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ORIGINAL ARTICLE

Altered status of antioxidant vitamins and fatty acids in patients with inactive inflammatory bowel disease[☆]

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Summary

Background & aims: Data regarding the nutritional status, antioxidant compounds and plasma fatty acid (FA) composition in inactive IBD are conflicting. We compared plasma levels of antioxidants and FA of patients with inactive IBD with active IBD and controls.

Methods: Plasma levels of vitamin C, vitamin E, carotenoids, saturated, monounsaturated and polyunsaturated FA, inflammatory markers and nutritional status were determined after an overnight fast in 132 patients with quiescent IBD (40.6 ± 13.2 years, 87F/45M), 35 patients with active disease (37.9 ± 12.1 years, 25F/10M) and 45 age- and BMI-matched healthy controls (38.1 ± 10.5 years, 39F/6M). Results are expressed as mean \pm SD or median [25th percentile;75th percentile].

Abbreviations: AAG, acid-alpha-1 glycoprotein; CAI, ulcerative colitis activity index; CD, Crohn's disease; CDAI, Crohn's disease activity index; DRI, daily reference intake; EFA, essential fatty acids; FA, fatty acids; IBD, inflammatory bowel disease; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SGA, subjective global assessment; UC, ulcerative colitis.

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Results: Body mass index (BMI) was normal in inactive ($23.9 \pm 4.7 \text{ kg/m}^2$), active IBD ($22.7 \pm 4.2 \text{ kg/m}^2$) and controls ($22.3 \pm 1.9 \text{ kg/m}^2$). Compared with controls patients with quiescent IBD showed significantly decreased plasma levels of carotenoids ($1.85 [1.37;2.56]$ vs $1.39 [0.88;1.87] \mu\text{mol/L}$) and vitamin C ($62.3 [48.7;75.0]$ vs $51.0 [36.4;77.6] \mu\text{mol/L}$), increased levels of saturated FA ($3879 [3380;4420]$ vs $3410 [3142;3989] \mu\text{mol/L}$) and monounsaturated FA ($2578 [2258;3089]$ vs $2044 [1836;2434] \mu\text{mol/L}$) and similar levels of vitamin E and polyunsaturated FA. Results in active disease were similar to inactive disease.

Conclusion: This study shows that antioxidant status and FA profile in a larger population of IBD patients are disturbed independently from disease activity and despite normal overall nutritional status.

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Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel disease entities (IBD) with still unknown aetiology. Past clinical trials indicated that oxidative stress plays an important role in the initiation and progression of these diseases.^{1,2} The increased generation of reactive oxygen species or the exhaust of endogenous and exogenous capacity in active disease may lead to a lack of antioxidants as well as to lipid peroxidation in cellular membranes. An impairment of membrane fluidity and membrane proteins is the consequence. Furthermore, an altered profile of polyunsaturated fatty acids, the main target of reactive oxygen species and precursors of eicosanoids, appears as a significant parameter in the inflammatory process.²⁻⁵

Previous studies investigated nutritional antioxidants and fatty acids mostly in patients with active IBD and with small sample sizes. Compared to healthy controls reduced levels of total plasma zinc,^{3,6} selenium⁶⁻⁸ as well as β -carotene,^{3,9} total carotenoids,¹⁰⁻¹² vitamin C^{2,3,9,12} and vitamin E¹³ have been reported. Abnormal serum and mucosal fatty acid patterns have been described in some publications, however, with inconsistent results.^{2,14-17} Some of the inconsistencies might be explained by differences in the inflammatory status and small sample sizes. In summary, data on antioxidant micronutrients and fatty acid profiles for inactive IBD are scarce and not conclusive. Additionally, recent studies suggest^{27,28} that improvement of medical therapies over the past decades led to a generally good nutritional status of IBD patients with inactive disease. Therefore, it is important to know whether micronutrient deficits and metabolic alterations still persist in the presence of apparently normal overall nutritional status.

The aim of the present study was to examine overall nutritional status, antioxidant micronutrients, plasma fatty acids and inflammatory markers in an adequately sized cohort of IBD patients in remission and to compare results with healthy controls and patients with active IBD. We hypothesized that even patients with quiescent IBD show alterations in antioxidant micronutrients and fatty acids as compared to healthy controls, though less pronounced than in active disease and that these alterations are associated with inflammatory activity.

