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ORIGINAL CONTRIBUTION

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Zeaxanthin is bioavailable from genetically modified zeaxanthin-rich potatoes

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Abstract The carotenoid zeaxanthin accumulates in the human macula lutea and protects retinal cells from blue light damage. However, zeaxanthin intake from food sources is low. Increasing zeaxanthin in common foods such as potatoes by traditional plant breeding or by genetic engineering could contribute to an increased intake of this carotenoid and, consequently, to a decreased risk of age-related macular degeneration. Our aim was to investigate whether zeaxanthin from genetically modified zeaxanthin-rich potatoes is bioavailable in humans. Three men participated in this randomized, controlled double-blinded, crossover pilot study. All subjects consumed 1,100 g of mashed potatoes, either genetically modified (*Solanum tuberosum* L. var. Baltica GM47/18; 3 mg zeaxanthin) or wild-type control potatoes (*Solanum tuberosum* L. var. Baltica; 0.14 mg zeaxanthin). A second treatment was followed after a 7-day wash-out period. The concentration of zeaxanthin was significantly increased in chylomicrons after consumption of genetically modified potatoes and 0.27 mg of the 3 mg zeaxanthin dose could be detected in chylomicrons. Consumption of control potatoes had no effect on concentrations of zeaxanthin in chylomicrons. After normalization of chylomicron zeaxanthin for plasma triacylglycerol, the time course of zeaxanthin concentrations peaked at 7 h after consumption of genetically modified potatoes. There were no significant differences in the concentrations of other major potato carotenoids such as lutein and β-carotene in chylomicrons after consumption of genetically modified and wild type control potatoes. Thus, consumption of zeaxanthin-rich potatoes significantly increases chylomicron zeaxanthin concentrations suggesting that potentially such potatoes could be used as an important dietary source of zeaxanthin.

Key words zeaxanthin – bioavailability – age-related macular degeneration – potato

Introduction

A large body of evidence suggests that carotenoids exert a broad range of health effects and protect from cardiovascular disease [26], cancer [24], skin damage [23], and certain eye disease like age-related macular degeneration (AMD). AMD is the leading cause of severe and irreversible loss of vision in developed countries [10] with increasing incidence in the elderly and a

prevalence in European countries [1, 15] ranging from 1 to 12%. In Germany AMD is responsible for 50% of all cases of blindness [4]. Currently, there is no effective treatment of AMD, which underlines the importance of preventive approaches to this disease. Several risk factors for AMD have been identified, amongst them the low intake of crucial food components. In particular a diet high in carotenoid-rich fruit and vegetables has been shown to reduce the risk of AMD [7, 9, 12]. Food and vegetable-borne carotenoids like lutein and zeaxanthin selectively accumulate in the *macula lutea* of the retina and protect pigment epithelial cells from blue light damage [16]. A high dietary intake of, or supplementation with these carotenoids increased plasma concentrations and were accompanied by a reduced risk of AMD [2, 3, 13, 17, 20]. Consumption of lutein-rich spinach and collard greens reduces the risk for neovascular AMD [22] by 86%, but the supply of zeaxanthin in the general population is inadequate. Recently, a zeaxanthin-rich potato has been developed by means of genetic modification [21]. Potatoes are a staple food in many countries. The average per capita consumption of potatoes in the UK is about 111 kg/year, in Germany 78 kg and in the US about 62 kg (National Potato Council 2003). Zeaxanthin-rich potatoes may become a widely distributed and easily available source of zeaxanthin and could, at least partly, meet the specific needs of groups at high risk of developing AMD. There is little or no information on uptake of zeaxanthin from potatoes. Therefore, the aim of the present study was to investigate whether zeaxanthin from these genetically modified and zeaxanthin-rich potatoes is incorporated into chylomicrons, as a measure of bioavailability in humans.

Subjects and methods

Subjects

For this pilot study four healthy men were recruited by advertisement at local universities and other institutions. Exclusion criteria were smoking, use of supplements or medication, and gastrointestinal diseases. All subjects were in good medical health as was determined by a screening history and medical examination. Subject characteristics are given in Table 1. The study was approved by the Medical Ethical Committee of the Landesärztekammer Baden-Württemberg and all participants gave their consent in writing.

