

Effects of Orlistat Therapy on Plasma Concentrations of Oxygenated and Hydrocarbon Carotenoids

Isabella Sundl^a, Elisabeth Pail^b, Karin Mellitzer^b,
Hermann Toplak^b, and Brigitte M. Winklhofer-Roob^{a,*}

^aHuman Nutrition & Metabolism Research and Training Center Graz, Institute of Molecular Biosciences, Karl-Franzens University, 8010 Graz, Austria, and ^bDepartment of Internal Medicine, Medical University, 8010 Graz, Austria

ABSTRACT: Orlistat is a lipase inhibitor that is applied for treating obesity. Lipases are required for digestion and absorption of dietary lipids and fat-soluble vitamins and carotenoids. The aim of this study was to compare the effects of orlistat therapy on plasma concentrations of oxygenated (β -cryptoxanthin, lutein/zeaxanthin) and hydrocarbon (α -, β -carotene, lycopene) carotenoids. Six patients with a body mass index (BMI) ≥ 30 kg/m² received 360 mg/d orlistat over 4.5 mon. Plasma carotenoid concentrations were determined at baseline (T_0) and after 3 (T_3) and 4.5 mon ($T_{4.5}$) along with anthropometric, dietary, and biochemical indices, including plasma lipids, retinol, α - and γ -tocopherols, and FA. Baseline BMI was 32.7 ± 1.97 kg/m². Five of six patients lost weight; the average weight loss was $3.6 \pm 2.4\%$ ($P = 0.47$). There were no significant changes in dietary carotenoid intakes. In contrast, plasma α - and β -carotene concentrations decreased significantly from T_0 to $T_{4.5}$ by 45% ($P = 0.006$) and 32% ($P = 0.013$), respectively. Plasma lycopene decreased from T_0 to T_3 but increased again from T_3 to $T_{4.5}$, while β -cryptoxanthin and lutein/zeaxanthin concentrations did not change. There were no significant alterations in tocopherols, retinol, and FA concentrations. In conclusion, even though weight loss was not significant, orlistat therapy was associated with significant decreases in plasma concentrations of the highly lipophilic hydrocarbon carotenoids, α - and β -carotene.

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The prevalence of overweight and obesity has grown progressively over the past two decades in both developing and industrial countries (1). Since 1991, the proportion of obese inhabitants increased from 12 to 21% in the United States, and there are 300 million obese adults worldwide (1,2). Given the strong association between obesity and several chronic diseases such as cardiovascular disease and diabetes mellitus (3), these changes will have major health implications in the near future.

One of the options for long-term treatment of obesity is orlistat, a potent inhibitor of gastric and pancreatic lipases. Lipases hydrolyze TG into absorbable MG and FFA and are thus instrumental in the digestion and absorption of dietary fats from the gastrointestinal tract (4). Orlistat binds to the active site of lipases, resulting in the inhibition of enzyme activity and, subsequently, impaired formation of mixed micelles. Along with

impaired absorption of dietary fat, the absorption of fat-soluble vitamins (5), carotenoids (6), and dietary FA (7) is affected.

The absorption of highly lipophilic compounds such as carotenoids is impaired in patients with clinically overt fat malabsorption, e.g., in patients with cystic fibrosis (8), and in the presence of minimally impaired lipase activity (9). Depending on the chemical structure and subsequent lipophilicity of different carotenoids, the absorption may be affected to different extents. As a consequence, the oxygenated carotenoids β -cryptoxanthin, lutein, and zeaxanthin may be more easily absorbed than the hydrocarbon carotenoids α - and β -carotene and lycopene (10,11).

Different carotenoids exert different biological actions and functions, including antioxidant and immunoenhancing effects, protection against cardiovascular disease and certain types of cancer (lycopene for prostate cancer), and maintenance of normal vision (lutein for age-related macular degeneration) (12). However, the relative importance of the individual carotenoids has not been clearly established.

The aim of our study was to investigate the effects of treatment with the lipase inhibitor orlistat on carotenoid status in general and, more specifically, to compare the effects on hydrocarbon carotenoids with those on oxygenated carotenoids. In addition, the effects on other fat-soluble compounds such as retinol, α - and γ -tocopherol, and FA were analyzed.