Materials and methods

Patients

In total, 167 IBD patients (CD: $n = 100$, 37.7 ± 11.3 years; UC: $n = 67$, 42.8 ± 14.6 years) between 18 and 70 years were recruited in three centers (Berlin, Germany, $n = 145$; Vienna, Austria, $n = 15$; Bari, Italy, $n = 7$) from September 2004 to February 2006.

The diagnosis was based on histological, endoscopic and radiological findings. One hundred and thirty two patients were in a quiescent phase of disease, which was defined by Crohn's Disease Activity Index (CDAI)¹⁸ <150 or Ulcerative Colitis Activity Index (CAI)¹⁹ ≤ 5 and 35 patients were in an active phase of disease, which was defined by CDAI ≥ 150 or CAI >5 . Disease duration ranged between 7.8 [3.2;13.8] years for CD and 9.0 [5.5;14.5] years for UC. The ileocolon (41.3%) or the upper gastrointestinal tract (30.4%) was the most common disease site in CD patients. Fifty-nine percent of UC patients had a pancolitis and 41% a distal colitis. None of the patients had an extensive small bowel resection; 44.2% of CD and 10% of UC patients had intestinal resections.

Exclusion criteria for patients were severe concomitant disease, celiac disease, pregnancy, ostomy, deliberate adherence to an extreme diet (vegan, macrobiotics, etc.) and supplementation of micronutrients or fatty acids of more than one DRI (daily reference intake) value per day.

Healthy controls

Forty-five age- and BMI-matched healthy controls were recruited from the general population of Berlin. Health was defined as absence of acute or chronic disease, no acute or chronic medication and all standard routine blood parameters within the normal range. Exclusion criteria were smoking, deliberate adherence to an extreme diet (vegan, macrobiotics, etc.) and supplementation of micronutrients or fatty acids of more than one DRI value per day.

The study was approved by the ethics committee of the coordinating center Charité – Universitätsmedizin Berlin, Germany, and by the local ethics committees of each study center. All IBD patients and healthy controls gave informed consent.

Nutritional status

Nutritional status was assessed by two trained investigators [LS, SH] according to body mass index (BMI), subjective global assessment (SGA)²⁰ and serum albumin. SGA assesses the nutritional status based on medical history (changes in weight, dietary intake, functional capacity, gastrointestinal symptoms), physical examination (loss of subcutaneous fat, muscle wasting, sacral and ankle edema, ascites) and the clinician's overall judgment of the patient's status. Patients were classified into well-nourished (SGA-A), mild-to-moderately malnourished (SGA-B) or severely malnourished (SGA-C). BMI <18.5 kg/m², SGA-B or SGA-C or serum albumin <36 g/L indicated malnutrition, respectively.

Food frequency questionnaire

Food intake was analysed by using a standard food frequency questionnaire. It contains 67 food items and the subjects were asked to indicate the frequency of intake for each item (0 points: never; 1 point: rarely to once a month, 2 points: once a week to every second week, 3 points: several times a week to daily). The food items were summarized into eight food groups for analysis (meat & fish, milk products, non-sweet carbohydrates, fruits & vegetables, sweets, fast food, alcohol, oils & fats).

Inflammatory status

C-reactive protein (CRP), alpha-1-acid glycoprotein (AAG) and albumin were analysed with routine methods.

Faecal calprotectin

Faecal samples (80–120 mg) were collected in plastic containers and stored at –20 °C until analysis. Samples from Vienna and Bari were dispatched with dry ice to Berlin. Faecal calprotectin was analysed at the Berlin center using a commercial enzyme linked immunoassay (ELISA) method (Calprotectin (mRP8-14), Immundiagnostik AG, Bensheim, Germany) following the manufacturer's instructions. Concentration >15 mg/L indicated an increased local intestinal inflammation.

Antioxidant micronutrients

Trace elements

Venous blood samples were obtained after an overnight fast. Copper, selenium and zinc were analysed with routine methods in use at the hospitals in Berlin, Vienna and Bari.

Vitamins, provitamins

For analyses of antioxidant vitamins and fatty acids blood was drawn and prepared under required conditions (overnight fast, light-protected vials, centrifugation at –6 °C and storage at –80 °C within 30 min). For analyses of vitamin C metaphosphoric acid was added 1:1 to EDTA plasma after centrifugation. Samples of all three centers were dispatched for analyses with dry ice to the "Human Nutrition & Metabolism Research and Training Center, University of Graz", Graz, Austria.