Study design

The study was a randomized controlled double-blinded intervention study and was performed at the Human Nutrition Ward of the Federal Research Centre for Nutrition and Food. Volunteers were provided with a list of zeaxanthin-rich fruits and vegetables, which they had to avoid for 2 weeks prior to the study and for its entire duration. The study consisted of two experimental days (experiments 1 and 2) separated by a 1-week wash out period. On the experimental days, after an overnight fast, an indwelling catheter was placed into an antecubital vein for multiple blood samplings. Before and then 2, 4, 7 and 10 h after consuming the experimental diet, blood was drawn for analysis. The experimental diet consisted of mashed potatoes only (1,100 g) either from genetically modified potatoes (GM) or wild type as control (Co). On exp1 each volunteer received a single dose of GM (1,100 g, equivalent to 3 mg zeaxanthin) or Co (1,100 g, 0.14 mg zeaxanthin). After a one-week wash out on a low zeaxanthin diet the intervention was repeated (experiment 2), but this time each volunteer consumed the other experimental diet and served as its own control. One subject did not complete experiment 1 and was excluded from the study. In the final analysis data from three participants are presented.

Table 1 Baseline characteristics of study participants

Subject	Age (years)	BMI (kg/m^2)	Plasma TG* (mmol/l)	Plasma glucose (mmol/l)
# 1	25	27.0	2.3	6.2
# 2	33	24.8	2.0	5.1
# 3	23	24.1	1.2	5.1

TG triacylglycerol

Mashed potatoes were prepared by a dietician from blinded batches of either Co (*Solanum tuberosum* L. var. Baltica) or GM (Baltica GM47/18; 18) under identical conditions (250 ml low fat milk, 50 g butter and salt per kilo of potatoes) and portions were frozen and stored at −30°C. On the experimental days, portions were thawed and heated in a microwave oven before consumption. The carotenoid content of the mashed potatoes (frozen and heated) is given in Table 2. Mashed potatoes (100 g) provided 2.2 g protein, 3.3 g fat and 12.5 carbohydrate.

Wild type and GM potatoes were grown under standard field conditions at the experimental station "Roggenstein" of the Technical University Munich (near Fürstenfeldbruck, Germany). The GMO experiment was approved by the responsible federal and local authorities (ref. ZG 6786-01-135).

Sample preparation and analysis

Blood samples were collected in 9 mL tubes containing EDTA (Sarstedt-Monovette, Nümbrecht, Germany). Plasma was collected after centrifugation at 1,500×*g* for

Table 2 Major carotenoids in mashed potatoes

	Control	GM 47/18[a]
µg/100 g[b]		
Lutein	66.1	67.1
Zeaxanthin	12.9	270
β-Carotene	13.3	9.9
µg/meal		
Lutein	727	738
Zeaxanthin	141	2970
β-Carotene	146	108

[a]GM 47/18: genetically modified potatoes based on the wildtype *Solanum tuberosum* L. var. Baltica (control)
[b]values are means from three independent determinations

10 min at 4°C. Chylomicrons were prepared by overlaying 2 ml plasma with 2 ml NaCl 0.9% after ultracentrifugation (100,000×*g* at 10°C for 40 min). Triacylglycerol concentrations in plasma were analysed enzymatically (Roche, Mannheim, Germany).

The carotenoid content in mashed potatoes, plasma and chylomicrons were detected by HPLC as described by Briviba et al. [6]. Carotenoids from mashed potatoes were extracted in an ultra-turrax by hexane/dichloromethane (5/1, V/V with 250 mg/l BHT) at least three times until the last extract was colourless. Carotenoids from plasma and chylomicrons were extracted according to Briviba et al. [6].

Statistics and data analysis

All data are presented as means and standard deviation. Areas under the curve (AUC; 0–10 h) were determined by the trapezoidal rule after subtracting carotenoid concentration at t_0. Group differences for carotenoid AUC were tested by paired *t* test. The fractional absorption (F) of zeaxanthin was estimated by dividing the mean AUC for zeaxanthin in chylomicrons after the test meal by a calculated AUC after a hypothetical intravenous dose of the same size according to van Vliet et al. [25] and Hu et al. [14]. The AUC i.v. calc. after an intravenous dose was calculated using equation: $\mathrm{AUC\,i.v.calc.} = \mathrm{Does}/\left(V \times \ln 2/t_{1/2}\right)$ [14], where it was assumed that elimination of zeaxanthin occurs by first order kinetics, the half-lives ($t_{1/2}$) of zeaxanthin is equivalent to that of chylomicrons: 0.192 h; plasma volume (V) was calculated according to V (liter) = 0.041 × body weight in kg + 1.53; dose of zeaxanthin in nmol (3 mg = 5,272 nmol); $k = \ln\ 2/t_{1/2}$, k is the first order elimination rate constant.