EXPERIMENTAL PROCEDURES

Patients. Consecutive patients who attended the Outpatient Clinic for Diabetes and Metabolism at the Department of Internal Medicine, Medical University of Graz, and who had a body mass index (BMI) > 30 kg/m² were eligible for enrollment in the study. Patients were excluded if one or more of the following criteria were present: pregnancy, lactation, cholestasis, chronic malabsorption, and chronic alcoholism. The study protocol was approved by the Ethics Committee of the Medical University of Graz, and written informed consent was obtained from the patients. The six patients enrolled included five postmenopausal women and one man, aged 59.5 ± 3.78 yr (min, 54 yr; max, 65 yr). One patient smoked on average four cigarettes per day, while the others were nonsmokers. Two patients suffered from combined hyperlipidemia and showed elevated TG and LDL cholesterol concentrations. Demographic data are presented in Table 1.

*To whom correspondence should be addressed at Nutrition & Metabolism Research and Training Center Graz, Institute of Molecular Biosciences, Karl-Franzens University, Schubertstrasse 1, A-8010 Graz, Austria. E-mail: brigitte.winklhoferroob@uni-graz.at

Abbreviations: BMI, body mass index; FFQ, Food frequency questionnaire.

TABLE 1
Anthropometric Data, Serum Lipids, Fat-Soluble Vitamins, and FA Concentrations (mean \pm SD) During Orlistat Therapy^a ($n = 6$).

	Time points		
	T_0	T_3	$T_{4.5}$
Weight (kg)	95.2 \pm 11.0	91.4 \pm 10.4	91.8 \pm 10.7
BMI (kg/m ²)	32.7 \pm 1.97	31.4 \pm 1.48	31.5 \pm 1.65
Waist (cm)	110 \pm 4.51	106 \pm 7.82	107 \pm 4.90
Hip (cm)	121 \pm 5.32	119 \pm 4.27	119 \pm 4.72
Waist/hip ratio	0.912 \pm 0.068	0.887 \pm 0.082	0.898 \pm 0.066
Body fat (%)	41.9 \pm 4.62	42.1 \pm 3.78 ^b	43.2 \pm 2.74 ^b
Total cholesterol	7.77 \pm 1.18	7.15 \pm 0.969	7.23 \pm 0.492

^aThere were no significant differences between T_0 , T_3 , and $T_{4.5}$ for any of the variables studied. BMI, body mass index.

^b $n = 5$.

Study design. In a single-center observational study, patients received 120 mg orlistat three times per day for 4.5 mon in addition to dietary advice and recommendations regarding physical activity as part of an established intervention program. Patients were advised to take the medication along with each fat-containing main meal during or up to 1 h after the meal. Patients were not allowed to take any vitamin and carotenoid supplements 2 mon prior to and during the trial. They were recommended to follow a weight-reducing diet including $\leq 30\%$ of calories from fat, 10–15% from protein, and $\geq 50\%$ from carbohydrates. Patients were monitored at the monthly visits for their dietary intake. Compliance with the study medication was checked by interview and pill count. The study period was based on the usual length of coverage of costs of obesity treatment with orlistat for patients with a BMI > 30 kg/m² by the major local health care provider.

Anthropometric assessment. At T_0 , T_3 , and $T_{4.5}$ anthropometric variables including weight, height, waist and hip circumference were measured, and the waist/hip ratio was calculated. Body fat was determined using Bodystat Quadscan 4000 (Eumedics, Purkersdorf, Austria).

Dietary assessment. At T_0 patients completed a food frequency questionnaire (FFQ) for assessment of dietary habits over the past 6 mon prior to study entry. At T_3 and $T_{4.5}$ they recorded all food items and beverages consumed during the preceding 5 d. Intake of carotenoids was calculated using the food composition databases of Souci, Fachmann, and Kraut (13) and REGAL (14). Intake of energy, protein, fat, carbohydrates, and vitamins was calculated using the Austrian database Ernährungswissenschaftliches Programm (dato Denkwerkzeuge, Vienna 1997).