Antioxidant vitamins and provitamins were assessed by plasma concentrations of vitamin C, vitamin E (α - and γ -tocopherols), lycopene, lutein/zeaxanthin, β -cryptoxanthin, α - and β -carotenes. Plasma ascorbate concentrations were determined according to Levine et al. and Lykkesfeldt et al.^{21,22} The mix of plasma and metaphosphoric acid was defrosted and centrifuged (12 000 \times g) for 3 min, injected onto the reverse-phase column (Zorbax SB C18 Stable Bond 4.6 \times 250.5 μ m), an electrochemical detector and an analytic cell (200 mV, E1 upstream, +150 mV, E2 downstream). Tocopherols and carotenoids were analysed by HPLC according to Aebischer et al.²³ EDTA plasma was deproteinised with 400 μ L ethanol and the extraction of the analytes of interest was performed using hexane. The residue was diluted with methanol and dioxane (1:1) and solved in acetonitrile. With the use of the reversed-phase-HPLC α - and γ -tocopherols were analysed by a fluorescence detector at 298 nm extinction and 328 nm emission, carotenoids were analysed with a UV detector at 472 nm (lycopene) and 450 nm (lutein/zeaxanthin, β -cryptoxanthin, α - and β -carotenes).²³

Fatty acid profile

The determination of the total fatty acid content in plasma was based on an esterification procedure and a subsequent gas-chromatographic (GC) analysis of the fatty acid methyl esters as described by Sattler et al.²⁴ All determinations were done in duplicate and in the same run. EDTA plasma was thawed slowly in the thermomixer and centrifuged in the microfuge centrifuge for 3 min. One hundred microliters of internal standard (10 mg heptadecanoic acid and 100 mg BHT in 10 mL methanol) and 450 μ L of the EDTA plasma were added to a Teflon screw-capped tube vortexed and then kept at –80 °C for a minimum of 30 min. The deep-fried suspension was freeze dried for about 15 h. After 1 mL boron trifluoride–methanol-complex and 500 μ L toluene were added, the Pyrex tubes were closed and transesterification was performed at 110 °C for 90 min. Following the transesterification step 2 mL aqua dest was added and the fatty acid methyl esters were extracted three times with *n*-hexane. The hexane extracts were dried in a Speed Vac at room temperature for 30 min, redissolved dichloromethane, and subjected to GC analysis. Heptadecanoic acid was used as internal standard and added to each tube with the plasma samples. Nineteen plasma samples of the patients were processed along with a control sample from the plasma pool. Separation of fatty acid methyl esters was achieved on a DB-23. The mobile phase was a mixture of helium and hydrogen gas. The oven temperature at injection was 150 °C and was raised stepwise to 255 °C (20 °C/min). The areas under the GC peaks were quantified by integration and the internal standard described above was used for calculation of the amounts of fatty acids (modification of the method of Sattler et al.).²⁴ Coefficients of variation (within-run) for the different fatty acids were between 0.38% and 8.28%.

In total, 21 fatty acids were analysed. The following fatty acid indices were recorded: Sum of all fatty acids (Σ FA), sum of saturated fatty acids (Σ SFA), sum of mono-unsaturated fatty acids (Σ MUFA), sum of polyunsaturated

fatty acids (Σ PUFA), sum of n-3 fatty acids (n-3) and sum of n-6 fatty acids (n-6). Furthermore, the ratio of n-3 and n-6 fatty acids was calculated as well as the sum of essential fatty acids (EFA index, Σ n-6 + Σ n-3/ Σ n-7 + Σ n-9).⁴

Statistical analysis

We used SPSS 12.0 for Windows (SPSS Inc., Chicago Ill., USA) for all statistical analyses. Results were considered statistically different at the $P < .05$ and data were analysed by mean \pm SD or median [25th percentile;75th percentile]. Data were analysed concerning normal distribution by the Kolmogorov–Smirnov test. Differences of inflammatory markers, antioxidants and fatty acids between patients from Berlin, Vienna and Bari as well as between patients with quiescent IBD, active disease and healthy controls were analysed with univariate analysis of variance (ANOVA). To determine associations between antioxidants and fatty acids as well as antioxidants/fatty acids and inflammatory markers or markers of nutritional status Spearman coefficient of correlation (r) and linear regression analysis was used.

According to detailed statistical analyses (ANOVA) there was no difference in nutritional status as well as biochemical markers of inflammation, antioxidants and fatty acids between the three centers. All calculations were therefore performed in the combined group.