Results

Three volunteers successfully finished the study. None complained of any discomfort or health problems due to potato consumption. Plasma TG time course did not differ between the two potato preparations (Fig. 1). Zeaxanthin was detected in human chylomicrons after consumption of genetically modified (GM) potatoes with increased zeaxanthin content (Fig. 2). Adjusting chylomicron zeaxanthin for plasma TG reduces variability [25] and time course of zeaxanthin peaked at 7 h after consumption of GM mashed potatoes, while the control (Co) did not change from baseline (Fig. 3). Other major potato carotenoids such as lutein and β-carotene were also detectable in chylomicrons (Table 3) after potato (GM, Co) consumption, but there was no net increase in lutein or β-carotene as determined by the area

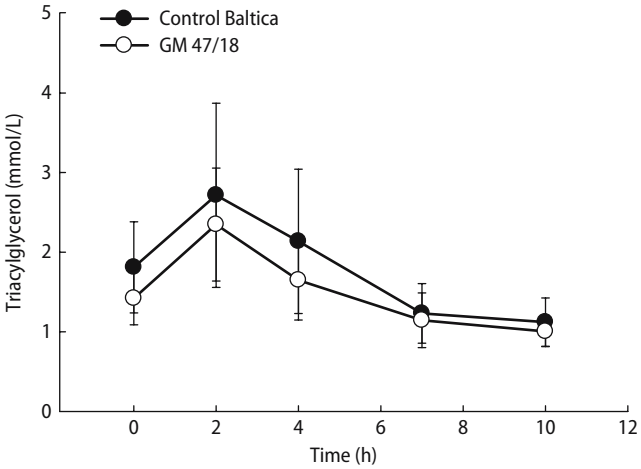

Fig. 1 Time course of plasma triacylglycerol after consumption of mashed potatoes prepared from either control Baltica potato (*filled circles*) or zeaxanthin-rich genetically modified GM 47/18 potato (*open circles*) (means ± SD; $n = 3$)

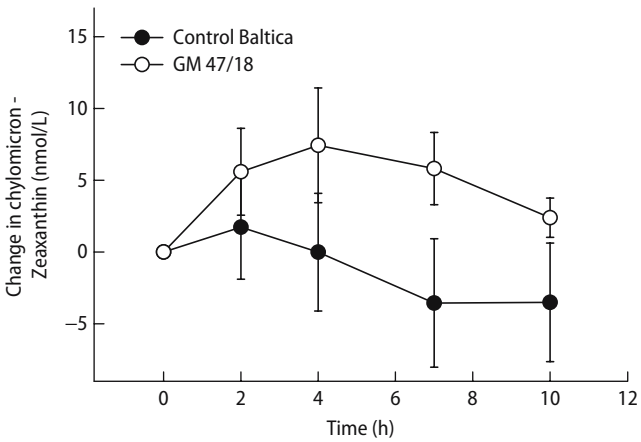

Fig. 2 Time course of relative zeaxanthin uptake in chylomicrons (change from baseline) after consumption of mashed potatoes prepared from either control Baltica potato (*filled circles*) or zeaxanthin-rich genetically modified GM 47/18 potato (*open circles*) (means ± SD; $n = 3$)

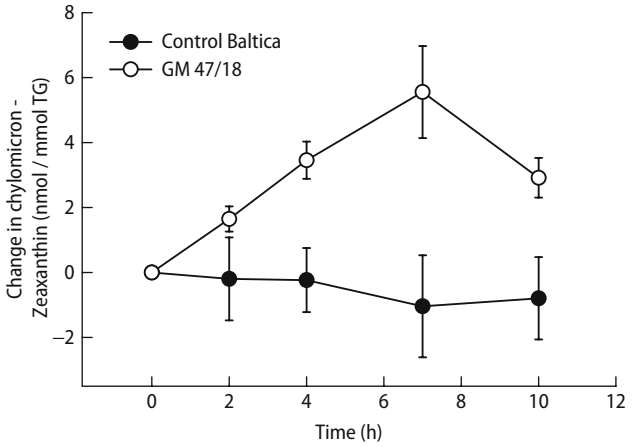

Fig. 3 Change in chylomicron zeaxanthin concentration adjusted for plasma triacylglycerol after consumption of mashed potatoes prepared from either control Baltica potato (*filled circles*) or zeaxanthin-rich genetically modified GM 47/18 potato (*open circles*) (means ± SD; $n = 3$)