Analytical methods. (i) **Sample collection.** At T_0 , T_3 , and $T_{4.5}$, venous blood was drawn after an overnight fast into plastic tubes containing either EDTA or lithium heparin (BD Vacutainer, Bellerive Industrial Estate, Plymouth, United Kingdom), protected from light using aluminum foil, and centrifuged immediately at $1500 \times g$ at 8°C for 10 min. Plasma was separated, divided into aliquots, collected in Eppendorf tubes, and stored at -80°C until determination of plasma carotenoids, retinol, tocopherols, and FA. Routine clinical chemistry indices, including total cholesterol, HDL, and TG, were determined in the

fresh samples at T_0 , T_3 , and $T_{4.5}$ at the laboratory core facility of the University hospital (Clinical Institute of Medical and Chemical Laboratory Diagnostics) using a Hitachi analyzer (COPD test kit; Roche Diagnostics, Vienna, Austria). LDL was calculated using the formula of Friedewald *et al.* (15).

(ii) **Determination of carotenoids, retinol, and tocopherols.** The determination of retinol, tocopherols, and carotenoids was performed as described by Aebischer *et al.* (16). Briefly, 200 μL of EDTA plasma was diluted with deionized distilled water and deproteinized with 400 μL absolute ethanol. To extract lipophilic compounds, 800 μL of *n*-hexane/BHT (350 mg BHT in 1000 mL *n*-hexane) were added, centrifuged, and the clear supernatant transferred by a dispenser/dilutor system (Micro Laboratory 500B Dilutor; Hamilton, Martinsried, Germany) to an Eppendorf tube to be dried on a Speed Vac (Savant, Farmingdale, NY). The residue was then redissolved in a mixture of methanol/1,4-dioxane (1:1), diluted with acetonitrile, and injected into the HPLC system (Hewlett-Packard 1100A; Agilent, Vienna, Austria). Separation was achieved on a reversed-phase column; the mobile phase was a mixture of acetonitrile, THF, methanol, 1% ammonium acetate solution, and 10 mg L(+)-ascorbate, and the flow rate was 1.6 mL/min. α -Carotene, β -carotene, lutein/zeaxanthin, and β -cryptoxanthin were detected by a UV detector (JASCO, Model UV-1570; Biolab, Vienna, Austria) at 450 nm, and lycopene was detected at 472 nm; retinol and tocopherols were detected by a fluorescence detector (JASCO, Model FP-920; Biolab) at 330 nm excitation and 470 nm emission wavelength (retinol) and at 298 nm excitation and 328 nm emission wavelength (γ - and α -tocopherol). The areas under the HPLC peaks were quantified on an HP Chemstation (Hewlett-Packard 35900E; Agilent). *All-trans*- and *cis*- β -carotene were measured, and total β -carotene was calculated as the sum of *all-trans*- and *cis*- β -carotene. The tocopherol and carotenoid standards were a kind gift of the Vitamin Research Department of Hoffmann La-Roche (now DSM Nutritional Products), Basel, Switzerland. The CV within-run was 3.9% for retinol, 5.2% for γ -tocopherol, 5.0% for α -tocopherol, 5.7% for lutein/zeaxanthin, 5.4% for β -cryptoxanthin, 5.6% for lycopene, 4.7% for α -carotene, 6.0% for *cis*- β -carotene, and 5.1% for *all-trans*- β -carotene. All determinations were performed in duplicate, and all samples were processed in the same run. Six plasma samples of the patients were processed along with two control samples from a plasma pool obtained from healthy subjects to be used for long-term quality control, and along with a standard solution.

(iii) **Determination of FA.** The determination was based on an esterification procedure and a subsequent GC analysis of the FAME as described by Sattler *et al.* (17). Briefly, 100 μL of internal standard (heptadecanoic acid) was added to 450 μL of the EDTA plasma and kept at -80°C for a minimum of 30 min. The deep-frozen suspension was freeze-dried on a lyophilizer (VirTis, Gardiner, NY). Then, boron trifluoride/methanol complex and toluene were added. After transesterification at 110°C for 90 min, the FAME were extracted three times with *n*-hexane. The extracts were dried in a Speed Vac (Bachhofer, Reutlingen, Germany), redissolved in dichloromethane, and subjected to

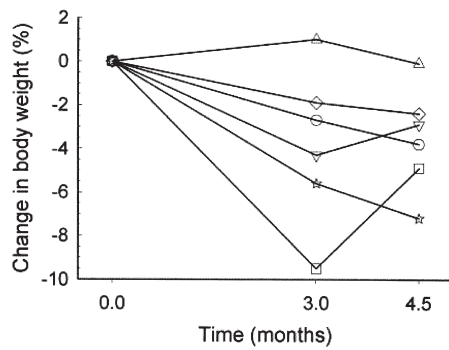


FIG. 1. Changes in body weight (% of baseline) in the individual patients during orlistat therapy.