Results

Nutritional status

The majority of patients with quiescent IBD were in normal nutritional status according to BMI (94.2%), SGA-A (86.2%) and albumin levels (96.3%) (Table 1). Active IBD, however, was associated with an impaired nutritional status in 11.4% (BMI <18.5 kg/m²), 60.0% (SGA-B + C) and 14.3% (albumin <36 g/L) of patients.

Food intake

Food frequency questionnaire analysis resulted in a significantly lower intake of fruits and vegetables in patients with quiescent IBD (11 [8;12] points, $P < 0.001$) and active IBD (11 [9;13] points, $P = 0.003$) as compared with healthy controls (13 [12;14] points). We could not find any differences in the remaining food groups.

Inflammatory status

CRP, AAG and faecal calprotectin were significantly higher in patients with quiescent IBD as compared with healthy controls (Table 1). Still, 83.8% and 89.1% of patients with quiescent IBD had normal CRP (<10 mg/L) or AAG (<1200 mg/L), respectively. Faecal calprotectin, however, was above the normal range in 55.7% of patients with quiescent IBD. Significantly higher levels of CRP, AAG and faecal calprotectin were found in patients with active IBD as compared with patients with quiescent IBD and healthy controls.

Antioxidant micronutrients

Plasma levels of vitamin C, lutein/zeaxanthin, β -cryptoxanthin, lycopene, α - and β -carotenes and Σ of carotenoids were decreased in patients with quiescent IBD as compared with healthy controls whereas no differences were found for vitamin E, copper, selenium and zinc values (Table 2). Antioxidant levels in active IBD were similar to those in patients with quiescent IBD, except for a further decrease in lycopene and total carotenoids and an increase in copper levels. Age, gender, disease location, intestinal resections, nutritional status and smoking had no influence on the investigated antioxidant levels.

Fatty acid profile

Aberrations of the fatty acid profile in patients with quiescent IBD were mainly seen for saturated FA (SFA) and monounsaturated FA (MUFA) as compared with healthy controls (Table 3). Plasma values of tetradecanoic (C15:0) and lignoceric acid (C24:0) were decreased, while increased values were seen for palmitic (C16:0), stearic (C18:0), vaccenic (C18:1n-7) and oleic acid (C18:1n-9) in patients with quiescent IBD. Polyunsaturated FA (PUFA) were similar to healthy controls, except for increased values of docosatetraenoic acid (C22:4n-6). Σ FA, Σ SFA and Σ MUFA were significantly higher and Σ PUFA similar, while EFA index was significantly lower in patients with quiescent IBD as compared to healthy controls. In contrast to our expectations, the FA profile in active IBD was similar to that in inactive IBD.

Age, gender, disease location, intestinal resections, nutritional status and smoking did not have an influence on the investigated fatty acids.

Correlations between inflammation, nutritional status, antioxidants and fatty acids

Interestingly, we found no significant differences in plasma antioxidants and fatty acids between patients with inactive and active diseases, except for copper, lycopene and total carotenoids. Nutritional status according to BMI and SGA did not influence plasma antioxidants or fatty acids. However, serum albumin <36 g/L was associated with lower levels of total carotenoids in both inactive ($P = 0.020$) and active IBD ($P = 0.009$).

In quiescent IBD, but not in active IBD and controls, total carotenoids and vitamin C correlated with PUFA and EFA index (Table 4). Furthermore, when dividing carotenoids and vitamin C into a low level ($<$ median) and a high level group ($>$ median) in inactive IBD, the low level group was associated with increased CRP and AAG levels.

Discussion

In the present study, antioxidant micronutrients, plasma fatty acids and inflammatory markers were investigated in a multi-national cohort of IBD patients with quiescent disease and compared to healthy controls and patients with active disease. The main result was that in patients with quiescent IBD aberrations of antioxidant micronutrients

Table 1 Inflammatory and nutritional status of patients with quiescent and active IBD and healthy controls

