted of α-carotene (0.69 mg), β-carotene (1.29 mg), γ-carotene (0.006 mg), lycopene (0.60 mg), lutein (0.41 mg) and zeaxanthin (0.003 mg) per 1500 kcal, with a

![Figure 1](patient_flow_chart.png)  
**Figure 1** Patient flow chart of the study.
total of 3 mg of carotenoids/1500 kcal. Patients received enteral feeding with fibers or without fibers according to their previous feeding regimen. The reported volume of the intake patients received prior to commencing to the study ranged from 700 to 2400 ml per day. The different formulas were packaged and labeled identically. Enteral tube feeding was administered via nasogastric tube or percutaneous endoscopic gastrostomy (PEG) using a peristaltic pump (Flocare 800 enteral feeding pump; Nutricia, Schiphol, The Netherlands).

Carotenoid levels

Fasting blood was collected and serum samples were frozen. Serum carotenoid status (β-carotene, lycopene, lutein, zeaxanthin, β-cryptoxanthin, lutein, zeaxanthin, respectively) was quantified by reversed phase HPLC using a modified version of a method described previously. Briefly, serum samples were extracted with hexane. The hexane layer was evaporated to dryness and dissolved in a mixture of methanol and acetonitril (1:1). This was injected on a reversed phase column (500 x 4.6 mm, 5 μM) that was eluted with a gradient consisting of methanol, acetonitril, 2-propanol and water at a flow rate of 1.5 ml/min. The detection was with a diode-array detector.

Oxidative stress

MDA levels and NF-κB activation were used as biomarkers for oxidative stress. MDA, one of the free radical breakdown products of lipid peroxidation derived from arachidonic acid hydroperoxide, was measured in plasma by HPLC as described by Pilz et al. NF-κB was measured in the nuclear extract of blood lymphocytes. NF-κB concentrations were assessed according to the instructions of the assay (TransAM NF-κB p50 Activation Assay (Active Motif, Rixensart, Belgium)). NF-κB activity was expressed as Jurkat whole cell extract NF-κB equivalents per mg nuclear protein. Protein concentrations were determined using the method of Bradford (Biorad) with BSA as standard.

Antioxidant vitamins

The enteral formulas contained 82 μg vitamin A, 1.3 mg vitamin E and 10 mg vitamin C per 100 ml, respectively. Vitamin A (retinol) and vitamin E (α-tocopherol) in plasma were analyzed by HPLC. Vitamin C was analyzed by HPLC using UV absorption as detection and uric acid was analyzed according to the method of Margolis and Duewer.

Statistical analysis

Data are presented as means ± SD and confidence intervals. The efficacy parameters were compared between the two treatment groups. The data were initially analyzed by using ANOVA including the factors treatment and fiber and the two-way interaction per visit (baseline, 3 weeks and 3 months). If no significant interaction and fiber effect were observed, the terms were removed from the ANOVA model. If the data were not normally distributed, the Mann–Whitney U-test (Wilcoxon rank sum test) was used to analyze per treatment group. Statistical significance was accepted as P < 0.05. The statistical analyses were performed by SPSS, version 12.0.1 for Windows, Rel. 11, 2003 Chicago: SPSS Inc.

Results

Study population

A total of 55 patients were included in the intention-to-treat analysis, and 51 patients in the per protocol analysis. The treatment group (enteral formula with carotenoids) consisted of 26 patients and the control group (enteral formula without carotenoids) of 25 patients. The intake of enteral feed in the treatment group was 1383 ± 296 ml per day and in the control group 1479 ± 279 ml per day at enrollment. The mean duration of enteral feeding prior to commencing the study was 29.2 months (Table 1). The type and composition of the enteral formulas used before the study was a reflection of the formulas on the market in Israel during the period 2003–2004. None of these formulas contained added carotenoids. Most patients at enrollment had a primary diagnosis reflecting neurological injury or disease including dementia, coma or hemiplegia (Table 1).

Carotenoid levels

The patients receiving long-term enteral tube feeding as the sole source of nutrition had extremely low serum carotenoid levels at baseline (Table 2). No significant differences between serum carotenoid levels of the intervention and control group were observed at baseline. All the supplemented carotenoids increased significantly (P < 0.01) in the treatment group after 3 months
of enteral feeding (Table 2). Lutein and zeaxanthin levels of the control group showed a significant decrease after 3 weeks and 3 months compared with baseline (P<0.001). The levels of \( \beta \)-carotene, lycopene, canthaxanthin and cryptoxanthin did not change significantly from baseline after 3 weeks and 3 months within the control group. Patients in both groups had significantly lower serum levels of carotenoids at baseline when compared with 13 age-matched healthy volunteers (P<0.001) who were included to obtain reference values. The serum carotenoid levels of the healthy volunteers were 539±302 ng/ml \( \beta \)-carotene, 127±55 ng/ml lycopene, 366±178 ng/ml lutein, 129±54 ng/ml zeaxanthin, 68±28 ng/ml canthaxanthin and 356±188 ng/ml cryptoxanthin.