GC analysis (Hewlett-Packard 5890 Series II; Agilent). Separation of FAME was achieved on a DB-23 column, 30 m length, 0.250 mm diameter; the mobile phase was a mixture of helium and hydrogen gas. The oven temperature at injection was 150°C and was then raised to 255°C. The areas under the GC peaks were quantified by integration, and the internal standard described above was used for calculation of the amounts of FA. CV (within-run) for the different FA were between 0.38 and 8.28%. All determinations were done in duplicate and in the same run, along with a control sample from the plasma pool.

Statistical analysis. All statistical analyses were performed on SigmaStat 3.0 (SPSS, Erkrath, Germany). Changes over time were analyzed using one-way repeated measures ANOVA. To isolate the time points that differed from the others, all-pairwise multiple comparison procedures (Tukey tests) were used.

RESULTS

Effects of intervention. All study subjects completed the 4.5-mon study period. Compliance with orlistat therapy was 100%, as checked by interview and pill count. One patient reported steatorrhea and diarrhea after she had not adhered to the dietary advice of reduced fat intake. Additional side effects were not reported.

TABLE 2
Dietary Intake^a (mean ± SD) of Carotenoids (mg/d) and Fruits and Vegetable Servings (servings/d) Estimated by FFQ (T_0) and 5-d Food Records (T_3 and $T_{4.5}$) ($n = 6$)

	FFQ T_0	5-d food records	
		T_3	$T_{4.5}$
α -Carotene	0.487 ± 0.298	0.671 ± 0.734	0.959 ± 0.881
β -Carotene	3.77 ± 2.56	3.11 ± 2.23	3.73 ± 3.40
Lycopene	3.50 ± 2.65	1.69 ± 1.25	1.38 ± 1.56
β -Cryptoxanthin	0.492 ± 0.296	0.587 ± 0.663	0.517 ± 0.868
Lutein/zeaxanthin	4.58 ± 3.91	2.64 ± 1.51	1.77 ± 1.42
Servings of fruits and vegetables per day	5.20 ± 3.61	2.77 ± 1.88	2.60 ± 1.37

^aThere were no significant differences between T_3 and $T_{4.5}$ for any of the variables studied. FFQ, food frequency questionnaire.

Anthropometry. Changes in body weight showed substantial interindividual variability. While all patients except one showed a significant weight loss during the initial 3 mon, only four of six lost weight during the following 1.5-mon of therapy (Fig. 1). Overall, changes in weight, BMI, body fat, and hip and waist circumference were not statistically significant (Table 1). Body fat could not be measured in one patient due to problems with the technical equipment (BIA analyzer; Eumetics) at T_3 and in another one due to phlebitis at $T_{4.5}$.

Dietary assessment. The study subjects showed a high percentage of energy intake from fat at T_3 (38.5 ± 5.95%) and at $T_{4.5}$ (37.3 ± 5.19%). Total calorie intake was 7729 ± 1639 kJ at T_3 and 8327 ± 2194 kJ at $T_{4.5}$. Dietary intakes of carotenoids calculated from FFQ at T_0 and from 5-d food records at T_3 and $T_{4.5}$ are presented in Table 2. There were no changes in dietary intakes of the different carotenoids during orlistat therapy, but wide ranges of consumed fruits and vegetables among the patients were observed (Table 2). There was no significant correlation between weight loss and energy intake ($P = 0.78$ at T_3 and $P = 0.26$ at $T_{4.5}$).