	Inactive IBD (n = 132)	P*	Active IBD (n = 35)	P†	Healthy controls (n = 45)	P‡
<i>Markers of disease activity</i>						
CDAI	62 [35;115]	<0.001	251 [221;316]			
CAI	3 [2;5]	<0.001	8 [7;11]			
CRP (mg/L)	2.0 [0.8;6.3]	<0.001	6.6 [1.8;26.7]	<0.001	0.8 [0.5;1.6]	0.009
AAG (mg/L)	770 [610;985]	<0.001	1080 [860;1380]	<0.001	630 [520;760]	0.006
Faecal calprotectin (mg/L)	17.3 [3.0;36.6]	<0.001	61.9 [15.2;102.3]	<0.001	0 [0;0]	0.010
<i>Nutritional status</i>						
BMI (kg/m ²)	23.9 ± 4.7	0.415	22.7 ± 4.2	1.0	22.3 ± 1.9	0.093
SGA-A (%)	86.2		40.0		100.0	
SGA-B (%)	13.0		42.9		0	
SGA-C (%)	0.7		17.1		0	
Albumin (g/L)	44.5 [42.2;47.0]	<0.001	41.0 [39.0;45.0]	<0.001	46.0 [44.0;47.0]	0.175
Smoking (%)	25.9		40.7		0.0	

Data are presented as mean ± SD or median [25th percentile;75th percentile], ANOVA and Bonferroni correction were used to detect significant differences between groups. *Inactive IBD vs active IBD, †active IBD vs controls, ‡inactive IBD vs controls, CDAI Crohn's disease activity index, CAI ulcerative colitis activity index, AAG alpha-1-acid glycoprotein, BMI body mass index, SGA subjective global assessment, SGA-A well-nourished, SGA-B mildly-to-moderate malnourished, SGA-C severely malnourished.

and fatty acid profile persisted at the same level as seen in patients with active IBD, despite markedly improved general nutritional status. These persisting alterations might be explained at least partly by the residual inflammatory activity.

Some of our findings are in agreement and some others in conflict with past investigations. Two factors might contribute to explaining the discrepancies to previous results and should therefore be mentioned first. Publications up to the year 2000 report a prevalence of malnutrition of about 40% for patients with quiescent IBD²⁵ and up to 80% for patients with active IBD.^{18,26} The majority of previous investigations on micronutrients and fatty acids in IBD patients fall in this category. Our patients, however, showed a lower prevalence of malnutrition especially in patients with quiescent IBD (13.7%) but also in active disease (60.0% of which the majority had only mild malnutrition). This finding is in agreement with more recent studies.^{27–29} This might reflect an improvement in the IBD therapy over

the past years with favorable effects on inflammatory and nutritional status.^{28,30}

Regarding trace elements with antioxidant functions, previous investigations reported reduced plasma selenium and zinc levels in IBD patients as compared to healthy controls.^{2,3,6–8} We could not find any differences in those trace elements, not even in active disease. In agreement with previous studies^{8,31} we found trends for elevated copper levels in active disease, an element which is known to be increased in many inflammatory conditions.³²

Data on vitamin C^{3,12,13} and carotenoids^{3,10–12} in quiescent IBD are scarce and controversial. We found decreased levels of vitamin C, lycopene, lutein/zeaxanthin, β-cryptoxanthin, α- and β-carotenes and total carotenoids despite minimal signs of inflammation in quiescent disease. Furthermore, smoking habits did not affect plasma levels and differences between quiescent and active disease were not significant, except for copper, lycopene and total carotenoids. It therefore appears that the decrease in

Table 2 Antioxidant micronutrients, vitamins and provitamins (μmol/L) of patients with quiescent and active IBD and healthy controls

	Inactive IBD (n = 132)	P*	Active IBD (n = 35)	P†	Healthy controls (n = 45)	P‡
Copper	19.1 [16.2;23.3]	0.040	21.9 [17.2;28.6]	0.018	17.8 [15.7;24.6]	1.000
Selenium	0.87 [0.73;1.00]	0.289	0.83 [0.65;0.95]	0.082	0.96 [0.77;1.05]	0.901
Zinc	14.3 [12.5;16.2]	0.139	13.5 [12.1;15.4]	1.000	14.0 [12.7;15.4]	0.525
Vitamin C	62.3 [48.7;75.0]	0.148	51.0 [36.4;77.6]	<0.001	73.6 [61.0;86.6]	0.001
Vitamin E	31.2 [26.2;36.3]	1.000	30.7 [27.3;36.0]	0.415	30.4 [23.9;33.1]	0.156
Lutein/zeaxanthin	0.379 [0.269;0.491]	0.052	0.295 [0.213;0.405]	<0.001	0.501 [0.394;0.630]	<0.001
β-Cryptoxanthin	0.200 [0.107;0.310]	1.000	0.162 [0.084;0.282]	0.014	0.350 [0.218;0.475]	0.002
Lycopene	0.562 [0.344;0.754]	0.009	0.383 [0.232;0.508]	<0.001	0.732 [0.508;0.799]	0.026
β-Carotene	0.512 [0.314;0.917]	0.104	0.330 [0.229;0.638]	<0.001	0.976 [0.682;1.466]	<0.001
α-Carotene	0.092 [0.055;0.162]	0.515	0.059 [0.036;0.119]	<0.001	0.223 [0.153;0.354]	<0.001
ΣCarotenoids	1.85 [1.37;2.56]	0.021	1.39 [0.88;1.87]	<0.001	2.91 [2.36;3.78]	<0.001