**Oxidative stress**

Oxidative stress as measured by NF-\( \kappa \)B activation in peripheral blood mononuclear cells significantly decreased in the carotenoid supplemented group after 3 months of enteral feeding when compared with the control group (P<0.05, result of Mann–Whitney test). Baseline values of NF-\( \kappa \)B of \( \beta \)-carotene were significantly different in the groups: 1.46±0.50 vs. 1.11±0.51 ng \( \mu \)g \( \kappa \)B equivalent/\( \mu \)g nuclear protein for the carotenoid-supplemented and control group (P<0.05), respectively. Post hoc analysis revealed a significant reduction of 30% in NF-\( \kappa \)B levels within the carotenoid-supplemented group (P=0.006; Table 3). No significant differences were observed regarding the MDA levels throughout the 3 months intervention in either group (Table 3).

**Antioxidant vitamin levels**

No significant differences were observed between the treatment and control group for vitamin A, vitamin C, and vitamin E levels at neither baseline nor the change in 3 weeks or 3 months. Mean baseline levels (±SD) for vitamin A were 2.35±0.73 \( \mu \)M and 2.47±0.53 \( \mu \)M for the treatment and control group, respectively. ANOVA with carotenoid supplementation and fiber effect on the uric acid levels revealed no effect of supplementation at baseline, 3 weeks and 3 months. Mean baseline levels were 236.7±14.9 \( \mu \)M for the treatment group vs. 250.9±12.2 \( \mu \)M for the control group.

**Discussion**

Most commercially available enteral formulas do not contain carotenoids. This results in low serum levels of carotenoids in patients receiving enteral feeding as their sole or primary source of nutrition. The present study examined the effects of an enteral formula supplemented with carotenoids on

<table>
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<th>Carotenoid</th>
<th>Treatment group</th>
<th>Control group</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>( \Delta 3 ) weeks</td>
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<tr>
<td>( \beta )-carotene</td>
<td>15.7 (11.0, 20.4)</td>
<td>+188.0a (145.4, 230.5)</td>
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<td>Lycopene</td>
<td>6.5 (4.9, 8.1)</td>
<td>+73.6a (53.3, 93.9)</td>
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<tr>
<td>Lutein</td>
<td>43.9 (35.3, 57.5)</td>
<td>+14.3a (7.8, 20.8)</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>10.6 (8.5, 12.7)</td>
<td>+2.7a (1.5, 3.8)</td>
</tr>
<tr>
<td>Canthaxanthinb,c</td>
<td>4.9 (4.9, 4.9)</td>
<td>+1.4 (0.0, 2.8)</td>
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<tr>
<td>Cryptoxanthinb,c</td>
<td>9.1 (4.9, 17.4)</td>
<td>+0.3d (0.0, 2.7)</td>
</tr>
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</table>

aSignificantly different between treatment group and control group; P<0.001. bMedian levels (25 and 75 percentiles). cSignificance levels of Mann–Whitney test. dSignificantly different between treatment group and control group; P<0.005. eSignificantly different between treatment group and control group; P<0.01.
Supplemented carotenoids in long-term enterally fed patients

Table 3  Mean levels and confidence interval of NF-κB activation (μg NF-κB equivalent/μg nuclear protein) and MDA (μM).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Baseline</th>
<th>Δ3 weeks</th>
<th>Δ3 months</th>
<th>Control group</th>
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<tr>
<td>(1.24, 1.68)</td>
<td>(-0.37, 0.02)</td>
<td>(-0.75, -0.14)</td>
<td>(0.89, 1.33)</td>
<td>(-0.22, 0.03)</td>
<td>(-0.34, 0.11)</td>
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</tr>
<tr>
<td>1.46*</td>
<td>-0.19*</td>
<td>-0.44*</td>
<td>1.11</td>
<td>-0.10</td>
<td>-0.11</td>
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<tr>
<td>(2.73, 4.66)</td>
<td>(0.89, 1.33)</td>
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<tr>
<td>MDA</td>
<td>0.33</td>
<td>0.18</td>
<td>0.44</td>
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<td>3.06</td>
<td>0.33</td>
<td>0.18</td>
<td>3.70</td>
<td>-0.41</td>
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<td>(2.13, 3.99)</td>
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<td>(0.89, 1.33)</td>
<td>(0.89, 1.33)</td>
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</tr>
</tbody>
</table>

P values between groups *0.01 < P < 0.05 (significance levels of Mann–Whitney test).
P values compared with baseline *0.01 < P < 0.05, *0.005 < P < 0.01.

the serum carotenoid levels and oxidative stress in patients receiving enteral nutrition for three months. The extremely low baseline carotenoid levels measured in this study population compare with findings of previous studies in similar patient groups.8,10,27 These studies reported low plasma β-carotene and lutein levels of about 0.04 and 0.05 μmol/l respectively in long-term enterally fed patients.