Plasma carotenoids. Mean plasma concentrations and standardization for cholesterol of the different carotenoids at the three time points are presented in Table 3, and changes in

TABLE 3
Changes in Plasma Carotenoid Concentrations (mean ± SD, $\mu\text{mol/L}$) During Orlistat Therapy ($n = 6$)

	Time points			P value ^a
	T_0	T_3	$T_{4.5}$	
Raw values				
α -Carotene	0.106 ± 0.058 ^b	0.081 ± 0.052	0.058 ± 0.043 ^b	0.006
β -Carotene	0.543 ± 0.310 ^{c,d}	0.392 ± 0.347 ^c	0.369 ± 0.227 ^d	0.013
Lycopene	0.362 ± 0.371	0.184 ± 0.272 ^e	0.338 ± 0.391 ^f	0.004
β -Cryptoxanthin	0.275 ± 0.123	0.215 ± 0.124	0.267 ± 0.183	NS
Lutein/zeaxanthin	0.343 ± 0.046	0.305 ± 0.091	0.341 ± 0.182	NS
Standardized values				
α -Carotene: cholesterol	0.015 ± 0.009 ^g	0.012 ± 0.008	0.008 ± 0.006 ^g	0.016
β -Carotene: cholesterol	0.072 ± 0.043 ^h	0.055 ± 0.047	0.051 ± 0.030 ^h	0.030
Lycopene: cholesterol	0.048 ± 0.050 ⁱ	0.025 ± 0.037 ^{i,j}	0.046 ± 0.051 ^j	0.005
β -Cryptoxanthin: cholesterol	0.036 ± 0.018	0.031 ± 0.018	0.037 ± 0.024	NS
Lutein/zeaxanthin: cholesterol	0.045 ± 0.005	0.043 ± 0.013	0.047 ± 0.025	NS

^aRepeated measures ANOVA.

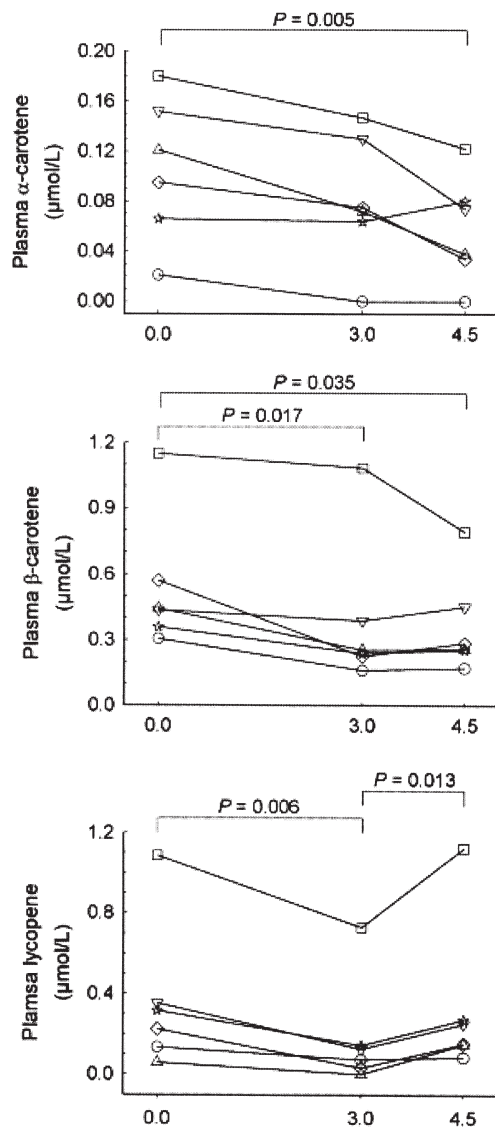


FIG. 2. Changes in plasma concentrations of α - and β -carotene and lycopene in the individual patients during orlistat therapy. P values were obtained by repeated measures ANOVA and Tukey all-pairwise multiple comparison procedures (for details see text and Table 2). Similar symbols indicate similar subjects.

plasma carotenoid concentrations of the individual patients are presented in Figure 2. Plasma α -carotene, β -carotene, and lycopene concentrations showed significant changes across the three time points (Fig. 2) (one-way repeated measures ANOVA), whereas β -cryptoxanthin and lutein/zeaxanthin did not. The Tukey all-pairwise multiple comparison procedure showed a significant decrease for α -carotene from T_0 to $T_{4.5}$ ($P = 0.005$), and for β -carotene from T_0 to T_3 ($P = 0.017$) and T_0 to $T_{4.5}$ ($P = 0.035$). Plasma lycopene concentrations decreased significantly from T_0 to T_3 ($P = 0.006$), and increased again from T_3 to $T_{4.5}$ ($P = 0.013$). Similar findings were observed for carotenoids standardized for cholesterol (Table 3). There were significant correlations between the carotenoid intakes and plasma concentrations for β -carotene at T_3 ($P = 0.04$), for ly-

copen at $T_{4.5}$ ($P = 0.02$), and for β -cryptoxanthin at T_3 ($P = 0.02$).