Data are presented as mean ± SD or median [25th percentile;75th percentile], ANOVA and Bonferroni correction were used to detect significant differences between groups. *Inactive IBD vs active IBD, †active IBD vs controls, ‡inactive IBD vs controls.

Table 3 Fatty acid profiles ($\mu\text{mol/L}$) of patients with quiescent and active IBD and healthy controls

$\mu\text{mol/L}$	Inactive IBD ($n = 132$)	P^*	Active IBD ($n = 35$)	P^\dagger	Controls ($n = 45$)	P^\ddagger
C14:0	141 [112;204]	1.000	140 [82;225]	0.149	127 [101;163]	0.092
C15:0	101 [84;119]	0.735	89 [74;109]	0.003	116 [97;134]	0.006
C16:0	2772 [2390;3193]	1.000	2768 [2177;3596]	0.020	2371 [2219;2896]	0.015
C18:0	693 [616;809]	1.000	648 [542;823]	0.668	645 [582;712]	0.042
C20:0	25 [21;29]	1.000	24 [20;30]	0.104	27 [24;30]	0.101
C22:0	56 [47;68]	0.971	52 [45;65]	0.164	35 [29;42]	0.438
C24:0	35 [29;42]	0.080	32 [26;40]	<0.001	41 [34;47]	0.004
C18:3n-3	57 [42;80]	1.000	58 [38;80]	1.000	51 [45;69]	1.000
C18:4n-3	8 [6;10]	1.000	8 [6;10]	1.000	7 [6;10]	1.000
C20:5n-3	42 [31;66]	0.786	40 [25;79]	1.000	49 [31;88]	0.289
C22:5n-3	28 [23;34]	1.000	26 [20;36]	1.000	26 [23;32]	0.684
C22:6n-3	101 [68;129]	1.000	90 [63;133]	1.000	114 [86;142]	0.67
C18:2n-6	2729 [2360;3220]	0.105	2612 [2185;2846]	0.742	2670 [2296;3131]	1.000
C18:3n-6	44 [33;58]	1.000	42 [24;56]	1.000	39 [32;48]	0.231
C20:3n-6	128 [110;160]	0.673	125 [86;167]	1.000	114 [99;147]	0.653
C20:4n-6	546 [457;659]	1.000	545 [444;636]	1.000	505 [410;658]	1.000
C22:4n-6	13 [11;16]	1.000	13 [10;16]	0.334	11 [10;14]	0.013
C16:1n-7	267 [204;381]	1.000	276 [192;414]	0.066	219 [173;290]	0.077
C18:1n-7	182 [154;215]	0.806	197 [150;209]	0.073	151 [129;178]	<0.001
C18:1n-9	2159 [1842;2597]	1.000	2263 [1846;2646]	0.001	1691 [1517;1858]	<0.001
C22:1n-9	5 [4;6]	1.000	6 [5;6]	1.000	5 [4;6]	1.000
FA	10354 [9138;11707]	1.000	10195 [8336;11974]	0.225	9460 [8237;10423]	0.011
SFA	3879 [3380;4420]	1.000	3786 [3066;4868]	0.067	3410 [3142;3989]	0.028
MUFA	2578 [2258;3089]	1.000	2810 [2196;3302]	<0.001	2044 [1836;2434]	<0.001
PUFA	3699 [3218;4339]	0.235	3646 [3087;4316]	1.000	3743 [3099;4143]	1.000
n-3	246 [185;297]	1.000	226 [175;347]	1.000	248 [202;329]	0.919
n-6	3482 [3019;4063]	0.142	3373 [2871;3840]	1.000	3379 [2903;3898]	0.967
n-3/n-6	14.9 [11.9;17.5]	0.300	13.9 [11.7;15.9]	1.000	13.6 [10.0;17.4]	0.476
EFA index	1.4 [1.2;1.6]	0.204	1.3 [1.1;1.5]	<0.001	1.8 [1.5;2.0]	<0.001