Low intake and low plasma levels of carotenoids are associated with a higher risk of morbidity and mortality from chronic diseases.12,14,15 De Waart et al.14 observed that the sum of serum levels of β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin were inversely associated with 7.2-year all-cause mortality in healthy independently living elderly persons. In cancer patients, high plasma lycopene was significantly correlated with a reduced risk of dying during chemoprevention.12 Plasma carotenoid levels have also been related in chronic diseases and complications such as age-related cataract and age-related macula degeneration,28 the incidence of acute respiratory infections,29 and risk of cardiovascular disease.13 Van de Horst-Graat et al.29 suggested that non-institutionalized elderly with a high plasma lycopene level have a lower occurrence of acute respiratory infections. Epidemiological studies have found inverse associations between serum and adipose β-carotene levels and cardiovascular disease.13 These studies emphasize the importance of normal dietary intake (and serum levels) of carotenoids. Our findings demonstrated that the addition of carotenoids to enteral formulas normalizes serum carotenoid levels in elderly patients receiving long-term enteral feeding to the lower end of the range found in age-matched controls, whereas the non-supplemented patients remained depleted throughout the study period of 3 months.

As outlined in the introduction, carotenoids are effective antioxidants. We measured MDA levels and NF-κB activation as indicators for oxidative stress. Some studies have shown a significant decrease in plasma MDA after β-carotene supplementation,30,31 while other studies found no MDA-reducing effect of β-carotene supplementation.32,33 In the present study no changes were observed within the two groups. It has to be noted that although MDA is used frequently as a biomarker for oxidative stress, the validity of using MDA in clinical trials to examine the efficacy of antioxidant supplementation has recently been criticized.34 It is becoming increasingly clear that this biomarker lacks specificity when applied to human plasma due to instability and cross-reactivity of MDA.

An increase in NF-κB activation has been reported in several chronic diseases compared with NF-κB activation in healthy age-matched controls.18–20 For example, NF-κB activation in sarcoidosis patients is twice as high as that in healthy subjects.19 Subclinical deficiencies in micronutrients, that are frequently observed in such a patient population, might lead to an increased activation of NF-κB with an increased production of pro-inflammatory cytokines.35 Additionally, various antioxidants such as vitamin E and C are known to inhibit NF-κB activation in vitro as well as in vivo.18,36–38 Interestingly, Bai et al.38 recently showed that β-carotene suppressed NF-κB activation and NF-κB-dependent expression of inflammatory genes in LPS-stimulated macrophages and LPS-treated mice. The results of the present study demonstrated a significant decrease in NF-κB activation when enterally fed patients were given
a formula supplemented with carotenoids. In this study, a randomization imbalance was observed between the baseline NF-κB value of the carotenoid-supplemented group (1.46 ± 0.50 μg NF-κB equivalent/μg protein) and the control group (1.11 ± 0.51 μg NF-κB equivalent/μg protein). Therefore, a post hoc analysis was performed to assess whether the activation at baseline influences dynamics of NF-κB. The control group was split in two sub-groups, namely patients with baseline levels of NF-κB either below or above the mean value (1.15 μg NF-κB equivalent/μg protein). In both control sub-groups (i.e. low and high baseline values) no decrease in NF-κB activation after 3 months was shown (P = 0.26 and 0.72, respectively). This indicates that, despite the randomization imbalance, it can be concluded that the carotenoid supplementation reduced NF-κB activation. Interestingly, the 30% decrease of NF-κB activation observed in the carotenoid-supplemented group in the present study is similar to the decrease shown after a 3-day intervention study with lipoic acid in diabetic patients with nephropathy. In accordance with our findings, the in vitro and in vivo observations indicate that antioxidants play an important role in NF-κB inhibition.

In conclusion, these results demonstrated that 3 months of carotenoid-enriched (3-mg/1500 kcal) enteral nutrition normalized serum carotenoid levels and significantly reduced the NF-κB activation in a group of enterally fed elderly patients deprived of carotenoids. The reduced NF-κB activation as biomarker indicates a lower oxidative stress in the supplemented group. Emerging evidence in the literature indicates that patients with low intake and low serum levels of carotenoids have a higher risk of morbidity and mortality from chronic diseases. These findings imply that enteral formulas intended for use over a significant period of time should provide a carotenoid intake comparable to the average content of a normal healthy diet.

Acknowledgments

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References