Serum lipids, plasma fat-soluble vitamins, and FA. Changes in total cholesterol (7.77 ± 1.18 at T_0 , 7.15 ± 0.969 at T_3 , and 7.23 ± 0.492 at $T_{4.5}$), HDL-cholesterol (1.45 ± 0.385 at T_0 , 1.42 ± 0.258 at T_3 , and 1.44 ± 0.395 at $T_{4.5}$), LDL-cholesterol (5.03 ± 0.705 at T_0 , 4.86 ± 1.20 at T_3 , and 4.64 ± 0.490 at $T_{4.5}$), and TG (2.84 ± 1.42 at T_0 , 2.89 ± 1.93 at T_3 , and 2.53 ± 0.991 at $T_{4.5}$) were not significant. Concentrations in plasma retinol (2.41 ± 1.13 at T_0 , 2.51 ± 0.801 at T_3 , and 2.29 ± 0.756 at $T_{4.5}$), α -tocopherol (37.2 ± 8.66 at T_0 , 37.6 ± 4.54 at T_3 , and 36.4 ± 6.15 at $T_{4.5}$) and γ -tocopherol (3.53 ± 2.27 at T_0 , 3.01 ± 1.10 at T_3 , and 2.62 ± 1.12 at $T_{4.5}$) were also not significant. In addition, plasma FA concentrations did not change significantly.

DISCUSSION

This is the first study showing plasma concentrations of the different hydrocarbon and oxygenated carotenoids during orlistat therapy and therefore allowing for comparison of the effects of orlistat based on the chemical structure of carotenoids.

Plasma α -carotene, β -carotene, and lycopene concentrations showed significant changes across the three time points, whereas β -cryptoxanthin and lutein/zeaxanthin did not. These results indicate that plasma concentrations of the hydrocarbon carotenoids α - and β -carotene and lycopene are more severely affected by orlistat-induced inhibition of gastrointestinal lipase activity than those of the less hydrophobic oxygenated carotenoids β -cryptoxanthin and lutein/zeaxanthin. The decrease in plasma α -carotene concentrations by 45% to $0.058 \mu\text{mol/L}$ and in plasma β -carotene by 32% to $0.369 \mu\text{mol/L}$ resulted in plasma concentrations that were about 76 and 60% lower for α -carotene and 59 and 43% lower for β -carotene compared with those of healthy Swiss (18) and Austrian subjects (19), respectively. In contrast, plasma concentrations of α - and β -carotene were comparable whereas plasma lycopene concentrations were lower during orlistat therapy in our study compared with serum concentrations of healthy subjects participating in the NHANES III (Third National Health and Nutrition Examination Survey) study (20). It seems unlikely that the changes in plasma concentrations in the present study were due to changes in intakes of α - and β -carotene, given that dietary recommendations focused on high intakes of fruits and vegetables, which are associated with higher intake of carotenoids. Unfortunately, prospective dietary records, in addition to FFQ, were not available at baseline to allow for assessment of prospective dietary intake data. There was a substantial difference in the consumption of fruits and vegetables recorded by FFQ and 5-d food records, respectively, suggesting that intakes might have been overestimated in FFQ.

Intestinal absorption of fat-soluble compounds such as carotenoids is facilitated when ingested along with dietary lipids (11). The study subjects showed a high percentage of energy intake from fat, which contrasted the recommended dietary regimen but is expected to have facilitated efficient absorption of lipophilic compounds. In the absence of changes in dietary habits and lack of significant weight loss, the decrease

in α - and β -carotene concentrations observed in our study can be explained by impaired lipase activity and subsequently reduced intestinal absorption during orlistat therapy. The significance of intestinal absorption of dietary lipids to facilitate specifically the absorption of carotenoids is further underlined by a recent study showing decreased plasma concentrations of carotenoids when olestra, an indigestible sucrose polyester containing 6–8 FA per molecule, was used to replace one-third of energy from fat. This effect could not be prevented by carotenoid supplements (21).