Data are presented as mean \pm SD or median [25th percentile;75th percentile], ANOVA and Bonferroni correction were used to detect significant differences between groups. *Inactive IBD vs active IBD, \dagger active IBD vs controls, \ddagger inactive IBD vs controls, FA fatty acid, SFA saturated FA, MUFA monounsaturated FA, PUFA polyunsaturated FA, EFA essential FA.

these antioxidants is not a sole consequence of inflammation and malnutrition but maybe rather due to different eating habits. Food frequency analyses resulted in a reduced intake of fruits and vegetables. This might even offer the opportunity of dietary recommendations for patients. The similar intake patterns of patients with quiescent disease as compared to active disease can be explained

by the nature of the food frequency questionnaire, which asks for usual and not actual intake pattern.

In the current investigation, patients with quiescent and active IBD had significantly increased plasma levels of total fatty acids (FA), saturated FA and monounsaturated FA and similar levels of polyunsaturated FA compared with healthy controls. In previous studies regarding FA, results for saturated FA and monounsaturated FA were inconsistent,^{2,13–15} but most of the studies reported decreased levels of polyunsaturated FA in inactive^{2,4} and active disease.^{13,15}

Increased levels of FA, saturated FA and monounsaturated FA might be attributed to increased lipolysis initiated by cytokines during the inflammatory response. Khovidhunkit et al. described higher hepatic fatty acid synthesis, activation of lipolysis as well as suppression of fatty acid oxidation in association with inflammation.³³ We mainly found elevated levels of saturated and monounsaturated FA of the C16 and C18 series, which account for the highest amount of endogenous synthesized FA in human metabolism and which are preferentially stored in biological cell membranes. Furthermore, Siguel and Leman reported a replacement of n-6 fatty acids by monounsaturated FA in cellular membranes to regulate cell function as well as membrane fluidity during n-6-fatty acid deficiency.³⁴

Table 4 Correlations between total carotenoids/vitamin C and fatty acids in patients with inactive IBD

	Total carotenoids ($n = 132$)		Vitamin C ($n = 132$)	
	r	P	r	P
Σ FA	0.124	0.108	0.038	0.669
Σ SFA	0.085	0.271	0.038	0.664
Σ MUFA	-0.095	0.221	-0.086	0.333
Σ PUFA	0.377	<0.001	0.163	0.063
Σ n-3	0.295	<0.001	0.239	0.006
Σ n-6	0.372	<0.001	0.145	0.100
EFA index	0.510	<0.001	0.235	0.007

FA fatty acid, SFA saturated FA, MUFA monounsaturated FA, PUFA polyunsaturated FA, EFA essential FA, r Spearman coefficient of correlation.

Therefore, it is tempting to speculate that the up-regulation of monounsaturated FA results in polyunsaturated FA sparing effect, which could explain the still balanced polyunsaturated FA levels in our patients, who had, as previously mentioned, a lower inflammatory burden than patients in older publications.

In conclusion, despite good nutritional status and low inflammatory activity in patients with quiescent IBD, antioxidant vitamins and fatty acids show significant alterations similar to those observed in active disease. Low levels of vitamin C and carotenoids can be explained by a combined effect of inflammation and low intake of fruits and vegetables. Aberrations in the fatty acid profile follow the pattern of increased lipolysis also observed in other inflammatory conditions. More research is necessary to investigate if the alterations observed are clinically meaningful and if they deteriorate the progress of disease. Since plasma levels of antioxidants are below normal ranges in most patients with IBD increased intake of fruits and vegetables as well as supplements may be beneficial as in a healthy population. However it cannot be concluded if such supplements would prevent relapses or reduce disease activity.

Conflict of interest statement

All authors stated having no conflict of interest related to the authorship of the submitted paper.

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SH and LV have equally contributed to the writing of the manuscript, SH has further done the data analyses and LV designed the study. SH, LS, TK collected the data in Berlin, WT in Vienna and NR in Bari. MM and BWR analysed the antioxidants and fatty acids and SB analysed faecal calprotectin. CB, WT, FG, BWR and HL provided their expertise in the interpretation and discussion of the results and made substantial suggestions on the manuscript.

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