under the chylomicron concentration versus time curve (AUC). The calculated fraction (F) of the zeaxanthin dose absorbed, ranged from 5.30 to 13.66%. The estimated average absorption efficiency was calculated to be 8.95 ± 4.28% or 0.27 ± 0.13 mg zeaxanthin.

Discussion

After successfully increasing the zeaxanthin content of potatoes by means of genetic engineering [21] we could show that zeaxanthin from GM potatoes is bioavailable in humans. Zeaxanthin was detected in chylomicrons after a single ingestion of mashed potatoes prepared under household conditions. Intestinal absorption of carotenoids involves the assembly of chylomicrons in the gut and transport via the lymph system into the circulation. Therefore, chylomicron composition and carotenoid content mainly reflect the uptake from the gut before entering the liver [8].

The time course of chylomicron zeaxanthin in our study is comparable to previous reports [5, 11, 19] although the type of zeaxanthin preparation (carotenoid mixture from algae—paprika oleoresin—milk based wolfberry formulation) and applied dose (approximately 1.8–5.3–15 mg) differ widely. The peak change in chylomicron zeaxanthin after GM consumption was slightly higher (7.5 nmol/l) compared to approximately 5.5 nmol/l after 5.3 mg zeaxanthin from paprika oleoresin [19] and 1.72 nmol/l after consumption of 15 mg zeaxanthin from a milk based wolfberry formulation [5]. In our study, the absorption efficiency was 8.95 ± 4.28% or 0.27 ± 0.13 mg. Taking into account a small number of subjects, several assumption used for the calculation and a relatively low dose of zeaxanthin (3 mg) the absorption efficiency estimated in this work should be interpreted carefully. However, the absorption efficacy of zeaxanthin in this work is comparable to that reported in the literature for β-carotene using water-soluble beadlets (11–17%), beadlets with a meal rich in fat (10.9–17.3%) and a palm oil extract (2.5–3.9%) [14, 18, 25].

In conclusion, zeaxanthin from GM potatoes is effectively absorbed from the human gut and incorporated into chylomicrons. Zeaxanthin-rich potatoes are potentially an easily accessible dietary source of zeaxanthin. Further studies are necessary to clarify whether repeated ingestions of zeaxanthin-rich potatoes lead to an accumulation of zeaxanthin in plasma and in the macula and therefore contribute to AMD risk reduction.

Table 3 Carotenoid concentrations in plasma and chylomicrons and area under the concentration-versus-time curve ($AUC_{(0-10\ h)}$) values for carotenoids after ingestion of mashed control and genetically modified (GM 47/18) potatoes (mean values and standard deviation, $n = 3$)

	Time (h)	Zeaxanthin				Lutein				β-Carotene			
		Control		GM 47/18[a]		Control		GM 47/18		Control		GM 47/18	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	mean	SD	mean	SD
Plasma (nmol/l)	0	90	32	62	38	306	102	296	187	381	94	444	188
Chylomicron (nmol/l)	0	6	6	2	2	18	17	12	2	17	8	11	8
	2	8	4	8	4	24	12	20	16	20	3	15	8
	4	6	4	10	3	19	9	14	9	16	3	12	5
	7	3	1	8	2	9	4	9	3	9	1	8	1
	10	3	2	5	2	8	5	8	5	8	0	8	4
$AUC_{(0-10\ h)}$ (nmol × h/mmol l)[b]	0–10	−12	35	51[c]	24	−34	97	−1	45	−34	63	−5	38

[a]GM 47/18: genetically modified potatoes based on the wild-type *Solanum tuberosum* L. var. Baltica (control)
[b]The time course was followed for 10 h: $AUC_{(0-10\ h)}$
[c]Significantly different from control ($P < 0.01$)

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