In the present 4.5-mon long-term study, a one-third reduction of β -carotene concentrations was observed during 360 mg/d orlistat therapy. These results are in agreement with a 6-mon long-term study showing a 22–40% reduction by using 90–720 mg/d orlistat (22). In contrast, 180 and 360 mg/d orlistat in a 2-yr long-term study did not show a significant effect (23).

Plasma concentrations of retinol were within the normal range and did not change during orlistat therapy. Both single-dosing (5,24) and long-term treatment with orlistat (22–25) did not alter plasma retinol concentrations, which can be explained by homeostatic control of plasma retinol concentrations.

The decrease in plasma α - and γ -tocopherol concentrations in the present 4.5-mon study was not significant. Other groups found impaired absorption of α -tocopherol taken along with orlistat in a single-dose test situation (5,24), whereas long-term treatment with orlistat did not alter plasma tocopherol concentrations (24,25).

In the present study, 4.5-mon orlistat therapy was not associated with statistically significant changes in plasma FA patterns, in contrast to 12-mon orlistat therapy in another study on 75 patients (7). There were also no significant changes in serum lipids during orlistat therapy. Although the cholesterol- and TG-lowering effect was smaller in our study compared with some other orlistat trials (23,26), it was comparable to those obtained in randomized placebo-controlled trials (7,27) that showed a decrease in plasma lipids that did not reach statistical significance. However, it cannot be ruled out that the findings of the present study could be due to the small number of study subjects (possible type II error), and extension of the study period might have allowed for significant changes to occur in FA patterns.

Taken together, significant changes in α - and β -carotene as well as in lycopene were observed even in the presence of relatively low weight losses of 0.1–7.2% ($3.6 \pm 2.4\%$) over 4.5 mon of orlistat therapy in the study patients compared with weight losses of 6.8–9.8% over 6 mon in other trials (20,26,28). However, there was a wide range in weight losses in the present as well as in previous studies (20,23,26,28), suggesting that some patients respond more favorably than others to orlistat (29).

Epidemiological evidence suggests that high intake of carotenoids and plasma concentrations in the upper normal range are associated with lower risk of cardiovascular disease and certain types of cancer (12). A recent study demonstrated that plasma α -carotene and γ -tocopherol concentrations partic-

ularly are lower in patients with coronary heart disease compared with healthy controls (30). Given the preventive effects of carotenoids, a decrease in plasma concentrations during orlistat therapy may add an additional risk factor for obese patients, who are at increased risk for degenerative diseases. This seems even more important in view of the fact that the study patients showed low carotenoid status already prior to orlistat therapy, which, at least in part, can be explained by unfavorable dietary habits. Dietary intake of both α - and β -carotene was considerably lower in our study patients than in nonobese healthy male subjects living in the same area (19). This is even more striking because with one exception the study patients were females, who, in general, consume a diet higher in carotenoids compared with males (31).

It has been suggested that plasma concentrations of fat-soluble vitamins should be monitored and multivitamin supplements should be taken during orlistat therapy (20,22–25). However, high intake of carotenoids from fruits and vegetables during orlistat therapy might prove even more beneficial and contribute to the overall success of orlistat therapy, given that it would avoid possible unwanted effects of β -carotene supplements on the one hand (32), and facilitate lower total calorie and fat intake on the other.

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Isabella Sundl developed the study design under the supervision of Brigitte M. Winklhofer-Roob and Hermann Toplak. Elisabeth Pail, Karin Mellitzer, and Hermann Toplak recruited and investigated the patients and collected the blood samples. Isabella Sundl carried out the analyses of the blood samples and the dietary records and performed the statistical analysis under the supervision of Brigitte M. Winklhofer-Roob. Isabella Sundl and Brigitte M. Winklhofer-Roob had primary responsibility for writing the manuscript, but all the other authors provided editorial comments on the draft. None of the authors had any conflict of interest.

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